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Research-in-Progress

Reviews

Nutrition-related needs and considerations in the transgender and gender non-conforming (TGNC) population: Current gaps and future directions in research

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Abstract

Transgender and gender non-conforming (TGNC) individuals face significant health disparities despite growing visibility and awareness. Nutrition-related disparities are particularly concerning, with TGNC individuals experiencing higher rates of food insecurity, eating disorders, body dissatisfaction, and overweight or obesity. Gender-affirming hormone therapy (GAHT) and other medical interventions lead to significant physiological changes that can influence nutritional needs, as well as body composition and bone mineral density, yet existing daily energy estimation equations do not account for TGNC individuals or those undergoing GAHT. Perioperative nutrition is also vital, as gender-affirming surgeries increase metabolic demands and risk of muscle loss due to immobility and catabolism. Moreover, TGNC individuals report higher rates of food insecurity, with transgender men being particularly affected, largely due to economic disparities and systemic discrimination. Eating disorders are likely under reported in this population due to limited access to competent, inclusive care. Most research combines transgender and gender non-conforming individuals, limiting insight into subgroup differences. To improve health outcomes among TGNC individuals, there is a critical need for more inclusive, longitudinal research, particularly in the areas of nutrition, metabolism, and post-surgical care. Such research could inform the development of tailored interventions, enhance healthcare provider competence, and support the creation of clinical guidelines that address the specific health and nutrition needs of TGNC individuals. Ultimately, this would help reduce disparities and promote long-term well-being for TGNC individuals.

Key words: nutrition assessment, healthcare disparities, health inequities, transgender persons, nutrition requirements

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Highlights

- Transgender and/or gender non-conforming (TGNC) individuals are underrepresented in research, and experience several significant health disparities due to lack of clinician knowledge and bias
- One such key gap in knowledge are nutrition-related needs and considerations for the TGNC population, such as changes in body composition, changes to basal metabolism and daily energy needs, changes to bone density, and increased risks for eating disorders and food insecurity
- Greater research and clinical exploration into these areas will allow for these gaps in knowledge and practice to be addressed, and to establish best practices when working with TGNC patients and populations

Introduction

Transgender and/or gender non-conforming (TGNC) individuals are those people whose gender identity does not conform from the sex that was assigned to them at birth. Within the USA, it is estimated that there are over 1 million adults identify as TGNC, with some estimates suggesting that the number could be closer to 1.4 million.[1–3] Although awareness and visibility of TGNC individuals continue to improve, significant health disparities and inequities persist, placing an ongoing burden on members of the TGNC community. These disparities exist across a wide spectrum of health outcomes and risk factors, including refusal or denial of care, gaps in provider knowledge and competence related to TGNC health needs and culture, and higher rates of bias and stigma when interacting with healthcare providers.[4] Despite having relatively similar levels of health insurance coverage compared to cisgender individuals (those whose gender identity aligns with their sex assigned at birth), TGNC individuals are still more likely to delay or avoid interactions with the healthcare system due to fears of stigma and bias.[5] An area in which there is often a lack of awareness and competence is the unique food and nutrition needs and considerations for TGNC people. The literature shows that TGNC experience disproportionate levels of eating disorders, body dissatisfaction, food insecurity, overweight and obese weight status, and limited overall access to nutrition-related services.[6,7] Additionally, significant gaps remain in understanding how gender-affirming hormone therapy (GAHT) impacts nutritional and metabolic needs, bone health, overall body composition, and perioperative nutrition support for individuals undergoing gender-affirming surgical interventions.

Objectives

The goal of this editorial is to synthesize and share findings and insights regarding what is shown in the current literature regarding these specific nutrition-related needs for supporting TGNC health and wellness, as well as identifying causes of nutrition and health-related disparities. Furthermore, it is our goal to identify gaps in knowledge that can be used to inform and guide future research to reduce these disparities.

Impacts on body composition, bone density and daily energy requirements

Research and practice related to the healthcare needs and considerations of TGNC patients represent an ever-expanding and evolving field. One emerging area of study is the impact of hormone replacement therapy (HRT) on body composition, metabolism and daily nutritional needs.

Changes to skeletal muscle mass and adipose tissue

Research shows that patients undergoing testosterone therapy experience an increase in lean body mass and skeletal muscle mass, along with a decrease in adipose (fat) tissue. Conversely, those undergoing estrogen therapy (with or without antiandrogen agents) tend to experience a decrease in lean body and skeletal muscle mass, accompanied by an increase in adipose tissue.[8] When beginning GAHT, efforts have been made to understand the extent to which skeletal muscle mass is altered. For those using masculinizing GAHT, lean skeletal muscle mass has been shown to increase by approx. 10% over the first year, as well as a decrease in fat mass by about 10%,[9–13] and an increase in skeletal muscle mass of 19% over the course of 3 years of using GAHT.[14] For those using feminizing GAHT, longitudinal cohort studies have shown that lean skeletal muscle mass has a moderate decrease in the first year of therapy of approx. 3–5%,[9,11,13,15–18] with an accompanying increase in body fat mass of approx. 28%.[11] With these alterations in skeletal muscle mass and adipose tissue mass, there is also the potential for an increased risk for overweight and obese status. Based on national survey data of 2,700 LGBTQIA+ people, 65.9% of transgender men were overweight or obese, with transgender men being

the group most likely to be obese within the lesbian, gay, bisexual, transgender, and queer (LGBTQ) community.[19] The increased rates of overweight and obesity among TGNC individuals may result not only from the physiological changes associated with GAHT, but also from the interaction of GAHT with minority stressors and barriers to healthcare access, both of which can further elevate the risk of developing overweight or obese weight status.[20]

Changes to bone density

Sex hormones play several important roles within the body, including bone modeling and bone remodeling. In adults utilizing GAHT, the impact of this form of gender-affirming therapy on bone health and mineralization is more mixed and uncertain. Some studies have associated GAHT in transgender women with an increase in bone mineral density (BMD).[21] However, several systematic reviews and meta-analyses have reported inconsistent findings, particularly regarding changes in bone mineral density in transgender women.[22,23] Additionally, potential changes to BMD cannot be solely attributed to the physiologic impact of GAHT, as changes in lean muscle mass and adipose tissue levels (and associated total body weight), as well as any potential changes in physical activity levels that an individual may undergo, will also impact BMD levels. An additional important area to note is that there remains a gap in long-term research on fracture risk in older transgender adults, so the exact potential impact of GAHT on BMD, osteoporosis and fracture risk remains unknown.

Estimating energy requirements

When it comes to estimating energy requirements for individuals, healthcare professionals will often rely on standard equations such as Harris–Benedict, Mifflin–St Jeor, FAO/WHO/UNU, and Owen. These equations have recently been updated and have demonstrated greater accuracy compared to earlier versions.[24] A significant limitation of these equations, however, is that they are based on sex assigned at birth (i.e., Male Equation and Female Equation), making them potentially inadequate and less applicable for TGNC or intersex individuals, particularly those undergoing gender-affirming hormone therapy (GAHT) as part of their care. To date, there are no standard equations, or adjustments to current basal metabolic rate formulas, that are recommended for use in the TGNC or intersex communities. Therefore, until equations or formulas specifically recommended for TGNC individuals are developed, it is advisable to use calorie (kcal) estimations based on body weight (kg). Some research has suggested that daily energy needs can also be influenced by the length of time receiving GAHT. In a study by Frenser and Fischer,[25] it was noted that the body composition of transgender women and men after 1 year of GAHT was within the middle/mean equivalent ranges for cisgender women and men.

As a result, individuals undergoing GAHT could, after 1 year of therapy, be advised to follow energy intake recommendations that align more closely with the midpoint of the existing reference values for caloric needs established for cisgender women and men during this initial period.

Perioperative nutrition support

For those who seek out gender-affirming surgeries, there are significant considerations related to diet and nutrition to support and aid in the body's healing process, potential changes to digestion and absorption, and to prevent potential complications. Nutritional status is a powerful predictor of postoperative outcomes and receiving proper dietary support and education pre- and postoperatively is critical. It has been estimated that anywhere between 24% and 65% of surgical patients are malnourished or at risk for malnourishment.[26] During surgery, there is an upregulation of cortisol, glucagon and proinflammatory cytokines that stimulate catabolism (breakdown) of liver and muscle glycogen stores to meet the energy demands of wound healing.[27] This process interferes with insulin secretion, which can lead to an increase in blood glucose levels, which can last for several hours, days or even several weeks.[28] Additionally, there is an elevation in protein catabolism postoperatively. Protein synthesis is reduced due to increased levels of cortisol, which leads to an impairment in the availability of amino acids to be used for gluconeogenesis, wound healing, and immune functioning.[28] Coupled with immobility that may be experienced postoperatively (depending on the kind of surgical procedure(s) used), this can lead to a significant loss of muscle mass, which can begin in as little as 48-h of post-surgical inactivity.[28,29]

Eating disorders and body dissatisfaction

Body dissatisfaction in TGNC individuals is complicated because body dissatisfaction can often be related to the difference between gender identity and sex assigned at birth. With gender-affirming care, however, body dissatisfaction and disordered eating behaviors appear to decrease. The actual rates of disordered eating and clinical diagnoses of eating disorders in this population are likely underreported, due to barriers such as limited access to care, avoidance of healthcare settings, discrimination, and a lack of competent providers. It is estimated that approx. 10% of transgender men and 8% of transgender women have been diagnosed with either anorexia nervosa or bulimia nervosa.[30] These figures reflect only confirmed and diagnosed cases; therefore, the actual prevalence of eating disorders and body dissatisfaction within the TGNC population is likely higher. Published research indicates that trans men report rates of binge eating and fasting as high as 35%.[31] In a small sample of transgender women, dietary restraint was found to be common, reported by just

over 25% of participants, while binge eating and excessive exercise were reported at lower rates of 13% and 8%, respectively.[30] It is important to note that most of the currently available research focuses on youth and young adults, while significantly less data are available on older TGNC individuals. It is also important to note that much of the available research groups transgender and gender non-conforming individuals together, making it difficult to distinguish differences in disordered eating behaviors between these 2 populations.

Food insecurity

Within the larger LGBTQ population, individuals are more likely to experience food insecurity than heterosexual and cisgender individuals. Utilization of the Supplemental Nutrition Assistance Program (SNAP) is higher among LGBTQ individuals than non-LGBTQ individuals, further highlighting the reality of food insecurity among this population. However, TGNC individuals experience food insecurity at especially higher levels. Almost 28% of transgender adults report food insecurity,[32] with approx. 65% of transgender men in particular experiencing food insecurity.[33] One potential contributing factor to the disproportionate rates of food insecurity among LGBTQ individuals is economic disadvantage, as this population experiences higher rates of poverty compared to the general population. A study using data from the Behavioral Risk Factor Surveillance System in the United States found that LGBTQ people experience poverty rates reaching over 21%, while heterosexual and cisgender people experience poverty at rates closer to 15%.[34] These poverty rates are especially high among transgender people and bisexual women, who have poverty rates over 29%.[34]

Conclusion

Transgender and gender non-conforming individuals experience several health disparities rooted in social stigma, bias, lack of provider training and competence, and significant gaps within the published research. While gender affirming care utilizing hormone replacement therapy and surgical interventions have been shown to be safe, there is limited long-term data on how these procedures impact basal metabolic rate, overall daily energy needs, and macronutrient and micronutrient needs due to changes in body composition. Additionally, there remains inconsistencies in how gender-affirming care can impact bone mineral density and skeletal health over time, as well as how nutritional considerations such as calcium, vitamin D, protein, daily energy needs, as well as potential impacts on nutrient absorption, may play a potential role. Researchers working both with TGNC populations, as well as those in metabolic research, can play a significant role in addressing these gaps in knowledge by developing prospective studies in TGNC who are beginning to utilize gender-affirming medical therapies and monitor changes in metabolism, nutrition status and nutrient needs over time. This kind of research could aid in the development of perioperative nutrition education, guidelines and support systems to reduce the risk for surgical complications and promote overall health and wellbeing. Additionally, most research on eating disorders and food security does not adequately include TGNC individuals. More purposeful research and sampling of this population may not only yield greater insight into the true burden of eating disorders and food insecurity within the TGNC population but also help to generate best practices in identifying and remedying these nutritional risk factors over time.

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References

1. Flores A, Herman J, Gates G, Brown T. How many adults identify as transgender in the United States? Los Angeles, USA: Williams Institute; 2022. https://williamsinstitute.law.ucla.edu/wp-content/uploads/Trans-Pop-Update-Jun-2022.pdf. Accessed April 10, 2025.
2. Herman J, Flores A, Brown T, Wilson B, Conron K. Age of individuals who identify as transgender in the United States. Los Angeles, USA: Williams Institute; 2017. https://williamsinstitute.law.ucla.edu/wp-content/uploads/Age-Trans-Individuals-Jan-2017.pdf. Accessed April 10, 2025.
3. Meerwijk EL, Sevelius JM. Transgender population size in the United States: A meta-regression of population-based probability samples. *Am J Public Health*. 2017;107(2):e1–e8. doi:10.2105/AJPH.2016.303578
4. James SE, Herman JL, Rankin S, Keisling M, Mottet L, Anafi M. *The Report of the 2015 U.S. Transgender Survey*. Washington, D.C, USA: National Center for Transgender Equality; 2016. https://transequality.org/sites/default/files/docs/usts/USTS-Full-Report-Dec17.pdf. Accessed April 10, 2025.
5. Feldman JL, Luhur WE, Herman JL, Poteat T, Meyer IH. Health and health care access in the US transgender population health (TransPop) survey. *Andrology*. 2021;9(6):1707–1718. doi:10.1111/andr.13052
6. Ferrero EM, Yunker AG, Cuffe S, et al. Nutrition and health in the lesbian, gay, bisexual, transgender, queer/questioning community: A narrative review. *Adv Nutr*. 2023;14(6):1297–1306. doi:10.1016/j.advnut.2023.07.009
7. Mittertreiner EJE, Hunter A, Lacroix E. Nutritional considerations for gender-diverse people: A qualitative mini review. *Front Nutr*. 2024;11:1332953. doi:10.3389/fnut.2024.1332953
8. Spanos C, Bretherton I, Zajac JD, Cheung AS. Effects of gender-affirming hormone therapy on insulin resistance and body composition in transgender individuals: A systematic review. *World J Diabetes*. 2020;11(3):66–77. doi:10.4239/wjd.v11.i3.66
9. Auer MK, Ebert T, Pietzner M, et al. Effects of sex hormone treatment on the metabolic syndrome in transgender individuals: Focus on metabolic cytokines. *J Clin Endocrinol Metab*. 2018;103(2):790–802. doi:10.1210/jc.2017-01559
10. Pelusi C, Costantino A, Martelli V, et al. Effects of three different testosterone formulations in female-to-male transsexual persons. *J Sex Med*. 2014;11(12):3002–3011. doi:10.1111/jsm.12698
11. Klaver M, De Blok CJM, Wiepjes CM, et al. Changes in regional body fat, lean body mass and body shape in trans persons using cross-sex hormonal therapy: Results from a multicenter prospective study. *Eur J Endocrinol*. 2018;178(2):163–171. doi:10.1530/EJE-17-0496
12. Tack LJW, Craen M, Lapauw B, et al. Proandrogenic and antiandrogenic progestins in transgender youth: Differential effects on body composition and bone metabolism. *J Clin Endocrinol Metab*. 2018;103(6):2147–2156. doi:10.1210/jc.2017-02316

13. Van Caenegem E, Wierckx K, Taes Y, et al. Body composition, bone turnover, and bone mass in trans men during testosterone treatment: 1-year follow-up data from a prospective case-controlled study (ENIGI). *Eur J Endocrinol*. 2015;172(2):163–171. doi:10.1530/EJE-14-0586
14. Gooren LJG, Bunck MCM. Transsexuals and competitive sports. *Eur J Endocrinol*. 2004;151(4):425–429. doi:10.1530/eje.0.1510425
15. Mueller A, Zollver H, Kronawitter D, et al. Body composition and bone mineral density in male-to-female transsexuals during cross-sex hormone therapy using gonadotrophin-releasing hormone agonist. *Exp Clin Endocrinol Diabetes*. 2011;119(2):95–100. doi:10.1055/s-0030-1255074
16. Gava G, Cerpolini S, Martelli V, Battista G, Seracchioli R, Meriggiola MC. Cyproterone acetate vs leuprolide acetate in combination with transdermal oestradiol in transwomen: A comparison of safety and effectiveness. *Clin Endocrinol (Oxf)*. 2016;85(2):239–246. doi:10.1111/cen.13050
17. Wierckx K, Van Caenegem E, Schreiner T, et al. Cross-sex hormone therapy in trans persons is safe and effective at short-time follow-up: Results from the European network for the investigation of gender incongruence. *J Sex Med*. 2014;11(8):1999–2011. doi:10.1111/jsm.12571
18. Yun Y, Kim D, Lee ES. Effect of cross-sex hormones on body composition, bone mineral density, and muscle strength in trans women. *J Bone Metab*. 2021;28(1):59–66. doi:10.11005/jbm.2021.28.1.59
19. Warren JC, Smalley KB, Barefoot KN. Differences in psychosocial predictors of obesity among LGBT subgroups. *LGBT Health*. 2016;3(4):283–291. doi:10.1089/lgbt.2015.0076
20. Linsenmeyer W, Drallmeier T, Thomure M. Towards gender-affirming nutrition assessment: A case series of adult transgender men with distinct nutrition considerations. *Nutr J*. 2020;19(1):74. doi:10.1186/s12937-020-00590-4
21. Jackson RD, Wactawski-Wende J, LaCroix AZ, et al. Effects of conjugated equine estrogen on risk of fractures and BMD in postmenopausal women with hysterectomy: Results from the Women's Health Initiative randomized trial. *J Bone Miner Res*. 2006;21(6):817–828. doi:10.1359/jbmr.060312
22. Delgado-Ruiz R, Swanson P, Romanos G. Systematic review of the long-term effects of transgender hormone therapy on bone markers and bone mineral density and their potential effects in implant therapy. *J Clin Med*. 2019;8(6):784. doi:10.3390/jcm8060784
23. Fighera TM, Ziegelmann PK, Rasia da Silva T, Spritzer PM. Bone mass effects of cross-sex hormone therapy in transgender people: Updated systematic review and meta-analysis. *J Endocr Soc*. 2019;3(5):943–964. doi:10.1210/js.2018-00413
24. Pavlidou E, Papadopoulou SK, Seroglou K, Giaginis C. Revised Harris–Benedict equation: New human resting metabolic rate equation. *Metabolites*. 2023;13(2):189. doi:10.3390/metabo13020189
25. Frenser M, Fischer T. Changes in body composition and nutritional requirements of transgender people undergoing gender-affirming hormone therapy. *Ernahrungs Umschau*. 2024;71(6):52–59. doi:10.4455/eu.2024.016
26. Thomas MN, Kufeldt J, Kisser U, et al. Effects of malnutrition on complication rates, length of hospital stay, and revenue in elective surgical patients in the G-DRG-system. *Nutrition*. 2016;32(2):249–254. doi:10.1016/j.nut.2015.08.021
27. Hirsch KR, Wolfe RR, Ferrando AA. Pre- and post-surgical nutrition for preservation of muscle mass, strength, and functionality following orthopedic surgery. *Nutrients*. 2021;13(5):1675. doi:10.3390/nu13051675
28. Gillis C, Carli F. Promoting perioperative metabolic and nutritional care. *Anesthesiology*. 2015;123(6):1455–1472. doi:10.1097/ALN.0000000000000795
29. Kilroe SP, Fulford J, Jackman SR, Van Loon LJC, Wall BT. Temporal muscle-specific disuse atrophy during one week of leg immobilization. *Med Sci Sports Exerc*. 2020;52(4):944–954. doi:10.1249/MSS.0000000000002200
30. Nagata JM, Murray SB, Compte EJ, et al. Community norms for the Eating Disorder Examination Questionnaire (EDE-Q) among transgender men and women. *Eat Behav*. 2020;37:101381. doi:10.1016/j.eatbeh.2020.101381
31. Watson RJ, Veale JF, Saewyc EM. Disordered eating behaviors among transgender youth: Probability profiles from risk and protective factors. *Int J Eat Disord*. 2017;50(5):515–522. doi:10.1002/eat.22627
32. Beaudoin S. Addressing LGBTQIA+ hunger, poverty, and discrimination. Washington D.C., USA: Food Research & Action Center; 2023. https://frac.org/blog/lgbtqia-hunger-2023. Accessed April 10, 2025.
33. Leslie IS, Carson J, Bruce A. LGBTQ+ food insufficiency in New England. *Agric Hum Values*. 2023;40(3):1039–1054. doi:10.1007/s10460-022-10403-5
34. Lampe NM, Barbee H, Tran NM, Bastow S, McKay T. Health disparities among lesbian, gay, bisexual, transgender, and queer older adults: A structural competency approach. *Int J Aging Hum Dev*. 2024;98(1):39–55. doi:10.1177/00914150231171838

Revolutionizing cancer treatment: Navigating the intricate landscape of cellular signaling networks

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Abstract

Cancer progression and therapeutic resistance are propelled by the remarkable plasticity of signaling networks, which dynamically rewire under selective pressures to maintain proliferation, enable immune evasion and promote metastasis. Despite advances in precision oncology, the dynamic crosstalk between tumor cells, non-coding genomes and the microenvironment continues to undermine treatment efficacy. This call for submissions, *Revolutionizing Cancer Treatment: Navigating the Intricate Landscape of Cellular Signaling Networks*, seeks cutting-edge research that dissects these adaptive mechanisms through innovative technologies – from single-cell multi-omics and spatial transcriptomics to AI-powered network modeling. We welcome studies leveraging physiomimetic models (e.g., organoids, 3D-bioprinted ecosystems) to decode tumor heterogeneity, as well as translational work targeting emergent vulnerabilities at the intersection of epigenetics, metabolic reprogramming and stromal interactions. By integrating systems biology with computational and experimental approaches, this collection aims to catalyze the design of adaptive therapies that outmaneuver cancer's evolutionary resilience.

Key words: cancer treatment, cellular signaling networks, biomarkers

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Highlights

- Cancer-signaling networks and therapy resistance: Mapping pathway redundancy and tumor-microenvironment cross-talk pinpoints how cancers evade targeted treatments.
- Multi-omics + AI + spatial biology: Integrating genomics, proteomics, and deep-learning analytics deciphers tumor heterogeneity and predicts patient-specific drug response.
- Organoid and 3D-bioprinted tumor models: Next-gen preclinical platforms deliver physiologic testing grounds for network-targeted therapies – accelerating bench-to-bedside translation.
- Non-coding RNAs, epigenetic reprogramming, and TME dynamics: Emerging regulators that drive metastasis and therapy escape reveal fresh intervention points.
- Systems-biology and computational oncology convergence: Interdisciplinary modeling designs adaptive, precision cancer treatments that keep pace with tumor evolution.

Introduction

Cancer, a disease of profound complexity, arises from the dysregulation of cellular signaling networks that orchestrate cell fate, immune evasion and metastatic dissemination.[1] While chemotherapy, immunotherapy and targeted therapies have transformed oncology, the adaptability of tumors – fueled by redundant pathways, microenvironmental interactions and evolutionary pressures – remains a formidable barrier. This call for submissions, *Revolutionizing Cancer Treatment: Navigating the Intricate Landscape of Cellular Signaling Networks*, invites the scientific community to confront the complex challenges of cancer research through a systems-level lens – bridging molecular discovery with translational innovation.

The double-edged sword of signaling complexity: From pathways to networks

Carcinogenesis is not merely the result of driver mutations, but rather a systemic collapse of signaling homeostasis. Canonical pathways such as PI3K/AKT/mTOR,[2] RAS/MAPK,[3] and Wnt/β-catenin[4] are frequently co-opted by tumors; however, their extensive crosstalk and functional redundancy often create escape routes that undermine therapeutic efficacy. For example, BRAF (B-Raf proto-oncogene, serine/threonine kinase) inhibitors in melanoma initially shrink tumors, only to see resistance emerge via NRAS (neuroblastoma RAS viral oncogene homolog) mutations or EGFR (epidermal growth factor receptor)-driven rewiring.[5] Similarly, EGFR-targeted therapies in lung cancer fail when tumors activate AXL (AXL receptor tyrosine kinase) or MET (MET proto-oncogene, receptor tyrosine kinase) signaling.[6,7] Even immunotherapy, which reinvigorates cytotoxic T cells by blocking PD-1 (programmed cell death protein 1)/PD-L1 (programmed death-ligand 1), is thwarted by compensatory immunosuppressive networks involving TGF-β (transforming growth factor beta), adenosine or regulatory T cells within the tumor microenvironment (TME).[8] The TME itself acts as a dynamic signaling hub. Cancer-associated fibroblasts (CAFs) secrete growth factors like FGF (fibroblast growth factor) and VEGF (vascular endothelial growth factor),[9] while tumor-associated macrophages (TAMs) release interleukin 10 (IL-10) and CCL22,[10] creating a pro-metastatic niche. Recent studies reveal that extracellular vesicles (EVs) shuttle oncogenic miRNAs (e.g., miR-122) between tumor and stromal cells, further entrenching resistance.[11] These interactions underscore the need to map signaling networks as adaptive circuits rather than static pathways.

Bridging the gap: From multi-omic insights to AI, spatial biology and beyond

Advances in multi-omics – proteogenomics,[12] spatial transcriptomics[13] and metabolomics[14] – have unmasked tumor heterogeneity and plasticity.[15] Single-cell RNA sequencing has identified rare subpopulations with enhanced tumor metastasis ability.[16] Furthermore, computational models now simulate network responses to perturbations, predicting resistance mechanisms and optimal drug sequences. Artificial intelligence (AI) and machine learning (ML) are accelerating discoveries. For instance, the deep learning model identified 8 core protein assemblies integrating multi-gene alterations to predict palbociclib response in breast cancer, outperforming single-gene biomarkers.[17] Spatial transcriptomics, spatial proteomics and computational approaches have been combined to reveal that gliomas organize into spatially structured cellular states, with local microenvironments dominated by single states and specific state pairs consistently co-localizing across tumors. Hypoxia emerges as a key driver of long-range tissue architecture, shaping a layered global organization absent in non-hypoxic tumors like low-grade IDH-mutant gliomas.[18] Patient-derived organoids[19] and three-domesnional (3D) bioprinted TME[20] models are also revolutionizing preclinical testing. These platforms recapitulate stromal interactions and drug penetration barriers, enabling high-throughput screening of network-targeted therapies.

Call for contributions

We invite contributions that explore the following emerging and advanced areas of cancer biology and therapy:

– Novel regulators of oncogenic hubs: Non-coding RNAs, phase-separated condensates and post-translational modifiers (e.g., ubiquitin ligases).

– TME-mediated network modulation: Role of exosomes, circadian rhythm disruptions and neural signaling in metastasis.

– Resistance mechanisms: Epigenetic plasticity, adaptive kinome reprogramming and persister cell states.

– AI/ML-driven discovery: Network-based drug repurposing and digital twins for personalized therapy.

– Biomarkers: Circulating tumor DNA (ctDNA) for real-time network monitoring, and metabolic imaging signatures.

Conclusions

The future of cancer therapy lies in decoding the rules that govern signaling networks – not just the molecules. By integrating systems biology, AI, and spatially resolved technologies, we can design therapies that anticipate and disrupt cancer's adaptive strategies. This call for submissions seeks to catalyze interdisciplinary collaboration, uniting basic researchers, computational biologists and clinicians to transform complexity into curability.

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References

1. Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. *Cell*. 2011;144(5):646–674. doi:10.1016/j.cell.2011.02.013
2. Glaviano A, Foo ASC, Lam HY, et al. PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Mol Cancer*. 2023; 22(1):138. doi:10.1186/s12943-023-01827-6
3. Bahar ME, Kim HJ, Kim DR. Targeting the RAS/RAF/MAPK pathway for cancer therapy: From mechanism to clinical studies. *Sig Transduct Target Ther*. 2023;8(1):455. doi:10.1038/s41392-023-01705-z
4. Liu J, Xiao Q, Xiao J, et al. Wnt/β-catenin signalling: Function, biological mechanisms, and therapeutic opportunities. *Sig Transduct Target Ther*. 2022;7(1):3. doi:10.1038/s41392-021-00762-6
5. Nazarian R, Shi H, Wang Q, et al. Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature*. 2010; 468(7326):973–977. doi:10.1038/nature09626
6. Engelman JA, Zejnullahu K, Mitsudomi T, et al. *MET* amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. *Science*. 2007;316(5827):1039–1043. doi:10.1126/science.1141478
7. Zhang Z, Lee JC, Lin L, et al. Activation of the AXL kinase causes resistance to EGFR-targeted therapy in lung cancer. *Nat Genet*. 2012;44(8): 852–860. doi:10.1038/ng.2330
8. Binnewies M, Roberts EW, Kersten K, et al. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*. 2018;24(5):541–550. doi:10.1038/s41591-018-0014-x
9. Kalluri R. The biology and function of fibroblasts in cancer. *Nat Rev Cancer*. 2016;16(9):582–598. doi:10.1038/nrc.2016.73
10. Mantovani A, Marchesi F, Malesci A, Laghi L, Allavena P. Tumour-associated macrophages as treatment targets in oncology. *Nat Rev Clin Oncol*. 2017;14(7):399–416. doi:10.1038/nrclinonc.2016.217
11. Fong MY, Zhou W, Liu L, et al. Breast-cancer-secreted miR-122 reprograms glucose metabolism in premetastatic niche to promote metastasis. *Nat Cell Biol*. 2015;17(2):183–194. doi:10.1038/ncb3094
12. Petralia F, Ma W, Yaron TM, et al. Pan-cancer proteogenomics characterization of tumor immunity. *Cell*. 2024;187(5):1255–1277.e27. doi:10.1016/j.cell.2024.01.027
13. Jin Y, Zuo Y, Li G, et al. Advances in spatial transcriptomics and its applications in cancer research. *Mol Cancer*. 2024;23(1):129. doi:10.1186 /s12943-024-02040-9
14. Kumar A, Misra BB. Challenges and opportunities in cancer metabolomics. *Proteomics*. 2019;19(21–22):1900042. doi:10.1002/pmic.201900042
15. Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic signaling pathways in The Cancer Genome Atlas. *Cell*. 2018;173(2):321–337.e10. doi:10.1016/j.cell.2018.03.035
16. Puram SV, Tirosh I, Parikh AS, et al. Single-cell transcriptomic analysis of primary and metastatic tumor ecosystems in head and neck cancer. *Cell*. 2017;171(7):1611–1624.e24. doi:10.1016/j.cell.2017.10.044
17. Park S, Silva E, Singhal A, et al. A deep learning model of tumor cell architecture elucidates response and resistance to CDK4/6 inhibitors. *Nat Cancer*. 2024;5(7):996–1009. doi:10.1038/s43018-024-00740-1
18. Greenwald AC, Darnell NG, Hoefflin R, et al. Integrative spatial analysis reveals a multi-layered organization of glioblastoma. *Cell*. 2024; 187(10):2485–2501.e26. doi:10.1016/j.cell.2024.03.029
19. Driehuis E, Kretzschmar K, Clevers H. Establishment of patient-derived cancer organoids for drug-screening applications. *Nat Protoc*. 2020;15(10):3380–3409. doi:10.1038/s41596-020-0379-4
20. Shukla P, Yeleswarapu S, Heinrich MA, Prakash J, Pati F. Mimicking tumor microenvironment by 3D bioprinting: 3D cancer modeling. *Biofabrication*. 2022;14(3):032002. doi:10.1088/1758-5090/ac6d11

Lectins and neurodegeneration: A glycobiologist's perspective

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Neurodegenerative diseases, including Alzheimer's and Parkinson's disease, affect an increasing number of people in aging societies, dramatically reducing the quality of life of those affected. Hence, intensive research efforts are aimed at understanding the molecular mechanisms of the disease progress, with the hope for developing effective therapeutic strategies. The progress of neurodegenerative diseases is associated with a complex activity of the immune system in the brain tissue. Carbohydrate-binding proteins (lectins) play a key role in the inflammation-related activation of microglia. Siglecs, maintained in an active state by binding to sialic acid-terminated glycoconjugates, help establish homeostasis by protecting nerve cells from phagocytosis and preventing triggering receptor expressed on myeloid cells 2 (TREM2) activation. Upon activation, microglia release sialidase, an enzyme that cleaves sialic acid residues from glycoconjugates, thereby exposing galactose as the next monosaccharide in the glycan chain. After losing siglec-mediated protection, the glycan becomes a ligand for Galectin-3 (Gal-3). Overexpression of this lectin under inflammatory conditions activates TREM2 and TLR4 signaling pathways, enhances the phagocytic activity of microglia and leads to tissue damage. Blocking Gal-3 interactions with the thiodigalactoside inhibitor (TD-139) appears to be a promising novel approach to pharmacologically alleviating neuroinflammation.

Key words: microglia, neurodegeneration, galectin, glycosylation, Siglec

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Highlights

- Involvement of specific lectin-carbohydrate interactions in the progress of neuroinflammation attracts increasing interest.
- Siglec-polysialic acid binding maintains homeostasis and protects brain tissue.
- Under inflammatory trigger, sialidase removes sialic acid residues from glycans.
- Truncated glycans become ligands for Galectin-3 (Gal-3), which promotes excessive inflammation.
- Gal-3 blockade may be a target for therapeutic intervention.

Introduction

Aging populations are experiencing a rising incidence of neurodegenerative diseases, including Alzheimer's disease (AD) and Parkinson's disease. The progressive degradation of the nervous tissue significantly reduces the quality of patient's life, inevitably leading to disability. The scale of the problem becomes a serious challenge for the healthcare systems. Despite extensive research, the precise mechanisms driving the onset and progression of these diseases remain poorly understood, and the search for regulatory targets to support the development of novel therapeutic strategies is ongoing.

Activation of the immune system is one of the mechanisms involved in the development of neurodegenerative diseases. When immune cells are unable to effectively remove deposits of misfolded proteins, they intensify their activity, which eventually leads to an attack on the body's own nervous tissue. Protein–carbohydrate interactions are important in regulation of these processes, and carbohydrate-recognizing proteins – lectins – act as pattern recognition receptors. In the recent years, attention has been paid to the interactions mediated by galectins, particularly Galectin-3 (Gal-3).[1–5] Galectins are a group of lectics, capable of recognizing β-galactosides in the oligosaccharides of glycoconjugates. Protein–sugar interactions mediated by lectins are involved in numerous physiological and pathological processes, including those related to cancer, infections and autoimmune diseases.[6] The key issue seems to be the regulation of the immune response, for which the subtle changes in the sugar structures of glycoproteins and glycolipids are essential.

A significant part of the brain tissue is made up of the immune system cells, mainly astrocytes and microglia. As in other tissues, they act as guardians, detecting compounds considered non-self in their surroundings.[7–9] In nervous tissue, the inflammatory process is most often independent of pathogens. The immune response can be triggered by tissue damage or the accumulation of misfolded protein deposits, such as β-amyloid in AD or fibrillar forms of α-synuclein in Parkinson's disease.[4] Such a trigger leads to the activation of microglia (Fig. 1). The resting ramified microglial cells change their shape, transcriptional profile and behavior, transforming into an amoeboid form capable of phagocytosis.[7] Such an inflammatory state is of the key importance in the pathology of neurodegenerative diseases. With the initial appearance of deposits of misfolded, insoluble protein plaques, the activation of microglial cells is aimed at the clearance of debris and thus maintaining a healthy tissue profile.[6–8] Later, the active microglia secrete significant amounts of pro-inflammatory cytokines, and the increasing inflammation leads to the progressive damage of the nervous tissue. An altered transcriptional profile of activated microglia leads to the massive release of cytokines and other inflammation-associated proteins, including Gal-3. This protein is weakly expressed in homeostatic, inactive microglia, but is highly overexpressed in activated cells (Fig. 1).[2,3,8–10]

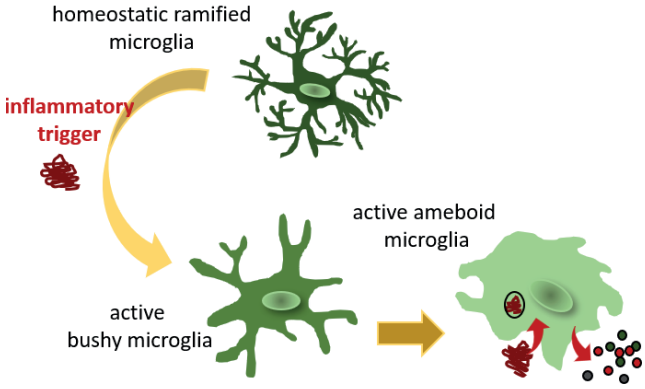

Fig. 1. Resting microglia and their activation: Upon full activation, amoeboid microglial cells phagocytose amyloid deposits and secrete pro-inflammatory cytokines along with Galectin-3 (Gal-3)

Glycosylation as an immune checkpoint

Glycosylation is a common post-translational modification of proteins. Oligosaccharide chains decorate the surface of more than half of the proteins in all living organisms. Membrane lipids, such as gangliosides, are also glycosylated and are particularly abundant in nervous tissue. Even subtle changes in structures of these oligosaccharides have essential impact on the dynamics of interactions with their receptors.[11]

Complex glycosylation pathways enable the creation of countless variants of oligosaccharide structures.

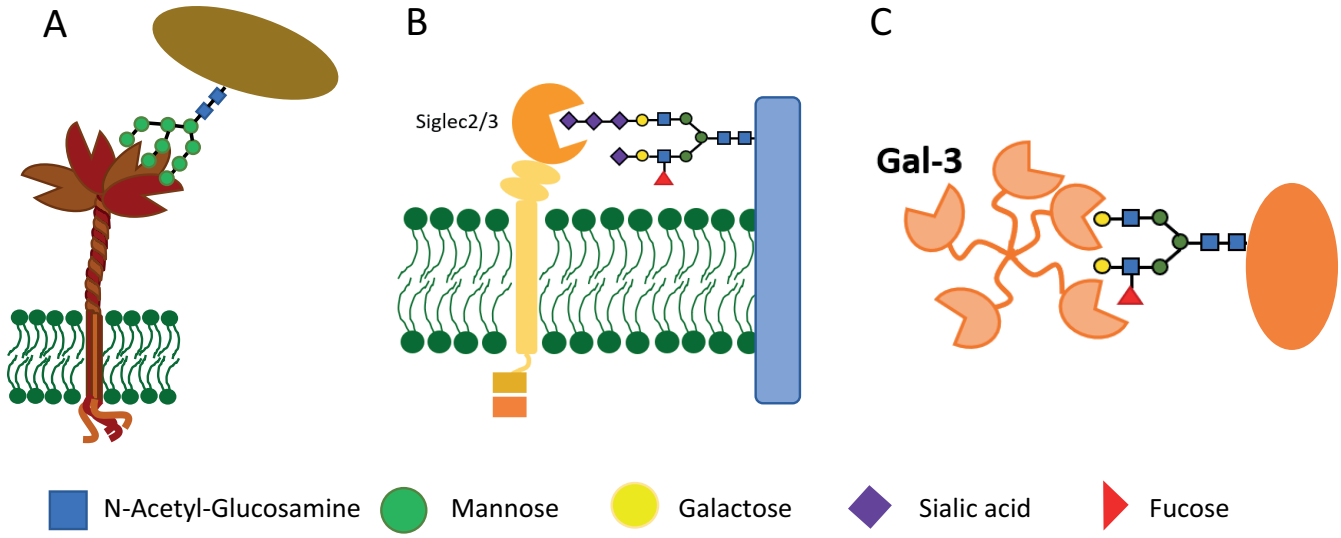

Fig. 2. Examples of lectins and their carbohydrate ligands. A. Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN) binds high-mannose glycans, recognizing them as pathogen-associated molecular patterns (PAMPs); B. Siglecs interact with sialylated glycans, which serve as self-associated molecular patterns (SAMPs); C. Galectins bind truncated, galactose-exposing glycans, which are characteristic of damage-associated molecular patterns (DAMPs)

As a result of evolution, these structures differ between lower organisms (including pathogens) and vertebrates. In vertebrates, tissue-specific and developmental (ontogenetic) differences also play a significant role. Structural changes in glycans are associated with numerous pathological processes, including carcinogenesis. The diverse variants of oligosaccharides are recognized by an equally rich array of proteins, collectively known as lectins (Fig. 2).[11] In addition to the previously mentioned galectins, there are groups of lectins that recognize high-mannose glycans and are responsible for detecting and binding pathogens. The 3rd major group comprises Siglecs, which specifically recognize sialic acid-containing structures. Lectins are classified as pattern recognition receptors (PRRs), with the "pattern" referring to specific glycosylation profiles.[12] Pattern recognition drives the appropriate immune response of the organism. Thus, mannose-specific lectins primarily recognize pathogen-associated molecular patterns (PAMPs).[13,14] In the context of the topics discussed here, 2 additional patterns are of particular interest: damage-associated molecular patterns (DAMPs), recognized by galectins, and self-associated molecular patterns (SAMPs), recognized by Siglecs, i.e., the sialic acid-binding immunoglobulin-like lectins. Glycosylated ligands for these lectins can be either anchored to the cell membrane or secreted into the extracellular space (Fig. 2).[4,15–17]

Sialylation, Siglecs and sialidase

The vast majority of glycoproteins and glycolipids in the human body are terminated with a sialic acid residue, a monosaccharide that consistently occupies the terminal position within oligosaccharide structures, although the mode of attachment can vary.[11] This characteristic also applies to nervous tissue. Moreover, particularly in the central nervous system (CNS), glycans often form unique structures characterized by the presence of multiple sialic acid residues at their termini (polysialylation).[18,19] Their receptors, the Siglecs, are expressed on the surface of both neurons and microglial cells.[17,18,20] This protein–carbohydrate interaction is responsible for maintaining homeostasis. Activation of CD22 (Siglec-2) and CD33 (Siglec-3) through binding to sialylated ligands inhibits phagocytosis, suppresses TLR4 activation and restricts complement receptor 3 (CR3) access to the cell surface.[18,19,21] The situation changes when a trigger signal emerges within the tissue, leading to microglial activation. The initial deposits of amyloid-β or other misfolded proteins may serve as such a trigger.[22] In response, microglial cells secrete sialidase (also known as neuraminidase), an enzyme that specifically cleaves terminal sialic acid residues from the surface glycans of both microglia and neurons.[16,19,21,23,24] The removal of sialic acid exposes the underlying galactose residue within the glycan chain, thereby disrupting the interaction with Siglecs that normally serves to protect cell integrity. Moreover, nerve fibers with exposed galactose residues become susceptible to phagocytosis and, additionally, serve as ligands for Gal-3.[19] Thus, in resting microglia, the interaction between Siglecs and their ligands is crucial for maintaining cellular homeostasis. Upon activation, sialidase-mediated modification of glycan structures disrupts this protective mechanism.

Galectins in the nervous tissue

Microglial activation is primarily directed toward the clearance of early deposits of misfolded proteins.[3,7] At this stage, the cells start to express and secrete Gal-3. Galectin-3, released from activated microglia, further

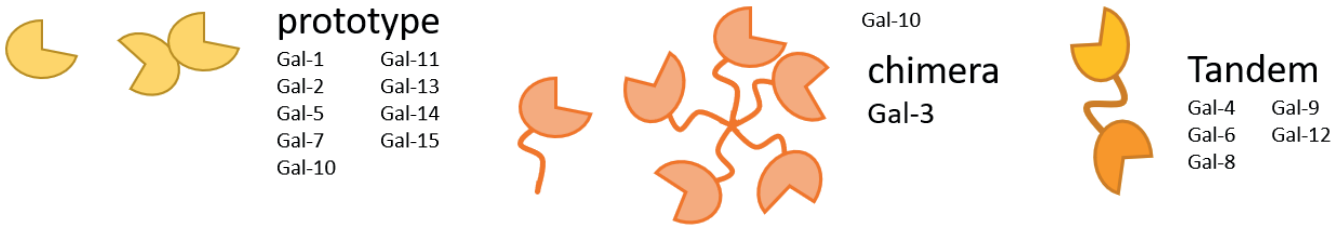

Fig. 3. Types of galectins

amplifies microglial activation by binding to TLR4 and TREM2, attaches to desialylated neurons to opsonize them for phagocytosis, and promotes amyloid-β aggregation and associated toxicity (Fig. 3).[1–4,25–27]

Galectin-3, the most extensively studied galectin in CNS pathology, is the sole representative of the chimeric type within this protein family (Fig. 3). In addition to its C-terminal carbohydrate recognition domain (CRD), Gal-3 possesses a flexible collagen-like domain and an N-terminal domain responsible for multiple protein–protein interactions.[3,4,10,28] This N-terminal domain facilitates oligomerization, allowing Gal-3 to form higher-order structures, including pentamers. Due to its unique structure, Gal-3 can therefore interact with both sugars and protein structures, which is an exception in this type of lectins. Multimeric CRD interactions, as well as simultaneous interactions of the CRD and N-terminal domains, give Gal-3 the ability to function as a bridge, connecting ligands on the cell surface, in the extracellular matrix (ECM), and elements secreted outside the cell with those anchored in the membrane. Additionally, Gal-3 is susceptible to 2 characteristic post-translational modifications: proteolytic cleavage by matrix metalloproteinases (MMPs), separating the CRD domain from the N-terminal fragment, and phosphorylation. N-terminal cleavage prevents oligomerization and simultaneous interactions with both carbohydrate and protein ligands. The role of potential phosphorylation remains unknown.

Galectin-3 levels in cerebrospinal fluid (CSF) and serum of AD patients are significantly elevated and correlate with disease stage and severity. A similar increase was observed in mouse models of AD, particularly in microglial regions associated with amyloid deposits.[19,29,30] Studies have demonstrated that the loss of Gal-3 function, via genetic deletion or inhibition, mitigates key features of neurodegenerative diseases, including decreased expression of pro-inflammatory genes, reduced amyloid burden and improved cognitive performance.[10,29] In the healthy CNS, the role of Gal-3 seems to be limited. Knockout mice (Gal-3$^{-/-}$) do not exhibit any significant functional impairments.[3,28] However, in healthy neural tissue, Gal-3 is physiologically expressed in the subventricular zone, where neurogenic stem cells reside. Microglia in this area are semi-activated despite the absence of tissue damage. This process is reported to be associated with neurogenesis and migration of new neurons to olfactory bulbs.[10]

Apart from Gal-3, expression of Galectin-1, -3, -8, and -9 has been described in the nervous tissue.[31–35] Galectins other than Gal-3 are considered neuroprotective rather than promoting a tissue damage. Galectin-1 belongs to the prototype group of galectins. These proteins contain a single CRD capable of dimerization, enabling bivalent interactions and cross-linking of glycosylated ligands. Galectin-1 is often co-expressed with Gal-3 but exerts opposing effects. It is highly expressed in astrocytes and plays a regulatory role by suppressing microglial activation. This is achieved through binding to O-glycans on CD45 and attenuating inflammatory signaling pathways. In Gal-1 knockout mice, microglia display a more pro-inflammatory phenotype, accompanied by exacerbated demyelination.[36] Galectins-4, -8 and -9 belong to the tandem-repeat type, characterized by the presence of 2 distinct CRDs connected by a polypeptide linker of variable length. This structure also allows ligands cross-linking. Galectin-8, similarly to Gal-9, has an immunosuppressive impact on the CNS. It is involved in the activation of autophagy in cells containing tau aggregates in AD. Galectin-8 detects luminal glycans of the damaged endomembranes caused by cytosolic aggregation of tau protein and directs them to autophagy. Inhibition of Gal-8 results in the higher amount of tau aggregates inside the cells.[34] Galectin-4 is localized in neurons and plays its role in axonal growth and transport. Its presence is also associated with the inhibition of myelination and the maturation of oligodendrocytes. However, no significant changes have been reported in the overall progression of neurodegeneration.[36]

Cross-talk of Siglecs, Galectin-3 and TREM2

Over the past decade, triggering receptor expressed on myeloid cells 2 (TREM2) has been considered implicated in the progression of AD.[37–39] Expressed by microglia, TREM2 is a key regulator of the innate immune response. It is a membrane protein composed of a large ectodomain that binds extracellular ligands, a transmembrane region consisting of a single α-helix, and a short cytoplasmic tail lacking intrinsic signaling motifs. TREM2 requires interaction with the adaptor proteins DAP12 or DAP10 to activate signaling pathways that promote the secretion of pro-inflammatory cytokines and enhance phagocytic activity.[39,40] Genetic variants of TREM2 have been associated with an increased risk of AD and other

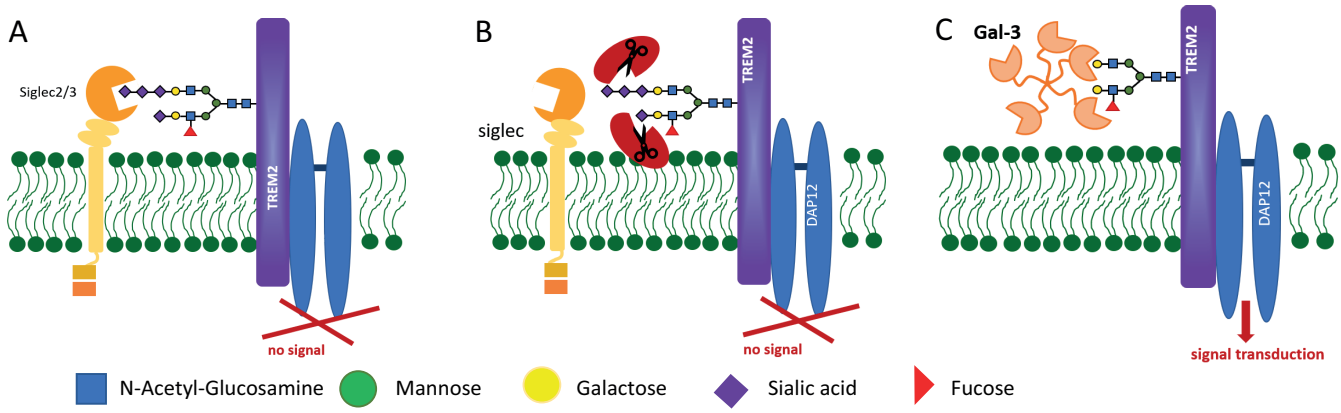

Fig. 4. Lectin interactions and TREM2/DAP12 signaling. A. In resting microglia, Siglec binding to sialylated glycans inhibits signal transduction, maintaining immune homeostasis; B. Upon activation, microglial cells secrete sialidase, which removes terminal sialic acids and disrupts Siglec–glycan interactions; C. As a result, truncated glycans expose galactose residues, allowing recognition and binding by Galectin-3 (Gal-3)

TREM2 – triggering receptor expressed on myeloid cells 2.

neurodegenerative disorders.[39] In the CNS, TREM2 expression increases with age and the progression of neurodegenerative diseases.[39] Studies using TREM2$^{-/-}$ mice have shown a decrease in microglial clustering around amyloid deposits, reduced microglial cell numbers and increased apoptosis.[41] The TREM2 ectodomain can be shed by ADAM-family metalloproteinases, generating a soluble form (sTREM2) that acts as a mediator of cellular interactions within the tissue (Fig. 4).[42,43]

Both Gal-3 and Siglec-3 (CD33) are endogenous ligands of the TREM2/DAP12 receptor complex, modulating its signaling, a key driver of microglial activation and neurodegeneration, which plays a crucial role in regulating phagocytosis and inflammation.[29,41,44,45] The binding of Siglec-3 with sialylated glycans suppresses TREM2-driven phagocytic activity in microglia (Fig. 4).[41,44,46] In turn, increased Gal-3 expression leads to the activation of TREM2 signaling pathways.[29,45] Notably, Gal-3 binding to TREM2 has been shown to involve its carbohydrate recognition domain.[29]

Therapeutic approach

The interaction between terminal sialic acids and Siglecs appears to play a key role in protecting brain tissue, suggesting that inhibiting sialidase may hold therapeutic potential.[47] However, current research is focused on low-molecular-weight inhibitors of the galectin CRD. Among a few digalactosyl analogues of lectin natural ligands,[48] TD-139 (olitygaltin) is the subject of most advanced studies. The compound, 3,3′-bis-(4-aryltriazol-1-yl)thio-digalactoside, consists of a core formed by 2 galactose units linked via a thiol bond, flanked by triazole rings and fluorine-substituted phenyl groups (Fig. 5). The reduction of pro-inflammatory Gal-3 activity by TD-139 has been tested in several animal models of nervous system diseases. Rombaut et al.[49] demonstrated a neuroprotective effect of intravitreally injected TD-139 in a rat ocular hypertensive model of glaucoma, reporting the prevention of retinal

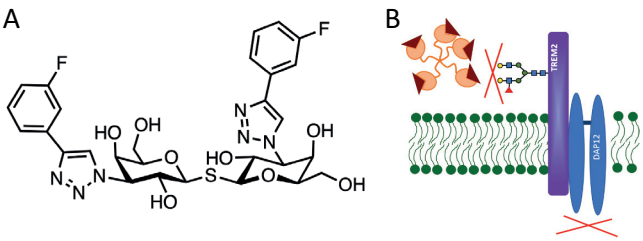

Fig. 5. TD-139 as a blocker of the galectin binding site, preventing TREM2/DAP12 signal transduction. A. Chemical structure of TD-139; B. Mechanism of TD-139 action: inhibition of galectin binding to truncated glycans, thereby preserving Siglec-mediated inhibition and preventing activation of the TREM2/DAP12 signaling pathway

TREM2 – triggering receptor expressed on myeloid cells 2.

ganglion cell degeneration. In a mouse model of an early brain injury due to subarachnoid hemorrhage, Shen et al.[50] demonstrated both the role of Gal-3 in the microglia activation and its efficient inhibition by TD-139, which prevented brain injury and excessive secretion of pro-inflammatory cytokines by the microglial cells. In a rat model of epilepsy, elevated Gal-3 activity was observed, and its inhibition by TD-139 was shown to reduce inflammation and associated neurodegenerative changes. This intervention reduced the expression of pro-inflammatory factors, the severity of seizures and hippocampal damage.[51] Experimental studies in animal models may be limited by the significantly lower binding affinity of the inhibitor to mouse and rat analogues of Gal-3 compared to the human form of the lectin.[52] Based on crystallographic studies, the authors indicate structural differences in the carbohydrate-binding site in all 3 cases. The key role is suggested to hA146, which holds the ligand in the correct position in the lectin binding pocket by interacting with the fluorophenyl flanking structure. The presence of larger V160 and T158 residues in mouse and rat proteins disrupted the alignment required for proper binding. Interestingly, targeted mutations replacing the aforementioned residues

with alanine in animal proteins led to improved alignment and increased binding affinity. Conversely, introducing a valine residue in place of alanine in human Gal-3 diminished its binding affinity. Such data are essential not only for further planning of animal model studies and their interpretation, but also for possible research on the advantageous structural modifications of digalactosyl compounds (Fig. 5).

Conclusions

Interactions between lectins and their carbohydrate ligands are gaining increasing attention as effective immune checkpoints that regulate the body's immune response. This applies to various diseases that contain an inflammatory component. It does not differ in disorders affecting the nervous tissue, although our knowledge here is much more limited. Further studies are important for a better understanding of the dynamics and regulation of neuroinflammation that leads to a tissue damage. Current data suggest that a deeper understanding of these interactions could pave the way for the development of new therapeutic agents aimed at slowing the progression of neurodegenerative diseases. The challenge for our understanding of these mechanisms comes from the possible pleiotropic effects of lectins, as their action may be context-dependent and vary in changing conditions of the microenvironment. Little is known about the possible interplay of different galectins, sometimes co-expressed but varying in function. The signaling pathways leading to galectin expression may be also influenced by external factors.

Current efforts to utilize the TD-139 inhibitor as a potential therapeutic agent originate primarily from its demonstrated efficacy in treating pulmonary fibrosis by limiting the Gal-3-induced excessive immune response. However, in the context of neurological diseases, an added challenge lies in the limited understanding of its ability to cross the blood–brain barrier. The molecule, in addition to the digalactoside structure recognized by the lectin, contains flanking regions composed of phenyl-derived moieties. Due to its relatively hydrophobic character, TD-139 may more easily penetrate biological membranes, including potentially the blood–brain barrier. This highlights the critical role of the flanking structures surrounding the galactoside core, which may serve as key targets for future modifications aimed at enhancing membrane permeability. Another advantage of low molecular weight compounds like TD-139 lies in their ability to circumvent potential side effects commonly associated with antibody-based therapies, such as immune system activation.

Although our understanding of the complex mechanisms underlying neuroinflammation and degeneration is still incomplete, the topic continues to garner significant research attention, and a surge of new, insightful studies is anticipated in the near future.

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References

1. Nio-Kobayashi J, Itabashi T. Galectins and their ligand glycoconjugates in the central nervous system under physiological and pathological conditions. *Front Neuroanat*. 2021;15:767330. doi:10.3389/fnana.2021.767330
2. Ramírez Hernández E, Alanis Olvera B, Carmona González D, et al. Neuroinflammation and galectins: A key relationship in neurodegenerative diseases. *Glycoconj J*. 2022;39(5):685–699. doi:10.1007/s10719-022-10064-w
3. García-Revilla J, Boza-Serrano A, Espinosa-Oliva AM, et al. Galectin-3, a rising star in modulating microglia activation under conditions of neurodegeneration. *Cell Death Dis*. 2022;13(7):628. doi:10.1038/s41419-022-05058-3
4. Srejovic I, Selakovic D, Jovicic N, Jakovljević V, Lukic ML, Rosic G. Galectin-3: Roles in neurodevelopment, neuroinflammation, and behavior. *Biomolecules*. 2020;10(5):798. doi:10.3390/biom10050798
5. Tan Y, Zheng Y, Xu D, Sun Z, Yang H, Yin Q. Galectin-3: A key player in microglia-mediated neuroinflammation and Alzheimer's disease. *Cell Biosci*. 2021;11(1):78. doi:10.1186/s13578-021-00592-7
6. Liu FT, Stowell SR. The role of galectins in immunity and infection. *Nat Rev Immunol*. 2023;23(8):479–494. doi:10.1038/s41577-022-00829-7
7. Butler CA, Popescu AS, Kitchener EJA, Allendorf DH, Puigdellívol M, Brown GC. Microglial phagocytosis of neurons in neurodegeneration, and its regulation. *J Neurochem*. 2021;158(3):621–639. doi:10.1111/jnc.15327
8. Wu KM, Zhang YR, Huang YY, Dong Q, Tan L, Yu JT. The role of the immune system in Alzheimer's disease. *Ageing Res Rev*. 2021;70:101409. doi:10.1016/j.arr.2021.101409
9. Kaur D, Sharma V, Deshmukh R. Activation of microglia and astrocytes: A roadway to neuroinflammation and Alzheimer's disease. *Inflammopharmacology*. 2019;27(4):663–677. doi:10.1007/s10787-019-00580-x
10. Soares LC, Al-Dalahmah O, Hillis J, et al. Novel galectin-3 roles in neurogenesis, inflammation and neurological diseases. *Cells*. 2021;10(11):3047. doi:10.3390/cells10113047
11. Varki A, Cummings RD, Esko JD, et al., eds. *Essentials of Glycobiology*. 2nd ed. Cold Spring Harbor, USA: Cold Spring Harbor Laboratory Press; 2009. ISBN:978-0-87969-770-9.
12. Dam TK, Brewer CF. Lectins as pattern recognition molecules: The effects of epitope density in innate immunity. *Glycobiology*. 2010;20(3):270–279. doi:10.1093/glycob/cwp186
13. Prado Acosta M, Lepenies B. Bacterial glycans and their interactions with lectins in the innate immune system. *Biochem Soc Trans*. 2019;47(6):1569–1579. doi:10.1042/BST20170410
14. Loke I, Kolarich D, Packer NH, Thaysen-Andersen M. Emerging roles of protein mannosylation in inflammation and infection. *Mol Aspects Med*. 2016;51:31–55. doi:10.1016/j.mam.2016.04.004
15. Vasta GR, Feng C, González-Montalbán N, et al. Functions of galectins as 'self/non-self'-recognition and effector factors. *Pathog Dis*. 2017;75(5):ftx046. doi:10.1093/femspd/ftx046
16. Klaus C, Hansen JN, Ginolhac A, et al. Reduced sialylation triggers homeostatic synapse and neuronal loss in middle-aged mice. *Neurobiol Aging*. 2020;88:91–107. doi:10.1016/j.neurobiolaging.2020.01.008
17. Liao H, Klaus C, Neumann H. Control of innate immunity by sialic acids in the nervous tissue. *Int J Mol Sci*. 2020;21(15):5494. doi:10.3390/ijms21155494
18. Klaus C, Liao H, Allendorf DH, Brown GC, Neumann H. Sialylation acts as a checkpoint for innate immune responses in the central nervous system. *Glia*. 2021;69(7):1619–1636. doi:10.1002/glia.23945
19. Puigdellívol M, Allendorf DH, Brown GC. Sialylation and galectin-3 in microglia-mediated neuroinflammation and neurodegeneration. *Front Cell Neurosci*. 2020;14:162. doi:10.3389/fncel.2020.00162
20. Pshezhetsky AV, Ashmarina M. Keeping it trim: Roles of neuraminidases in CNS function. *Glycoconj J*. 2018;35(4):375–386. doi:10.1007/s10719-018-9837-4
21. Allendorf DH, Puigdellívol M, Brown GC. Activated microglia desialylate their surface, stimulating complement receptor 3-mediated phagocytosis of neurons. *Glia*. 2020;68(5):989–998. doi:10.1002/glia.23757

22. Sgambati E, Tani A, Leri M, et al. Correlation between sialylation status and cell susceptibility to amyloid toxicity. *Cells*. 2022;11(4):601. doi:10.3390/cells11040601
23. Fremuth LE, Hu H, Van De Vlekkert D, Annunziata I, Weesner JA, D'Azzo A. Neuraminidase 1 regulates neuropathogenesis by governing the cellular state of microglia via modulation of Trem2 sialylation. *Cell Rep*. 2025;44(1):115204. doi:10.1016/j.celrep.2024.115204
24. Rawal P, Zhao L. Sialometabolism in brain health and Alzheimer's disease. *Front Neurosci*. 2021;15:648617. doi:10.3389/fnins.2021.648617
25. Barake F, Soza A, González A. Galectins in the brain: Advances in neuroinflammation, neuroprotection and therapeutic opportunities. *Curr Opin Neurol*. 2020;33(3):381–390. doi:10.1097/WCO.0000000000000812
26. Lerman BJ, Hoffman EP, Sutherland ML, et al. Deletion of galectin-3 exacerbates microglial activation and accelerates disease progression and demise in a SOD1^{G93A} mouse model of amyotrophic lateral sclerosis. *Brain Behav*. 2012;2(5):563–575. doi:10.1002/brb3.75
27. Rahimian R, Béland L, Sato S, Kriz J. Microglia-derived galectin-3 in neuroinflammation: A bittersweet ligand? *Med Res Rev*. 2021;41(4):2582–2589. doi:10.1002/med.21784
28. Lozinski BM, Ta K, Dong Y. Emerging role of galectin 3 in neuroinflammation and neurodegeneration. *Neural Regen Res*. 2024;19(9):2004–2009. doi:10.4103/1673-5374.391181
29. Boza-Serrano A, Ruiz R, Sanchez-Varo R, et al. Galectin-3, a novel endogenous TREM2 ligand, detrimentally regulates inflammatory response in Alzheimer's disease. *Acta Neuropathol*. 2019;138(2):251–273. doi:10.1007/s00401-019-02013-z
30. Ramos-Martínez E, Ramos-Martínez I, Sánchez-Betancourt I, et al. Association between galectin levels and neurodegenerative diseases: Systematic review and meta-analysis. *Biomolecules*. 2022;12(8):1062. doi:10.3390/biom12081062
31. Camby I, Le Mercier M, Lefranc F, Kiss R. Galectin-1: A small protein with major functions. *Glycobiology*. 2006;16(11):137R–157R. doi:10.1093/glycob/cwl025
32. Endo T. Glycans and glycan-binding proteins in brain: Galectin-1-induced expression of neurotrophic factors in astrocytes. *Curr Drug Targets*. 2005;6(4):427–436. doi:10.2174/1389450054021909
33. Cengiz T, Türkboyları S, Gençler OS, Anlar Ö. The roles of galectin-3 and galectin-4 in the idiopathic Parkinson disease and its progression. *Clin Neurol Neurosurg*. 2019;184:105373. doi:10.1016/j.clineuro.2019.105373
34. Falcon B, Noad J, McMahon H, Randow F, Goedert M. Galectin-8-mediated selective autophagy protects against seeded tau aggregation. *J Biol Chem*. 2018;293(7):2438–2451. doi:10.1074/jbc.M117.809293
35. Jujic A, Vieira JPP, Matuskova H, et al. Plasma galectin-4 levels are increased after stroke in mice and humans. *Int J Mol Sci*. 2023;24(12):10064. doi:10.3390/ijms241210064
36. Siew JJ, Chern Y. Microglial lectins in health and neurological diseases. *Front Mol Neurosci*. 2018;11:158. doi:10.3389/fnmol.2018.00158
37. Ulland TK, Song WM, Huang SCC, et al. TREM2 maintains microglial metabolic fitness in Alzheimer's disease. *Cell*. 2017;170(4):649–663.e13. doi:10.1016/j.cell.2017.07.023
38. Qin Q, Teng Z, Liu C, Li Q, Yin Y, Tang Y. TREM2, microglia, and Alzheimer's disease. *Mech Ageing Dev*. 2021;195:111438. doi:10.1016/j.mad.2021.111438
39. Kober DL, Brett TJ. TREM2-ligand interactions in health and disease. *J Mol Biol*. 2017;429(11):1607–1629. doi:10.1016/j.jmb.2017.04.004
40. Yin P, Su Z, Shu X, Dong Z, Tian Y. Role of TREM2 in immune and neurological diseases: Structure, function, and implications. *Int Immunopharmacol*. 2024;143:113286. doi:10.1016/j.intimp.2024.113286
41. Griciuc A, Patel S, Federico AN, et al. TREM2 acts downstream of CD33 in modulating microglial pathology in Alzheimer's disease. *Neuron*. 2019;103(5):820–835.e7. doi:10.1016/j.neuron.2019.06.010
42. Yang J, Fu Z, Zhang X, Xiong M, Meng L, Zhang Z. TREM2 ectodomain and its soluble form in Alzheimer's disease. *J Neuroinflammation*. 2020;17(1):204. doi:10.1186/s12974-020-01878-2
43. Zhong L, Xu Y, Zhuo R, et al. Soluble TREM2 ameliorates pathological phenotypes by modulating microglial functions in an Alzheimer's disease model. *Nat Commun*. 2019;10(1):1365. doi:10.1038/s41467-019-09118-9
44. Chan G, White CC, Winn PA, et al. CD33 modulates TREM2: Convergence of Alzheimer loci. *Nat Neurosci*. 2015;18(11):1556–1558. doi:10.1038/nn.4126
45. Fan Q, Wu YZ, Jia XX, et al. Increased Gal-3 mediates microglia activation and neuroinflammation via the TREM2 signaling pathway in prion infection. *ACS Chem Neurosci*. 2023;14(20):3772–3793. doi:10.1021/acschemneuro.3c00344
46. Salminen A, Kaarniranta K, Kauppinen A. Hypoxia/ischemia impairs CD33 (Siglec-3)/TREM2 signaling: Potential role in Alzheimer's pathogenesis. *Neurochem Int*. 2021;150:105186. doi:10.1016/j.neuint.2021.105186
47. Wißfeld J, Abou Assale T, Cuevas-Rios G, Liao H, Neumann H. Therapeutic potential to target sialylation and SIGLECs in neurodegenerative and psychiatric diseases. *Front Neurol*. 2024;15:1330874. doi:10.3389/fneur.2024.1330874
48. Mijailović NR, Vesic K, Arsenijevic D, Milojević-Rakić M, Borovcanin MM. Galectin-3 involvement in cognitive processes for new therapeutic considerations. *Front Cell Neurosci*. 2022;16:923811. doi:10.3389/fncel.2022.923811
49. Rombaut A, Brautaset R, Williams PA, Tribble JR. Intravitreal injection of the galectin-3 inhibitor TD139 provides neuroprotection in a rat model of ocular hypertensive glaucoma. *Mol Brain*. 2024;17(1):84. doi:10.1186/s13041-024-01160-z
50. Shen Y, Zhang W, Chang H, et al. Galectin-3 modulates microglial activation and neuroinflammation in early brain injury after subarachnoid hemorrhage. *Exp Neurol*. 2024;377:114777. doi:10.1016/j.expneurol.2024.114777
51. Sun W, Hao Y, Li C, Zhao Y, Yu H, Wang L. Inhibition of galectin-3 in a rat model of epilepsy and Kainate-activated BV2 cells limits microglial activation through the NLRP3/pyroptosis pathway. *Neuroimmunomodulation*. 2023;30(1):325–337. doi:10.1159/000534833
52. Kumar A, Paul M, Panda M, et al. Molecular mechanism of interspecies differences in the binding affinity of TD139 to galectin-3. *Glycobiology*. 2021;31(10):1390–1400. doi:10.1093/glycob/cwab072

The impact of systemic and topical antimicrobial therapy combined with non-surgical periodontal therapy: A meta-analysis

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Combining antibiotics with non-surgical periodontal therapy has a beneficial impact in case of infection while its role for dental-related outcomes is still unclear.

Objectives. The current study's main objective was to evaluate the impact of adding adjuvant systemic and topical antimicrobial therapy to non-surgical periodontal therapy.

Materials and methods. A systematic literature search was accomplished and 1,093 study participants with periodontal diseases were recruited to the current study; 541 of them were treated with adjuvant systemic or topical antimicrobial agents and 552 with non-surgical interventions. The inclusion criteria of the current study took into account only randomized clinical trials.

Results. Adding systemic antibiotics to non-surgical intervention resulted in a significant enhancement regarding probing pocket depth reduction (PPD). Metronidazole/amoxicillin showed a significant impact on PPD and the clinical attachment level (CAL), while doxycycline showed no significant impact regarding CAL. Using topical antimicrobial agents showed a significant beneficial role in reducing PPD regarding doxycycline, while non-significant effects were seen with metronidazole.

Conclusions. Adding adjuvant systemic and topical antimicrobial agents to non-surgical periodontal therapy showed a beneficial impact regarding PPD and CAL (metronidazole/amoxicillin and doxycycline). In addition, using doxycycline as a topical agent showed a beneficial impact on the reduction of PPD.

Key words: periodontal therapy, antimicrobials, CAL, PPD

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Background

Periodontitis is a condition marked by an imbalance in the microbial community, leading to the breakdown of the tissues supporting the teeth, including the alveolar bone, cementum, periodontal ligament, and gingiva. It is widely recognized as the primary reason for tooth loss.[1] Periodontitis affects almost 50% of the world's population, with 11% experiencing a severe version of the disease.[2] Periodontitis is linked to a transition from a mutually beneficial periodontal microbiome to an imbalanced one. The presence of dysbiosis is linked to a greater occurrence of anaerobic bacteria, specifically *Porphyromonas gingivalis*, which is recognized as a major pathogen.[3,4] This pathogen has the ability to penetrate tissues and trigger inflammatory signaling pathways, leading to inflammation. Periodontitis is characterized by the loss of attachment between the teeth and gums, an increase in the depth of the pockets around the teeth, bleeding when probed, and can be accompanied by purulence, pain and swelling of the gums. These symptoms can negatively impact the long-term health of the affected teeth.[5,6] The current objective of periodontal treatment is to restore the balance between organisms living in the periodontium and reduce inflammation, while also enhancing the clinical attachment level (CAL). In addition to providing guidance on oral hygiene and making adjustments to potential local and systemic risk factors, the primary focus of the current treatment strategy is non-surgical periodontal treatment, specifically scaling and root planing (SRP).[7] Nevertheless, in instances of severe infections, the effectiveness of treatment may be hindered by challenges in gaining instrumental access to the affected area, bacterial infiltration of the surrounding soft tissues and a persistent inflammatory response.[8] This underscores the necessity of employing additional treatments, such as surgical interventions. Adjuvants have been suggested in the early stages of periodontal therapy to enhance the response to treatment, given specific circumstances.[9]

To minimize the indications for surgical operations that are invasive and need advanced technical skills, various additional methods of SRP have been suggested, including the administration of antibiotics or anti-inflammatory medications. These additional therapies enhanced treatment outcomes by reducing probing pocket depth (PPD) and increasing CAL acquisition.[10] Nevertheless, the administration of these pharmaceuticals through enteral routes necessitates the utilization of large amounts of active substances to achieve optimal concentration at the targeted location. Additionally, patient adherence to the prescribed administration schedule is crucial, and there is a possibility of encountering adverse effects. As a result, many localized, topical therapies, such as gels, fibers or chips containing diverse, active compounds and medications, have been created and assessed in clinical environments. The primary benefits of these treatments include targeted administration of potent medications directly to the affected areas, minimized potential for adverse reactions, and the potential for 3-dimensional stability of the blood clot.[11] Periodontitis (which comes in a variety of forms) is one of the leading causes of tooth loss in adults. The most damaging kind of periodontitis among them is called aggressive periodontitis (AgP). Periodontitis is often characterized by 3 factors: A decrease in CAL, radiographic bone loss, and 1 or more sites with inflammation (bleeding on probing). In patients with AgP, SRP treatment by itself does not produce satisfactory outcomes. Thus, it is advised to treat this condition with additional systemic antibiotics. Hence, analysis of previous studies could provide clinical guidance that would aid in the practical management of periodontitis and enhance clinical outcomes. In addition, the findings of the current study would be valuable for determining the impact of different antimicrobial therapies on the clinical outcomes of periodontitis and enhance the ability to select specific antimicrobial therapies.

Objectives

The primary purpose of this investigation was to assess the effects of including adjuvant systemic and topical antimicrobial medications in non-surgical periodontal care on clinical parameters such as CAL and PPD reduction.

Method

Study design

For this meta-analysis, researchers looked at studies that followed a specific protocol to determine the epidemiological impact.[12] Several scientific databases, including Ovid, PubMed, Google Scholar, Cochrane Library, and Embase,[13] were used to collect and analyze data from the included studies, following the inclusion criteria provided. The entire study sequence is shown in Fig. 1.

Eligibility and inclusion

The inclusion criteria were examination of the effects of adjuvant systemic and topical antimicrobial treatment as an adjunct to non-surgical periodontal therapy. Only articles that specifically looked at how various therapies (such as systemic or topical adjuvant antibacterial medication or non-surgical periodontal therapy) reduced the PPD and CAL were included in the sensitivity research. To do subclass and sensitivity analysis, the medical interventional groups were compared to various types of antimicrobials and routes of delivery.

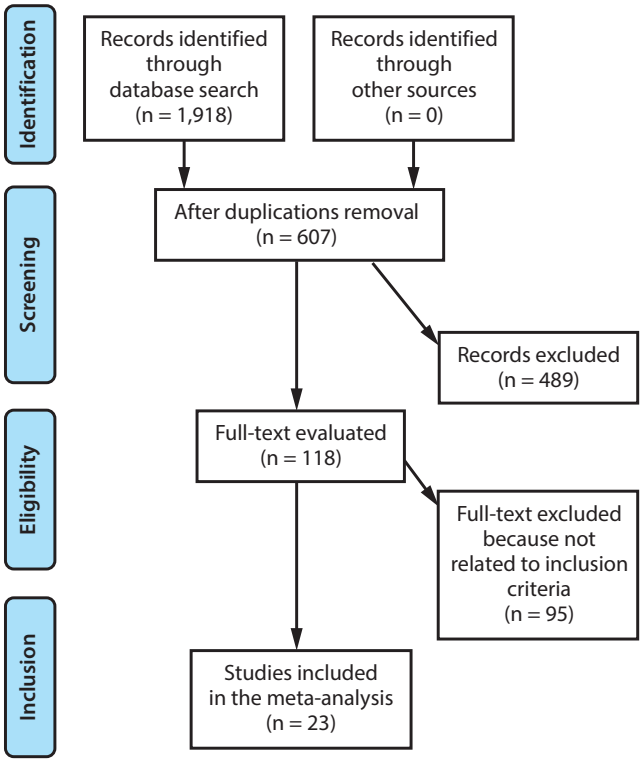

Fig. 1. Study inclusion flowchart

Inclusion criteria

The current study only incorporated randomized clinical trials that were published up until December 2023. In addition, studies including patients who had periodontal conditions treated with SRP were subject to the study. Study designs that compared the effects of 2 interventions (adjuvant antimicrobial agent + non-surgical interventions compared to non-surgical intervention alone) were required for inclusion.

Exclusion criteria

Articles that were unable to convey the results of a comparison between different interventions in a suitable manner, such as interquartile range or median, were excluded. Failure to use mean (± standard deviation (±SD)) to express all results of diverse outcomes was excluded. Finally, we excluded cases where the research was published as a book chapter, letter or review article.

Identification

First, we conducted our research up until December 2023 utilizing a set of terms related to periodontal disorders, antibiotics, CAL, reduction of probing pockets, doxycycline, metronidazole, and amoxicillin. A protocol for the search strategies was defined in accordance with the PICOS principle as follows: P (population) – periodontal conditions; I (intervention/exposure) – non-surgical periodontal therapy and adjuvant antimicrobial agents (topical or systemic); C (comparison) – non-surgical periodontal therapy plus adjuvant antimicrobial agents (topical or systemic) compared with control; O (outcome) – PPD reduction and CAL; S (study design) – randomized clinical trial.

The author did a thorough search of the PubMed, Cochrane Library, Embase, Ovid, and Google Scholar databases until December 2023 using the keywords and related terms. Any article that did not discuss and evaluate the role of early supported discharge compared to traditional care was disregarded after an evaluation of the titles and abstracts of the articles that had been collected into Endnote v. 20 (Clarivate Analytics, London, UK).

Screening

To narrow down the data, certain criteria were used. These criteria included the following: the author's surname, year of publication, country, type of study, length of the study, demographic information, clinical and treatment characteristics, total number of study participants, methods used, information sources, and outcomes. The writer examined the selected papers' methodological quality and checked each study for possible bias.

Through the use of Review Manager v. 5.3 (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark), we were able to determine whether the included pieces of research had a low, medium or high potential for bias.

Statistical analyses

The mean difference (MD) was calculated with a 95% confidence interval (95% CI) using a random-effect model with continuous analysis.[14] The data were fitted with a random-effects model due to a lack of high-level similarity between the included studies. Results of the analysis were expressed in the form of forest plots, which indicated the 95% CI of each group and displayed a visual direction (positive or negative) of the different studies in the same analysis. Group and subgroup analysis were performed for different models; the group analysis reflected the overall results of all included studies, while the subgroup analysis showed the effect of additional factors shared between a small number of studies with the same outcome under investigation.

Tau^2, the degree of heterogeneity, was determined using the constrained maximum-likelihood estimator. A numerical value between 0 and 100, known as the I^2 index, was calculated. Jamovi software (https://www.jamovi.org/) was used to obtain this index. Percentages representing low, moderate and high levels of heterogeneity were also used to display the heterogeneity level, which can range from 0% to 100%. Quantitative research on publication bias was carried out using Begg's and Egger's tests and visual evaluation of funnel plots.

Table 1. Characteristics of clinical trials recruited in the analysis

Study	Country	Year	Intervention type	Intervention	Control	Total
Vyas et al.[15]	India	2019	doxycycline	26	26	52
Al-Nowaiser et al.[16]	Saudi Arabia	2014	doxycycline	35	33	68
Al-Zahran et al.[17]	Saudi Arabia	2009	doxycycline	14	15	29
Gaikwad et al.[18]	India	2013	doxycycline	25	25	50
O'Connel et al.[19]	Brazil	2008	doxycycline	15	15	30
Rodrigues et al.[20]	Brazil	2003	amoxicillin/clavulanic acid	15	15	30
Singh et al.[21]	India	2008	doxycycline	15	15	30
Tsalikis et al.[22]	Greece	2014	doxycycline	31	35	66
Gupta et al.[23]	India	2008	doxycycline	30	30	60
Pandit et al.[24]	India	2013	metronidazole	20	20	40
Lie et al.[25]	China	1998	metronidazole	18	18	36
Eickholz et al.[26]	Germany	2002	doxycycline	110	110	220
Srirangarajan et al.[27]	India	2011	doxycycline	10	10	20
Kinane et al.[28]	UK	1999	metronidazole	19	20	39
Aimetti et al.[29]	Italy	2012	metronidazole/amoxicillin	19	20	39
Guerrero et al.[30]	USA	2005	metronidazole/amoxicillin	20	21	41
Xajigeorgiou et al.[31]	Greece	2006	metronidazole/amoxicillin	10	11	21
Yek et al.[32]	Turkey	2010	metronidazole/amoxicillin	12	16	28
Mestnik et al.[33]	Brazil	2012	metronidazole/amoxicillin	15	15	30
Madi et al.[34]	Saudi Arabia	2018	doxycycline	15	15	30
Tamashiro et al.[35]	Brazil	2016	metronidazole/amoxicillin	29	27	56
Taiete et al. [36]	Brazil	2016	metronidazole/amoxicillin	21	18	39
Grossi et al.[37]	USA	1997	doxycycline	17	22	39

Table 2. Analysis results of all models

Model		Estimate	p-value	95% CI lower limit	95% CI upper limit	I^2	Begg's test	Egger's test
Systemic antimicrobial therapy impact on PPD reduction		0.79	0.006	0.225	1.356	91.45%	0.51	0.001
Subgroup 1	doxycycline	0.947	0.128	–0.273	2.167	96.56%	0.72	0.001
Subgroup 2	metronidazole/amoxicillin	0.796	<0.001	0.539	1.053	0%	1	0.808
Systemic antimicrobial therapy impacts on CAL		0.688	0.128	–0.197	1.573	96.5%	0.19	0.001
Subgroup 1	doxycycline	1.03	0.303	–0.929	2.987	98.61%	0.399	0.001
Subgroup 2	metronidazole/amoxicillin	0.482	<0.001	0.226	0.738	2.87%	0.562	0.665
Adjuvant topical antimicrobial agents' impact on PPD		0.363	<0.001	0.181	0.545	0%	0.548	0.708
Subgroup 1	doxycycline	0.442	0.002	0.165	0.719	23.8%	1.000	0.682
Subgroup 2	metronidazole	0.254	0.175	–0.113	0.622	0%	0.333	0.411

95% CI – 95% confidence interval; PPD – pocket probing depth; CAL – clinical attachment level.

Results

After reviewing 1,918 pertinent studies, 23 research papers from the period between 1997 and 2019, including 1,093 study participants with periodontal disease receiving non-surgical interventions, were selected for the meta-analysis.[15–37] The results of these investigations are compiled in Tables 1,2. In addition, analysis models and publication bias were reported in Fig. 2–6.

Systemic antimicrobial therapy's impact on probing pocket depth reduction

Continuous analysis using the random-effects model of 16 clinical trials for evaluation of the impact of adjuvant antimicrobial therapy on non-surgical periodontal therapy regarding the PPD reduction (Fig. 2A). The outcomes of the analysis showed a significant ($p < 0.01$) beneficial impact of combining systemic antimicrobial therapy with

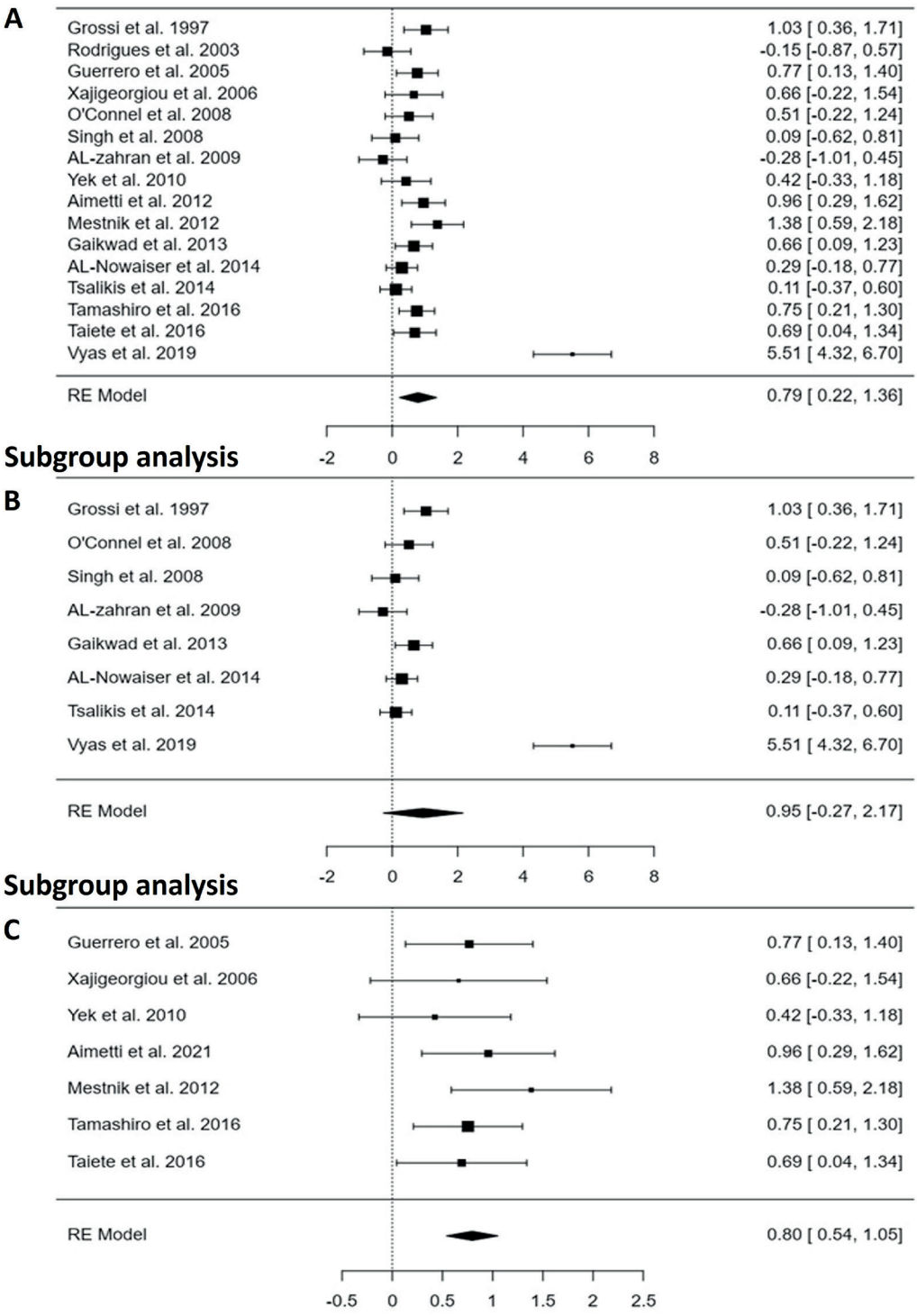

Fig. 2. Forest plot for the analysis of the impact of systemic adjuvant antimicrobial therapy on non-surgical periodontal therapy regarding pocket depth reduction (PPD) (A), subgroup analysis including doxycycline (B) and subgroup analysis including the metronidazole/amoxicillin combination (C)

SRP compared to controls (MD = 0.79, 95% CI: [0.22–1.36], I^2 = 91.4%). A single study (Vyas et al.) may be deemed unduly influential based on Cook's distances.[15]

Systemic doxycycline impact on probing pocket depth reduction

Subgroup continuous analysis using the random-effects model of 8 clinical trials for evaluation of the impact of adjuvant doxycycline therapy on non-surgical periodontal therapy regarding the PPD reduction (Fig. 2B). The outcomes of the analysis showed a non-significant ($p < 0.13$) impact of combining systemic antimicrobial therapy with SCP compared with controls (MD = 0.95, 95% CI:–0.27–2.17, I^2 = 96.5%). One study (Vyas et al.) had a value larger than ±2.73, suggesting that it might be an outlier in the context of this model, according to an analysis of the studentized residuals. By excluding this outlier study, the finding showed a significant ($p = 0.01$) beneficial difference in favor of doxycycline intervention.[15]

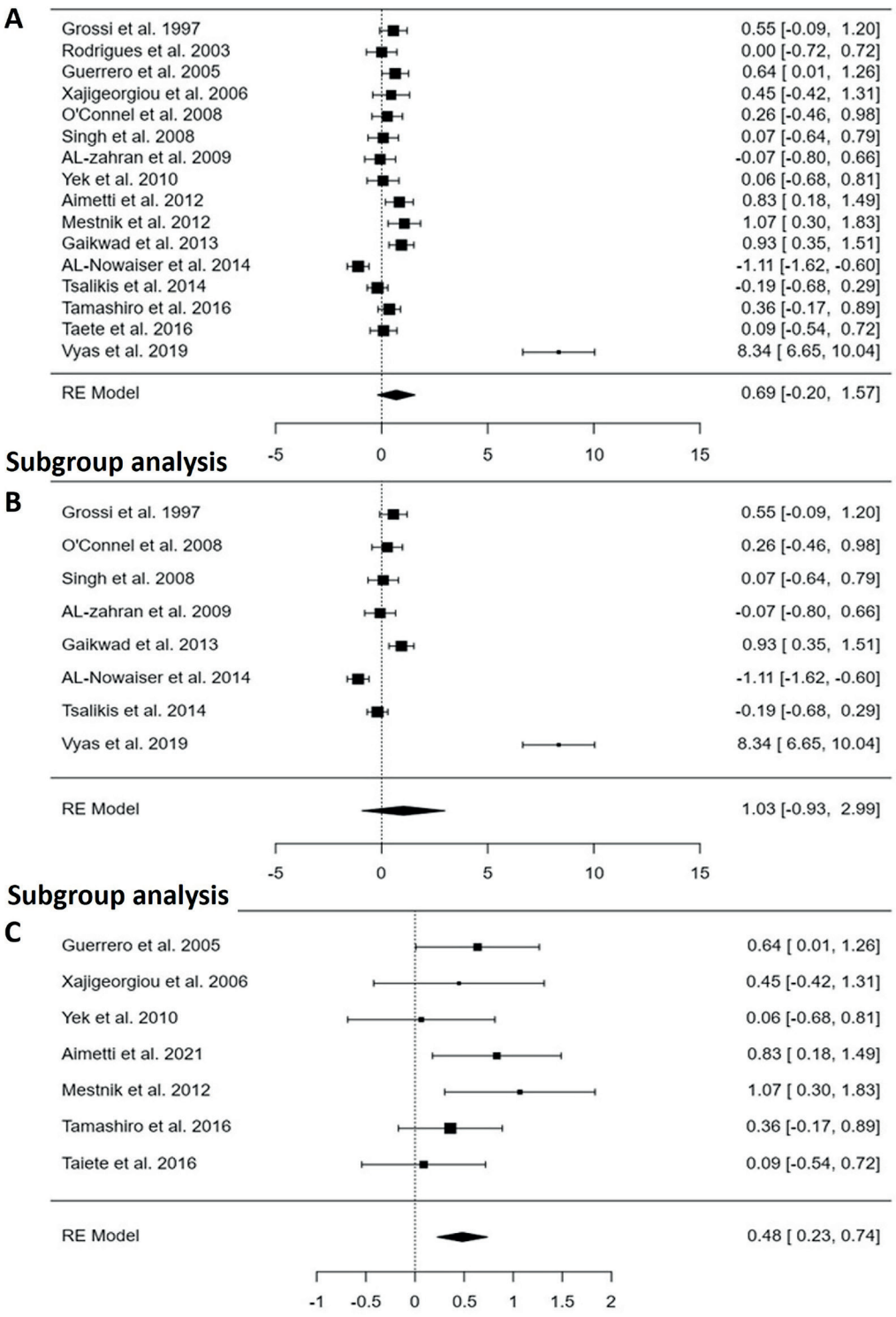

Fig. 3. Forest plot for the analysis of the impact of systemic adjuvant antimicrobial therapy on non-surgical periodontal therapy regarding clinical attachment level (CAL) (A), subgroup analysis including doxycycline (B) and subgroup analysis including the metronidazole/amoxicillin combination (C)

Systemic metronidazole/amoxicillin impact on probing pocket depth reduction

Subgroup analysis using the random-effects continuous analysis model for 7 clinical trials for evaluation of the impact of adjuvant metronidazole/amoxicillin on non-surgical periodontal therapy regarding the PPD reduction (Fig. 2C). The outcomes of the analysis showed a significant ($p < 0.001$), beneficial impact of combining systemic antimicrobial therapy with SRP compared to control (MD = 0.80, 95% CI: 0.54–1.05, $I^2 = 0\%$).

Systemic antimicrobial therapy impacts on clinical attachment level

In contrast to the analysis of 16 studies for PPD, analysis of the impact of systemic administration of adjuvant antimicrobial therapy on CAL (Fig. 3A) using 16 studies

revealed that the addition of antimicrobial drugs did not result in significant (p = 0.13) changes in CAL compared to controls (MD = 0.69, 95% CI: –0.20–1.57], I^2 = 96.5%).

Systemic doxycycline impact on clinical attachment level

Regarding subgroup analysis of the impact of systemic administration of adjuvant antimicrobial therapy on CAL (Fig. 3B), results from the analysis of 8 studies revealed that the addition of doxycycline did not result in significant (p = 0.3) changes in the CAL compared to controls (MD = 1.03, 95% CI: –0.93–2.99, I^2 = 98.6%).

Systemic metronidazole/amoxicillin impact on clinical attachment level

Concerning subgroup analysis of the impact of systemic administration of adjuvant antimicrobial therapy on CAL (Fig. 3C), results from the analysis of 7 studies revealed that the addition of systemic metronidazole/amoxicillin resulted in significant (p < 0.001) changes in the CAL compared to controls, represented as a higher CAL (MD = 0.48, 95% CI: 0.23–0.74, I^2 = 2.87%).

Adjuvant topical antimicrobial agents impact on probing pocket depth

Rgarding topical administration of adjuvant antimicrobial therapy (doxycycline or metronidazole) on PPD, results from the analysis of 8 studies revealed that the addition of topical antimicrobial agents to non-surgical periodontal therapy resulted in significant (p < 0.001) changes in PPD compared to controls, represented as a higher PPD level (MD = 0.36, 95% CI: 0.18–0.55, I^2 = 0%). Among the 8 studies, 5 studies demonstrated the impact of topical doxycycline and 3 papers demonstrated the role of metronidazole. Subgroup analysis according to the type of topical antimicrobial agent showed a different result for doxycycline compared to metronidazole. Doxycycline showed a significantly (p = 0.002) higher impact on PPD compared to controls, while metronidazole did not (p = 0.17). The heterogeneity level for both subgroup analyses was 0% (Fig. 4).

Publication bias

Analysis of publication bias using Begg's and Egger's tests, in addition to the visual evaluation of funnel plots, showed differences between groups regarding the level of publication bias, as shown in Table 2 and Fig. 5,6. Groups that showed low levels or no publication bias were metronidazole/amoxicillin therapy's impact on PPD reduction (Begg's test = 1, Egger's test = 0.808), metronidazole/amoxicillin therapy's impact on PPD (Begg's test = 0.56, Egger's test = 0.66) and models for analysis of topical antimicrobial therapy's impact on PPD (Table 2, Fig. 6). However, it is important to note that some of the analyzed groups contain sample size less than 10; in these cases, the Begg's and Egger's tests may not have the necessary power to identify bias.

Discussion

Twenty-three randomized clinical trials published between the period of 1997 to 2019, including 1,093 subjects with periodontal disease receiving non-surgical intervention, were selected for the current meta-analysis.[15–36]

The addition of a systemic antibiotic to a non-surgical intervention resulted in a significant improvement in terms of the reduction in the PPD. On the other hand, metronidazole/amoxicillin demonstrated a significant impact on the CAL and PPD, whereas doxycycline did not demonstrate any significant impact on the CAL. The use of topical antimicrobial drugs had a substantial favorable impact in reducing pocket depth (PD) in relation to doxycycline; however, the effect connected to metronidazole was not significant.

Three recent systematic reviews have examined the effects of a specific combination of antibiotics, metronidazole plus amoxicillin, in treating chronic periodontitis (ChP), AgP or both. Systematic reviews previously conducted[6,38,39] have shown that using this antibiotic protocol as an adjunct to treatment provides significant benefits. The prevailing method employed in these investigations involved producing aggregated estimations by utilizing the average alterations from the initial state to various time periods after therapy in clinical outcomes resulting from SRP alone or in conjunction with systemic antibiotics. A previous study by Teughels et al. indicated that systemic antimicrobials, when used as an adjuvant in periodontal therapy, produced statistically significant improvements in clinical outcomes, while the intervention groups that received systemic antimicrobials experienced more frequent side effects compared to controls.[40] In contrast, when compared to surgical therapy alone, the use of systemic or local antimicrobials during surgical therapy does not appear to increase the clinical efficacy in patients with peri-implantitis.[41] While another study demonstrated the influence of topical antimicrobial agents showing that the PPD decreased and the CAL gained with statistically significant effects when the adjunctive was locally applied to subgingival antimicrobials.[42] During surgical periodontal therapy, locally given antibiotics lead to longer-lasting post-surgical improvements for CAL, PPD and bleeding on probing (≤6 months).[43]

Typically, when comparing the effectiveness of different antibiotics (as the experimental group) with conventional therapy (SRP as the control group), the studies are combined using a pairwise meta-analysis.[44,45] However, a traditional pairwise meta-analysis is unable to directly compare 3 or more treatment regimens at the same time.

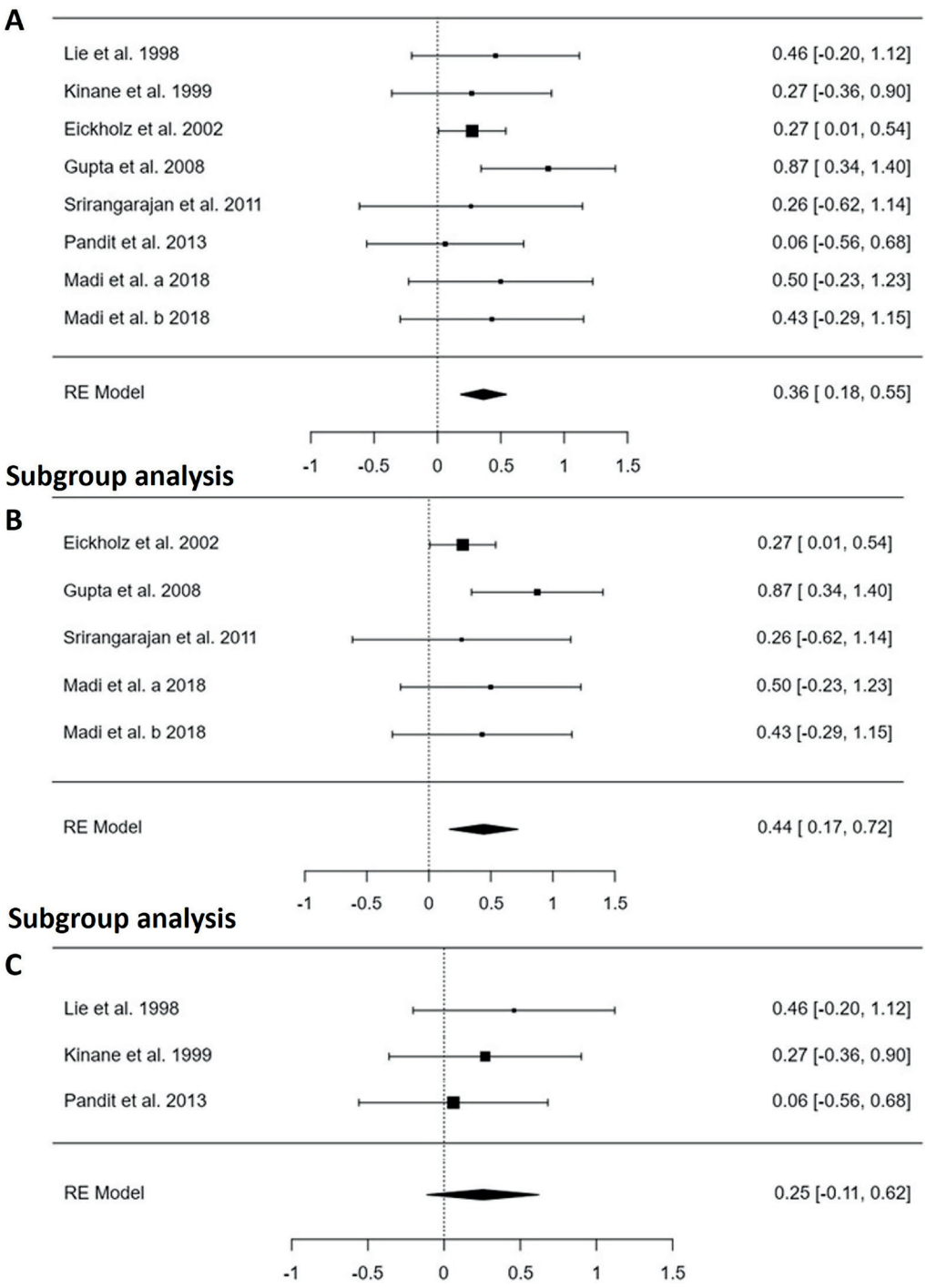

Fig. 4. Forest plot for the analysis of the impact of topical adjuvant antimicrobial therapy on non-surgical periodontal therapy regarding pocket depth reduction (PPD) (A), subgroup analysis including topical doxycycline (B) and subgroup analysis including topical metronidazole (C)

To evaluate the impact of various treatments, it is necessary to do several pairwise analyses for each outcome measured. These analyses are based on studies [46–48] employing a Bayesian network meta-analysis to address this limitation. Instead of doing numerous individual comparisons, this form of meta-analysis evaluates all existing treatment regimens under a single statistical model.[46,49,50]

The results of this meta-analysis align with previous systematic reviews[51–53] that have suggested the clinical effectiveness of systemic antibiotics, particularly metronidazole + amoxicillin, in treating individuals with AgP.[38,45] Nevertheless, a noteworthy outcome of the current study, worth considering, was the substantial therapeutic advantage shown when utilizing additive metronidazole to treat patients with AgP. This pairwise meta-analysis has shown that the combination of SRP and metronidazole resulted in a statistically significant increase in CAL and a decrease in probing depth by 1.08 mm and 1.05 mm, respectively, compared to 0.45 mm and 0.53 mm for the combination of SRP, metronidazole and amoxicillin. It is crucial to highlight that the increased gain in CAL and reduction in PPD seen in the SRP + metronidazole group compared to the SRP + metronidazole/amoxicillin group may be attributed to the severe periodontal damage

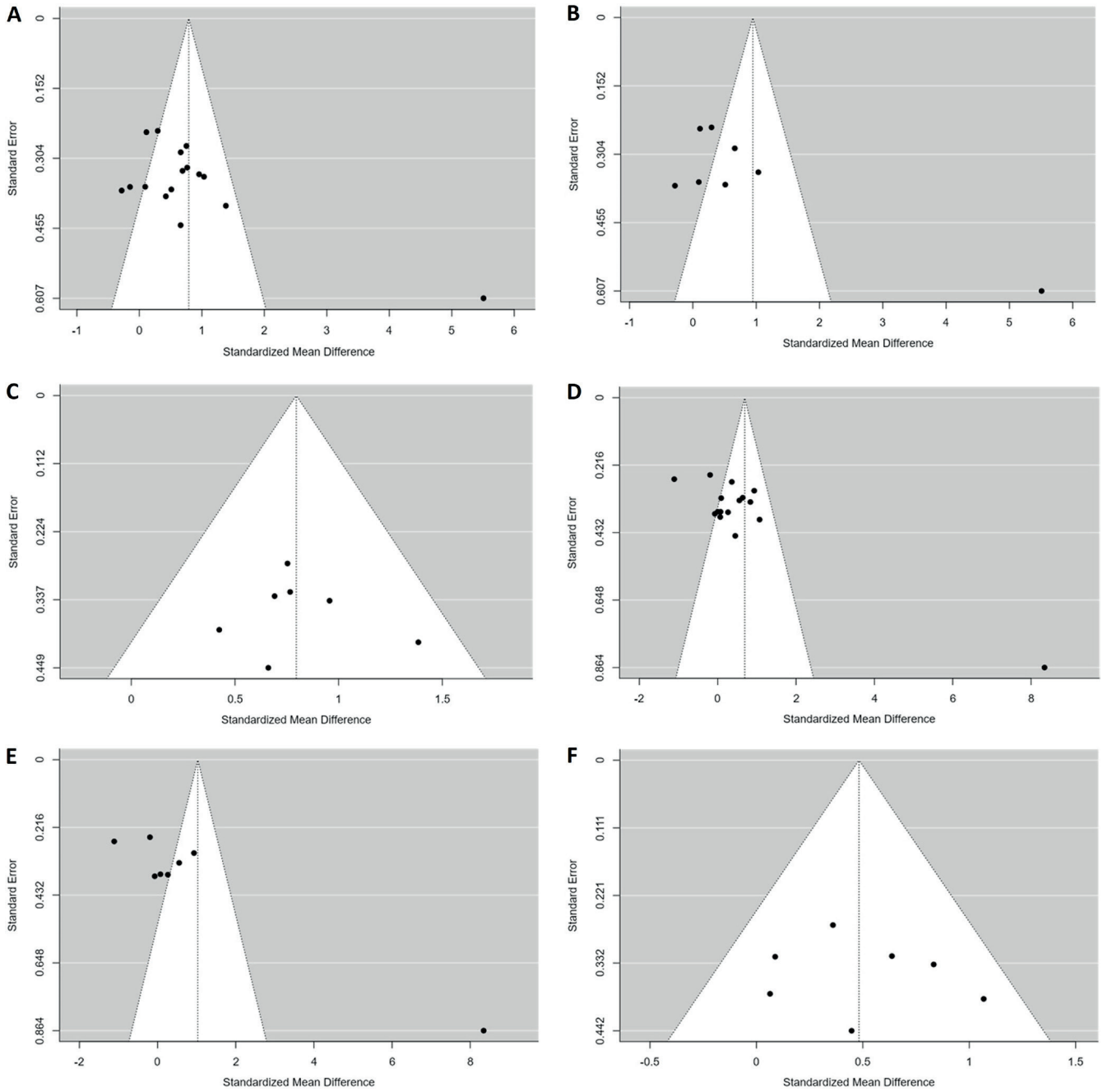

Fig. 5. Funnel plot showing the possibility of publication bias for studies included for the analysis models of the impact of systemic adjuvant antimicrobial therapy to non-surgical periodontal therapy regarding pocket depth reduction (PPD) (A), subgroup analysis of doxycycline (B), subgroup analysis of the metronidazole/amoxicillin combination (C), the impact of systemic adjuvant antimicrobial therapy to non-surgical periodontal therapy regarding clinical attachment level (CAL) (D), subgroup analysis including doxycycline (E), and subgroup analysis including the metronidazole/amoxicillin combination (F)

present in the study population of one of the research studies investigating the effects of metronidazole.[39] The average PD and CAL values at the beginning of the trial were 5.8 mm and 6.2 mm, respectively, when measured across all teeth in the mouth. By comparison, the groups examined in the 5 trials that evaluated the impact of SRP + metronidazole/amoxicillin had average initial PD values ranging from 4.1 mm to 4.63 mm and CAL values from 4.5 mm to 4.97 mm. Therefore, these initial clinical variations may have had a role in the more significant level of recovery seen in participants who were treated with metronidazole compared to those who received metronidazole and amoxicillin. Furthermore, it is crucial to acknowledge that the favorable outcomes found for metronidazole were derived solely from the analysis of data from 2 randomized controlled trials.[31,39] Hence, the data regarding the significant advantages of metronidazole in treating AgP, as seen in the current study, should be approached with caution, especially when compared to the benefits observed for metronidazole + amoxicillin.

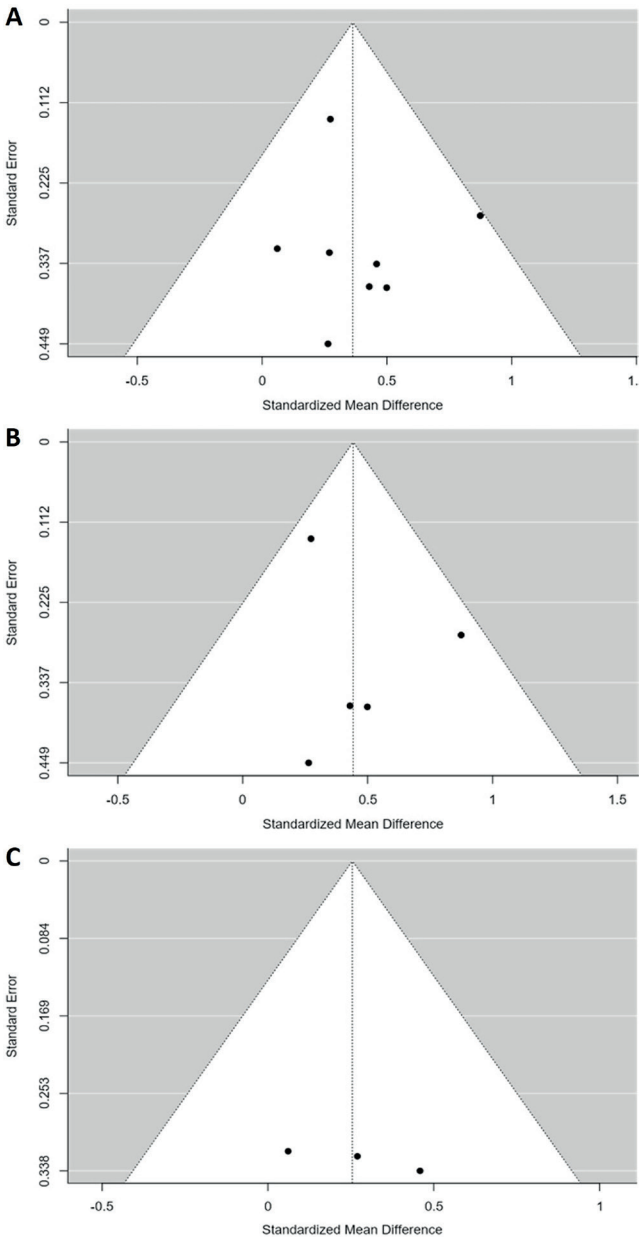

Fig. 6. Funnel plot for the analysis of the publication bias for models used for assessing the impact of topical adjuvant antimicrobial therapy on non-surgical periodontal therapy regarding pocket depth reduction (PPD) (A), subgroup analysis including topical doxycycline (B) and subgroup analysis including topical metronidazole (C)

The primary constraints associated with the utilization of locally administered supplementary treatment are the gel's stability within the defect, the amount of active medication accessible at the site and the duration of drug release. Local antimicrobials used as an adjuvant increased the effectiveness of non-surgical periodontal therapy in lowering PPD and raising CAL at sites where the PD present was ≥5 mm prior to treatment. The available data does not support comparable benefits when systemic antibiotics or antimicrobials are combined with SRP as part of therapy.[54] Various hydrogels, including chitosan, xanthan and hyaluronic acid, have been examined. However, these hydrogels lack long-term stability of their 3-dimensional (3D) shape when compared to in-situ forming gel.[55] No control group was utilized for the duration of contact when topical antimicrobial gels were injected into the periodontal pocket in the experiments included. Thus, in multiple investigations, the application of gel was repeated at a 1-week interval on multiple occasions. This could be perceived as an inconvenience due to the time requirement and patient compliance needed for successful treatment. Furthermore, the majority of the studies included a follow-up period ranging from 3 months to 1 year. This follow-up period aligns with the duration required to achieve a state of stable healing. It is worth noting that the study did not evaluate pocket closure as a primary outcome. Enabling an objective comparison of trials and accurately evaluating the clinical efficacy of the tested treatment would be a highly useful measure. The majority of the papers analyzed exhibited significant heterogeneity, underscoring the necessity for additional verification of these findings.

Limitations

The absence of large multicenter studies and studies with large sample sizes are considered limitations of this analysis. Comparing different doses and therapy durations should be taken into consideration in further studies as most of the analyzed trials did not compare the impact of drug dose and duration.

Conclusions

When combined with non-surgical periodontal care, adjuvant systemic and topical antimicrobial medicines demonstrated a favorable influence on PPD reduction and CAL in cases of periodontal disease (metronidazole/amoxicillin). In addition, the application of doxycycline as a topical treatment has shown a positive impact on the decrease in PPD. Further multicenter randomized studies are needed to evaluate the influence of different medication doses on clinical outcomes.

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References

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. *Lancet*. 2005;366(9499):1809–1820. doi:10.1016/S0140-6736(05)67728-8
2. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJL, Marcenes W. Global burden of severe periodontitis in 1990–2010: A systematic review and meta-regression. *J Dent Res*. 2014;93(11):1045–1053. doi:10.1177/0022034514552491
3. Darveau RP, Hajishengallis G, Curtis MA. *Porphyromonas gingivalis* as a potential community activist for disease. *J Dent Res*. 2012;91(9):816–820. doi:10.1177/0022034512453589
4. Hajishengallis G. The inflammophilic character of the periodontitis-associated microbiota. *Mol Oral Microbiol*. 2014;29(6):248–257. doi:10.1111/omi.12065

5. Matuliene G, Pjetursson BE, Salvi GE, et al. Influence of residual pockets on progression of periodontitis and tooth loss: Results after 11 years of maintenance. *J Clin Periodontol*. 2008;35(8):685–695. doi:10.1111/j.1600-051X.2008.01245.x
6. Petit C, Schmeltz S, Burgy A, Tenenbaum H, Huck O, Davideau JL. Risk factors associated with long-term outcomes after active and supporting periodontal treatments: Impact of various compliance definitions on tooth loss. *Clin Oral Invest*. 2019;23(11):4123–4131. doi:10.1007/s00784-019-02851-x
7. Suvan J, Leira Y, Moreno Sancho FM, Graziani F, Derks J, Tomasi C. Subgingival instrumentation for treatment of periodontitis: A systematic review. *J Clin Periodontol*. 2020;47(Suppl 22):155–175. doi:10.1111/jcpe.13245
8. Sanz M, Herrera D, Kebschull M, et al. Treatment of stage I–III periodontitis: The EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2020;47(Suppl 22):4–60. doi:10.1111/jcpe.13290
9. Herrera D, Sanz M, Kebschull M, et al. Treatment of stage IV periodontitis: The EFP S3 level clinical practice guideline. *J Clin Periodontol*. 2022;49(Suppl 4):4–71. doi:10.1111/jcpe.13639
10. Sgolastra F, Petrucci A, Ciarrocchi I, Masci C, Spadaro A. Adjunctive systemic antimicrobials in the treatment of chronic periodontitis: A systematic review and network meta-analysis. *J Periodontal Res*. 2021;56(2):236–248. doi:10.1111/jre.12821
11. Li J, Mooney DJ. Designing hydrogels for controlled drug delivery. *Nat Rev Mater*. 2016;1(12):16071. doi:10.1038/natrevmats.2016.71
12. Zangeneh MM, Zangeneh A. Prevalence of wound infection following right anterolateral thoracotomy and median sternotomy for resection of benign atrial masses that induce heart failure, arrhythmia, or thromboembolic events: A meta-analysis. *Int J Clin Med Res*. 2023;2(1):37–48. doi:10.61466/ijcmr2010004
13. Singh RK. A meta-analysis of the impact on gastrectomy versus endoscopic submucosal dissection for early stomach cancer. *Int J Clin Med Res*. 2023;1(3):37–48. doi:10.61466/ijcmr1030011
14. Sheikhbahaei S, Trahan TJ, Xiao J, et al. FDG-PET/CT and MRI for evaluation of pathologic response to neoadjuvant chemotherapy in patients with breast cancer: A meta-analysis of diagnostic accuracy studies. *Oncologist*. 2016;21(8):931–939. doi:10.1634/theoncologist.2015-0353
15. Vyas D, Deshpande N, Dave D. Effect of systemic doxycycline on scaling and root planing in chronic periodontitis. *J Integr Health Sci*. 2019;7(1):8. doi:10.4103/JIHS.JIHS_4_18
16. Al-Nowaiser AM, Al-Zoman H, Baskaradoss JK, et al. Evaluation of adjunctive systemic doxycycline with non-surgical periodontal therapy within type 2 diabetic patients. *Saudi Med J*. 2014;35(10):1203–1209. PMID:25316464. PMCID:PMC4362117.
17. Al-Zahrani MS, Bamshmous SO, Alhassani AA, Al-Sherbini MM. Short-term effects of photodynamic therapy on periodontal status and glycemic control of patients with diabetes. *J Periodontol*. 2009;80(10):1568–1573. doi:10.1902/jop.2009.090206
18. Gaikwad SP, Gurav AN, Shete AR, Desarda HM. Effect of scaling and root planing combined with systemic doxycycline therapy on glycemic control in diabetes mellitus subjects with chronic generalized periodontitis: A clinical study. *J Periodontal Implant Sci*. 2013;43(2):79. doi:10.5051/jpis.2013.43.2.79
19. O'Connell PAA, Taba M, Nomizo A, et al. Effects of periodontal therapy on glycemic control and inflammatory markers. *J Periodontol*. 2008;79(5):774–783. doi:10.1902/jop.2008.070250
20. Rodrigues DC, Taba M, Novaes AB, Souza SLS, Grisi MFM. Effect of non-surgical periodontal therapy on glycemic control in patients with type 2 diabetes mellitus. *J Periodontol*. 2003;74(9):1361–1367. doi:10.1902/jop.2003.74.9.1361
21. Singh S, Kumar V, Kumar S, Subbappa A. The effect of periodontal therapy on the improvement of glycemic control in patients with type 2 diabetes mellitus: A randomized controlled clinical trial. *Int J Diab Dev Ctries*. 2008;28(2):38. doi:10.4103/0973-3930.43097
22. Tsalikis L, Sakellari D, Dagalis P, Boura P, Konstantinidis A. Effects of doxycycline on clinical, microbiological and immunological parameters in well-controlled diabetes type-2 patients with periodontal disease: A randomized, controlled clinical trial. *J Clin Periodontol*. 2014;41(10):972–980. doi:10.1111/jcpe.12287
23. Gupta R, Pandit N, Aggarwal S, Verma A. Comparative evaluation of subgingivally delivered 10% doxycycline hyclate and xanthan-based chlorhexidine gels in the treatment of chronic periodontitis. *J Contemp Dent Pract*. 2008;9(7):25–32. PMID:18997913.
24. Pandit N, Dahiya R, Gupta R, Bali D, Kathuria A. Comparative evaluation of locally delivered minocycline and metronidazole in the treatment of periodontitis. *Contemp Clin Dent*. 2013;4(1):48. doi:10.4103/0976-237X.111615
25. Lie T, Bruun G, Böe OE. Effects of topical metronidazole and tetracycline in treatment of adult periodontitis. *J Periodontol*. 1998;69(7):819–827. doi:10.1902/jop.1998.69.7.819
26. Eickholz P, Kim T, Bürklin T, et al. Non-surgical periodontal therapy with adjunctive topical doxycycline: A double-blind randomized controlled multicenter study. I. Study design and clinical results. *J Clin Periodontol*. 2002;29(2):108–117. doi:10.1034/j.1600-051x.2002.290204.x
27. Srirangarajan S, Mundargi RC, Ravindra S, Setty SB, Aminabhavi TM, Thakur S. Randomized, controlled, single-masked, clinical study to compare and evaluate the efficacy of microspheres and gel in periodontal pocket therapy. *J Periodontol* 2011;82(1):114–121. doi:10.1902/jop.2010.100324
28. Kinane DF, Radvar M. A six-month comparison of three periodontal local antimicrobial therapies in persistent periodontal pockets. *J Periodontol*. 1999;70(1):1–7. doi:10.1902/jop.1999.70.1.1
29. Aimetti M, Romano F, Guzzi N, Carnevale G. Full-mouth disinfection and systemic antimicrobial therapy in generalized aggressive periodontitis: A randomized, placebo-controlled trial. *J Clin Periodontol*. 2012;39(3):284–294. doi:10.1111/j.1600-051X.2011.01795.x
30. Guerrero A, Griffiths GS, Nibali L, et al. Adjunctive benefits of systemic amoxicillin and metronidazole in non-surgical treatment of generalized aggressive periodontitis: A randomized placebo-controlled clinical trial. *J Clin Periodontol*. 2005;32(10):1096–1107. doi:10.1111/j.1600-051X.2005.00814.x
31. Xajigeorgiou C, Sakellari D, Slini T, Baka A, Konstantinidis A. Clinical and microbiological effects of different antimicrobials on generalized aggressive periodontitis. *J Clin Periodontol*. 2006;33(4):254–264. doi:10.1111/j.1600-051X.2006.00905.x
32. Yek EC, Cintan S, Topcuoglu N, Kulekci G, Halim İssever, Kantarci A. Efficacy of amoxicillin and metronidazole combination for the management of generalized aggressive periodontitis. *J Periodontol*. 2010;81(7):964–974. doi:10.1902/jop.2010.090522
33. Mestnik MJ, Feres M, Figueiredo LC, et al. The effects of adjunctive metronidazole plus amoxicillin in the treatment of generalized aggressive periodontitis: A 1-year double-blinded, placebo-controlled, randomized clinical trial. *J Clin Periodontol*. 2012;39(10):955–961. doi:10.1111/j.1600-051X.2012.01932.x
34. Madi M, Pavlic V, Samy W, Alagl A. The anti-inflammatory effect of locally delivered nano-doxycycline gel in therapy of chronic periodontitis. *Acta Odontol Scand*. 2018;76(1):71–76. doi:10.1080/00016357.2017.1385096
35. Tamashiro NS, Duarte PM, Miranda TS, et al. Amoxicillin plus metronidazole therapy for patients with periodontitis and type 2 diabetes: A 2-year randomized controlled trial. *J Dent Res*. 2016;95(7):829–836. doi:10.1177/0022034516639274
36. Taiete T, Casati MZ, Ribeiro Édel P, Sallum EA, Nociti Júnior FH, Casarin RC. Amoxicillin/metronidazole associated with nonsurgical therapy did not promote additional benefits in immunologic parameters in generalized aggressive periodontitis: A randomized controlled clinical trial. *Quintessence Int*. 2016;47(4):281–292. doi:10.3290/j.qi.a34723
37. Grossi SG, Skrepcinski FB, DeCaro T, et al. Treatment of periodontal disease in diabetics reduces glycated hemoglobin. *J Periodontol*. 1997;68(8):713–719. doi:10.1902/jop.1997.68.8.713
38. Sgolastra F, Petrucci A, Gatto R, Monaco A. Effectiveness of systemic amoxicillin/metronidazole as an adjunctive therapy to full-mouth scaling and root planing in the treatment of aggressive periodontitis: A systematic review and meta-analysis. *J Periodontol*. 2012;83(6):731–743. doi:10.1902/jop.2011.110432
39. Sigusch B, Beier M, Klinger G, Pfister W, Glockmann E. A 2-step nonsurgical procedure and systemic antibiotics in the treatment of rapidly progressive periodontitis. *J Periodontol*. 2001;72(3):275–283. doi:10.1902/jop.2001.72.3.275

40. Teughels W, Feres M, Oud V, Martín C, Matesanz P, Herrera D. Adjunctive effect of systemic antimicrobials in periodontitis therapy: A systematic review and meta-analysis. *J Clin Periodontol*. 2020;47(Suppl 22): 257–281. doi:10.1111/jcpe.13264
41. Teughels W, Seyssens L, Christiaens V, Temmerman A, Castro AB, Cosyn J. Adjunctive locally and systemically delivered antimicrobials during surgical treatment of peri-implantitis: A systematic review. *J Clin Periodontol*. 2023;50(Suppl 26):359–372. doi:10.1111/jcpe.13773
42. Herrera D, Matesanz P, Martín C, Oud V, Feres M, Teughels W. Adjunctive effect of locally delivered antimicrobials in periodontitis therapy: A systematic review and meta-analysis. *J Clin Periodontol*. 2020;47 (Suppl 22):239–256. doi:10.1111/jcpe.13230
43. Yusri S, Elfana A, Elbattawy W, Fawzy El-Sayed KM. Effect of locally delivered adjunctive antibiotics during surgical periodontal therapy: A systematic review and meta-analysis. *Clin Oral Invest*. 2021;25(9): 5127–5138. doi:10.1007/s00784-021-04056-7
44. Sgolastra F, Gatto R, Petrucci A, Monaco A. Effectiveness of systemic amoxicillin/metronidazole as adjunctive therapy to scaling and root planing in the treatment of chronic periodontitis: A systematic review and meta-analysis. *J Periodontol*. 2012;83(10):1257–1269. doi:10.1902/jop.2012.110625
45. Zandbergen D, Slot DE, Cobb CM, Van Der Weijden FA. The clinical effect of scaling and root planing and the concomitant administration of systemic amoxicillin and metronidazole: A systematic review. *J Periodontol*. 2013;84(3):332–351. doi:10.1902/jop.2012.120040
46. Faggion CM, Chambrone L, Listl S, Tu Y. Network meta-analysis for evaluating interventions in implant dentistry: The case of peri-implantitis treatment. *Clin Implant Dent Rel Res*. 2013;15(4):576–588. doi:10.1111/j.1708-8208.2011.00384.x
47. Chambrone L, Pannuti CM, Tu Y, Chambrone LA. Evidence-based periodontal plastic surgery. II. An individual data meta-analysis for evaluating factors in achieving complete root coverage. *J Periodontol*. 2012;83(4):477–490. doi:10.1902/jop.2011.110382
48. Chambrone L, Preshaw PM, Rosa EF, et al. Effects of smoking cessation on the outcomes of non-surgical periodontal therapy: A systematic review and individual patient data meta-analysis. *J Clin Periodontol*. 2013;40(6):607–615. doi:10.1111/jcpe.12106
49. Ades AE, Sculpher M, Sutton A, et al. Bayesian methods for evidence synthesis in cost-effectiveness analysis. *PharmacoEconomics*. 2006; 24(1):1–19. doi:10.2165/00019053-200624010-00001
50. Sutton A, Ades AE, Cooper N, Abrams K. Use of indirect and mixed treatment comparisons for technology assessment. *PharmacoEconomics*. 2008;26(9):753–767. doi:10.2165/00019053-200826090-00006
51. Herrera D, Sanz M, Jepsen S, Needleman I, Roldán S. A systematic review on the effect of systemic antimicrobials as an adjunct to scaling and root planing in periodontitis patients. *J Clin Periodontol*. 2002; 29(Suppl 3):136–159. doi:10.1034/j.1600-051X.29.s3.8.x
52. Haffajee AD, Socransky SS, Gunsolley JC. Systemic anti-infective periodontal therapy: A systematic review. *Ann Periodontol*. 2003;8(1): 115–181. doi:10.1902/annals.2003.8.1.115
53. Keestra JAJ, Grosjean I, Coucke W, Quirynen M, Teughels W. Non-surgical periodontal therapy with systemic antibiotics in patients with untreated aggressive periodontitis: A systematic review and meta-analysis. *J Periodontal Res*. 2015;50(6):689–706. doi:10.1111/jre.12252
54. Chambrone L, Vargas M, Arboleda S, et al. Efficacy of local and systemic antimicrobials in the non-surgical treatment of smokers with chronic periodontitis: A systematic review. *J Periodontol*. 2016;87(11): 1320–1332. doi:10.1902/jop.2016.160268
55. Dubar M, Lizambard M, Delcourt-Debruyne E, et al. In-situ forming drug-delivery systems for periodontal treatment: Current knowledge and perspectives. *Biomed Mater*. 2021;16(6):062003. doi:10.1088/1748-605X/ac254c

Effectiveness of nursing care intervention on the management of patients with chronic obstructive pulmonary disease: A systematic review and meta-analysis of randomized controlled trials

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Abstract

Background. Chronic obstructive pulmonary disease (COPD) contributes considerably to morbidity and mortality worldwide, necessitating innovative interventions to enhance patient outcomes.

Objectives. The present synthesis aimed to discern the impact of nursing interventions on physical, mental and social health outcomes among COPD patients, focusing on 6-minute walk distance (6MWD), self-efficacy, anxiety, depression, dyspnea, hospitalization, St. George's Respiratory Questionnaire score, patient satisfaction, and all-cause mortality.

Materials and methods. This review was conducted to include randomized controlled trials exploring nursing interventions for COPD patients without demographic restriction and sourced from several databases (MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, CINAHL (Cumulative Index to Nursing and Allied Health Literature), and OpenGrey) until September 2023. Quality assessments were done using the Cochrane Risk of Bias 2 (RoB 2) tool, followed by meta-analysis using a random-effects model with continuous outcomes interpreted as standardized mean difference (SMD) and categorical outcomes as risk ratio (RR).

Results. Thirty-six studies were incorporated, revealing nursing interventions to notably enhance 6MWD (SMD: 0.628, $p = 0.001$) and self-efficacy (SMD: 0.800, $p < 0.001$), and significantly decrease anxiety (SMD: −0.952, $p = 0.015$) and depression levels (SMD: −0.952, $p = 0.006$). However, the effects of hospitalization, quality of life (QoL) and dyspnea did not reach statistical significance. Notably, high heterogeneity was observed in several outcomes.

Conclusions. Nursing interventions yielded significant improvements for 6MWD, self-efficacy, anxiety, and depression among COPD patients. However, their impact on hospitalization and QoL remains indeterminate, necessitating further nuanced research to optimize and tailor nursing care strategies for this demographic. Enhanced intervention standardization and larger, multicenter trials are warranted to confirm and expand these findings.

Key words: quality of life, nursing, meta-analysis, chronic obstructive pulmonary disease

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Background

Chronic obstructive pulmonary disease (COPD) represents one of the most prevalent and impactful respiratory diseases globally. The World Health Organization (WHO) lists it as the 3rd leading cause of death, causing approx. 3.3 million deaths worldwide.[1] The global burden of COPD continues to increase, particularly in low- and middle-income countries, with a significant economic impact on healthcare systems.[2]

Defined by persistent respiratory symptoms and airflow limitation due to airway and/or alveolar abnormalities, COPD is typically caused by significant exposure to noxious particles or gases.[3] Tobacco smoke, occupational dust and chemicals, along with indoor and outdoor air pollution, have been identified as primary risk factors for the development of COPD.[4]

Clinical management of COPD poses numerous challenges. Patients with COPD often face recurrent exacerbations, which not only compromise their quality of life (QoL) but also lead to hospital admissions, contributing to the disease's economic burden. Furthermore, the comorbidities associated with COPD, such as cardiovascular diseases, osteoporosis and anxiety or depression, compound the complexity of its management.[5]

Nursing care, as an integral component of COPD management, has evolved over the years. The shift towards a patient-centered care approach has highlighted the pivotal role of nursing interventions in improving the QoL of COPD patients, reducing hospitalizations and managing symptoms.[6] These interventions encompass a broad spectrum, from health education and exercise training to behavioral therapy and self-management programs.

Health education is one of the cornerstones of nursing care interventions. Patients with COPD, when adequately educated about their disease, its progression and potential triggers, are more likely to adhere to treatment regimens and actively participate in their care. Such education also enables early recognition of exacerbations, thereby preventing hospital admissions.[7]

Exercise training, especially pulmonary rehabilitation, has proven to be highly beneficial for COPD patients. These programs, often managed by nurses, focus on strengthening respiratory muscles, improving exercise tolerance and enhancing overall wellbeing. Studies have consistently shown that patients undergoing pulmonary rehabilitation experience fewer hospital admissions, improved QoL and better exercise capacity.[8,9]

Behavioral therapies, including cognitive-behavioral therapy, are also being integrated into nursing care interventions for COPD patients. Such therapies, aiming at addressing the psychological comorbidities of COPD, have demonstrated improved mental health outcomes and reduced hospital admissions.[10]

Self-management programs that empower patients to take an active role in their care have gained significant traction in recent years. These programs, often facilitated by nurses, provide COPD patients with tools and strategies to manage their symptoms, monitor medication and promptly respond to exacerbations.[11] The importance of nurse-led management programs for COPD patients cannot be overstated. Nurses, with their detailed knowledge and regular patient interactions, are ideally positioned to lead these programs. They can provide continuous, comprehensive education on the disease process, inhaler techniques and lifestyle modifications. Moreover, nurse-led programs facilitate improved communication between patients and healthcare providers, enabling timely interventions and adjustments to treatment plans.

Given the diverse array of nursing interventions available for COPD management, it becomes imperative to ascertain their efficacy through rigorous research. While individual randomized controlled trials (RCTs) have provided insights into specific interventions, a comprehensive synthesis through a systematic review and meta-analysis can provide a clearer picture of the overall effectiveness of nursing care interventions in managing COPD.

The importance of synthesizing existing evidence lies in the fact that COPD, given its chronic nature, requires long-term, holistic care. While pharmacological interventions play an essential role, non-pharmacological strategies, especially those delivered by nursing professionals, can significantly impact patient outcomes, QoL and self-efficacy. With the increasing healthcare costs and the rising global burden of COPD, there is a pressing need to identify cost-effective and efficient strategies that can be integrated into daily clinical practice.

Objectives

This systematic review and meta-analysis aims to synthesize the evidence from RCTs on the effectiveness of various nursing care interventions in COPD management, providing clinicians, policymakers and researchers with valuable insights and directions for future research.

Methods

In alignment with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines,[12] we systematically constructed and executed this meta-analysis to assess the effect of nursing interventions for COPD patients.

This review included studies involving patients diagnosed with chronic obstructive pulmonary disease (COPD), regardless of age, gender, ethnicity, or geographical location. Eligible interventions focused on nursing or nurse-led approaches provided in hospital or primary care settings, compared to conventional care or non-nurse-led interventions. The outcomes assessed encompassed

physical parameters such as all-cause mortality, 6-minute walk distance (6MWD), dyspnea, and hospitalization rates. Mental health-related outcomes, including depression and anxiety, were also considered, alongside measures of QoL assessed through the St. George's Respiratory Questionnaire (SGRQ), self-efficacy scores, and patient satisfaction.

Study design

Randomized clinical trials written in English from the inception of the database until September 2023 were included. Randomized clinical trials remain the gold standard for evaluating the efficacy of therapeutic interventions. By synthesizing data from various RCTs on nursing care interventions for COPD, we can draw more robust conclusions, validate findings and potentially identify areas for future research. It is especially crucial given the heterogeneity in nursing interventions, patient populations, healthcare settings, and outcomes measured across different studies.

Both peer-reviewed articles and grey literature were sought to circumvent publication bias.

Information sources and search strategy

A comprehensive search was initiated on databases such as MEDLINE, Cochrane Central Register of Controlled Trials (CENTRAL), Scopus, CINAHL (Cumulative Index to Nursing and Allied Health Literature), and OpenGrey. Reference lists of relevant articles and reviews were manually scrutinized. Correspondence with authors was established as required to obtain additional information or clarity. Search terms combined "nursing," "chronic obstructive pulmonary disease" and each of the outcomes listed along with appropriate Boolean operators like "AND," "OR" and "NOT." Language (only publications in English) and time restrictions (up to September 2023) were applied. The full search strategy can be found in the Supplementary Appendix.

Selection process

Rayyan was utilized to manage and categorize identified studies. After discarding duplicates, the remaining articles were critically evaluated for inclusion. Two authors (Y.D. and L.Z.) independently assessed titles and abstracts of captured articles. They subsequently reviewed the full text of potential inclusions. Consensus was reached either via discussion or, if necessary, by involving a 3rd author (Y.W.).

Data collection process and data items

A standardized manual form was used by the same researchers (Y.D. and L.Z.) for independent data extraction. The information retrieved encompassed study attributes like author information; journal; study title; year of publication; participant demographics like age, gender distribution, and comorbidities; intervention details in terms of nursing type, frequency, duration, intensity, and follow-up; and all of the outcome measures mentioned above.

Study risk of bias assessment

The Cochrane Risk of Bias 2 (RoB 2) tool was employed to evaluate the risk of bias in included RCTs.[13] Differences in assessments between authors (X.X. and B.W.) were resolved through dialogue or consultation with a 3rd authors (Y.W.).

This tool assesses the bias in 5 key domains. The 1st domain evaluates the bias arising from the randomization process. It considers the random sequence generation and allocation concealment mechanisms to determine if they introduce any systematic differences between intervention and comparison groups. The 2nd domain examines bias due to deviations from the intended interventions, which might result from blinding inadequacies or other reasons. The analysis is conducted for both participants and study personnel to understand if the outcome measurement is affected by knowledge of the received intervention. The 3rd domain addresses bias arising from incomplete outcome data. Studies are scrutinized for dropout rates, reasons for missing data, and whether appropriate methods were used to handle such missing data. The 4th domain focuses on bias introduced during outcome measurement. It checks for blinding of outcome assessors and determines if the outcomes were measured in the same way across intervention groups. Bias in the final domain arises when the reported outcome is selected from multiple outcome measurements or analysis methods. It checks for pre-specification and reporting consistency to ensure that selective reporting does not affect the results.

Each domain is rated as "low risk," "some concerns" or "high risk" of bias. The overall risk for each study is then categorized based on the domain with the highest risk. For instance, if 1 domain is rated as "high risk," the overall risk for the study is also deemed "high."

Effect measures and synthesis methods

The collected data were systematically synthesized to draw consolidated findings. Meta-analysis was performed, and pooled effect sizes were computed using a random-effects model, given the expected variability in populations, interventions and outcome measurement.[14] The primary measure of treatment effect for continuous outcomes was the mean difference (MD) or standardized mean difference (SMD) when different scales were used. For dichotomous outcomes, risk ratios (RR) with 95% confidence intervals (95% CIs) were computed. Each outcome was presented in individual forest plots, showing the effect size and CIs of individual studies and the pooled effect size. All the analyses were conducted utilizing STATA v. 14.2 (StataCorp LLC, College Station, USA).

The I^2 statistic was employed to quantify the extent of variability in effect sizes that could not be explained by sampling error alone. An I^2 value above 50% indicated substantial heterogeneity.[14] To assess the robustness of our findings, sensitivity analyses were performed. This involved excluding studies with a high risk of bias, conducting analyses with different statistical models or removing studies with outlier results.

The symmetry of funnel plots was visually assessed to detect potential biases, with specific consideration for the distortions associated with using SMD as the effect size measure. To mitigate these distortions and ensure a more accurate assessment of publication bias and small-study effects, we employed an alternative precision estimate of $1/\sqrt{n}$ (i.e., n is the total sample size) in our funnel plot analyses. This adjustment, based on sample size, was chosen in response to documented concerns over SMD-related funnel plot asymmetry, providing a more reliable foundation for our statistical appraisal. Consequently, Egger's regression test was applied using this revised precision estimate, offering a more statistically robust evaluation of funnel plot symmetry and the potential presence of publication bias.

Results

Search results and study selection

From an initial identification of 2,435 records in databases, 621 duplicates were removed. Of the 1,814 records screened, 1,707 were excluded, leading to 107 full-text articles assessed for eligibility. Finally, 34 studies met the inclusion criteria (Fig. 1).[15–48]

Characteristics of the included studies

The included 34 studies investigating nurse-led interventions for patients with COPD originated from varied global locales, including the USA, Turkey, China, and the UK, between 2003 and 2022. Notably, the sample size within the intervention arm across studies fluctuates between 8 and 217 participants. While most studies focused on patients aged 40 years and above, interventions primarily revolved around telephonic support, home visits and various nurse-led programs, contrasting with control groups typically receiving usual

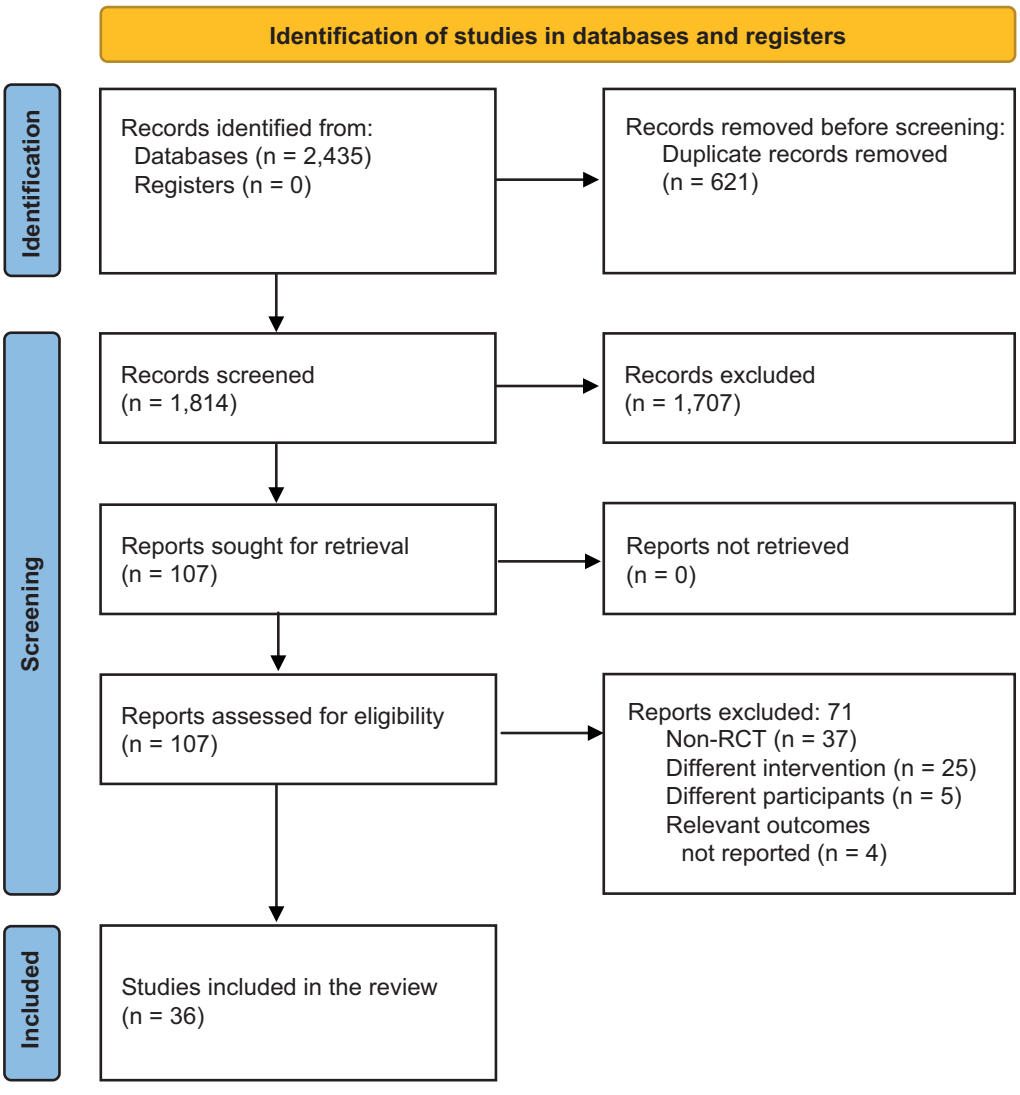

Fig. 1. Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) 2020 flowchart

RCT – randomized controlled trial.

Table 1. Characteristics of included studies (n = 34)

Authors and year of publication	Country	Study period	Follow-up duration	Sample size in intervention arm	Sample size in control arm	Study participants	Intervention details	Control group details	Risk of bias
Shany et al., 2017[15]	Australia	2009–2010	12 months	11	18	patients with COPD with at least 1 hospital admission for COPD exacerbation in previous year	telephonic nursing support and home visits	usual care	some concerns
Chau et al., 2012[16]	China	NR	2 months	22	18	patients aged >60 years with moderate or severe COPD	use of telecare system with community nurse	usual care	some concerns
Li et al., 2015[17]	China	2012–2013	19 months	30	31	COPD patients with FEV1/FVC < 70% and no change in the preceding 4 weeks of therapy	education management of disease by expert hospital nurse	usual care	high risk
Cameron-Tucker et al., 2016[18]	Australia	NR	8–12 weeks	35	30	patients aged >18 years with COPD and present an exacerbation at least 2 months before data collection	tele-rehabilitation	usual care	high risk
Padilla-Zárate et al., 2013[19]	Mexico	NR	12 months	64	60	patients with COPD with GOLD I, II, III, or IV	nursing intervention based on personalized counseling	usual medical care	some concerns
Wood-Baker et al., 2012[20]	Australia	NR	12 months	55	51	patients with COPD aged >45 years and hospitalized for acute exacerbations of COPD	health education program by community nurses	usual care	high risk
Bischoff et al., 2012[21]	the Netherlands	2004–2006	24 months	55	55	patients aged >35 years with COPD and FVC < 0.7	monitoring by primary care nurse	usual care	low risk
Utens et al., 2012[22]	the Netherlands	NR	3 months	70	69	patients with COPD with GOLD I and hospitalized for exacerbations	discharge assisted by community nurses	usual hospital care	high risk
Scheerens et al., 2020[23]	Belgium	NR	6 months	12	13	patients with COPD who are oxygen dependent and 3 or more hospitalization for COPD in last 3 years	integrated palliative home care plan from nurses	usual care	some concerns
Wang et al., 2014[24]	China	NR	3 months	42	46	patients with COPD <45 years with FEV1/FVC < 70% and FEV1 between 30% and 80%	model of beliefs in health after hospitalization from nurses	routine nursing care	some concerns
Li et al., 2020[25]	China	2017	4 months	35	35	patients with COPD with GOLD I, II, III, IV and FEV1/FVC < 70%	nursing care based on information theory	standard nursing care	some concerns
Benzo and McEvoy, 2019[26]	USA	NR	12 months	108	107	COPD patients with ability to communicate over phone	post-discharge nurse training with home visits and phone calls	standard care	high risk

Table 1. Characteristics of included studies (n = 34) – cont.

Authors and year of publication	Country	Study period	Follow-up duration	Sample size in intervention arm	Sample size in control arm	Study participants	Intervention details	Control group details	Risk of bias
Song et al., 2014[27]	South Korea	NR	2 months	20	20	moderate COPD patients aged 65–75 years	face-to-face and telephonic sessions from nurses	usual care	low risk
Jonsdottir et al., 2015[28]	Iceland	NR	12 months	45	47	COPD patients aged 45–65 years	face-to-face and telephonic sessions from nurses	traditional healthcare	high risk
Walters et al., 2013[29]	Australia	NR	12 months	74	80	COPD patient with age >45 years and smoking history >10 pack-years	telephonic mentoring sessions by nurses	standard care plus non-interventional phone calls	low risk
Billington et al., 2014[30]	UK	NR	12 weeks	34	35	patients with COPD with previous spirometry results of FEV1/FVC ratio of 70% or less	telephonic nursing support	self-care plan only	some concerns
Karasu and Aylaz, 2020[31]	Turkey	2017	8 months	25	25	patients with COPD for at least 6 months	home care following Health Promotion Model	no additional nursing care	some concerns
Bucknall et al., 2012[32]	UK	NR	12 months	69	53	patients with COPD admitted to hospital with an acute exacerbation of COPD	individual training sessions at home from a study nurse with further home visits	usual care	low risk
Bal Özkaptan and Kapucu, 2016[33]	Turkey	2012–2013	12 months	53	53	patients with COPD for at least 1 year	home nursing care with self-efficacy self-care model with COPD	standard care	some concerns
Lamers et al., 2010[34]	the Netherlands	2003–2005	20 months	96	91	COPD patients aged >60 years	nursing management of minimal psychological intervention	standard nursing treatment	some concerns
Jurado-Gámez et al., 2012[35]	Spain	2010–2011	12 months	36	35	patients aged <75 years with COPD	nursing home visit 48–72 h after hospital discharge	usual care	some concerns
Lavesen et al., 2016[36]	Denmark	2010–2012	18 months	101	73	COPD patients with acute pneumonia exacerbation	telephonic nurse led follow-up	usual treatment	some concerns
Wang et al., 2018[37]	China	2016–2017	12 months	60	60	patients with COPD	humanistic nursing care	regular nursing care	high risk
Cumming et al., 2010[38]	Australia	NR	12 months	36	32	patients aged >45 years with COPD and who had at least 1 exacerbation	electronic monitoring techniques and tutoring by community nurses	usual care	some concerns
Nguyen, 2009[39]	USA	NR	6 months	8	9	patients with COPD at severe condition as per GOLD criteria: FEV1/FVC < 70%, FEV1% < 80% and receiving supplemental oxygen	long term exercise support mobilization with the help of nurse	no help from the nurse	low risk
Heslop-Marshall et al., 2018[40]	UK	NR	12 months	93	79	patients with COPD with FEV1/FVC < 70%	nurse led cognitive behavioral therapy	regular care	high risk
Jolly et al., 2018[41]	UK	NR	12 months	217	256	COPD patients with MRC ½ scale in primary care	telephonic intervention of health training by nurses	usual care	some concerns

Table 1. Characteristics of included studies (n = 34) – cont.

Authors and year of publication	Country	Study period	Follow-up duration	Sample size in intervention arm	Sample size in control arm	Study participants	Intervention details	Control group details	Risk of bias
De San Miguel et al., 2013[42]	Australia	NR	6 months	36	35	COPD patients with O_2 at home	remote monitoring of vital parameters with a telemedicine team assisted by nurse	nursing assistance data collection of vital parameters only	high risk
Wang et al., 2020[43]	China	NR	12 months	77	77	patients with COPD with GOLD II, III or IV and hospitalized for acute exacerbations of COPD	nurse-led self-management program	usual care	high risk
Khoshkesht et al., 2015[44]	Iran	2010–2011	3 months	35	35	moderate or severe COPD patients aged >65 years	pulmonary rehabilitation by nurses applying Bandura technique self-efficacy theory	routine nursing care	high risk
Deng et al., 2013[45]	China	2010–2011	6 months	32	32	patients with COPD with FEV1 60–25% post-bronchodilator	nurse-led psychological, cognitive, behavioral, physical, and functional therapy	usual therapy	high risk
Lee et al., 2015[46]	South Korea	2010–2011	6 months	78	73	COPD patients with age between 40 and 80 years	nurse-led problem solving therapy	usual care	high risk
Sorknaes et al., 2013[47]	Denmark	NR	12 months	132	134	patients with COPD with FEV1/FVC < 70%	teleconsultations from hospital nurses	usual care	some concerns
Akinci and Olgun, 2011[48]	Turkey	2005–2007	3 months	16	16	GOLD III and IV with no history of infections or exacerbation of respiratory symptoms	nurse-led home pulmonary rehabilitation	absence of rehabilitation program	high risk

COPD – chronic obstructive pulmonary disease; FEV1 – forced expiratory volume in 1 s; FVC – forced vital capacity; GOLD – Global Initiative for Chronic Obstructive Lung Disease; MRC – medical research council; NR – not reported.

or standard care. Risk of bias assessment showed varied integrity with 13 studies labeled "high risk," 17 entailing "some concerns" and merely 6 assessed as "low risk." The intervention duration predominantly ranged from 2 to 24 months, with 7 studies not reporting the exact study period (Table 1).

6MWD

A total of 7 studies encompassing 456 participants were meticulously analyzed to discern the effectiveness of nursing interventions against standard care, focusing on 6MWD. The pooled analysis unveiled an overall SMD of 0.628 (95% CI: 0.261 to 0.996; z = 3.348, p = 0.001), implying a statistically significant change in the 6MWD, attributable to the nursing interventions when compared with the standard care (Fig. 2). Cochran's Q statistic was 19.57 (p = 0.003), and the I^2 statistic was observed to be 69.3%, which further underscores the notable variability among the enlisted studies. Sensitivity analysis did not reveal any substantial variation in the estimates (Supplementary Fig. 1). The funnel plot was slightly asymmetrical, but Egger's test indicated no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (–3.18, p = 0.199) (Supplementary Fig. 2).

Anxiety

A total of 9 studies, enrolling 1,544 participants, were meticulously analyzed to discern the effectiveness of nursing interventions against standard care on anxiety scores amongst COPD patients. The pooled analysis revealed an overall SMD of –0.952 (95% CI: –1.719 to –0.186; z = –2.434, p = 0.015). This indicated a statistically significant reduction in anxiety, attributable to the nursing interventions, compared with standard care (Fig. 3). Pertinently, Cochran's Q statistic was reported as 357.35 (p < 0.001) and I^2 statistic at 97.8%, pointing towards a substantial level of heterogeneity among the included studies.

Upon executing a leave-one-out sensitivity analysis, omitting each study in turn, the overall SMD in anxiety outcomes due to nursing interventions among COPD patients ranged from –0.5259 to –1.0948. These findings affirm that the observed reduction in anxiety, SMD = –0.952 (95% CI: –1.719 to –0.186), remained robust and statistically significant across the analyzed studies (Supplementary Fig. 3). The funnel plot was slightly asymmetrical, but Egger's test indicated no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (–8.15, p = 0.161) (Supplementary Fig. 4).

Depression

A systematic examination of 9 studies, incorporating 1,529 participants, was undertaken to discern the efficacy of nursing interventions relative to standard care in managing depression among COPD patients. The meta-analysis, employing a random-effects model, resulted in an overall SMD of –0.952, substantiating a statistically significant decrement in depression scores attributable to nursing interventions (95% CI: –1.631 to –0.272; z = –2.746, p = 0.006) (Fig. 4). Substantial heterogeneity was manifested across studies (Cochran's Q = 284.36, p < 0.001; I^2 = 97.2%).

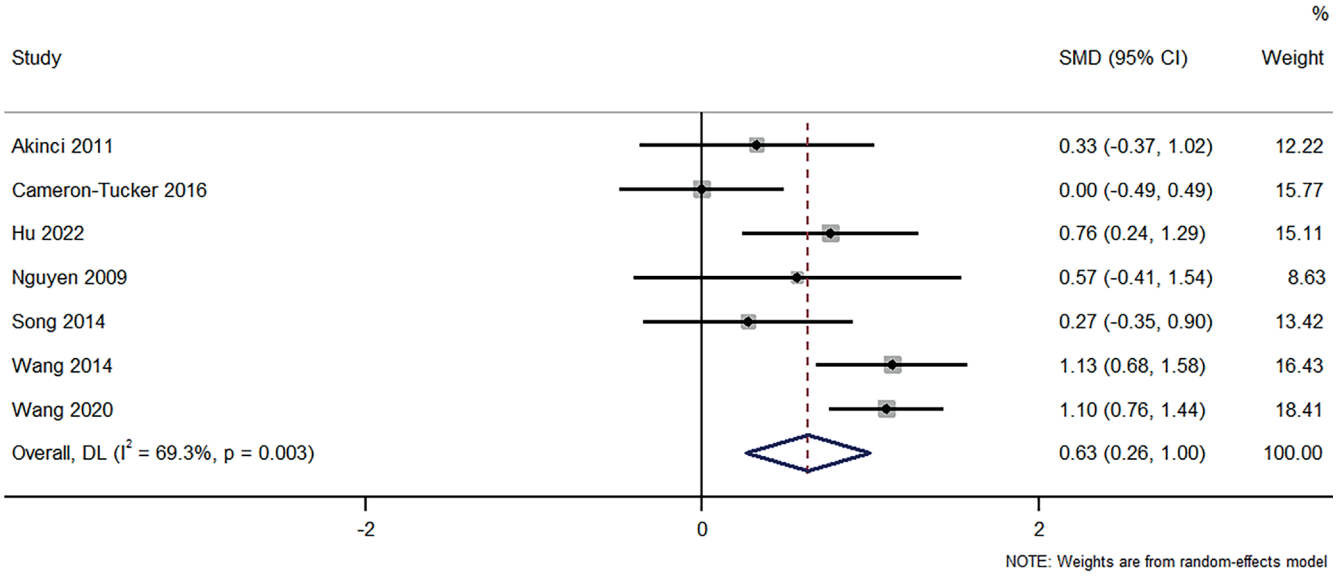

Fig. 2. Forest plot for 6-minute walk distance (6MWD)

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

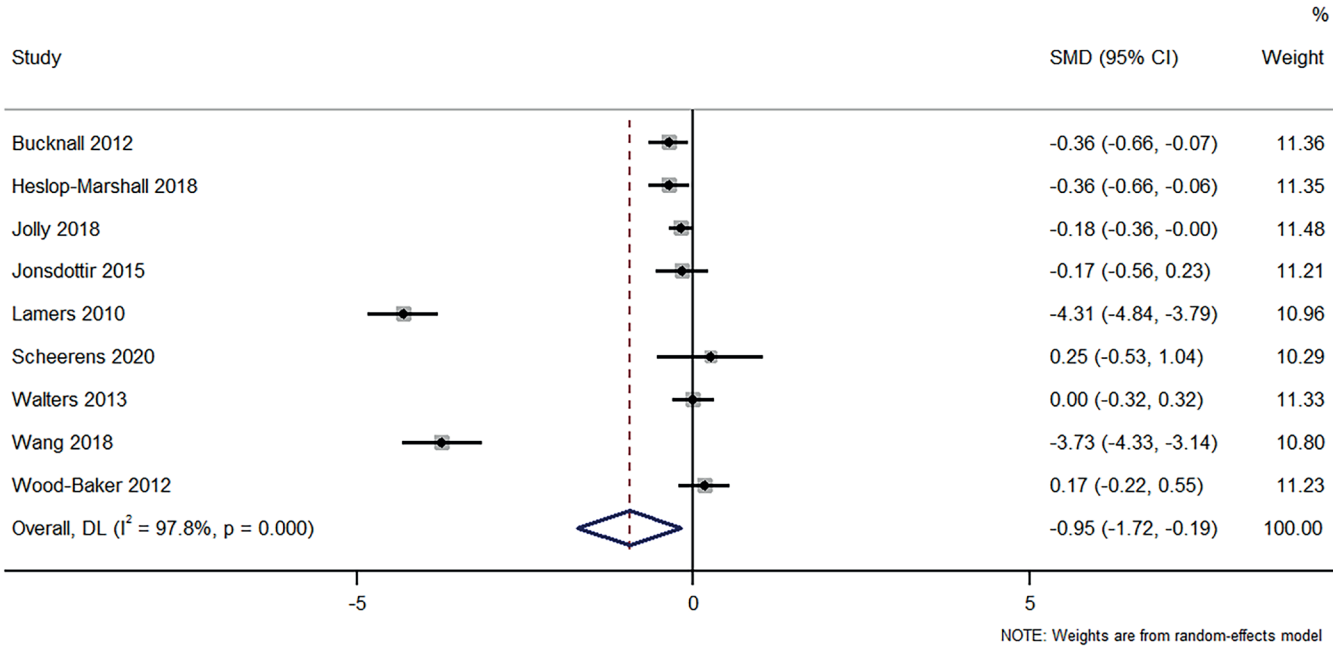

Fig. 3. Forest plot for anxiety

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

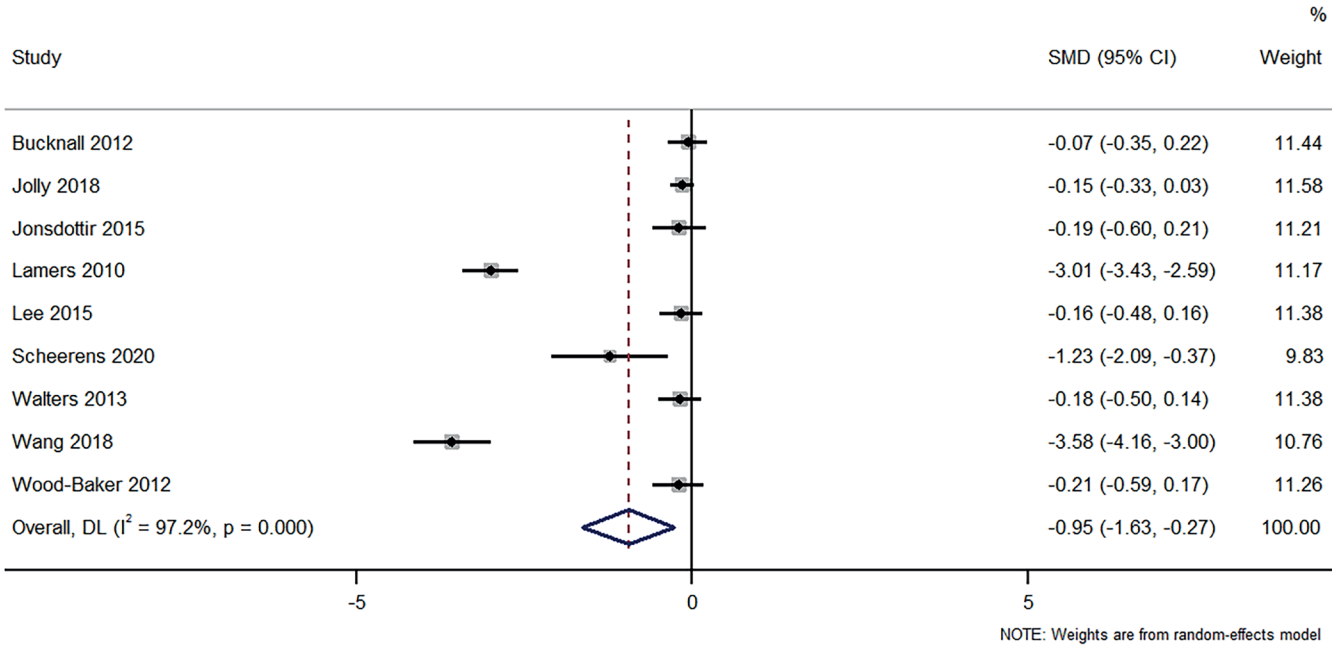

Fig. 4. Forest plot for depression

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

Upon excluding each study one by one to assess the robustness of the results in a leave-one-out sensitivity analysis, the consolidated effect size (SMD) varied from –0.627 to –1.069, consistently underscoring a statistically significant reduction in depression scores attributed to nursing interventions across various iterations (Supplementary Fig. 5). The funnel plot was slightly asymmetrical, but Egger's test indicated no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (–8.44, p = 0.101) (Supplementary Fig. 6).

Self-efficacy

The meta-analysis involving 12 studies and 1,695 participants indicated a statistically significant improvement in self-efficacy outcome, with an SMD of 0.800 (95% CI: 0.361 to 1.240; z = 3.567, p < 0.001; Fig. 5) However, high heterogeneity was observed among the studies (Cochran's Q = 192.12, degrees of freedom (df) = 11, p < 0.0001; I^2 = 94.3%). The sensitivity analysis, omitting one study at a time and recalculating the pooled SMD for self-efficacy outcomes, still revealed a consistent effect size, indicating that the overall SMD (0.800; 95% CI: 0.0361 to 1.240) was not highly dependent on any single study (Supplementary Fig. 7). The funnel plot was symmetrical, and Egger's test indicated no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (14.25, p = 0.124) (Supplementary Fig. 8).

Hospitalization

The pooled SMD from the meta-analysis, which included 8 studies and 1,464 participants, suggests a trend towards reduced hospitalization outcomes (SMD = –0.797, 95% CI: –1.611 to 0.018; p = 0.055), though it did not reach statistical significance (Fig. 6). The high heterogeneity among studies (I^2 = 97.8%, p < 0.001) indicated substantial variability in effect sizes across the included studies. Leave-one-out sensitivity analysis suggests that the overall pooled estimate was relatively stable and not overly influenced by any single study (Supplementary Fig. 9). The funnel plot was symmetrical, and Egger's test indicated no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (–6.06, p = 0.133) (Supplementary Fig. 10).

Quality of life

This analysis synthesizes the findings of 18 studies involving 2,179 participants, investigating the impact of nursing interventions on the QoL of COPD patients using the SGRQ as an outcome measure. The overall effect size (SMD) was –0.299, but was not statistically significant (p = 0.311), and there was substantial heterogeneity among study results (I^2 = 97.3%), suggesting that the interventions' impacts on respiratory QoL varied widely across studies (Fig. 7).

The sensitivity analysis reveals that the overall estimate of the impact on QoL marginally fluctuated when each study was omitted one at a time, with a combined estimate of –0.2989. The 95% CI ranged from approx. –0.877 to 0.279, crossing 0, which indicates a non-significant overall effect

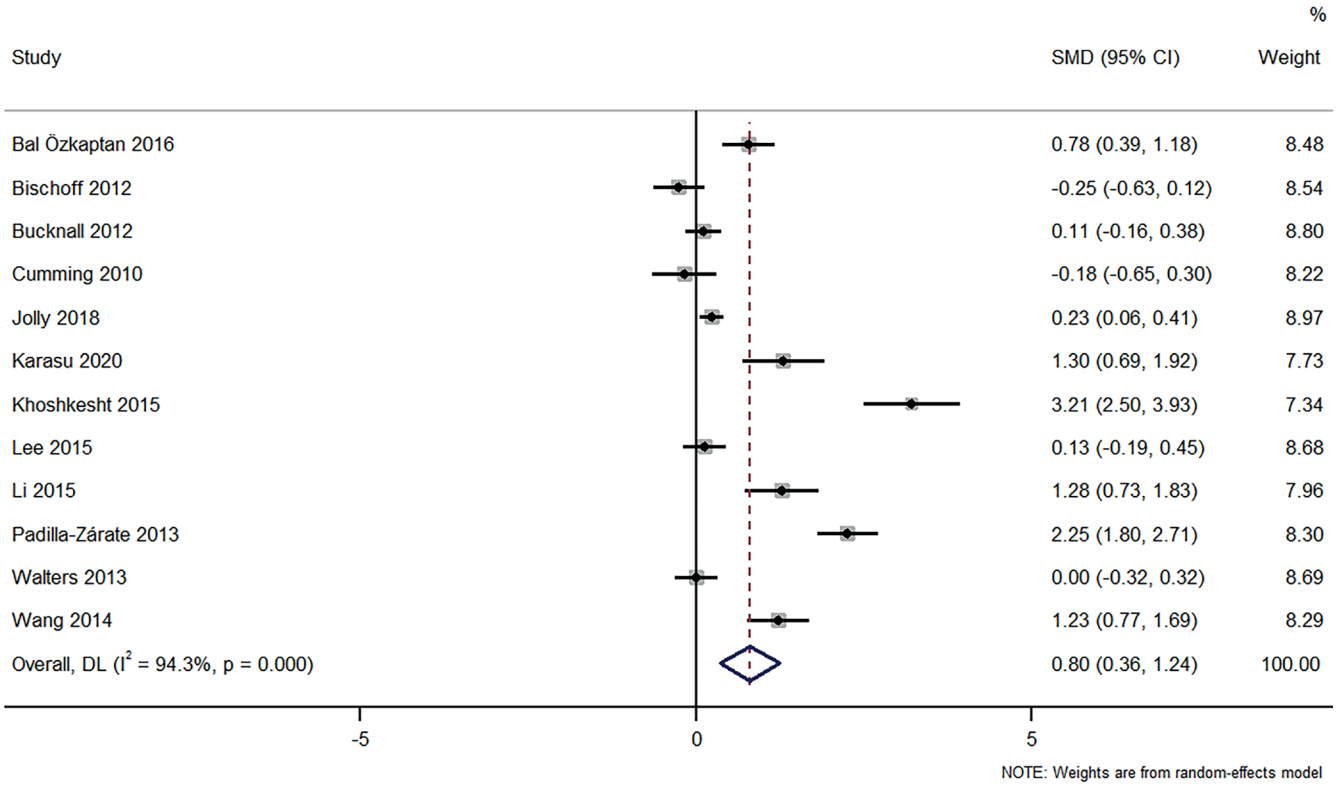

Fig. 5. Forest plot for self-efficacy

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

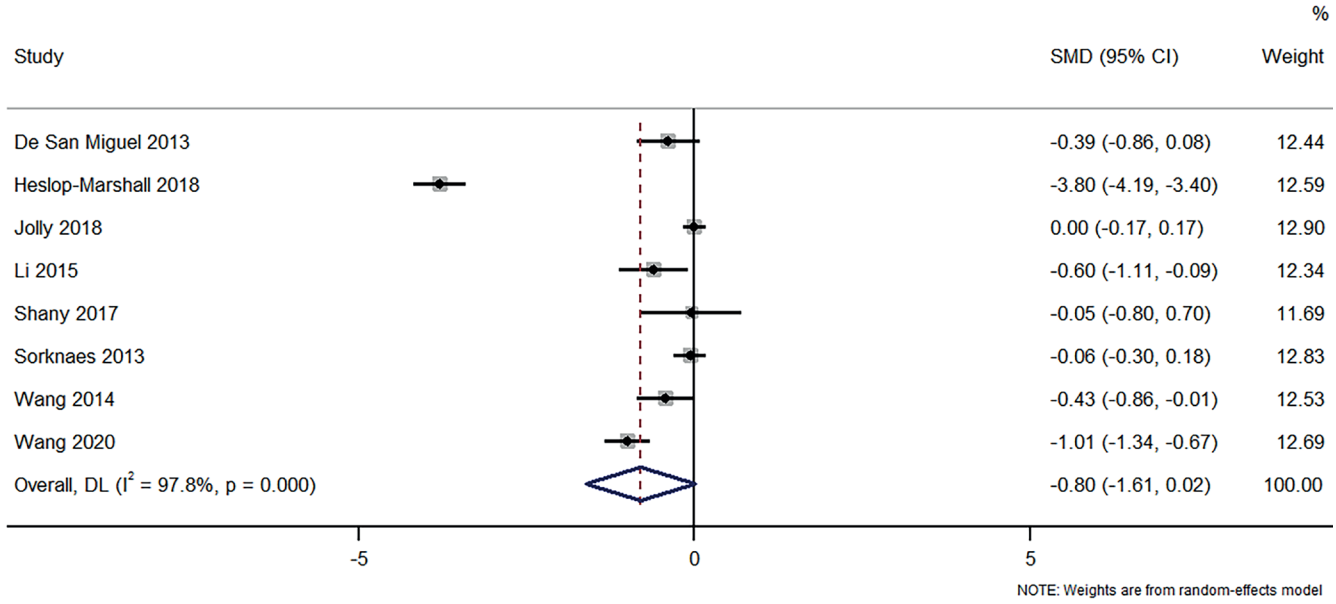

Fig. 6. Forest plot for hospitalization

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

(Supplementary Fig. 11). This insensitivity to the omission of individual studies suggests that the meta-analysis was relatively robust. The funnel plot was symmetrical, and Egger's test output showed that the intercept (bias) was −4.356, with a p-value of 0.727, which does not indicate a significant publication bias (Supplementary Fig. 12).

Dyspnea

Data from 6 studies, totaling 419 participants, were used to evaluate the effect of nursing interventions on dyspnea in managing COPD patients using a random-effects model. The overall SMD was −0.102 (95% CI: −0.529 to 0.326;

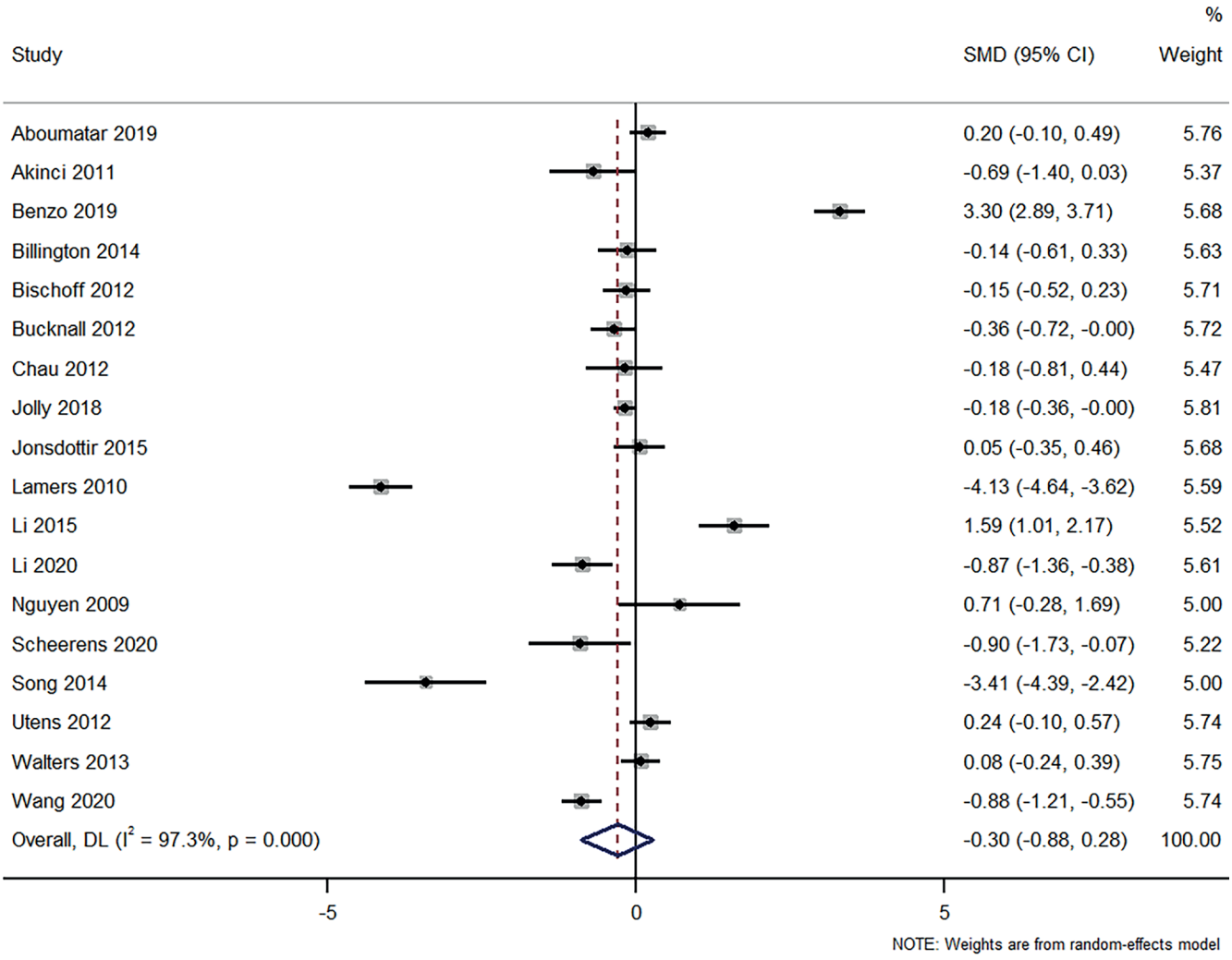

Fig. 7. Forest plot for quality of life

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

p = 0.641), indicating no statistically significant effect of the nursing interventions on dyspnea compared to usual care across the included studies (Fig. 8). Notably, there was significant heterogeneity among the studies (I^2 = 77.6%, p < 0.001). Sensitivity analysis revealed that the overall combined effect size, including all studies, was −0.102 (95% CI: −0.529 to 0.326). This CI crossed 0, indicating that the overall effect size was not statistically significant. However, the analysis revealed that the omission of the study by Akinci and Olgun[48] changed the pooled effect size to a significant value, suggesting that this particular study might hold some weight or influence on the overall combined results (Supplementary Fig. 13). The funnel plot was symmetrical, and Egger's test indicated no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (4.83, p = 0.229) (Supplementary Fig. 14).

Patient satisfaction

This meta-analysis incorporated findings from 3 studies, cumulatively analyzing data from 286 participants, to investigate patient satisfaction in COPD patients. The pooled RR across the included studies was 1.151 (95% CI: 0.987 to 1.343) (Fig. 9). The test of the overall effect size was calculated with a z-value of 1.795 and an associated p-value of 0.073. Cochran's Q was calculated to be 6.24, with an associated p-value of 0.044 and I^2 statistic of 67.9%, indicating a statistically significant level of heterogeneity. The sensitivity analysis revealed that the omission of the study by Billington et al.[30] changed the pooled effect size to a significant value, suggesting that this particular study might hold some weight or influence on the overall combined results (Supplementary Fig. 15). The funnel plot was symmetrical, and Egger's test indicates no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (−0.20, p = 0.973) (Supplementary Fig. 16).

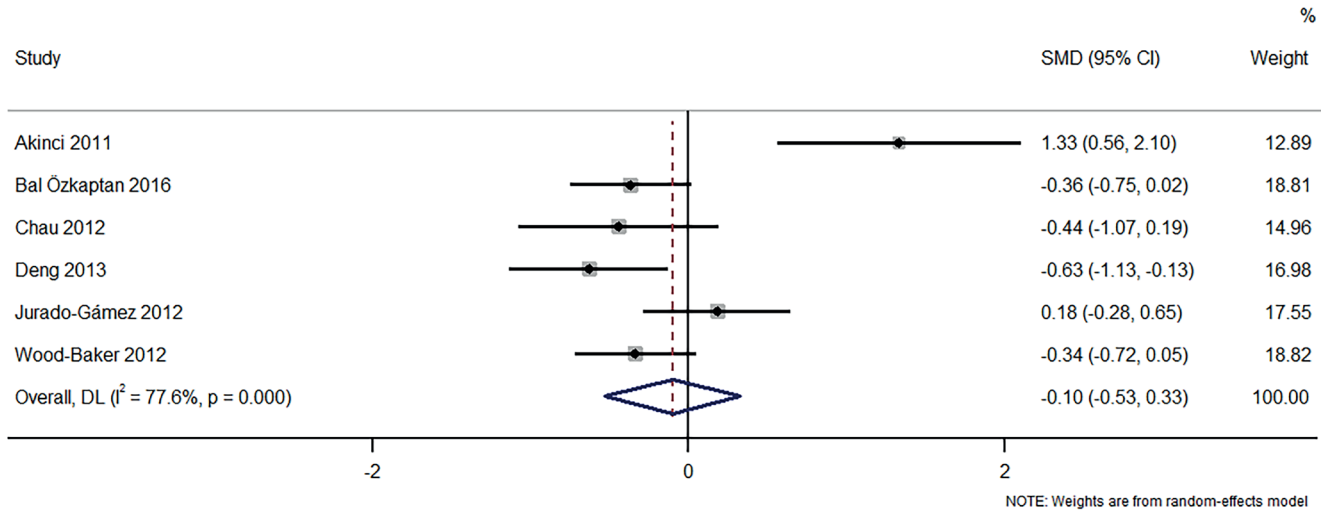

Fig. 8. Forest plot for dyspnea

DL – DerSimonian and Laird; 95% CI – 95% confidence interval; SMD – standardized mean difference.

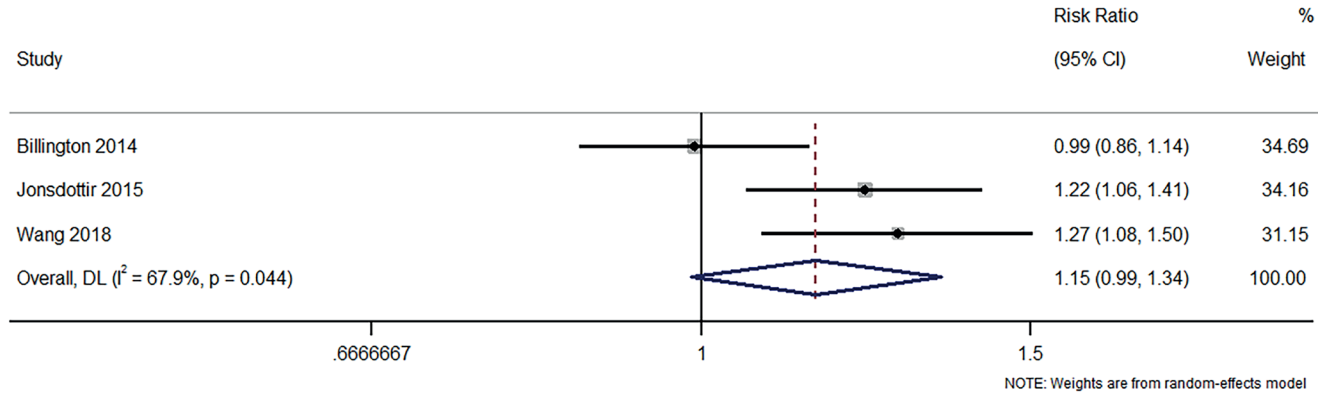

Fig. 9. Forest plot for patient satisfaction

DL – DerSimonian and Laird; 95% CI – 95% confidence interval.

All-cause mortality

This analysis comprehensively synthesized the findings from a total of 5 studies involving 848 participants to investigate the impact of various interventions on all-cause mortality. The pooled RR across the analyzed studies was 1.206, with a 95% CI ranging from 0.749 to 1.943 (Fig. 10). The overall effect was tested against the null hypothesis of RR = 1 and did not reach statistical significance (z = 0.771, p = 0.441). The Cochran's Q value was 1.76 with df of 4, translating to a p-value of 0.780, suggesting that there was no statistically significant heterogeneity. Further, the I^2 statistic, which describes the percentage of variation across studies due to heterogeneity rather than chance, was 0%, indicating no observed heterogeneity (with its 95% CI ranging from 0.0% to 41.6%). The sensitivity analysis did not reveal any substantial variation in the estimates (Supplementary Fig. 17). The funnel plot was symmetrical, and Egger's test indicates no potential publication bias or other small-study effects in the meta-analysis, evidenced by a nonsignificant bias coefficient (–0.76, p = 0.109) (Supplementary Fig. 18).

Discussion

In light of the critical role of nursing interventions in the management of COPD, this comprehensive review was conducted to illuminate the impact of these interventions on multiple clinical and psychosocial outcomes. The cumulative findings indicate a variable impact of nursing interventions on distinct domains of patient outcomes among individuals with COPD.

Regarding physical and functional capacity, there was a significant improvement in the 6MWD, with an SMD of 0.628. This underscores the potential benefit of nursing interventions in bolstering the exercise capacity of individuals with COPD, which is pivotal, considering the integral role of functional capacity in sustaining autonomy and QoL.

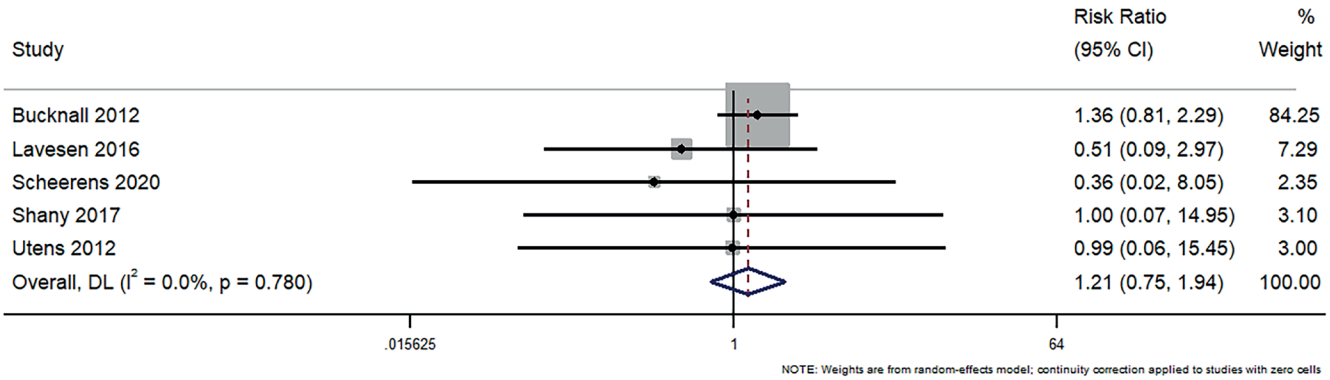

Fig. 10. Forest plot for all-cause mortality

DL – DerSimonian and Laird; 95% CI – 95% confidence interval.

Anxiety and depression are paramount considerations in COPD management due to their prevalence and detrimental influence on adherence to treatment, health status and clinical outcomes. The evidence from this meta-analysis elucidates a statistically significant reduction in both anxiety and depression, aligning with some previous literature that has emphasized the efficacy of nursing care in enhancing psychological wellbeing through various strategies, such as patient education, behavioral interventions and self-management facilitation.[49,50]

Contrastingly, although there was a trend toward reduced hospitalization (SMD = −0.797, p = 0.055) and improved QoL (SMD = −0.299, p = 0.311), these effects did not reach statistical significance.

Compared with prior research,[49,50] these results may reflect the inherent complexity and multifactorial nature of these outcomes, which can be influenced by numerous variables, including disease severity, comorbidities and social determinants of health, which are not solely contingent upon the quality or extent of nursing interventions.

In the context of self-efficacy, our findings illustrate a significant positive impact – an outcome that aligns with the theoretical underpinnings of self-management interventions, which often empower patients with skills and knowledge that foster a greater sense of control over their condition. Comparatively, the finding that nursing interventions did not exert a statistically significant impact on dyspnea diverges from some prior studies, which may be attributed to the variance in intervention types, delivery and the patient populations involved. This affirms the necessity for a nuanced understanding and application of nursing strategies, ensuring they are adeptly tailored to the multifaceted needs of COPD patients, potentially involving a multidisciplinary approach.

Given the importance of self-management in COPD care, it is imperative to highlight the interplay between self-management and the comorbidities often encountered by these patients. Effective self-management in COPD is not solely about managing the pulmonary symptoms but also entails a comprehensive approach that includes managing coexisting conditions such as cardiovascular disease, diabetes and anxiety/depression. This holistic approach is crucial, as these comorbidities can significantly impact patients' overall health status, their ability to engage in self-management practices and their responses to nursing interventions.

Furthermore, the effectiveness of nurse-led interventions is intrinsically linked to their ability to enhance self-management capabilities in patients with COPD. Nursing interventions that focus on education, skill development and psychological support are designed to empower patients, enabling them to manage not only their respiratory symptoms but also the broader aspects of their health. This encompasses adherence to medication, recognition of exacerbation signs, lifestyle modifications, and coping strategies in dealing with the psychological burdens of the disease and its comorbidities.

The results from this meta-analysis necessitate a judicious interpretation. A critical assessment of the efficacy of nursing interventions underscores their potential role in enhancing physical, psychological and functional outcomes in COPD management, thereby advocating for their integration into routine clinical practice. These findings underscore the value of nursing professionals in the management of COPD, propelling the potential for targeted, patient-centered care, and also advocate for the integration of nursing interventions into conventional management protocols for COPD while emphasizing the indispensable role of nurses in enhancing patient-centered outcomes.

For nursing professionals and clinical practice, this study reaffirms the importance of targeted interventions for COPD patients and highlights domains such as psychological wellbeing and functional capacity as particularly responsive to such interventions. Furthermore, it reiterates the need for an individualized, patient-centered approach, considering the varied responses across different outcome domains.

This meta-analysis leverages robust methodological rigor and comprehensive data synthesis across numerous studies to provide a broad perspective on the impact of nursing interventions across various outcome domains.

The sensitivity analysis also revealed that the findings of the study were credible and not sensitive to single study effects. This meta-analysis included only RCTs, the highest level of evidence to provide more reliable estimates, essential for making decisions regarding the implementation of the nursing interventions into the routine practice.

The relationship between nurse-led interventions and patient self-management in COPD is a critical area for exploration. The success of these interventions often hinges on their ability to foster an environment where patients feel capable and confident in managing their condition. This includes navigating the complexities introduced by comorbid conditions, which can complicate the management of COPD. By addressing these multifaceted needs, nurse-led programs can significantly contribute to the effectiveness of self-management practices among COPD patients. This, in turn, underscores the necessity for these interventions to be patient-centered and tailored to the individual's specific health profile, including comorbidities.

While providing valuable insights, this study also paves the way for future research. A more in-depth exploration is needed to decipher the elements within nursing interventions that are most potent in driving positive outcomes in COPD management. Additionally, research investigating the longitudinal impacts of nursing interventions, the optimization of their implementation in varied healthcare contexts and the identification of patient subgroups who derive maximal benefit would be worthwhile. Future research with rigorous design, adequate power and meticulous reporting will further contribute to the evidence base, making it possible to delineate the role and optimization of nursing interventions in COPD management.

Limitations

Nevertheless, it is imperative to acknowledge the present study's limitations. The evident heterogeneity among some of the included studies, particularly in areas such as anxiety and depression, is indicative of variability in study designs, populations and interventions, potentially influencing the collective findings. Furthermore, the risk of bias assessment unveiled varied integrity among the studies, with several marked as "high risk," potentially affecting the credibility and generalizability of the findings.

Another limitation resides in the potential influence of unaccounted confounding variables such as varying healthcare systems, practitioner expertise, intensity of the management program, follow-up, method of conducting the patient, and patient adherence, which might have influenced the observed outcomes and heterogeneity. Furthermore, the varying duration of interventions across the studies presents a potential variable that could influence the results and is not systematically evaluated within this paper.

Conclusions

This review underlines the significant potential of nursing interventions in enhancing certain domains of outcomes for individuals with COPD, specifically in areas such as exercise capacity, anxiety, depression, and self-efficacy. Although variable impacts are observed across different outcome domains, these findings herald the value of nursing interventions as a crucial component of comprehensive COPD management. Harnessing these insights and refining and understanding these interventions will be pivotal in evolving the holistic, patient-centered management of COPD, thus optimizing patient outcomes and QoL in this prevalent and impactful condition.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of The First Hospital of Hebei Medical University (approval No. 20230238).

Supplementary data

The supplementary materials are available at https://doi.org/10.5281/zenodo.11295652. The package includes the following files:

Supplementary Appendix. Search strategy.
Supplementary Fig. 1. Sensitivity analysis for 6MWD.
Supplementary Fig. 2. Funnel plot for 6MWD.
Supplementary Fig. 3. Sensitivity analysis for anxiety.
Supplementary Fig. 4. Funnel plot for anxiety.
Supplementary Fig. 5. Sensitivity analysis for depression.
Supplementary Fig. 6. Funnel plot for depression.
Supplementary Fig. 7. Sensitivity analysis for self-efficacy.
Supplementary Fig. 8. Funnel plot for self-efficacy outcome.
Supplementary Fig. 9. Sensitivity analysis for hospitalization.
Supplementary Fig. 10. Funnel plot for hospitalization.
Supplementary Fig. 11. Sensitivity analysis for QoL.
Supplementary Fig. 12. Funnel plot for QoL outcome.
Supplementary Fig. 13. Sensitivity analysis for dyspnea.
Supplementary Fig. 14. Funnel plot for dyspnea.
Supplementary Fig. 15. Sensitivity analysis for patient satisfaction.
Supplementary Fig. 16. Funnel plot for patient satisfaction.
Supplementary Fig. 17. Sensitivity analysis for all-cause mortality.
Supplementary Fig. 18. Funnel plot for all-cause mortality.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Use of AI and AI-assisted technologies

Not applicable.

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References

1. Safiri S, Carson-Chahhoud K, Noori M, et al. Burden of chronic obstructive pulmonary disease and its attributable risk factors in 204 countries and territories, 1990–2019: Results from the Global Burden of Disease Study 2019. *BMJ*. 2022;378:e069679. doi:10.1136/bmj-2021-069679
2. Adeloye D, Song P, Zhu Y, Campbell H, Sheikh A, Rudan I. Global, regional, and national prevalence of, and risk factors for, chronic obstructive pulmonary disease (COPD) in 2019: A systematic review and modelling analysis. *Lancet Respir Med*. 2022;10(5):447–458. doi:10.1016/S2213-2600(21)00511-7
3. Halpin DMG, Criner GJ, Papi A, et al. Global initiative for the diagnosis, management, and prevention of chronic obstructive lung disease: The 2020 GOLD Science Committee Report on COVID-19 and Chronic Obstructive Pulmonary Disease. *Am J Respir Crit Care Med*. 2021;203(1):24–36. doi:10.1164/rccm.202009-3533SO
4. Choi JY, Kim JW, Kim YH, et al. Clinical characteristics of non-smoking chronic obstructive pulmonary disease patients: Findings from the KOCOSS cohort. *COPD*. 2022;19(1):174–181. doi:10.1080/15412555.2022.2053088
5. Divo M, Cote C, De Torres JP, et al. Comorbidities and risk of mortality in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012;186(2):155–161. doi:10.1164/rccm.201201-0034OC
6. Sahin H, Varol Y, Naz I, Tuksavul F. Effectiveness of pulmonary rehabilitation in COPD patients receiving long-term oxygen therapy. *Clin Respir J*. 2018;12(4):1439–1446. doi:10.1111/crj.12680
7. Newham J, Presseau J, Heslop-Marshall K, et al. Features of self-management interventions for people with COPD associated with improved health-related quality of life and reduced emergency department visits: A systematic review and meta-analysis. *COPD*. 2017;12:1705–1720. doi:10.2147/COPD.S133317
8. McCarthy B, Casey D, Devane D, Murphy K, Murphy E, Lacasse Y. Pulmonary rehabilitation for chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*. 2015;2015(2):CD003793. doi:10.1002/14651858.CD003793.pub3
9. Arnold MT, Dolezal BA, Cooper CB. Pulmonary rehabilitation for chronic obstructive pulmonary disease: Highly effective but often overlooked. *Tuberc Respir Dis*. 2020;83(4):257–267. doi:10.4046/trd.2020.0064
10. Williams MT, Johnston KN, Paquet C. Cognitive behavioral therapy for people with chronic obstructive pulmonary disease: Rapid review. *COPD*. 2020;15:903–919. doi:10.2147/COPD.S178049
11. Schrijver J, Lenferink A, Brusse-Keizer M, et al. Self-management interventions for people with chronic obstructive pulmonary disease. *Cochrane Database Syst Rev*. 2022;2022(1):CD002990. doi:10.1002/14651858.CD002990.pub4
12. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71. doi:10.1136/bmj.n71
13. Sterne JAC, Savović J, Page MJ, et al. RoB 2: A revised tool for assessing risk of bias in randomised trials. *BMJ*. 2019;366:l4898. doi:10.1136/bmj.l4898
14. Higgins JPT, Thomas J, Chandler J, et al., eds. *Cochrane Handbook for Systematic Reviews of Interventions*. 2nd ed. Chichester, UK: Wiley & Sons; 2019. doi:10.1002/9781119536604
15. Shany T, Hession M, Pryce D, et al. A small-scale randomised controlled trial of home telemonitoring in patients with severe chronic obstructive pulmonary disease. *J Telemed Telecare*. 2017;23(7):650–656. doi:10.1177/1357633X16659410
16. Chau JPC, Lee DTF, Yu DSF, et al. A feasibility study to investigate the acceptability and potential effectiveness of a telecare service for older people with chronic obstructive pulmonary disease. *Int J Med Inform*. 2012;81(10):674–682. doi:10.1016/j.ijmedinf.2012.06.003
17. Li P, Gong Y, Zeng G, Ruan L, Li G. A new mode of community continuing care service for COPD patients in China: Participation of respiratory nurse specialists. *Int J Clin Exp Med*. 2015;8(9):15878–15888. PMID:26629091. PMCID:PMC4658980.
18. Cameron-Tucker H, Wood-Baker R, Joseph L, Walters J, Schuz N, Walters EH. A randomized controlled trial of telephone-mentoring with home-based walking preceding rehabilitation in COPD. *COPD*. 2016;11:1991–2000. doi:10.2147/COPD.S109820
19. Padilla-Zárate MP, Cortés-Poza D, Martínez-Soto JM, et al. Autocuidado y calidad de vida posterior a la consejería de enfermería en pacientes con enfermedad pulmonar obstructiva crónica. *Rev Mex Enf Cardiol*. 2013;21(1):15–23. https://www.medigraphic.com/cgi-bin/new/resumen.cgi?IDARTICULO=43086. Accessed August 15, 2023.
20. Wood-Baker R, Robinson A, Reid D, Walters. Clinical trial of community nurse mentoring to improve self-management in patients with chronic obstructive pulmonary disease. *COPD*. 2012;7:407. doi:10.2147/COPD.S32220
21. Bischoff EWMA, Akkermans R, Bourbeau J, Van Weel C, Vercoulen JH, Schermer TRJ. Comprehensive self management and routine monitoring in chronic obstructive pulmonary disease patients in general practice: Randomised controlled trial. *BMJ*. 2012;345:e7642. doi:10.1136/bmj.e7642
22. Utens CMA, Goossens LMA, Smeenk FWJM, et al. Early assisted discharge with generic community nursing for chronic obstructive pulmonary disease exacerbations: Results of a randomised controlled trial. *BMJ Open*. 2012;2(5):e001684. doi:10.1136/bmjopen-2012-001684
23. Scheerens C, Pype P, Van Cauwenberg J, et al. Early Integrated Palliative Home Care and Standard Care for End-Stage COPD (EPIC): A phase II pilot RCT testing feasibility, acceptability, and effectiveness. *J Pain Symptom Manage*. 2020;59(2):206–224.e7. doi:10.1016/j.jpainsymman.2019.09.012
24. Wang Y, Zang X, Bai J, Liu S, Zhao Y, Zhang Q. Effect of a health belief model-based nursing intervention on Chinese patients with moderate to severe chronic obstructive pulmonary disease: A randomised controlled trial. *J Clin Nurs*. 2014;23(9–10):1342–1353. doi:10.1111/jocn.12394
25. Li XX, Du XW, Song W, Lu C, Hao WN. Effect of continuous nursing care based on the IKAP theory on the quality of life of patients with chronic obstructive pulmonary disease: A randomized controlled study. *Medicine (Baltimore)*. 2020;99(11):e19543. doi:10.1097/MD.0000000000019543
26. Benzo R, McEvoy C. Effect of health coaching delivered by a respiratory therapist or nurse on self-management abilities in severe COPD: Analysis of a large randomized study. *Respir Care*. 2019;64(9):1065–1072. doi:10.4187/respcare.05927
27. Song HY, Yong SJ, Hur HK. Effectiveness of a brief self-care support intervention for pulmonary rehabilitation among the elderly patients with chronic obstructive pulmonary disease in Korea. *Rehabil Nurs*. 2014;39(3):147–156. doi:10.1002/rnj.92
28. Jonsdottir H, Amundadottir OR, Gudmundsson G, et al. Effectiveness of a partnership-based self-management programme for patients with mild and moderate chronic obstructive pulmonary disease: A pragmatic randomized controlled trial. *J Adv Nurs*. 2015;71(11):2634–2649. doi:10.1111/jan.12728
29. Walters J, Cameron-Tucker H, Wills K, et al. Effects of telephone health mentoring in community-recruited chronic obstructive pulmonary disease on self-management capacity, quality of life and psychological morbidity: A randomised controlled trial. *BMJ Open*. 2013;3(9):e003097. doi:10.1136/bmjopen-2013-003097

30. Billington J, Coster S, Murrells T, Norman I. Evaluation of a nurse-led educational telephone intervention to support self-management of patients with chronic obstructive pulmonary disease: A randomized feasibility study. *COPD*. 2015;12(4):395–403. doi:10.3109/15412555.2014.974735
31. Karasu F, Aylaz R. Evaluation of meaning of life and self-care agency in nursing care given to chronic obstructive pulmonary patients according to health promotion model. *Appl Nurs Res*. 2020;51:151208. doi:10.1016/j.apnr.2019.151208
32. Bucknall CE, Miller G, Lloyd SM, et al. Glasgow supported self-management trial (GSuST) for patients with moderate to severe COPD: Randomised controlled trial. *BMJ*. 2012;344:e1060. doi:10.1136/bmj.e1060
33. Bal Özkaptan B, Kapucu S. Home nursing care with the self-care model improves self-efficacy of patients with chronic obstructive pulmonary disease. *Jpn J Nurs Sci*. 2016;13(3):365–377. doi:10.1111/jjns.12118
34. Lamers F, Jonkers CCM, Bosma H, Chavannes NH, Knottnerus JA, Van Eijk JThM. Improving quality of life in depressed COPD patients: Effectiveness of a minimal psychological intervention. *COPD*. 2010;7(5):315–322. doi:10.3109/15412555.2010.510156
35. Jurado Gámez B, Feu Collado N, Jurado García JC, et al. Home intervention and predictor variables for rehospitalization in chronic obstructive pulmonary disease exacerbations [in Spanish]. *Arch Bronconeumol*. 2013;49(1):10–14. doi:10.1016/j.arbres.2012.08.003
36. Lavesen M, Ladelund S, Frederiksen AJ, Lindhardt BØ, Overgaard D. Nurse-initiated telephone follow-up on patients with chronic obstructive pulmonary disease improves patient empowerment, but cannot prevent readmissions. *Dan Med J*. 2016;63(10):A5276. PMID:27697128.
37. Yan Wang X, Hua Liu L. Observation on the clinical effects of humanistic nursing on the psychological status of patients with COPD in acute phase. *Biomed Res*. 2018;29(2):296–299. doi:10.4066/biomedicalresearch.29-17-2965
38. Cummings E, Robinson A, Pratt HC, et al. Pathways home: Comparing voluntary IT and non-IT users participating in a mentored self-management project. *Stud Health Technol Inform*. 2010;160(Pt 1):23–27. PMID:20841643.
39. Nguyen H. Pilot study of a cell phone-based exercise persistence intervention post-rehabilitation for COPD. *COPD*. 2009;4:301–313. doi:10.2147/COPD.S6643
40. Heslop-Marshall K, Baker C, Carrick-Sen D, et al. Randomised controlled trial of cognitive behavioural therapy in COPD. *ERJ Open Res*. 2018;4(4):00094–02018. doi:10.1183/23120541.00094-2018
41. Jolly K, Sidhu MS, Hewitt CA, et al. Self management of patients with mild COPD in primary care: Randomised controlled trial. *BMJ*. 2018;361:k2241. doi:10.1136/bmj.k2241
42. De San Miguel K, Smith J, Lewin G. Telehealth remote monitoring for community-dwelling older adults with chronic obstructive pulmonary disease. *Telemed J E Health*. 2013;19(9):652–657. doi:10.1089/tmj.2012.0244
43. Wang LH, Zhao Y, Chen LY, Zhang L, Zhang YM. The effect of a nurse-led self-management program on outcomes of patients with chronic obstructive pulmonary disease. *Clin Respir J*. 2020;14(2):148–157. doi:10.1111/crj.13112
44. Khoshkesht S, Zakerimoghadam M, Ghiyasvandian S, Kazemnejad A, Hashemian M. The effect of home-based pulmonary rehabilitation on self-efficacy in chronic obstructive pulmonary disease patients. *J Pak Med Assoc*. 2015;65(10):1041–1046. PMID:26440829.
45. Deng G, Liu F, Zhong Q, Chen J, Yang M, He H. The effect of non-pharmacological staged interventions on fatigue and dyspnoea in patients with chronic obstructive pulmonary disease: A randomized controlled trial. *Int J Nurs Pract*. 2013;19(6):636–643. doi:10.1111/ijn.12116
46. Lee H, Yoon JY, Lim Y, et al. The effect of nurse-led problem-solving therapy on coping, self-efficacy and depressive symptoms for patients with chronic obstructive pulmonary disease: A randomised controlled trial. *Age Ageing*. 2015;44(3):397–403. doi:10.1093/ageing/afu201
47. Sorknaes AD, Bech M, Madsen H, et al. The effect of real-time teleconsultations between hospital-based nurses and patients with severe COPD discharged after an exacerbation. *J Telemed Telecare*. 2013;19(8):466–474. doi:10.1177/1357633X13512067
48. Akinci AC, Olgun N. The effectiveness of nurse-led, home-based pulmonary rehabilitation in patients with COPD in Turkey. *Rehabil Nurs*. 2011;36(4):159–165. doi:10.1002/j.2048-7940.2011.tb00084.x
49. Aranburu-Imatz A, López-Carrasco JDLC, Moreno-Luque A, et al. Nurse-led interventions in chronic obstructive pulmonary disease patients: A systematic review and meta-analysis. *Int J Environ Res Public Health*. 2022;19(15):9101. doi:10.3390/ijerph19159101
50. Helvaci A, Gok Metin Z. The effects of nurse-driven self-management programs on chronic obstructive pulmonary disease: A systematic review and meta-analysis. *J Adv Nurs*. 2020;76(11):2849–2871. doi:10.1111/jan.14505

The relationship between rheumatoid arthritis and epicardial fat thickness, and serum levels of chemerin, adropin, and betatrophin

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Abstract

Background. Cardiovascular diseases (CVDs) are highly prevalent among patients with rheumatoid arthritis (RA). Epicardial adipose tissue, serum betatrophin, chemerin, and adropin levels are factors associated with atherosclerosis and cardiovascular involvement.

Objectives. This study aimed to investigate the relationship between RA and epicardial fat thickness (EFT), as well as serum betatrophin, chemerin and adropin levels.

Materials and methods. This cross-sectional study included 80 patients (62 women and 18 men) diagnosed with RA according to the American College of Rheumatology/The European Alliance of Associations for Rheumatology (ACR/EULAR) 2010 RA classification criteria and 80 healthy controls (64 women and 16 men). Exclusion criteria comprised other autoimmune diseases, CVDs, diabetes mellitus, other endocrine disorders, acute or chronic pancreatic disorders, malignancy, pregnancy, breastfeeding, or antihyperlipidemic drug usage. Serum betatrophin, chemerin and adropin concentrations were measured. Epicardial fat thickness was evaluated with transthoracic echocardiography.

Results. Adropin levels were significantly lower in the RA group compared to the control group ($p < 0.001$). Chemerin levels and EFT were significantly higher in the RA group than in the control group ($p = 0.016$, $p < 0.001$, respectively). When assessing the relationship between biomarkers and EFT in RA patients, a strong positive correlation was observed between chemerin and EFT ($r = 0.73$, $p = 0.046$) in patients with high disease activity.

Conclusions. Epicardial fat thickness, as an indicator of cardiovascular involvement, is higher in patients with RA. Moreover, high chemerin levels and low adropin levels in these patients may be indicative of cardiovascular involvement.

Key words: rheumatoid arthritis, chemerin, epicardial fat thickness, betatrophin, adropin

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Background

Rheumatoid arthritis (RA) is a degenerative chronic rheumatic disease that affects the small peripheral synovial joints symmetrically, resulting in joint abnormalities and loss of function.[1] According to traditional cardiovascular risk factors, the prevalence of cardiovascular disease (CVD) is higher in RA patients than in the general population.[2] Patients with RA have a limited understanding of the factors associated with their condition that place them at increased risk of CVD.[3] The increased mortality associated with RA is due to severe comorbidities that frequently induce inflammation and are inadequately treated.[4] Numerous studies have demonstrated that RA is associated with a higher risk of death from cardiovascular causes.[5,6]

Up to 80% of the heart's surface is covered with epicardial fat tissue, which is located between the visceral pericardium and myocardium.[7] Visceral adipose tissue plays a significant role in the pathophysiology of coronary artery disease (CAD).[8] This disease may develop due to factors such as epicardial fat tissue being adjacent to the coronary vessels or sharing the same microcirculation as the myocardium, local inflammation or paracrine effects.[9]

Betatrophin (also known as C190RF80, RIFL, ANGPTL8, or lipasin) is a newly discovered circulatory hormone synthesized in the human liver that promotes glucose and lipid metabolism.[10,11] Betatrophin, which is thought to play a role in lipid metabolism and glucose homeostasis, may be associated with high cardiovascular risk and dysfunctional lipid metabolism.[10] Chemerin is a recently discovered adipokine that regulates inflammation, angiogenesis and adipogenesis. It is a chemoattractant adipokine identified in immune cells and white adipose tissue, potentially triggering multiple proinflammatory processes in RA, possibly by stimulating synovial fibroblasts.[12] Adropin is a newly identified peptide consisting of 76 amino acids with a molecular weight of 4,499.9 Da. It has been studied for its hormonal role in preserving endothelial cell function. It has been found in the brain and liver of rats, and its expression is associated with a gene that regulates energy homeostasis.[13] Adropin has been shown to independently suppress atherosclerosis, irrespective of glucose and lipid metabolism and blood pressure.[14]

Objectives

The objective of this study was to investigate the relationship between RA and epicardial fat thickness (EFT), as well as betatrophin, chemerin and adropin levels in the blood of study participants.

Materials and methods

Study population

The study was conducted with patients admitted to the Physical Medicine and Rehabilitation outpatient clinic of Mengucek Gazi Training and Research Hospital (Erzincan, Turkey) between June 2020 and June 2021. Eighty RA patients (62 women and 18 men) diagnosed according to the ACR/EULAR 2010 RA classification criteria and 80 healthy controls (64 women and 16 men) were included in the study. The ACR/EULAR RA classification criteria included duration of symptoms, joint involvement, anti-cyclic citrullinated peptide (anti-CCP) and rheumatoid factor (RF) positivity, and acute phase reactants.[15] Patients' gender, age, waist circumference, and body mass index (BMI) were recorded. Informed consent was obtained from all subjects, and permission to conduct the study was granted by the Clinical Research Ethics Committee of Erzincan Binali Yildirim University, Turkey (dated March 3, 2020, No. 11665).

The DAS-28 remission criteria, including C-reactive protein (CRP), swollen and tender joint counts, and assessments of general health, were used to determine disease activity.[16] The Steinbrocker classification of functional capacity was used to assess functional status.[17] The healthy controls consisted of outpatients with acute musculoskeletal pain but no chronic inflammatory disease. They had no clinical history, laboratory or examination findings suggestive of RA. Both groups completed the Short Form-36 Health Survey Questionnaire (SF-36) to assess quality of life, and the visual analogue scale (VAS) to measure pain intensity.

Measurement of EFT

Epicardial fat thickness was measured from the echolucent area between the epicardial surface in front of the free wall of the right ventricle and the parietal pericardium. Measurements were taken at the end-diastole. During EFT measurement, each patient was placed in the left lateral decubitus position to obtain an optimal parasternal long-axis view. The aortic root and the interventricular septum were used as reference points for measurement from the parasternal long-axis view. Measurements were made by placing the aortic annulus and right ventricular free wall on the midline of the ultrasound waves and using the aortic root as a reference.[18,19] All measurements were performed transthoracically using a Philips HD 11XE echocardiography device (Koninklijke Philips N.V., Eindhoven, the Netherlands). According to a systematic review by Bertaso et al., a cutoff value of >5 mm was accepted for EFT.[18]

Patients with other autoimmune diseases, CVD, diabetes mellitus, endocrine disorders, acute and chronic pancreatic disorders, malignancy, pregnancy, breastfeeding, and those using antihyperlipidemic drugs were excluded from the study.

Measurement of plasma adropin, chemerin and betatrophin levels

After 8 h of fasting, blood samples were obtained from the antecubital vein in the morning and collected into vacuum gel tubes. The serum was separated by centrifugation of the samples at 5,000 rpm for 20 min at 4°C within 1 h of collection. Samples were stored at –80°C until analysis and were thawed only once before the analysis. Complete blood count, CRP, erythrocyte sedimentation rate (ESR), triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol, and glucose levels were assessed from fresh blood samples. The levels of plasma adropin, chemerin and betatrophin (USCN, human adropin, chemerin, betatrophin ELISA kit) were determined using an enzyme-linked immunosorbent assay (ELISA). The absorbances of standards and samples were read at 450 nm (with correction at 540 nm) using an Epoch spectrophotometer (BioTek Instruments, Inc., Winooski, USA).

Statistical analyses

The statistical analyses were performed using IBM SPSS for Windows, v. 22.0 (IBM Corp., Armonk, USA). Results for categorical data are presented as numbers and percentages, and for continuous variables as mean ± standard deviation (±SD). The χ^2 test was used for the analysis of categorical data. The assumption of normality for continuous variables was checked using the Kolmogorov–Smirnov test. Depending on the normality of the variables, either the Mann–Whitney U test or Student's t-test was applied. Pearson's or Spearman's correlation tests were used to evaluate the relationship between variables. A p-value less than 0.05 was considered statistically significant.

Results

Table 1 displays the baseline characteristics of 80 RA patients and 80 healthy controls. The RA patients and healthy controls did not differ significantly in terms of age, gender, BMI, waist circumference, and smoking status.

The RA group had a mean disease duration of 120.5 ±98.6 months, with a mean RF value of 70.7 ±127.5 months. Anti-CCP was positive in 44 (55%) patients and negative in 36 (45%) patients. The mean DAS-28 score was 3.81 ±0.66. According to their DAS-28 scores, 18 patients (22.5%) had low disease activity, 54 patients (67.5%) had moderate disease activity and 8 patients (10%) had high disease activity.

Compared to the control group, the RA patients had significantly higher platelet-to-lymphocyte ratio, neutrophil-to-lymphocyte ratio, CRP, ESR, monocytes, and neutrophils. The RA group also had significantly lower red blood cell counts and mean platelet volume compared to the control group. There were no significant differences between the 2 groups for glucose, total cholesterol, HDL, LDL, triglycerides, white blood cell count, hemoglobin, hematocrit, platelet count, and lymphocytes (Table 2).

There was no difference between RA patients and the control group in terms of betatrophin serum levels (0.28 ±0.24 vs 0.23 ±0.20, respectively; p = 0.466). Adropin

Table 1. Demographic and clinical features of patients

Parameters		Group		p-value
		RA	control	
Sex	female	62 (77.5)	64 (80.0)	0.699
	male	18 (22.5)	16 (20.0)	
Age [year]		51.9 ±11.3	49.7 ±9.9	0.215
Weight [kg]		70.1 ±8.6	69.8 ±10.8	0.279
Height [cm]		162.2 ±6.7	163.6 ±7.5	0.346
BMI [kg/m^2]		26.7 ±3.0	26.0 ±3.1	0.680*
Waist circumference [cm]		91.4 ±8.3	87.4 ±10.2	0.793*
Smoking status	non-smoker	74 (92.5)	78 (97.5)	0.147
	smoker	6 (7.5)	2 (2.5)	
VAS [mm]		61.9 ±17.6	46.0 ±14.4	<0.001*
SF-36		73.8 ±9.7	93.7 ±5.2	<0.001

RA – rheumatoid arthritis; BMI – body mass index; VAS – visual analogue scale; SF-36 – short form-36; *independent samples Student's t-test; otherwise, Mann–Whitney U-test was performed.

Table 2. Laboratory findings of study groups

Parameters	Group		p-value
	RA	control	
CRP [mg/L]	10.5 ±12.2	3.7 ±1.1	<0.001
ESR [mm/h]	23.0 ±19.2	8.2 ±5.7	<0.001
Glucose [mg/dL]	99.8 ±30.9	95.2 ±13.5	0.618
Total cholesterol [mg/dL]	192.7 ±35.9	186.8 ±35.9	0.092
HDL [mg/dL]	51.7 ±10.9	53.1 ±11.2	0.235
LDL [mg/dL]	122.5 ±28.6	120.8 ±29.9	0.706
Triglyceride [mg/dL]	134.0 ±72.1	113.3 ±53.9	0.090
WBC [×10^3/uL]	7.3 ±2.0	6.8 ±1.4	0.128
RBC [×10^6]	4.7 ±0.5	4.8 ±0.4	0.044*
Hb [g/dL]	13.3 ±1.4	13.7 ±1.5	0.063
Hct [%]	40.6 ±3.9	40.9 ±3.6	0.608*
Mpv [fl]	10.2 ±0.9	10.4 ±0.9	0.026
Plt [×10^3]	276.8 ±64.4	263. 0 ±50.3	0.134*
Lymphocytes [×10^3]	2.1 ±0.8	2.3 ±0.6	0.087
Monocytes [×10^3]	0.58 ±0.16	0.51 ±0.13	0.010
Neutrophils [×10^3]	4.5 ±1.6	3.8 ±1.1	0.008
NLR	2.4 ±1.7	1.8 ±0.7	0.002
PLR	146.5 ±60.9	122.0 ±36.7	0.024

RA – rheumatoid arthritis; CRP – C-reactive protein; ESR – erythrocyte sedimentation rate; WBC – white blood cells count; RBC – red blood cells count; Hb – hemoglobin; Hct – hematocrit, Mpv – mean platelet volume; Plt – platelets; NLR – neutrophils/lymphocytes rate; PLR – platelets/lymphocytes rate; *independent samples t-test; otherwise, Mann–Whitney U test was performed.

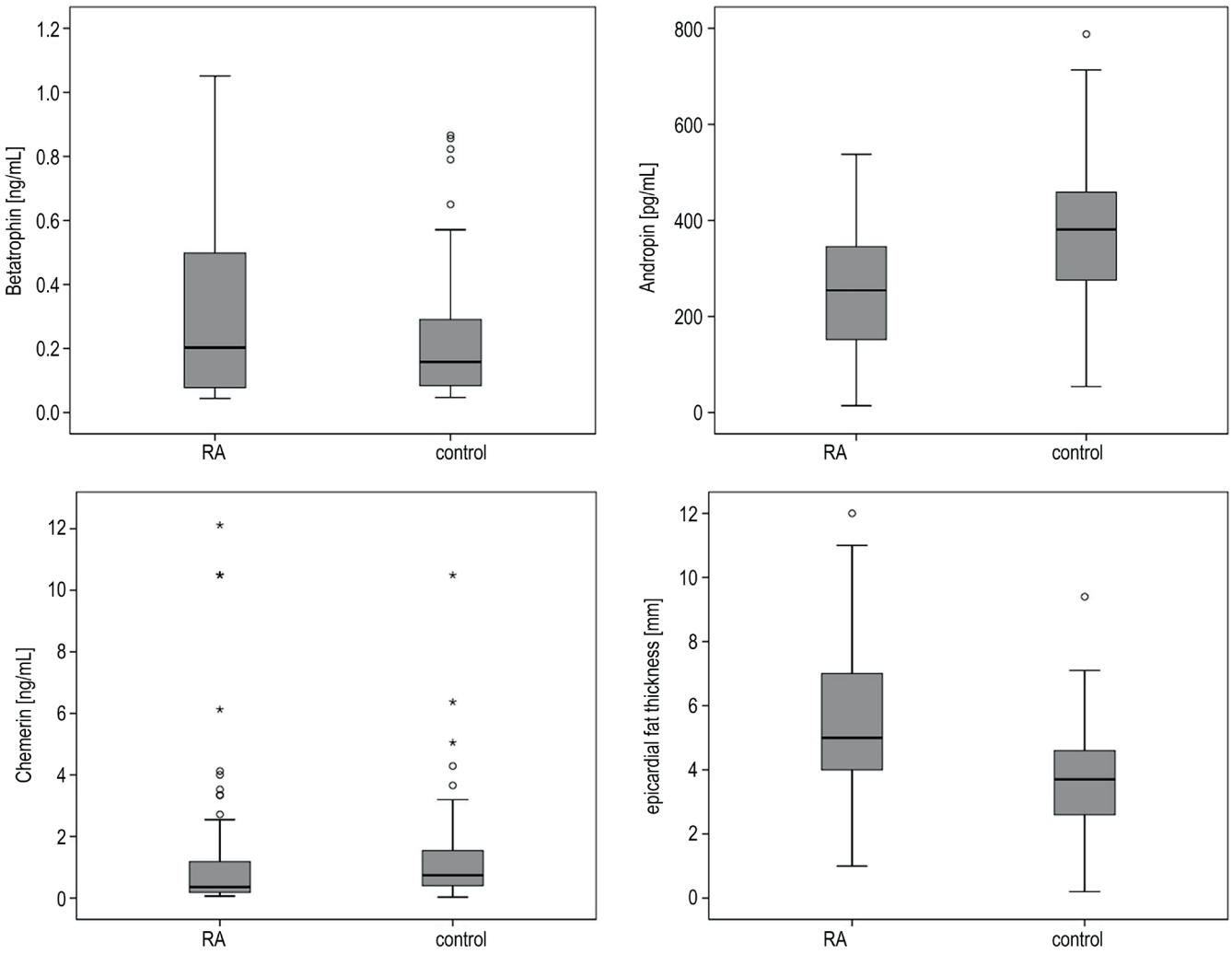

Fig. 1. Betatrophin, adropin, chemerin, and epicardial fat thickness (EFT) levels in the study groups

RA – rheumatoid arthritis.

Table 3. The serum values of biomarkers and EFT in study groups

Parameters	Group		p-value
	RA	control	
Betatrophin [ng/mL]	0.28 ±0.24	0.23 ±0.20	0.466
Adropin [pg/mL]	253.3 ±132.5	384.3 ±152.0	<0.001*
Chemerin [ng/mL]	1.37 ±2.48	1.25 ±1.55	0.016
EFT [mm]	5.7 ±2.6	3.7 ±1.6	<0.001

EFT – epicardial fat thickness; RA – rheumatoid arthritis; *independent samples Student's t-test; otherwise, Mann–Whitney U test was performed.

levels were lower in the RA group compared to controls ($p < 0.001$). Chemerin levels and EFT were higher in the RA group compared to the control group ($p = 0.016$, $p < 0.001$, respectively) (Table 3, Fig. 1).

In the RA group, patients with low disease activity according to the DAS-28 had lower EFT (4.4 ±1.76 mm) compared to those with moderate/high disease activity (6.0 ±2.7 mm; $p = 0.040$). Levels of betatrophin, adropin and chemerin did not differ significantly based on disease activity.

The study groups were also compared according to the median value of EFT (EFT < 5 mm or EFT ≥ 5 mm). The proportion of patients with high EFT (EFT ≥ 5 mm) was 57.5% in the RA group and 20% in the control group. The proportion of patients with EFT ≥ 5 mm was significantly higher in the RA group compared to the control group ($p < 0.001$).

A weak positive correlation was found between BMI and betatrophin in the RA group ($r = 0.28$, $p = 0.011$). Both the RA and control groups showed a weak positive correlation between BMI and EFT (for RA: $r = 0.24$, $p = 0.029$; for control: $r = 0.27$, $p = 0.016$). There was no correlation between CRP and the biomarkers. There was a moderate positive correlation between age and EFT ($r = 0.49$, $p < 0.001$) in the RA group, whereas this correlation was weak ($r = 0.29$, $p = 0.010$) in the control group.

When evaluating the relationship between biomarkers and EFT in RA patients, a strong positive correlation was found between chemerin and EFT ($r = 0.73$, $p = 0.046$) in patients with high disease activity.

Discussion

In this study, EFT was higher in RA patients than in controls. Serum chemerin levels were also higher in RA patients. Conversely, serum adropin levels were lower in RA patients. There was a correlation between chemerin and EFT, and a relationship was found between disease activity and EFT.

Ormseth et al. reported that patients with RA had a higher EFT associated with cardiometabolic risk factors and metabolic syndrome compared to the control group. Similar to the present study, they also reported correlations between EFT, waist circumference and BMI.[20] On the other hand, Kitagawa et al. reported that macrophage infiltration and neoangiogenesis, demonstrated with immunohistochemical staining on EFT, correlated with calcific and non-calcific plaque formation in the coronary arteries on cardiac computed tomography (CT).[21]

Karpouzas et al. evaluated epicardial fat tissue volumes (EFTVs) in RA patients and controls using CT angiography. They reported a higher plaque load and the presence of non-calcified plaques in the EFTV of RA patients, although similar EFTVs were found between RA patients and controls.[22] This demonstrates that epicardial fat tissue promotes atherogenesis through inflammation, biological dysfunction and paracrine effects through a mechanism other than traditional risk factors (e.g., metabolic syndrome, insulin resistance and abdominal visceral fat). Epicardial fat thickness may have a more pathogenic effect on the development of subclinical atherosclerosis and cardiovascular risk in RA. The relationship between severe disease activity and EFT could indicate an increased cardiovascular risk in these patients.

Chemerin, a proinflammatory adipokine, activates the chemotaxis of macrophages, natural killer cells and dendritic cells. It increases the production of tumor necrosis factor alpha (TNF-α), interleukin (IL)-6 , IL-1β, matrix metalloproteinase (MMP)-1, and MMP-8 in human chondrocytes.[23] ChemR23, or the CMKLR receptor, is expressed in macrophages, dendritic cells and fibroblast-like synoviocytes, and has been associated with both adaptive and innate immunity.[12] Tolusso et al. found that plasma chemerin values were correlated with disease activity and BMI in RA patients.[24] They also found that a reduction in BMI of at least 5% improved disease control in obese RA patients without changing the RA treatment. Vazquez-Villegas et al. demonstrated a relationship between high chemerin levels and functional disability in RA patients and found a correlation between functional disability and DAS-28.[25] Leiherer et al. demonstrated that chemerin was a strong predictor of cardiovascular events in individuals with metabolic syndrome.[26] The present study found that chemerin levels increased in patients with RA, and there was a correlation between serum chemerin levels and EFT. This suggests that patients with RA have an increased risk of CVD.

Adropin plays a role in lipid metabolism (by suppressing carnitine palmitoyl-transferase) and glucose metabolism (by activating pyruvate dehydrogenase).[27] Gao et al. reported that in a diet-dependent mouse model, adropin 34-76 suppressed cAMP-activated protein kinase A activity and reduced the phosphorylation of inositol triphosphate receptor and element-binding protein. Thus, they stated that adropin increased intracellular signaling activities in insulin-mediated glucose homeostasis.[28] Lovren et al. showed that adropin decreased the level of apoptosis caused by TNF-α in human umbilical vein endothelial cells.[29] Impaired endothelial function is the triggering factor for the development and progression of cardiovascular, metabolic, inflammatory, renal, and infectious diseases, with atherothrombosis having the most notable pathological effect. Several investigations have revealed that adropin levels are lower in the blood of people with CAD, coronary slow flow phenomenon and hypertension compared to those in control groups.[30–32]

Wu et al. included individuals with and without type 2 diabetes in a study to evaluate the link between blood adropin levels and the angiographic severity of coronary atherosclerosis. They found that serum adropin levels were lower in patients with type 2 diabetes. Furthermore, they discovered that these levels were inversely and independently associated with the angiographic severity of coronary atherosclerosis.[33] Butler et al. found that rats fed a high-fat diet had significantly elevated adiponectin levels in their blood, along with significant changes in insulin sensitivity and glucose intolerance.[34] They also stated that adropin plays a role in protecting the endothelium and maintaining its functions. Similarly, the present study revealed that adropin levels were found to be low in patients with RA.

Erman et al. reported that obese patients had low serum adropin levels, with 216.7 ng/L being the optimal cutoff point to detect insulin resistance.[35] Fujie et al. reported that adropin levels decreased with age and increased with an aerobic exercise program.[36] Tuleab et al. reported that adropin levels in the serum of RA patients were noticeably lower than in the control group.[37] Similarly, low serum levels of adropin were observed in individuals with RA in the current study.

We found that patients with RA had higher EFT levels than the control group. There was a connection between age, BMI, waist circumference, and exercise intensity. It was discovered that patients experiencing intense illness activity had higher EFT. Patients diagnosed with RA had a higher EFT, indicating cardiovascular involvement.[38] Additionally, serum chemerin levels were higher in patients with RA. Conversely, serum adropin levels were lower in RA patients. Low serum adropin levels may reduce endothelium protection and may induce or accelerate the progression of atherosclerosis.[33] Recent studies suggest that chemerin is important in the pathogenesis of CVD, particularly CAD.[39,40]

Limitations

There were 3 significant limitations to our investigation. First, this was a cross-sectional analysis focusing on the relationship between RA and EFT, serum betatrophin, chemerin, and adropin levels. Second, the sample size was somewhat limited. Third, because this was not a prospective controlled trial, causal relationships could not be inferred from our findings.

Conclusions

Measurement of EFT in patients with RA may assist in determining cardiovascular risk and enable early precautions to be taken. Given that patients with RA have a higher risk of developing CVD, it is hypothesized that elevated serum chemerin levels combined with decreased adropin levels contribute to the pathophysiology of this condition.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Aletaha D, Neogi T, Silman AJ, et al. 2010 rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum*. 2010;62(9):2569–2581. doi:10.1002/art.27584
2. Chung CP, Oeser A, Solus JF, et al. Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. *Atherosclerosis*. 2008;196(2):756–763. doi:10.1016/j.atherosclerosis.2007.01.004
3. Teoh BC, Syed Sulaiman SA, Tan BE. Knowledge of cardiovascular disease risk in rheumatoid arthritis patients before and after educational intervention from a Southeast Asia country: Malaysia. *Arch Rheumatol*. 2020;36(1):1159. doi:10.46497/ArchRheumatol.2021.7726
4. Gabriel SE. Why do people with rheumatoid arthritis still die prematurely? *Ann Rheum Dis*. 2008;67(Suppl 3):iii30–iii34. doi:10.1136/ard.2008.098038
5. Aviña-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D. Risk of cardiovascular mortality in patients with rheumatoid arthritis: A meta-analysis of observational studies. *Arthritis Care Res (Hoboken)*. 2008;59(12):1690–1697. doi:10.1002/art.24092
6. Meune C, Touze E, Trinquart L, Allanore Y. Trends in cardiovascular mortality in patients with rheumatoid arthritis over 50 years: A systematic review and meta-analysis of cohort studies. *Rheumatology*. 2009;48(10):1309–1313. doi:10.1093/rheumatology/kep252
7. Monti M, Monti A, Murdolo G, et al. Correlation between epicardial fat and cigarette smoking: CT imaging in patients with metabolic syndrome. *Scand Cardiovasc J*. 2014;48(5):317–322. doi:10.3109/14017431.2014.942872
8. Öhman MK, Luo W, Wang H, et al. Perivascular visceral adipose tissue induces atherosclerosis in apolipoprotein E deficient mice. *Atherosclerosis*. 2011;219(1):33–39. doi:10.1016/j.atherosclerosis.2011.07.012
9. Sacks HS, Fain JN, Cheema P, et al. Inflammatory genes in epicardial fat contiguous with coronary atherosclerosis in the metabolic syndrome and type 2 diabetes. *Diabetes Care*. 2011;34(3):730–733. doi:10.2337/dc10-2083
10. Quagliarini F, Wang Y, Kozlitina J, et al. Atypical angiopoietin-like protein that regulates ANGPTL3. *Proc Natl Acad Sci U S A*. 2012;109(48):19751–19756. doi:10.1073/pnas.1217552109
11. Fu Z, Yao F, Abou-Samra AB, Zhang R. Lipasin, thermoregulated in brown fat, is a novel but atypical member of the angiopoietin-like protein family. *Biochem Biophys Res Commun*. 2013;430(3):1126–1131. doi:10.1016/j.bbrc.2012.12.025
12. Kaneko K, Miyabe Y, Takayasu A, et al. Chemerin activates fibroblast-like synoviocytes in patients with rheumatoid arthritis. *Arthritis Res Ther*. 2011;13(5):R158. doi:10.1186/ar3475
13. Kumar KG, Trevaskis JL, Lam DD, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell Metab*. 2008;8(6):468–481. doi:10.1016/j.cmet.2008.10.011
14. Sato K, Yamashita T, Shirai R, et al. Adropin contributes to anti-atherosclerosis by suppressing monocyte-endothelial cell adhesion and smooth muscle cell proliferation. *Int J Mol Sci*. 2018;19(5):1293. doi:10.3390/ijms19051293
15. Neogi T, Aletaha D, Silman AJ, et al. The 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis: Phase 2 methodological report. *Arthritis Rheum*. 2010;62(9):2582–2591. doi:10.1002/art.27580
16. Wells G, Becker JC, Teng J, et al. Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann Rheum Dis*. 2009;68(6):954–960. doi:10.1136/ard.2007.084459
17. Steinbrocker O, Traeger CH, Batterman RC. Therapeutic criteria in rheumatoid arthritis. *JAMA*. 1949;140(8):659. doi:10.1001/jama.1949.02900430001001
18. Bertaso AG, Bertol D, Duncan BB, Foppa M. Epicardial fat: Definition, measurements and systematic review of main outcomes. *Arq Bras Cardiol*. 2013;101(1):e18–28. doi:10.5935/abc.20130138
19. Eroglu S. How do we measure epicardial adipose tissue thickness by transthoracic echocardiography? *Anatol J Cardiol*. 2015;15(5):416–419. doi:10.5152/akd.2015.5991
20. Ormseth MJ, Lipson A, Alexopoulos N, et al. Association of epicardial adipose tissue with cardiometabolic risk and metabolic syndrome in patients with rheumatoid arthritis. *Arthritis Care Res (Hoboken)*. 2013;65(9):1410–1415. doi:10.1002/acr.22027
21. Kitagawa T, Yamamoto H, Sentani K, et al. The relationship between inflammation and neoangiogenesis of epicardial adipose tissue and coronary atherosclerosis based on computed tomography analysis. *Atherosclerosis*. 2015;243(1):293–299. doi:10.1016/j.atherosclerosis.2015.09.013
22. Karpouzas GA, Rezaeian P, Ormseth SR, Hollan I, Budoff MJ. Epicardial adipose tissue volume as a marker of subclinical coronary atherosclerosis in rheumatoid arthritis. *Arthritis Rheumatol*. 2021;73(8):1412–1420. doi:10.1002/art.41693
23. Berg V, Sveinbjörnsson B, Bendiksen S, Brox J, Meknas K, Figenschau Y. Human articular chondrocytes express ChemR23 and chemerin: ChemR23 promotes inflammatory signalling upon binding the ligand chemerin21-157. *Arthritis Res Ther*. 2010;12(6):R228. doi:10.1186/ar3215
24. Tolusso B, Gigante MR, Alivernini S, et al. Chemerin and PEDF are metaflammation-related biomarkers of disease activity and obesity in rheumatoid arthritis. *Front Med (Lausanne)*. 2018;5:207. doi:10.3389/fmed.2018.00207
25. Vazquez-Villegas ML, Gamez-Nava JI, Saldaña-Cruz AM, et al. Functional disability is related to serum chemerin levels in rheumatoid arthritis. *Sci Rep*. 2021;11(1):8360. doi:10.1038/s41598-021-87235-6

26. Leiherer A, Muendlein A, Rein P, et al. Plasma chemerin is a strong and independent predictor of cardiovascular event risk. *Atherosclerosis*. 2015;241(1):e24. doi:10.1016/j.atherosclerosis.2015.04.095
27. Gao S, McMillan RP, Jacas J, et al. Regulation of substrate oxidation preferences in muscle by the peptide hormone adropin. *Diabetes*. 2014;63(10):3242–3252. doi:10.2337/db14-0388
28. Gao S, Ghoshal S, Zhang L, et al. The peptide hormone adropin regulates signal transduction pathways controlling hepatic glucose metabolism in a mouse model of diet-induced obesity. *J Biol Chem*. 2019;294(36):13366–13377. doi:10.1074/jbc.RA119.008967
29. Lovren F, Pan Y, Quan A, et al. Adropin is a novel regulator of endothelial function. *Circulation*. 2010;122(11 Suppl 1):S185–S192. doi:10.1161/CIRCULATIONAHA.109.931782
30. Zhang C, Zhao L, Xu W, et al. Correlation of serum adropin level with coronary artery disease [in Chinese]. *Zhonghua Yi Xue Za Zhi*. 2014; 94(16):1255–1257. PMID:24924892.
31. Zi-Wen Zhao, Yin-Gang Ren, Jun Liu. Low serum adropin levels are associated with coronary slow flow phenomenon. *Acta Cardiol Sin*. 2018;34(4):307–312. doi:10.6515/ACS.201807_34(4).20180306B
32. Gu X, Li H, Zhu X, et al. Inverse correlation between plasma adropin and ET-1 levels in essential hypertension: A cross-sectional study. *Medicine (Baltimore)*. 2015;94(40):e1712. doi:10.1097/MD.0000000000001712
33. Wu L, Fang J, Chen L, et al. Low serum adropin is associated with coronary atherosclerosis in type 2 diabetic and non-diabetic patients. *Clin Chem Lab Med*. 2014;52(5):751–758. doi:10.1515/cclm-2013-0844
34. Butler AA, Zhang J, Price CA, et al. Low plasma adropin concentrations increase risks of weight gain and metabolic dysregulation in response to a high-sugar diet in male nonhuman primates. *J Biol Chem*. 2019;294(25):9706–9719. doi:10.1074/jbc.RA119.007528
35. Erman H, Ozdemir A, Sitar ME, Cetin SI, Boyuk B. Role of serum adropin measurement in the assessment of insulin resistance in obesity. *J Invest Med*. 2021;69(7):1318–1323. doi:10.1136/jim-2021-001796
36. Fujie S, Hasegawa N, Horii N, et al. Aerobic exercise restores aging-associated reductions in arterial adropin levels and improves adropin-induced nitric oxide-dependent vasorelaxation. *JAHA*. 2021; 10(10):e020641. doi:10.1161/JAHA.120.020641
37. Tuleab SF. Correlation of calprotectin with galectin 33, adropin and CTLA-4 in Iraqi rheumatoid arthritis patients. *Int J Pharm Res*. 2020; 12(1):932–939. doi:10.31838/ijpr/2020.12.01.052
38. Fatma E, Bunyamin K, Savas S, et al. Epicardial fat thickness in patients with rheumatoid arthritis. *Afr Health Sci*. 2015;15(2):489. doi:10.4314/ahs.v15i2.23
39. Wu Q, Chen Y, Chen S, Wu X, Nong W. Correlation between adiponectin, chemerin, vascular endothelial growth factor and epicardial fat volume in patients with coronary artery disease. *Exp Ther Med*. 2019;19(2):1095–1102. doi:10.3892/etm.2019.8299
40. Kaur J, Mattu HS, Chatha K, Randeva HS. Chemerin in human cardiovascular disease. *Vascul Pharmacol*. 2018;110:1–6. doi:10.1016/j.vph.2018.06.018

Neutrophil-to-lymphocyte ratio and prognostic nutritional index in predicting composite endpoint of early safety following transcatheter aortic valve replacement

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Abstract

Background. The incidence of composite endpoint of early safety (CEES) after transcatheter aortic valve replacement (TAVR) has been a topic of focus within the cardiovascular field due to its impact on long-term patient outcomes. Timely prophylactic interventions are crucial for patients identified as high risk for CEES through preoperative risk stratification.

Objectives. This study aimed to explore the connection between inflammatory and nutritional markers, specifically the neutrophil-to-lymphocyte ratio (NLR) and prognostic nutritional index (PNI), and CEES occurrence.

Materials and methods. A cohort of 134 patients undergoing TAVR in a single center was studied. The study endpoint was the occurrence of CEES, which was defined according to the Valve Academic Research Consortium 3.

Results. The CEES was reached in 25.4% of patients at 30 days. A high NLR was associated with a 5.55-fold increased risk of CEES (95% confidence interval (95% CI): 1.52–20.29; $p < 0.05$), while a low PNI was linked to a 4.43-fold increased risk (95% CI: 1.55–12.65; $p < 0.01$). Combining NLR and PNI provided additional risk stratification for high-risk patients (hazard ratio (HR), 95% CI: 2.24–43.37; $p < 0.005$).

Conclusions. A high NLR and low PNI were shown to be significant predictors of CEES following TAVR. These findings underscore the significance of NLR and PNI in the risk assessment of TAVR patients, offering valuable insights for preventive measures.

Key words: prognostic nutritional index, neutrophil-to-lymphocyte ratio, transcatheter aortic valve replacement

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Background

In recent years, there has been a significant focus on clinical endpoints observed in patients who undergo transcatheter aortic valve replacement (TAVR). The concept of composite endpoints was initially introduced in the Valve Academic Research Consortium (VARC) Consensus Report in 2011.[1] With the continuous accumulation of TAVR experience and advancements in related studies, the indications for TAVR have broadened to include low-risk patients,[2,3] and the latest definition of composite endpoints was introduced in the VARC-3 in 2021.[4] Within this definition, the composite endpoint of early safety (CEES) at 30 days serves as a measure of the procedure's early safety and effectiveness, encompassing a variety of adverse events that can have a significant impact on patient outcomes.[5–9] Consequently, an early prediction of these adverse events and the implementation of prophylactic interventions are crucial for improving patient outcomes.

In the development and occurrence of atherosclerosis and calcific aortic valve disease, inflammation is a crucial factor.[10–12] The neutrophil-to-lymphocyte ratio (NLR), an emerging inflammatory biomarker, has gained widespread attention in the past decade due to its inexpensiveness and easy accessibility. Several studies have illustrated a significant correlation between elevated preoperative NLR levels and increased postoperative mortality as well as acute kidney injury (AKI) in patients undergoing TAVR.[13,14]

Furthermore, malnutrition also has an adverse impact on the prognosis following TAVR. Elderly patients comprise a considerable proportion of the population undergoing TAVR treatment, and malnutrition is particularly prevalent in this group. Previous studies have indicated that malnourished patients have higher mortality rates following TAVR procedures.[15] The prognostic nutritional index (PNI) is a useful indicator for assessing nutritional status and has been validated for its predictive value in cardiovascular diseases such as heart failure and acute coronary syndromes.[16–19] Recent research has also suggested that PNI exhibits good predictive ability for post-TAVR survival.[20]

Neutrophil-to-lymphocyte ratio and PNI serve as predictors of post-TAVR complications by reflecting the systemic inflammatory status and nutritional level. However, there is currently a lack of research demonstrating the predictive value of these 2 indicators for composite endpoints such as CEES.

Objectives

This research aimed to investigate the impact of the systemic inflammatory response and nutritional status on the CEES after TAVR, specifically looking at NLR and PNI. Additionally, the study assessed the predictive accuracy of NLR and PNI in identifying the occurrence of post-TAVR complications.

Materials and methods

Study design

The study retrospectively gathered common inflammatory and nutritional indicators for the study population. Baseline characteristics for the entire population and differences between subgroups divided according to the occurrence of CEES were analyzed. The prognostic significance of inflammatory and nutritional indicators in predicting CEES following TAVR was studied.

Study patients

This retrospective and observational study included 145 consecutive patients who underwent TAVR at the Second Affiliated Hospital of Nanchang University (Nanchang, China) between January 2018 and February 2023. Eleven individuals were excluded from the study because of a preoperative red blood cell transfusion, cancer, sick sinus syndrome, and high-degree atrioventricular block. As this was a retrospective study, the need for informed consent was not required.

Data collection

Demographic characteristics, clinical concomitant diseases, laboratory parameters, and pre-procedural imaging parameters were collected. Furthermore, the preprocedural Society of Thoracic Surgeons (STS) scores were calculated. In terms of inflammation parameters, we calculated the NLR, platelet-to-lymphocyte ratio (PLR) and monocyte-to-high-density lipoprotein ratio (MHR). Neutrophil-to-lymphocyte ratio is calculated as the ratio of neutrophils to lymphocytes, PLR as the ratio of platelets to lymphocytes, and MHR as the ratio of monocytes to high-density lipoprotein concentrations. For nutritional parameters, we calculated the body mass index (BMI) and the PNI. Prognostic nutritional index is measured as follows: (10 × serum albumin [g/dL]) + (0.005 × total lymphocytes [1000/μL]).[21]

TAVR procedure

The multidisciplinary Heart Team in our center evaluated all patients and formulated operation plans after ruling out definite contraindications. Preoperative transthoracic echocardiography and multidetector computed tomography (MDCT) were performed to assess the anatomy of the aortic valve and root. Based on the anatomical parameters and clinical characteristics, a suitable valve type and size were selected. All valves in this study used self-expanding valves and included the VENUS-A (Venus MedTech, Hangzhou, China) and VitaFlow (MicroPort, Shanghai, China) valves. All patients underwent general anesthesia by endotracheal intubation in the cardiac catheterization laboratory and monitoring using transesophageal echocardiography.

The preferred access for TAVR was transfemoral with other alternative accesses, including transcarotid or transaxillary. Following the procedure, all patients were transferred to the cardiac intensive care unit for observation.

Endpoint

The endpoint of this study was the occurrence of the CEES at 30 days, which was defined with VARC-3. This comprises all-cause mortality; stroke; VARC type 2–4 bleeding; major vascular, access-related, or cardiac structural complications; acute kidney injury stage 3 or 4; moderate or severe aortic regurgitation; new permanent pacemaker placement due to procedure-related conduction abnormalities; surgery or intervention related to the device.

Statistical analyses

The data analysis was performed using IBM SPSS v. 26.0 software (IBM Corp., Armonk, USA). Medians (interquartile ranges) or means ± standard deviations (±SD) were used to represent continuous variables, while frequencies (percentages) were used for categorical variables. Statistical significance was defined as a p-value of less than 0.05. Continuous variables were compared using t-tests or Mann–Whitney U tests, while categorical variables were compared using the χ^2 test or Fisher's test. The risk factors associated with the study endpoints were identified through univariate and multivariate logistic regression analyses conducted in sequence. For multivariate analysis, we adjusted for age, gender, STS score, New York Heart Association (NYHA) classification, hypertension, diabetes mellitus, coronary heart disease (CHD), atrial fibrillation, cerebrovascular disease, chronic obstructive pulmonary disease (COPD), chronic kidney disease (CKD), hemoglobin, platelet, hematocrit, red blood cell distribution width (RDW), mean platelet volume (MPV), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), non-high-density lipoprotein cholesterol (NHDL-c), lipoprotein(a) (Lp(a)), B-type natriuretic peptide (BNP), serum creatinine, left ventricular ejection fraction (LVEF), peak aortic jet velocity, annulus diameter, left ventricular outflow tract diameter, and ascending aorta diameter. The results were presented in terms of odds ratios (ORs) along with their corresponding 95% confidence intervals (95% CIs). A receiver operating characteristic (ROC) curve was generated to assess the predictive value of the risk factor for the occurrence of CEES, and the optimal cutoff values for NLR and PNI were identified.

Results

Baseline characteristic

A total of 134 patients undergoing TAVR at our center were included in this study. Among this cohort, the mean age was 71.39 ±6.92 years, with 38.8% of the population being female. The median (range) STS score was 2.36 (1.60, 3.90) and the median (range) LVEF was 56.5 (43.75, 64). Thirty-one patients (23.1%) were classified as a NYHA class IV. The occurrence of CEES at 30 days in all patients was 25.4% (34/134). We also analyzed the differences between patients who experienced CEES and those who did not. Compared to the non-CEES group, patients in the CEES group tended to have higher STS scores and BNP levels. Additionally, these patients had a higher proportion of NYHA class IV (41.2%) and intraoperative use of mechanical circulatory support devices (26.5%), but a lower baseline LVEF. In terms of inflammatory markers, the CEES group had higher NLR and MHR values compared to the non-CEES group, although PLR showed no significant differences. Regarding nutritional markers, patients in the CEES group had a lower PNI compared to the non-CEES group, but there was no significant difference in BMI between the groups. Further details are outlined in Table 1.

Comparison of baseline characteristics according to NLR/PNI binaries

Based on the ROC curve analysis for CEES incidence, the optimal NLR and PNI cutoff values were established at 2.27 and 42.9, respectively. Subsequently, patients were divided into 2 groups according to NLR and PNI binaries, and the baseline characteristics were analyzed (Table 2). Notably, the patients with high NLR values or low PNI values had a higher incidence of CEES and exhibited higher STS scores, BNP levels, PLR values, and RDW values, but had lower LVEF measurements. Additionally, higher NLR levels were associated with higher baseline creatinine levels, as well as a higher prevalence of hypertension and diabetes. On the other hand, patients with low PNI levels had lower baseline levels of hemoglobin and high-density lipoprotein cholesterol, a higher proportion of NYHA class IV, and a higher requirement rate of intraoperative mechanical circulatory support.

Prognostic significance in CEES

The levels of NLR or PNI were associated with the occurrence of CEES (Table 3). The risk of CEES in patients with a high NLR or low PNI was 5.55 (95% CI: 1.52–20.29) and 4.43 (95% CI: 1.55–12.65), respectively, compared to the patients with a low NLR or high PNI. To investigate whether the combination of NLR and PNI provides additional prognostic value, we further divided the patients into 3 groups: 1) NLR-low and PNI-high, 2) NLR-high or PNI-low and 3) NLR-high and PNI-low. We included the new categorical variables in the model for analysis. This simple and effective stratification successfully categorized patients into low-, intermediate- (OR = 4.07, (95% CI: 1.06–15.68)), and

Table 1. Demographic, clinical and procedural characteristics

Variable	Total population	CEES		
		CEES group (n = 34)	Non-CEES group (n = 100)	p-value
Age [years]	71.39 ±6.92	69.68 ±8.26	71.97 ±6.34	0.146
Female, n (%)	52 (38.8)	11 (32.4)	41 (41)	0.371
BSA [m^2]	1.57 ±0.16	1.60 ±0.17	1.56 ±0.16	0.213
BMI [kg/m^2]	22.08 (20.29–24.06)	21.76 (19.81–25.02)	22.24 (20.31–23.90)	0.988
STS score	2.36 (1.60–3.90)	3.13 (1.80–5.57)	2.22 (1.59–3.35)	0.028
NYHA class IV, n (%)	31 (23.1)	14 (41.2)	17 (17)	0.004
Hypertension, n (%)	70 (52.2)	21 (61.8)	49 (49)	0.198
Diabetes mellitus, n (%)	17 (12.7)	5 (14.7)	12 (12)	0.682
CHD, n (%)	35 (26.1)	9 (26.5)	26 (26.0)	0.957
Atrial fibrillation, n (%)	19 (14.2)	7 (20.6)	12 (12)	0.215
CVD, n (%)	16 (11.9)	2 (5.9)	14 (14)	0.207
COPD, n (%)	14 (10.4)	3 (8.8)	11 (11.0)	0.720
CKD, n (%)	15 (11.2)	5 (14.7)	10 (10)	0.530
Laboratory index				
RBC [$\times 10^9$/L]	4.11 ±0.55	4.02 ±0.55	4.14 ±0.55	0.307
Hemoglobin [g/L]	124.79 ±16.05	121.91 ±17.47	125.77 ±15.50	0.227
Platelet count [$\times 10^9$/L]	167 (132.75–198.25)	168.00 (140.75–199.25)	160.5 (131.25–197.25)	0.476
NLR	2.96 (2.11–4.61)	3.74 (2.65–7.03)	2.71 (1.99–4.13)	0.001
PLR	129.13 (94.41–162.65)	149.82 (94.41–193.81)	124.44 (93.01–161.06)	0.161
MHR	0.37 (0.27–0.57)	0.48 (0.31–0.70)	0.36 (0.26–0.55)	0.027
Hematocrit [%]	38.12 ±4.49	37.54 ±5.14	38.32 ±4.25	0.379
RDW [%]	13.30 (12.58–14.30)	13.70 (12.60–14.65)	13.2 (12.5–14.08)	0.122
MPV [fL]	11.05 (10.40–11.83)	11.15 (10.48–11.93)	11.0 (10.2–11.78)	0.350
HDL-c [mmol/L]	1.18 (0.99–1.43)	1.15 (0.86–1.43)	1.18 (1.02–1.43)	0.597
LDL-c [mmol/L]	2.27 (1.68–3.09)	1.95 (1.54–3.01)	2.34 (1.77–3.10)	0.093
NHDL-c [mmol/L]	3.04 (2.30–3.72)	2.74 (2.15–3.56)	3.07 (2.36–3.78)	0.121
Lp (a) [mg/L]	159.25 (78.08–355.85)	159.25 (62.13–375.65)	160.25 (83.00–315.10)	0.904
PNI	45.87 ±5.26	43.28 ±6.25	46.74 ±4.59	0.005
BNP [pg/mL]	679.72 (261.88–1811.09)	1203.26 (252.00–5000)	664.57 (259.96–1520.77)	0.034
Creatinine [µmol/L]	81.70 (70.69–110.58)	87.60 (74.43–127.70)	81.10 (68.95–106.08)	0.146
Baseline echocardiographic parameters				
Aortic regurgitation ≥moderate, n (%)	18 (13.4)	4 (11.8)	14 (14)	0.741
LVEDD [mm]	52.00 (46.00–59.00)	54.5 (45.5–64.25)	50.5 (46.0–57.0)	0.083
LVESD [mm]	36.0 (29.75–44.0)	42.00 (29.75–54.50)	36.00 (29.25–42.00)	0.031
LVEF [%]	56.5 (43.75–64)	49.5 (31.25–61.00)	58.00 (45.25–64.00)	0.018
Peak aortic jet velocity [m/s]	4.43 (3.98–5.01)	4.33 (3.90–4.82)	4.49 (4.02–5.08)	0.390
Baseline MDCT parameters				
Annulus diameter [mm]	24.93 ±2.65	25.56 ±2.90	24.71 ±2.55	0.108
Annulus area [mm^2]	477.97 ±102.25	25.56 ±2.90	24.71 ±2.55	0.153
LVOT diameter [mm]	25.65 (23.68, 28.9)	26.75 (23.25, 30.13)	25.55 (23.70, 28.60)	0.284
Ascending aorta diameter [mm]	37.85 ±4.56	38.70 ±5.63	37.56 ±4.13	0.209
Procedural details				
Circulatory support, n (%)	9 (6.7)	9 (26.5)	0 (0)	<0.001
Valve size >26 mm, n (%)	50 (37.3)	14 (41.2)	36 (36)	0.590
2nd valve needed, n (%)	34 (25.4)	10 (29.4)	24 (24)	0.531

Values are presented as counts (percentages) or median (IQR). CEES – composite endpoints of early safety; BSA – body surface area; STS – Society of Thoracic Surgery Risk Score; NYHA – New York Heart Association; CHD – coronary heart disease; CVD – cerebrovascular disease; COPD – chronic obstructive pulmonary disease; CKD – chronic kidney disease; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MHR – monocyte-to-high density lipoprotein cholesterol ratio; RDW – red blood cell distribution width; MPV – mean platelet volume; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; NHDL-c – non-high-density lipoprotein cholesterol; Lp(a) – lipoprotein(a); PNI – prognostic nutritional index; BNP – B-type natriuretic peptide; LVEDD – left ventricular end-diastolic dimension; LVESD – left ventricular end-systolic dimension; LVEF – left ventricular ejection fraction; MDCT – multidetector computed tomography; LVOT – left ventricular outflow tract.

Table 2. Demographic, clinical and procedural characteristics and outcomes in patients according to NLR and PNI

Variable	NLR			PNI		
	NLR-low group (n = 42)	NLR-high group (n = 92)	p-value	PNI-high group (n = 102)	PNI-low group (n = 32)	p-value
Age [years]	71.31 ±5.57	71.42 ±7.48	0.930	71.32 ±6.43	71.59 ±8.41	0.848
Female, n (%)	21 (50)	31 (33.7)	0.072	43 (42.2)	9 (28.1)	0.155
BSA [m^2]	1.54 ±0.18	1.59 ±0.15	0.153	1.57 ±0.17	1.57 ±0.14	0.991
BMI [kg/m^2]	22.28 (20.23, 23.38)	22.04 (20.27, 24.15)	0.694	22.35 (20.31, 24.22)	21.73 (18.82, 23.13)	0.186
STS score	1.90 (1.52, 3.23)	2.50 (1.70, 4.03)	0.032	2.12 (1.53, 3.25)	3.57 (2.25, 5.65)	<0.001
NYHA IV, n (%)	6 (14.3,)	25 (27.2,)	0.101	16 (15.7,)	15 (46.9,)	<0.001
Hypertension, n (%)	14 (33.3,)	56 (60.9,)	0.003	54 (52.9,)	16 (50,)	0.771
Diabetes mellitus, n (%)	1 (2.4,)	16 (17.4,)	0.015	13 (12.7,)	4 (12.5,)	0.971
CHD, n (%)	8 (19,)	27 (29.3,)	0.208	25 (24.5,)	10 (31.3,)	0.449
Atrial fibrillation, n (%)	4 (9.5,)	15 (16.3,)	0.297	14 (13.7,)	5 (15.6,)	0.788
CVD, n (%)	7 (16.7,)	9 (9.8,)	0.254	13 (12.7,)	3 (9.4,)	0.761
COPD, n (%)	1 (2.4,)	13 (14.1,)	0.064	8 (7.8,)	6 (18.8,)	0.099
CKD, n (%)	2 (4.8,)	13 (14.1,)	0.145	10 (9.8,)	5 (15.6,)	0.351
Laboratory index						
RBC [$\times 10^9/L$]	4.07 ±0.55	4.12 ±0.55	0.621	4.17 ±0.54	3.92 ±0.53	0.027
Hemoglobin [g/L]	123.95 ±13.87	125.17 ±17.00	0.684	126.47 ±15.14	119.44 ±17.87	0.030
Platelet count [$\times 10^9/L$]	162.0 (135.5, 192.0)	168.0 (131.3, 200.0)	0.631	167.5 (135.5, 198)	161 (118.25, 203)	0.395
NLR	1.87 (1.53, 2.07)	3.73 (2.83, 6.12)	<0.001	2.65 (1.96, 3.66)	5.62 (3.2, 8.78)	<0.001
PLR	104.02 (71.09, 132.03)	145.02 (109.07, 188.57)	<0.001	115.6 (88.87, 159.04)	159.04 (126.53, 210.86)	0.000
MHR	0.31 (0.24, 0.45)	0.40 (0.29, 0.63)	0.016	0.36 (0.27, 0.54)	0.45 (0.32, 0.72)	0.052
Hematocrit [%]	37.76 ±3.74	38.28 ±4.80	0.527	38.59 ±4.17	36.63 ±5.18	0.030
RDW [%]	12.95 (12.30, 13.63)	13.70 (12.80, 14.48)	0.002	13.2 (12.48, 13.93)	14.05 (13.63, 15.53)	<0.001
MPV [fL]	10.95 (10.43, 11.60)	11.10 (10.40, 11.90)	0.494	11.05 (10.35, 11.83)	11.05 (10.4, 11.85)	0.909
HDL-c [mmol/L]	1.22 (1.03, 1.52)	1.13 (0.95, 1.38)	0.152	1.2 (1.04, 1.49)	1.05 (0.84, 1.22)	0.003
LDL-c [mmol/L]	2.46 (1.62, 3.05)	2.23 (1.73, 3.10)	0.842	2.37 (1.7, 3.1)	2.18 (1.58, 2.94)	0.149
NHDL-c [mmol/L]	3.07 (2.20, 3.77)	2.97 (2.43, 3.10)	0.558	3.04 (2.29, 3.94)	3.01 (2.33, 3.49)	0.357
Lp (a) [mg/L]	140.45 (69.90, 262.87)	180.60 (79.83, 385.53)	0.415	177.05 (78.08, 351.78)	142.95 (67.08, 312.45)	0.661
PNI	48.68 ±3.72	44.58 ±5.37	<0.001	48.04 ±3.61	38.93 ±3.3	<0.001
BNP [pg/mL]	387.57 (203.50, 789.01)	806.73 (311.00, 2611.49)	0.003	634.54 (240.25, 1428.84)	2215.46 (360.41, 5000)	0.002
Creatinine [µmol/L]	75.00 (66.49, 92.30)	86.70 (74.29, 113.16)	0.006	80.47 (69.25, 106.43)	86.7 (72.11, 128.3)	0.098
Baseline echocardiographic parameters						
Aortic regurgitation ≥moderate, n (%)	12 (28.6)	24 (26.1)	0.763	28 (27.5)	8 (25)	0.785
LVEDD [mm]	51.00 (42.75, 58.25)	52.00 (46.00, 59.00)	0.226	52 (45.75, 57.25)	52.5 (46, 63.75)	0.255
LVESD [mm]	34.50 (27.00, 42.25)	37.00 (31.00, 45.75)	0.053	36 (29, 42)	39 (30.25, 49.5)	0.098
LVEF [%]	59.0 (51.0, 66.0)	54.0 (42.0, 62.0)	0.017	58 (45, 64)	49 (30.25, 63.75)	0.044
Peak aortic jet velocity [m/s]	4.74 (4.13, 5.36)	4.33 (3.95, 4.88)	0.081	4.44 (4.01, 5.14)	4.32 (3.95, 4.72)	0.287
Baseline MDCT parameters						
Annulus diameter [mm]	24.60 ±2.83	25.08 ±2.57	0.335	24.75 ±2.62	25.48 ±2.74	0.175
Annulus area [mm^2]	465.98 ±110.26	483.44 ±98.52	0.361	473.39 ±101.42	492.56 ±105.13	0.357
LVOT diameter [mm]	24.95 (22.95, 28.30)	26.30 (23.80, 29.20)	0.131	25.65 (23.55, 28.73)	25.85 (23.73, 29.2)	0.699
Ascending aorta diameter [mm]	37.77 ±4.57	37.89 ±4.58	0.886	37.51 ±4.35	38.95±5.1	0.119
Procedural details						
Circulatory support, n (%)	0 (0,)	9 (9.8,)	0.057	1 (1,)	8 (25,)	<0.001
Valve size >26 mm, n (%)	13 (31.0,)	37 (40.2,)	0.304	39 (38.2,)	11 (34.4,)	0.694
2nd valve needed, n (%)	9 (21.4,)	25 (27.2,)	0.478	25 (24.5,)	9 (28.1,)	0.682
CEES, n (%)	3 (7.1,)	31 (33.7,)	0.001	18 (17.6,)	16 (50,)	<0.001

Values are presented as counts (percentages) or median (IQR). CEES – composite endpoints of early safety; BSA – body surface area; STS – Society of Thoracic Surgery Risk Score; NYHA – New York Heart Association; CHD – coronary heart disease; CVD – cerebrovascular disease; COPD – chronic obstructive pulmonary disease; CKD – chronic kidney disease; NLR – neutrophil-to-lymphocyte ratio; PLR – platelet-to-lymphocyte ratio; MHR – monocyte-to-high density lipoprotein cholesterol ratio; RDW – red blood cell distribution width; MPV – mean platelet volume; HDL-c – high-density lipoprotein cholesterol; LDL-c – low-density lipoprotein cholesterol; NHDL-c – non-high-density lipoprotein cholesterol; Lp(a) – lipoprotein(a); PNI – prognostic nutritional index; BNP – B-type natriuretic peptide; LVEDD – left ventricular end-diastolic dimension; LVESD – left ventricular end- systolic dimension; LVEF – left ventricular ejection fraction; MDCT – multidetector computed tomography; LVOT – left ventricular outflow tract.

Table 3. Univariate and multivariate logistic regression analysis of NLR and PNI to predict CEES

Variable		Unadjusted		Adjusted	
		OR (95% CI)	p-value	OR (95% CI)	p-value
NLR	low (<2.27)	1 (reference)	–	1 (reference)	–
	high (≥2.27)	6.61 (1.89–23.09)	0.003	5.55 (1.52–20.29)	0.010
PNI	high (>42.9)	1 (reference)	–	1 (reference)	–
	low (≤42.9)	4.67 (1.98–11.23)	<0.001	4.43 (1.55–12.65)	0.005
Combined NLR + PNI	NLR-low + PNI-high	1 (reference)	–	1 (reference)	–
	NLR-high or PNI-low	4.04 (1.09–15.00)	0.037	4.07 (1.06–15.68)	0.041
	NLR-high + PNI-low	13.51 (3.43–53.19)	<0.001	9.85 (2.24–43.37)	0.003

NLR – neutrophil-to-lymphocyte ratio; PNI – prognostic nutritional index; CEES – composite endpoints of early safety; OR – odds ratio; 95% CI – 95% confidence interval.

high-risk (OR = 9.85 (95% CI: 2.24–43.37)) groups. These results also indicated that the combination of a high NLR and low PNI significantly heightened the risk of CEES occurrence compared to the presence of either variable alone.

Ability to predict CEES

We further evaluated the prognostic abilities of NLR and PNI in predicting CEES using a ROC curve. The area under the ROC curve (AUC) for the NLR in predicting the occurrence of CEES was 0.691 (95% CI: 0.593–0.790), and the optimal cutoff value was 2.27, with 91% sensitivity and 39% specificity (Fig. 1A). The optimal cutoff value of PNI to predict freedom from CEES was 42.9, with a sensitivity of 84%, a specificity of 47% and an AUC of 0.660 (95% CI: 0.593–0.790, Fig. 1B). Furthermore, Kaplan–Meier survival curves were plotted based on the optimal cutoff values for NLR and PNI, indicating significant differences in the occurrence of outcome events within 30 days between the 2 groups divided by the optimal cutoff values of NLR and PNI (Fig. 2).

Discussion

The impact of NLR and PNI on CEES following TAVR was retrospectively analyzed in this study. The primary study findings were as follows: 1) A high NLR or low PNI at baseline is associated with an increased incidence of CEES at 30 days following TAVR; 2) The presence of a high NLR or low PNI at baseline could serve as an independent risk factor for CEES; 3) Combining high NLR and low PNI may offer additional prognosis information for predicting the risk of CEES.

In previous studies, inflammatory biomarkers such as C-reactive protein (CRP) and interleukin 6 (IL-6) have

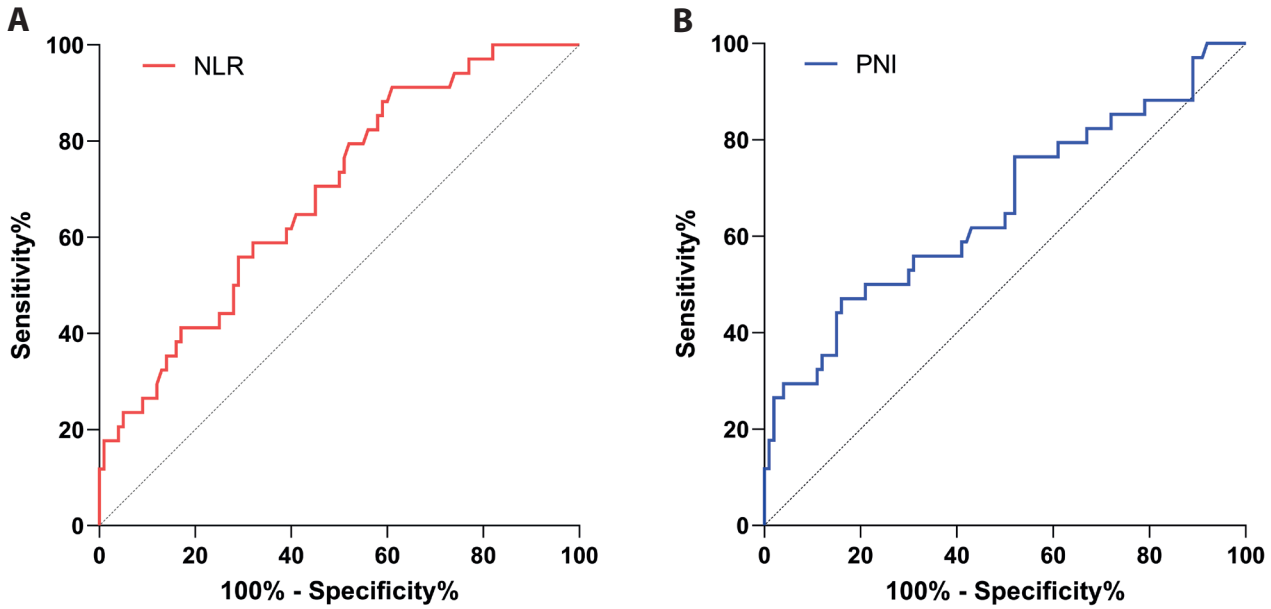

Fig. 1. ROC analysis of NLR and PNI in predicting CEES. A. ROC curve of NLR in predicting the occurrence of CEES; B. ROC curve of PNI in predicting freedom from CEES

ROC – receiver operating characteristic; NLR – neutrophil-to-lymphocyte ratio; PNI – prognostic nutritional index; CEES – composite endpoint of early safety.

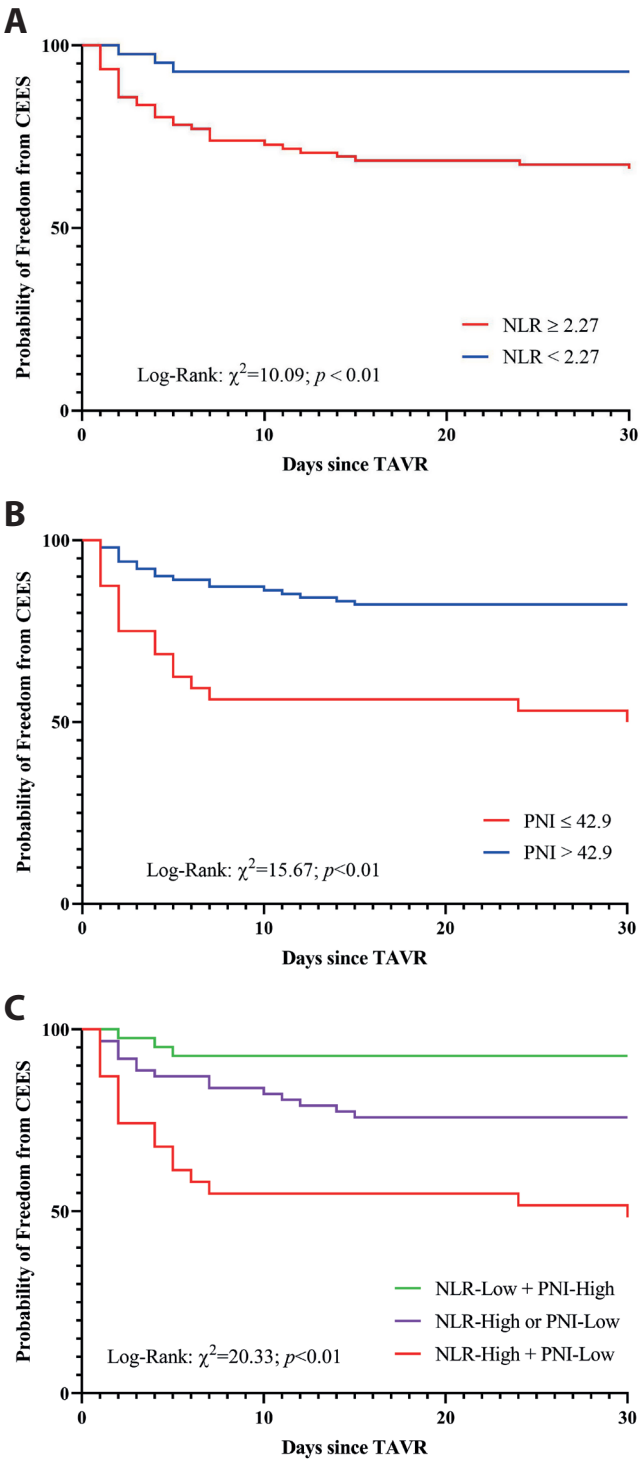

Fig. 2. Kaplan–Meier curves for CEES. A. Kaplan–Meier curves for CEES according to the cutoff value of NLR; B. Kaplan–Meier curves for CEES according to the cutoff value of PNI; C. Kaplan–Meier curves for CEES according to the combination of NLR and PNI

NLR – neutrophil-to-lymphocyte ratio; PNI – prognostic nutritional index; CEES – composite endpoint of early safety.

been utilized to predict the prognosis of patients who underwent TAVR and have shown promising results.[22–24] Although we recognize the importance of inflammation biomarkers in determining prognosis, these biomarkers are not routinely included in preoperative laboratory examinations for TAVR. Therefore, exploring inflammatory indicators in routine blood tests for risk stratification is of paramount importance. It should be noted thatNLR, PLR, MHR, and RDW are a class of inflammation biomarkers that can be easily obtained from routine blood tests and have demonstrated their predictive value for the long-term prognosis and complications following TAVR.[13,14,25] Previous studies have also confirmed significant correlations between inflammatory indicators, such as NLR and PLR, and early safety composite outcomes after TAVR.[26] However, thus far, the existing research on the risk factors associated with composite endpoints of early safety following TAVR has relied on the definition of composite endpoints outlined in VARC-2.[27] With the introduction of the latest VARC-3 definition for CEES, which includes new permanent pacemaker implantation due to procedure-related conduction abnormalities and moderate or severe aortic regurgitation, there is a lack of studies that investigate the risk factors for these updated composite endpoints. In this study, in addition to inflammatory markers, we also included the PNI, an indicator reflecting nutritional status. Similar to NLR and PLR, PNI can also be indirectly inferred from routine blood tests and offers similar advantages.

In our study, we observed significant differences in NLR and PNI between the CEES group and the non-CEES group. Therefore, we further analyzed these 2 indicators by dividing them into high- and low-level groups and comparing their baseline characteristics. We found that a high NLR was associated with an increased rate of CEES, which is consistent with prior studies that reported the correlation between NLR and CEES defined by VARC-2. Similarly, we obtained the same result for low PNI. In the multivariate analysis, we confirmed that a high NLR and low PNI were independent risk factors for an increased rate of CEES following TAVR. After adjusting for multiple potential confounders, patients with a NLR ≥2.27 had a 5.55 times higher risk of CEES following TAVR compared to patients with low NLR, while patients with a PNI ≤42.9 had a 4.43 times higher risk compared to patients with high PNIs. These results prove that NLR and PNI, which are routine blood parameters representing inflammation and nutritional levels, respectively, can provide valuable prognostic information for predicting the risk of CEES following TAVR. To further investigate whether their combination increases the risk of CEES, we stratified patients into low-, intermediate- and high-risk groups based on the NLR and PNI. The high-risk group (i.e., high NLR combined with low PNI) had a higher risk of CEES compared to patients with a high NLR or low PNI alone. This indicates a promising potential of combining NLR and PNI for the risk stratification of CEES following TAVR.

Neutrophil-to-lymphocyte ratio, as an inflammation marker, has previously been shown to be linked to various cardiovascular diseases.[28] A high NLR may reflect a systemic inflammatory state, which may be attributed

to a significant elevation of neutrophils as a marker of ongoing inflammation and relatively diminished lymphocytes, which play an essential role in immune regulation and inhibition of inflammation.[29]

Recent studies have revealed a correlation between NLR and negative outcomes in patients undergoing TAVR. Our study provides additional evidence supporting a notable link between high NLR and early adverse clinical outcomes after TAVR. A previous study by Condado et al. demonstrated the correlation between high NLR and PLR with the occurrence of CEES 30 days after TAVR.[26] However, as TAVR experience advanced and large-scale clinical studies were conducted worldwide, the VARC-3 had updated the definitions of CEES, including additional specifications and improvements in the definition of postoperative bleeding and vascular complications. Notably, the VARC-3 also included new permanent pacemaker implantation due to procedure-related conduction abnormalities in the definition of CEES, necessitating further investigation into the correlation between NLR and this updated definition. In our study, we found a significant correlation between NLR and the latest definition, providing further support for the importance of NLR in predicting adverse prognosis after TAVR. However, other biomarkers such as PLR, MHR and RDW did not show a similar predictive ability compared to NLR in our study.

Another indicator studied in our research is PNI, which serves as an important marker reflecting nutritional status. Given the TAVR patient population is predominantly comprised of the elderly, where malnutrition is common in this population, it is crucial to evaluate the potential risks of malnourished patients undergoing TAVR. Recent studies reported that comorbidities such as weakness and malnutrition increase the risk of an adverse prognosis after TAVR.[15,30,31] Therefore, assessing the nutritional status of patients in a reasonable and appropriate way before the procedure is beneficial for early risk stratification of TAVR patients. However, there is currently no research demonstrating the correlation between nutritional status and the occurrence of early safety composite outcomes after TAVR.

Prognostic nutritional index combines albumin levels, reflecting nutritional status, and lymphocyte count, reflecting immune function, both having an adverse impact on mortality.[26,32] Previous research has demonstrated a notable association between low PNI and increased mortality following TAVR.[20,33] In our study, we confirmed that a low PNI increases the risk of CEES after TAVR, although the specific mechanism has yet to be determined. Magri et al. found that the logistic EuroSCORE was an important predictor of CEES, which is a useful tool in assessing comorbidities and weakness.[34] This may explain the association between PNI, an indicator reflecting weakness, and the occurrence of CEES. Additionally, a low PNI may itself be a potential intermediate factor for CEES. However, further research is needed to identify potential confounders and the detailed relationship between PNI and CEES.

Recently, Conners et al.[35] used the metabolic vulnerability index (MVX), a novel biomarker that reflects both systemic inflammation and metabolic malnutrition, to explore its prognostic value in patients with heart failure. The results showed that in the heart failure population, a higher MVX was associated with mortality independently of existing chronic heart failure scores and other biomarkers, and MVX had additional value in the stratification of death risk in patients with heart failure. These results suggest such indicators that reflect both inflammation and malnutrition may have special value in evaluating the prognosis of patients with cardiovascular disease. However, the complexity of obtaining MVX may impede its widespread adoption in clinical applications. Thus, the combination of NLR and PNI has certain advantages because of the easily accessible and inexpensive characteristics. This study has preliminarily confirmed the prognostic value of the combination of NLR and PNI in the occurrence of adverse events following TAVR. Future research can further explore its value in other cardiovascular diseases.

Limitations

There are several limitations in our study. Firstly, this is a single-center, retrospective study, which restricts the generalizability of the findings to the entire TAVR patient population. Although we have proposed preliminary cutoff values for NLR and PNI in the TAVR population, these values need to be validated in larger cohorts due to the small sample size of this study. Secondly, certain potential confounding factors, such as some special inflammatory cytokines or other markers of nutritional status, were not included in this study. Moreover, the study population overall consisted primarily of low/moderate-risk individuals based on STS scores. However, 7% of patients required mechanical circulatory support, and 25% underwent 2nd valve implantation. It may be due to the prophylactic use of mechanical circulatory support in some high-risk patients to ensure procedural safety. Regarding the 2nd valve, besides the early lack of experience in our center, it may also be attributed to the enrollment of patients with pure aortic regurgitation in our study cohort. These patients typically have a higher risk of 2nd valve requirement due to anatomical factors. This also implies that caution should be exercised when extrapolating the results of this study to other populations. Lastly, due to the limited sample size of this study, subgroup analyses of various endpoints within the CEES were not performed, and larger patient populations will be required in the future to refine this study.

Conclusions

This study investigated the impact of inflammation status and nutritional level on CEES at 30 days following TAVR. The results demonstrated that baseline high NLR and low PNI are important independent predictors of CEES. These routine and inexpensive indicators may provide valuable references in the early risk stratification of TAVR patients.

Ethical approval

This study was approved by the Institutional Review Board of the Second Affiliated Hospital of Nanchang University (approval 2022.No.07).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Leon MB, Piazza N, Nikolsky E, et al. Standardized endpoint definitions for transcatheter aortic valve implantation clinical trials. *J Am Coll Cardiol*. 2011;57(3):253–269. doi:10.1016/j.jacc.2010.12.005
2. Popma JJ, Deeb GM, Yakubov SJ, et al. Transcatheter aortic-valve replacement with a self-expanding valve in low-risk patients. *N Engl J Med*. 2019;380(18):1706–1715. doi:10.1056/NEJMoa1816885
3. Mack MJ, Leon MB, Thourani VH, et al. Transcatheter aortic-valve replacement with a balloon-expandable valve in low-risk patients. *N Engl J Med*. 2019;380(18):1695–1705. doi:10.1056/NEJMoa1814052
4. Généreux P, Piazza N, Alu MC, et al. Valve Academic Research Consortium 3: Updated endpoint definitions for aortic valve clinical research. *Eur Heart J*. 2021;42(19):1825–1857. doi:10.1093/eurheartj/ehaa799
5. Pilgrim T, Stortecky S, Luterbacher F, Windecker S, Wenaweser P. Transcatheter aortic valve implantation and bleeding: Incidence, predictors and prognosis. *J Thromb Thrombolysis*. 2013;35(4):456–462. doi:10.1007/s11239-012-0842-3
6. Ribeiro HB, Rodés-Cabau J, Blanke P, et al. Incidence, predictors, and clinical outcomes of coronary obstruction following transcatheter aortic valve replacement for degenerative bioprosthetic surgical valves: Insights from the VIVID registry. *Eur Heart J*. 2018;39(8):687–695. doi:10.1093/eurheartj/ehx455
7. Gargiulo G, Sannino A, Capodanno D, et al. Impact of postoperative acute kidney injury on clinical outcomes after transcatheter aortic valve implantation: A meta-analysis of 5,971 patients. *Catheter Cardiovasc Interv*. 2015;86(3):518–527. doi:10.1002/ccd.25867
8. Houthuizen P, Van Der Boon RMA, Urena M, et al. Occurrence, fate and consequences of ventricular conduction abnormalities after transcatheter aortic valve implantation. *EuroIntervention*. 2014;9(10):1142–1150. doi:10.4244/EIJV9I10A194
9. Tamburino C, Capodanno D, Ramondo A, et al. Incidence and predictors of early and late mortality after transcatheter aortic valve implantation in 663 patients with severe aortic stenosis. *Circulation*. 2011;123(3):299–308. doi:10.1161/CIRCULATIONAHA.110.946533
10. Coté N, Mahmut A, Bosse Y, et al. Inflammation is associated with the remodeling of calcific aortic valve disease. *Inflammation*. 2013;36(3):573–581. doi:10.1007/s10753-012-9579-6
11. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med*. 2005;352(16):1685–1695. doi:10.1056/NEJMra043430
12. Uhle F, Castrup C, Necaev A, et al. Inflammation and its consequences after surgical versus transcatheter aortic valve replacement. *Artif Organs*. 2018;42(2):E1–E12. doi:10.1111/aor.13051
13. Olasińska-Wiśniewska A, Perek B, Grygier M, et al. Increased neutrophil-to-lymphocyte ratio is associated with higher incidence of acute kidney injury and worse survival after transcatheter aortic valve implantation. *Cardiol J*. 2021;30(6):938–945. doi:10.5603/CJ.a2021.0149
14. Merdler I, Frydman S, Sirota S, et al. Neutrophil-to-lymphocyte ratio as a prognostic marker in transcatheter aortic valve implantation (TAVI) patients. *Isr Med Assoc J*. 2022;24(4):229–234. PMID:35415981.
15. Eichler S, Salzwedel A, Harnath A, et al. Nutrition and mobility predict all-cause mortality in patients 12 months after transcatheter aortic valve implantation. *Clin Res Cardiol*. 2018;107(4):304–311. doi:10.1007/s00392-017-1183-1
16. Zencirkiran Agus H, Kahraman S. Prognostic nutritional index predicts one-year outcome in heart failure with preserved ejection fraction. *Acta Cardiol*. 2020;75(5):450–455. doi:10.1080/00015385.2019.1661139
17. Cheng Y, Sung S, Cheng H, et al. Prognostic nutritional index and the risk of mortality in patients with acute heart failure. *J Am Heart Assoc*. 2017;6(6):e004876. doi:10.1161/JAHA.116.004876
18. Basta G, Chatzianagnostou K, Paradossi U, et al. The prognostic impact of objective nutritional indices in elderly patients with ST-elevation myocardial infarction undergoing primary coronary intervention. *Int J Cardiol*. 2016;221:987–992. doi:10.1016/j.ijcard.2016.07.039
19. Chen QJ, Qu HJ, Li DZ, et al. Prognostic nutritional index predicts clinical outcome in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Sci Rep*. 2017;7(1):3285. doi:10.1038/s41598-017-03364-x
20. Mas-Peiro S, Hoffmann J, Seppelt PC, et al. Value of prognostic nutritional index for survival prediction in trans-catheter aortic valve replacement compared to other common nutritional indexes. *Acta Cardiol*. 2021;76(6):615–622. doi:10.1080/00015385.2020.1757854
21. Onodera T, Goseki N, Kosaki G. Prognostic nutritional index in gastrointestinal surgery of malnourished cancer patients [in Japanese]. *Nihon Geka Gakkai Zasshi*. 1984;85(9):1001–1005. PMID:6438478.
22. Sousa ALS, Carvalho LAF, Salgado CG, et al. C-reactive protein as a prognostic marker of 1-year mortality after transcatheter aortic valve implantation in aortic stenosis. *Arq Bras Cir Dig*. 2021;117(5):1018–1027. doi:10.36660/abc.20190715
23. Hioki H, Watanabe Y, Kozuma K, et al. Effect of serum C-reactive protein level on admission to predict mortality after transcatheter aortic valve implantation. *Am J Cardiol*. 2018;122(2):294–301. doi:10.1016/j.amjcard.2018.04.005
24. Sexton TR, Wallace EL, Chen A, et al. Thromboinflammatory response and predictors of outcomes in patients undergoing transcatheter aortic valve replacement. *J Thromb Thrombolysis*. 2016;41(3):384–393. doi:10.1007/s11239-015-1326-z
25. Karahan S, Okuyan E. Monocyte-to-high density lipoprotein cholesterol ratio as a predictor of mortality in patients with transcatheter aortic valve replacement. *Eur Rev Med Pharmacol Sci*. 2021;25(16):5153–5162. doi:10.26355/eurrev_202108_26529
26. Condado JF, Junpaparp P, Binongo JN, et al. Neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) can risk stratify patients in transcatheter aortic-valve replacement (TAVR). *Int J Cardiol*. 2016;223:444–449. doi:10.1016/j.ijcard.2016.08.260
27. Kappetein AP, Head SJ, Généreux P, et al. Updated standardized endpoint definitions for transcatheter aortic valve implantation: The Valve Academic Research Consortium-2 consensus document. *Eur Heart J*. 2012;33(19):2403–2418. doi:10.1093/eurheartj/ehs255
28. Afari ME, Bhat T. Neutrophil to lymphocyte ratio (NLR) and cardiovascular diseases: An update. *Exp Rev Cardiovasc Ther*. 2016;14(5):573–577. doi:10.1586/14779072.2016.1154788

29. Arbel Y, Shacham Y, Ziv-Baran T, et al. Higher neutrophil/lymphocyte ratio is related to lower ejection fraction and higher long-term all-cause mortality in ST-elevation myocardial infarction patients. *Can J Cardiol*. 2014;30(10):1177–1182. doi:10.1016/j.cjca.2014.05.010
30. Emami S, Rudasill S, Bellamkonda N, et al. Impact of malnutrition on outcomes following transcatheter aortic valve implantation (from a national cohort). *Am J Cardiol*. 2020;125(7):1096–1101. doi:10.1016/j.amjcard.2019.12.038
31. Goldfarb M, Lauck S, Webb JG, et al. Malnutrition and mortality in frail and non-frail older adults undergoing aortic valve replacement. *Circulation*. 2018;138(20):2202–2211. doi:10.1161/CIRCULATIONAHA.118.033887
32. Hebeler KR, Baumgarten H, Squiers JJ, et al. Albumin is predictive of 1-year mortality after transcatheter aortic valve replacement. *Ann Thorac Surg*. 2018;106(5):1302–1307. doi:10.1016/j.athoracsur.2018.06.024
33. Ishizu K, Shirai S, Tashiro H, et al. Prevalence and prognostic significance of malnutrition in older Japanese adults at high surgical risk undergoing transcatheter aortic valve implantation. *J Am Heart Assoc*. 2022;11(19):e026294. doi:10.1161/JAHA.122.026294
34. Magri CJ, Chieffo A, Durante A, et al. Impact of mean platelet volume on combined safety endpoint and vascular and bleeding complications following percutaneous transfemoral transcatheter aortic valve implantation. *Biomed Res Int*. 2013;2013:645265. doi:10.1155/2013/645265
35. Conners KM, Shearer JJ, Joo J, et al. The Metabolic Vulnerability Index. *JACC Heart Fail*. 2024;12(2):290–300. doi:10.1016/j.jchf.2023.06.013

The influence of the structure of the masticatory system on the presence and severity of the gag reflex in children with cerebral palsy

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Abstract

Background. Dysphagia, prevalent in 90% of children with neurological disorders, poses risks of medical complications and is associated with cognitive and psychosocial challenges. The absence of the sucking-swallowing reflex and variations in the gag reflex contribute to feeding difficulties.

Objectives. This study focuses on examining the impact of the gag reflex on the masticatory system structure in children with cerebral palsy, aiming to assess its significance.

Materials and methods. This observational study investigated the gag reflex and soft palate shape in 25 children with cerebral palsy (average age: 14 years). Inclusion criteria considered specific levels of the Eating and Drinking Ability Classification System (EDACS) and the Gross Motor Function Classification System (GMFCS). Exclusion criteria comprised hypotension, inflammation and tumors. The Castillo–Morales questionnaire assessed variables and statistical analysis (Spearman's rank correlation and non-parametric tests) utilizing PQStat v. 1.8.6.120 software.

Results. Findings did not reveal an association between the absence of the gag reflex and abnormal palate structure in children. Our results showed a correlation between higher tension of the buccinator muscles and mobility of the tongue on the structure of the palate.

Conclusions. Children with neurological disorders, such as cerebral palsy, are a diverse group requiring specialized orthodontic treatment and close interdisciplinary collaboration.

Key words: children, neurological disorders, gag reflex, masticatory system

Background

Dysphagia occurs in about 90% of children with neurological disorders.[1] Although feeding problems may lead to medical disorders such as malnutrition or gastroenterological dysfunction, some other aspects should also be taken into account. There are many research studies showing a connection between feeding difficulties and problems with cognitive skills, such as communication difficulties and psychosocial problems: lower self-esteem, social isolation and poor quality of life.[2–4] That is why specialists who take care of children with feeding problems should be aware of how the treatment of children with dysphagia is important for their patients and their surroundings. For instance, the Oxford Feeding Study showed strong correlations between the severity of motor delay and dysphagia.[5] It could be explained by the fact that disorders of the central nervous system may affect the frontal/insular–basal ganglia–brainstem swallowing pathway.[6] Therefore, children with dysphagia may have difficulty feeding due to the absence of the suck-swallow reflex or the absence of a gag reflex or hypersensitive reflex.[7] The diagnostic methods and therapy for the sucking reflex seem to be well known.[8] We think that the gag reflex should be explored and taken into account during the process of diagnosing and treating feeding difficulties in children.

The gag reflex, also known as the pharyngeal reflex, should be fully developed at about the 32nd week of gestation. The role of this reflex is to prevent swallowing foreign objects and the aspiration of a bolus into the respiratory tract.[9] The gag reflex is controlled by both the glossopharyngeal (IX) and vagus (X) nerves, in which the nerve roots exit the medulla through the jugular foramen and descend on both sides of the pharynx to innervate the posterior pharynx, posterior 1/3 of the tongue, soft palate, and the stylopharyngeus muscle.[9] In the first few months of life, the sucking reflex and gag reflex seem to be very strong. Starting around 6 months, the gag reflex diminishes but does not disappear completely, allowing infants to swallow more solid foods. Similarly, the sucking reflex is replaced by the function of the masticatory system.[10] Children with neurological disorders may have problems in eating because of problems with neurodevelopment in general, which can be a result of brain injuries during the perinatal period of life. This causes difficulties in neurodevelopmental skills and reflexes such as the suck-swallow and gag reflexes and with functions such as mastication and chewing. Sensorimotor dysfunction, as well as difficulties with maintaining the position of the trunk and head, may accompany dysphagia.[5–11] What is more, children with neurological diseases may suffer from gastrointestinal disorders leading to malnutrition and inadequate growth.[7]

Swallowing and speech use similar oral muscles and structures; therefore, it is possible that early patterns of eating impairment may impact speech and sound development.[12] That is why indirect problems with eating may lead to problems with cognitive skills.

Therefore, we think that reflexes that are involved in eating should be monitored as early as possible to improve not only safe feeding but also psychosocial skills.[13,14] Feeding problems may also lead to structural disorders such as temporomandibular joint dysfunction (TMD).[15,16] Abnormal oral reflexes and functions are correlated with hyperactivity of suprahyoid muscles.[17–19] Nowadays, the association between the suck-swallow reflex and the posture during eating seems to be satisfactory explored.[20,21]

Objectives

The purpose of this article was to analyze how the gag reflex influences the structure of the masticatory system in children with cerebral palsy. We hope that answering this question will allow the development of an easy and valid screening assessment and therapy regimen for children with feeding disorders.

Materials and methods

Study design

This observational study was approved by the Bioethics Committee (approval No. 481/21) on June 21, 2021. Informed consent was obtained from all subjects involved in the study.

Setting

The study was conducted in a controlled clinical setting.

Participants

We assessed 25 children with neurological diseases whose main diagnosis was cerebral palsy (16 girls and 9 boys with a median age of 11 years (range: 8–17 years)). The main inclusion criterion was the 5th (V) level in the Eating and Drinking Ability Classification System (EDACS). The EDACS enables the assessment of the functional capacity to eat and drink. There are 5 levels of the EDACS:

– Level I: eats and drinks safely and efficiently;

– Level II: eats and drinks safely but with some limitations to efficiency;

– Level III: eats and drinks with some limitations to safety;

– Level IV: eats and drinks with significant limitations to safety;

– Level V: unable to eat or drink safely. Tube feeding may be considered to provide nutrition.[22]

Others inclusion criteria were the presence of the suck reflex, the diagnosis of neurological disorders (cerebral palsy) and a 5th level of Gross Motor Function Classification System (GMFCS), which meant complete dependence on mobility, function and locomotion of the child. GMFCS I means that

a child can walk, run and jump on their own; GMFCS II – that a child sometimes needs physical assistance; GMFCS III – that for long distances, children needed wheeled mobility and they also walk short distances with a handheld mobility device; GMFCS IV – that physical assistance or powered mobility is required in almost all settings; and GMFCS V qualifiers require a wheelchair for mobility and have limited ability to control head and trunk positioning. Exclusion criteria in the present research study included hypotension, active inflammation and tumors.

Variables

The variables of interest in this study were the gag reflex and the shape of the soft palate. The gag reflex was checked by stimulating the posterior pharynx using a tongue blade when a child was in a semi-lying position. If a child produced a gagging reaction, we assessed it as 1 point, while when it was absent in the child, we gave 0 points. An orthodontist examined the shape of the soft palate and classified it as either narrow, typical or wide.

Data sources/measurement

We used the Castillo–Morales questionnaire to assess the gag reflex and soft palate. In compliance with ethical guidelines, all participants were thoroughly briefed about the study's purpose, procedures and potential risks. They had the opportunity to ask questions and could withdraw at any time. Prior to participating, each the parents or legal guardians of each participant provided their written informed consent, affirming their understanding and voluntary agreement to participate. All consent forms have been retained for verification purposes.

Study size

The study size was determined by the availability of participants who met the inclusion criteria.

Statistical analyses

Calculations were performed using PQStat v. 1.8.6.120 software (PQStat, Poznań, Poland). A significance level of $\alpha = 0.05$ was adopted. The result was considered statistically significant when $p < \alpha$. The normality of the age distribution was examined using the Shapiro–Wilk test. The relationship between variables was examined using Fisher's exact test or the Fisher–Freeman–Halton exact test with Bonferroni correction for multiple comparisons.

Results

We observed that children who did not have gag reflexes also did not have an abnormal structure to the palate. On the other hand, children who had a gag reflex had normal palates as well. There was no correlation between the gag reflex and the structure of the palate.

We also checked whether there was any correlation between the paralysis of the soft palate and the presence of a gag reflex. No correlations were noted.

Buccinator muscles are the muscular portion of the cheek responsible for helping compress the cheek against the teeth during actions such as chewing and blowing – cheek muscles are involved with the shape of the palate ($p = 0.015$, Table 1). If the tone of the buccinator muscles was higher, then a narrow palate was more often observed ($p = 0.015$) or even a gothic palate was seen ($p = 0.017$, Table 2,3). The palatal folds were observed more

Table 1. Fisher's exact test for association between the tension of the cheek muscles and the structure of the palate

Tension of the cheek muscles	Summary 2-way table: Observed frequencies		
	Palate structure: Normal? 1. Yes	Palate structure: Normal? 2. No	Total row
1. Increased	0	7	7
2. Normal	8	5	13
Total	8	12	20

Fisher's exact test $p = 0.015$ – there is an association. A statistically significant association with $p < 0.05$ was noted between the tension of the cheek muscles and the structure of the palate. In cases of increased tension in the cheek muscles, abnormal palate structure was more common.

Table 2. Fisher's exact test for association between the tension of the cheek muscles and the presence of a narrow palate

Tension of the cheek muscles	Summary 2-way table: Observed frequencies		
	Palate structure: Narrow? 1. Yes	Palate structure: Narrow? 2. No	Total row
1. Increased	7	0	7
2. Normal	5	8	13
Total	12	8	20

Fisher's exact test $p = 0.015$ – there is an association. A statistically significant association with $p < 0.05$ was noted between the tension of the cheek muscles and the presence of a narrow palate. Increased tension in the cheek muscles was associated with a narrow palate.

Table 3. Fisher's exact test for association between the tension of the cheek muscles and the presence of a gothic palate

Tension of the cheek muscles	Summary 2-way table: Observed frequencies		
	Palate structure: Gothic? 1. Yes	Palate structure: Gothic? 2. No	Total row
1. Increased	6	1	7
2. Normal	3	10	13
Total	9	11	20

A statistically significant association with $p < 0.05$ was noted between the tension of the cheek muscles and the presence of a gothic palate. Increased muscle tension was associated with the presence of a gothic palate.

Table 4. Fisher's exact test for association between tongue muscle mobility and the presence of very distinct palate folds

Tongue muscles: Tongue mobility	Summary 2-way table: Observed frequencies		
	Are palate folds very distinct? 1. Yes	Are palate folds very distinct? 2. No	Total row
1. Abnormal	0	5	5
2. Normal	11	4	15
Total	11	9	20

Fisher's exact test p = 0.008, there is an association. A statistically significant association with p < 0.05 was noted between tongue muscle mobility and the presence of very distinct palate folds. The presence of pronounced palate folds was more common in individuals with impaired tongue muscle function.

often in children with abnormal tongue muscle mobility (p = 0.008, Table 4). We also examined the rotator and flexor muscles of the head and neck, and noted that sternocleidomastoid muscles (SCM) were involved with the shape of the palate (p = 0.031, Table 5,6). If the tone of the SCM was higher, then a narrow palate was more often observed (p = 0.031, Table 7,8). The palatal folds were observed more often in children with hypertonic SCM (p = 0.046, Table 9,10). However, statistical tests involving the SCM were unable to determine any differing groups.

Discussion

Brainstem control is pivotal in orchestrating the complex mechanisms of the gag reflex. Centered in the medulla oblongata, the nucleus tractus solitarius (NTS) assumes a central role, receiving sensory input from the oropharynx via the glossopharyngeal (cranial nerve IX) and vagus nerves (cranial nerve X). Afferent fibers convey touch, pressure and taste sensations, crucial for triggering the gag reflex. The trigeminal (cranial nerve V) and facial nerves (cranial nerve VII) may also contribute to sensory modulation. The efferent fibers from the glossopharyngeal and vagus nerves relay signals to the muscles involved in the reflex, while the hypoglossal nerve (cranial nerve XII) aids in coordinating motor responses. The NTS acts as an integrating center for sensory information, not only from the oropharynx but also from visceral organs, influencing

Table 5. Fisher–Freeman–Halton exact test for association between rotator and flexor muscles of the head and neck and palate structure

Rotator and flexor muscles of the head and neck	Summary 2-way table: observed frequencies		
	Palate structure – normal? 1. Yes	Palate structure – normal? 2. No	Total row
1. Asymmetrically increased	6	7	13
2. Symmetrically increased	2	0	2
3. Normal	0	5	5
Total	8	12	20

Fisher–Freeman–Halton exact test p = 0.031, there is an association.

Table 6. Bonferroni multiple comparison for association between rotator and flexor muscles of the head and neck and palate structure

Rotator and flexor muscles of the head and neck	Bonferroni multiple comparison: p-value, χ2/*Fisher (Cochran's nonspecific)		
	3. Normal	2. Symmetrically increased	1. Asymmetrically increased
1. Asymmetrically increased	0.342437*	1*	–
2. Symmetrically increased	0.142857*	–	1*
3. Normal	–	0.142857*	0.342437*

Since Table 5 presents the Fisher–Freeman–Halton exact test indicating an association between the rotator and flexor muscles of the head and neck and the palate structure (p = 0.031), it was necessary to identify which groups differed. However, after applying the Bonferroni multiple comparison method, it was not possible to determine specific group differences.

Table 7. Fisher–Freeman–Halton exact test for association between rotator and flexor muscles of the head and neck and palate shape

Rotator and flexor muscles of the head and neck	Summary 2-way table: Observed frequencies		
	Palate structure: Normal? 1. Yes	Palate structure: Normal? 2. No	Total row
1. Asymmetrically increased	7	6	13
2. Symmetrically increased	0	2	2
3. Normal	5	0	5
Total	12	8	20

Fisher–Freeman–Halton exact test p = 0.031, there is an association.

Table 8. Bonferroni multiple comparison for association between rotator and flexor muscles of the head and neck and palate shape

Rotator and flexor muscles of the head and neck	Bonferroni multiple comparison: p-value, χ2/*Fisher (Cochran's nonspecific)		
	3. Normal	2. Symmetrically increased	1. Asymmetrically increased
1. Asymmetrically increased	0.342437*	1*	–
2. Symmetrically increased	0.142857*	–	1*
3. Normal	–	0.142857*	0.342437*

Since Table 7 presents the Fisher–Freeman–Halton exact test indicating an association between rotator and flexor muscles of the head and neck and palate shape (p = 0.031), it was necessary to identify which groups differed. However, after applying the Bonferroni multiple comparison method, it was not possible to determine specific group differences.

Table 9. Fisher–Freeman–Halton exact test for association between rotator and flexor muscles of the head and neck and palate folds

Rotator and flexor muscles of the head and neck	Summary 2-way table: Observed frequencies		
	Are palate folds very distinct? 1. Yes	Are palate folds very distinct? 2. No	Total row
1. Asymmetrically increased	5	8	13
2. Symmetrically increased	1	1	2
3. Normal	5	0	5
Total	11	9	20

Fisher–Freeman–Halton exact test p = 0.046, there is an association.

Table 10. Bonferroni multiple comparison for association between rotator and flexor muscles of the head and neck and palate folds

Rotator and flexor muscles of the head and neck	Bonferroni multiple comparison: p-value, χ2/*Fisher (Cochran's nonspecific)		
	3. Normal	2. Symmetrically increased	1. Asymmetrically increased
1. Asymmetrically increased	0.107843*	1*	–
2. Symmetrically increased	0.857143*	–	1*
3. Normal	–	0.857143*	0.107843*

Since Table 9 presents the Fisher–Freeman–Halton exact test indicating an association between rotator and flexor muscles of the head and neck and palate folds (p = 0.046), it was necessary to identify which groups differed. However, after applying the Bonferroni multiple comparison method, it was not possible to determine specific group differences.

reflex modulation. Beyond the brainstem, higher brain regions, particularly the cerebral cortex, exert influence on emotional and cognitive factors impacting the reflex. Neurotransmitters like serotonin, GABA and glutamate play a role in modulating the gag reflex by influencing neuronal excitability. Neural plasticity in the central nervous system contributes to the adaptation or impairment of the reflex over time, with changes in synaptic strength influenced by experiences, trauma or neurological disorders.[23–26] Understanding these intricate neural pathways not only sheds light on variations in the gag reflex but also enhances the comprehension of feeding challenges and clinical implications like dysphagia in neurological disorders.

Brainstem lesions and associated neurological disorders can lead to dysfunction of the gag reflex. Damage to the NTS or the glossopharyngeal and vagus nerves, commonly seen in brainstem lesions, may result in either an impaired or hyperactive gag reflex. Beyond the brainstem, dysregulation of broader neural networks, including the cerebellum and basal ganglia, can disrupt the coordination of motor responses during the gag reflex, contributing to feeding difficulties. Imbalances in neurotransmitter systems, such as serotonin and GABA, further complicate the scenario, impacting neural activity in the brainstem and associated areas. Considering developmental aspects, children with neurological disorders face challenges as their nervous systems are still maturing, potentially influencing the establishment of normal feeding behaviors. Additionally, higher brain regions involved in cognitive and emotional processing may exacerbate or modulate the gag reflex in the context of neurological disorders.

The muscles of the head and neck are involved in structural and functional development of the masticatory system and primarily with reflexes of this system. We presented for the first time a study that showed that hypertonic SCM (cranial nerve XI) is related to the abnormal shape of the palate in children with cerebral palsy. Similar correlations were found between temporal (cranial nerve V) and buccinator muscles (cranial nerve VII).[27] The gag reflex involves bilateral pharyngeal muscle contractions and elevation of the soft palate.[9] That is why reflexes such as sucking, swallowing and gagging may be responsible for the shape of the palate. If the reflexes are retained then the lack of pressure may affect the structure of the palate.[28] During the initiation time of oral functions to full oral

feeding, various internal and external factors influence palatal development and lead to a higher risk for facial abnormalities and other orthodontic consequences. Children who have cerebral palsy may have disorders in the shape of the palate because of intubation and positioning. For instance, children who were not intubated have a lower palatal depth compared to intubated children.[29]

Recognizing the variability in the nature and extent of neurological deficits among affected children is crucial for tailoring interventions and management strategies. Targeted rehabilitative approaches, encompassing physical therapy, sensory integration strategies and interventions focused on neural plasticity, offer promise in improving the gag reflex and overall feeding abilities.[30–32] A multidisciplinary collaboration involving neurologists, speech therapists, occupational therapists, and nutritionists is indispensable for addressing the multifaceted nature of neurological deficits impacting the gag reflex. By delving into the intricate connections between neurological deficits and the gag reflex, researchers and clinicians can develop nuanced strategies for the assessment and management of feeding difficulties in children with neurological disorders, ultimately enhancing the quality of life for both the affected child and their families.

Children with neurological diseases may not present a gag reflex upon stimulation.[7,33] On the other hand, gagging during feeding in early childhood is one risk factor for developing dysphagia.[34] There is a study that showed that the specificity of the gag reflex in detecting dysphagia was 96%, with a sensitivity of 39%. The presence of the gag reflex means that a person has protection against long-term swallowing problems.[35] That is why the gag reflex is called a protective reflex. One of the functions of the gag reflex is to prevent swallowing foreign objects and prevent choking or aspiration.[9] That is why patients with severe cerebral palsy who do not have a gag reflex may suffer from aspiration pneumonia as a major complication, which is dangerous because of its association with their survival prognosis.[36] Gagging is sometimes called a vital reflex because its role is to stop unwanted bolus entry into the oropharynx, which helps in avoiding aspiration of food into the airways since may lead to death.[37] It is sometimes called a "must part" of the assessment of brainstem function.[35] The gag reflex, which is the most easily and commonly tested airway reflex and the absence of which is recognized as one of the criteria for brainstem death, should be a part of the typical assessment of children with feeding difficulties. Neurons controlling gagging or vomiting are associated with nuclei within the brainstem controlling vasomotor, respiratory, salivary, vestibular, and cardiac activity.[38] The afferents of the gag reflex go to the medulla oblongata. That is why the heart rate may be indirectly influenced by the gag reflex.[39] A normal gag reflex is controlled mainly by the parasympathetic division of the autonomic nervous system. Children with cerebral palsy may present higher activity of the sympathetic nervous system and higher vasomotor tonicity resulting from lesions in specific brain sites that can cause peripheral blood vessel vasoconstriction and constrain cutaneous blood flow. This is because contracting muscles need to be able to increase their blood flow.[40] Although the gag reflex is governed mostly by the parasympathetic nervous system, both parasympathetic and sympathetic activations are observed during the stimulation of the gag reflex.[41] Therefore, some children with cerebral palsy have absent gag reflex, while others have a pronounced one.[37] Certainly, further studies are needed to describe the relationship between the occurrence of the gag reflex and the activation of the autonomic nervous system in children with neurological disorders.

The muscles of the head and neck are involved in structural and functional development of the masticatory system and primarily with reflexes of this system. It is the first study to show that hypertonic SCM is associated with the abnormal shape of the palate in children with cerebral palsy. Similar correlations were found with the temporal and buccinator muscles. The gag reflex involves bilateral pharyngeal muscle contraction and elevation of the soft palate.[9] That is why reflexes such as sucking, swallowing and gagging may be responsible for the shape of the palate. If the reflexes are retained, then the lack of pressure may influence the structure of the palate.[28] During the time of oral function initiation to full oral feeding, various internal and external factors influences may affect palatal development and lead to a higher risk for facial abnormalities and other orthodontic consequences. Children who have cerebral palsy may have disorders in the shape of the palate because of intubation or positioning. For instance, children who were not intubated revealed a lower palatal depth compared to intubated children.[29] Our observations indicated that the gag reflex may also influence the shape of the palate, in a manner analogous to the findings of Asdaghi Mamaghani et al., who demonstrated that patients with cerebral palsy exhibited an abnormal flattening of the palatal arch.[28] Abnormalities in palate formation in children may have serious functional and cognitive consequences in terms of speech, mastication, the mode of breathing, swallowing, and Eustachian tube function. The most fragile months in the growth of the palate are from birth up to 3 months when the sagittal direction changes the most and the round arch changes into oval arch forms.[42] This is the rationale behind the previous studies, which demonstrated the necessity of examining neurobehavioral disorders, including the gag reflex in neonates or children aged 3 months, in order to provide the most appropriate therapy, which can help children to eat (when fed orally) without any problems.[43,44]

The gag reflex may be evoked by stimulation of the posterior pharyngeal wall, tonsillar area or the base of the tongue. It is an essential component of evaluating the medullary brainstem and plays a role in the declaration of brain death.[9,43,44]

Gagging is responsible not only for the structure but also for the function of such structures as the tongue, hyoid bone and soft palate. Glossopharyngeal and vagus nerves control the gag reflex as well as innervate the soft palate. We showed that children who did not have a gag reflex also had paresis of the soft palate. Normally, intraoral stimulation of the gag reflex is connected with the afferent reaction of fibers from the trigeminal, glossopharyngeal and vagus nerves, which report to the medulla oblongata.[45] The neural control of the soft palate is similar. Both the glossopharyngeal (IX) and vagus (X) nerves contribute to the innervation of the palatoglossus and palatopharyngeus.[46] The functions of the soft palate depend on reflexes mediated by sensory nerve endings. The soft palate is also engaged in such motor activities as respiration, swallowing and speech.[47] This relationship shows the importance of functions or reflexes on the structure of the soft palate and vice versa. The orofacial complex comprises the oral cavity organs as well as the facial part – muscles of the masticatory system and the motor coordination areas of the nervous system.[48] The gag reflex is regarded as a part of neurocognitive behaviors. An abnormal gag reflex, together with abnormal volitional cough, dysphonia, dysarthria, coughing after swallowing, and voice changes after swallowing, are known as clinical features of the risk of aspiration. The gag reflex is one of the clinical and cognitive predictors of swallowing recovery in patients with brain injuries.[49] The gag reflex may be initiated by either somatic stimuli (i.e., sensory nerve stimulation from direct contact) or psychogenic stimuli (i.e., by higher centers in the central nervous system). Not only typical physical stimuli such as tactile stimulation of the oropharynx by placing an object next to the uvula, the posterior pharyngeal wall, the tonsillar area or on the base of the tongue may evoke the gag reflex, but also such psychological factors as fear, stress or phobia can trigger gagging. As a result, efferent impulses give rise to spasmodic and uncoordinated muscle movement. This results in the center in the medulla oblongata being activated during gagging. That is why the management strategies for optimizing the gag reflex may be different – both conventional and complementary, such as behavioral techniques, pharmacological techniques and complementary therapies.[50] Future studies should be developed to describe the treatment of hyper and hyporeflexia of the gag reflex. This study aimed to show how important the gag reflex is as a vital reflex as well as a reaction that has influences on the structure and psychomotor function. One of the psychomotor and cognitive aspects which is associated with the gag reflex is communication. Therefore, all professionals working with children with developmental disabilities should know how to check the gag reflex.

What is more, because of the period of dominating reflexes lasts from birth to approx. 6–7 months, early intervention, examination and therapy seem to be very important, especially in the first months of life.[10] This is why it is important to examine the orofacial region, including the gag reflex, during the child's development.

The management of feeding dysfunction in children with neurological disorders should be complex and requires well-coordinated multidisciplinary teams who clearly communicate with families.[33]

Limitations

The limitation was that only 25 children with neurological diseases were evaluated and the main diagnosis was cerebral palsy. Although the condition is rare, future studies should consider increasing the number of study participants to make the results more representative.

Conclusions

Children with neurological disorders, such as cerebral palsy, are a very heterogeneous group that requires special orthodontic treatment as well as close interdisciplinary cooperation.[38] Proper complex care of children with neurological diseases is possible only if they are properly examined. One of the elements of this examination should be checking the gag reflex.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Barton C, Bickell M, Fucile S. Pediatric oral motor feeding assessments: A systematic review. *Phys Occup Ther Pediatr.* 2018;38(2):190–209. doi:10.1080/01942638.2017.1290734
2. Speyer R, Cordier R, Kim J, Cocks N, Michou E, Wilkes-Gillan S. Prevalence of drooling, swallowing, and feeding problems in cerebral palsy across the lifespan: A systematic review and meta-analyses. *Dev Med Child Neurol.* 2019;61(11):1249–1258. doi:10.1111/dmcn.14316

3. Bell KL, Benfer KA, Ware RS, et al. Development and validation of a screening tool for feeding/swallowing difficulties and undernutrition in children with cerebral palsy. *Dev Med Child Neurol*. 2019; 61(10):1175–1181. doi:10.1111/dmcn.14220
4. Schwemmle C, Arens C. Feeding, eating, and swallowing disorders in infants and children: An overview [in German]. *HNO*. 2018;66(7): 515–526. doi:10.1007/s00106-017-0388-y
5. Ferluga ED, Archer KR, Sathe NA, et al. Introduction. In: *Interventions for Feeding and Nutrition in Cerebral Palsy*. AHRQ Comparative Effectiveness Reviews. Rockville, USA: Agency for Healthcare Research and Quality (US); 2013:Bookshelf ID NBK132442. http://www.ncbi.nlm.nih.gov/books/NBK132442/. Accessed May 23, 2024.
6. Erasmus CE, Van Hulst K, Rotteveel JJ, Willemsen MAAP, Jongerius PH. Clinical practice: Swallowing problems in cerebral palsy. *Eur J Pediatr*. 2012;171(3):409–414. doi:10.1007/s00431-011-1570-y
7. Arvedson JC. Feeding children with cerebral palsy and swallowing difficulties. *Eur J Clin Nutr*. 2013;67(Suppl 2):S9–S12. doi:10.1038/ejcn.2013.224
8. Malak R, Sikorska D, Rosołek M, et al. Impact of umbilical cord arterial pH, gestational age, and birth weight on neurodevelopmental outcomes for preterm neonates. *PeerJ*. 2021;9:e12043. doi:10.7717/peerj.12043
9. Sivakumar S, Prabhu A. Physiology. Gag reflex. In: *StatPearls*. Treasure Island, USA: StatPearls Publishing; 2024:Bookshelf ID NBK554502. http://www.ncbi.nlm.nih.gov/books/NBK554502. Accessed May 23, 2024.
10. Watanabe S. Development of masticatory function. *Dent Oral Craniofac Res*. 2017;3(7):1–2. https://www.oatext.com/pdf/DOCR-S2-004.pdf
11. Michalska A, Panasiuk J, Pogorzelska J, Połatyńska K, Wendorff J. Eating and drinking ability classification system in cerebral palsy. *Child Neurol*. 2017;26(52):63–67. doi:10.20966/chn.2017.52.394
12. Burr S, Harding S, Wren Y, Deave T. The relationship between feeding and non-nutritive sucking behaviours and speech sound development: A systematic review. *Folia Phoniatr Logop*. 2021;73(2):75–88. doi:10.1159/000505266
13. Nguyen T, Stewart D, Rosenbaum P, Baptiste S, Kraus De Camargo O, Gorter JW. Using the ICF in transition research and practice? Lessons from a scoping review. *Res Dev Disabil*. 2018;72:225–239. doi:10.1016/j.ridd.2017.11.003
14. Richards CL, Malouin F. Cerebral palsy. *Handb Clin Neurol*. 2013;111: 183–195. doi:10.1016/B978-0-444-52891-9.00018-X
15. Ortega AOL, Guimarães AS, Ciamponi AL, Marie SKN. Frequency of temporomandibular disorder signs in individuals with cerebral palsy. *J Oral Rehabil*. 2008;35(3):191–195. doi:10.1111/j.1365-2842.2007.01766.x
16. Miamoto CB, Ramos-Jorge ML, Pereira LJ, Paiva SM, Pordeus IA, Marques LS. Severity of malocclusion in patients with cerebral palsy: Determinant factors. *Am J Orthod Dentofacial Orthop*. 2010;138(4):394.e1–394.e5. doi:10.1016/j.ajodo.2010.03.025
17. Mishra A, Sheppard JJ, Kantarcigil C, Gordon AM, Malandraki GA. Novel mealtime duration measures: Reliability and preliminary associations with clinical feeding and swallowing performance in self-feeding children with cerebral palsy. *Am J Speech Lang Pathol*. 2018; 27(1):99–107. doi:10.1044/2017_AJSLP-16-0224
18. Lacerda DC, Manhães-de-Castro R, Gouveia HJCB, et al. Treatment with the essential amino acid L-tryptophan reduces masticatory impairments in experimental cerebral palsy. *Nutr Neurosci*. 2021;24(12): 927–939. doi:10.1080/1028415X.2019.1695360
19. Hashida N, Shamoto H, Maeda K, Wakabayashi H. Impact of geniohyoid and masseter muscle masses on dysphagia after salvage surgery and radiotherapy in head and neck cancer. *Sci Rep*. 2021;11(1):2278. doi:10.1038/s41598-021-82039-0
20. Kaviyani Baghbadorani M, Soleymani Z, Dadgar H, Salehi M. The effect of oral sensorimotor stimulations on feeding performance in children with spastic cerebral palsy. *Acta Med Iran*. 2014;52(12):899–904. PMID:25530052.
21. Larnert G, Ekberg O. Positioning improves the oral and pharyngeal swallowing function in children with cerebral palsy. *Acta Paediatr*. 1995;84(6):689–693. doi:10.1111/j.1651-2227.1995.tb13730.x
22. Ertekin C, Keskin A, Kiylioglu N, et al. The effect of head and neck positions on oropharyngeal swallowing: A clinical and electrophysiologic study. *Arch Phys Med Rehabil*. 2001;82(9):1255–1260. doi:10.1053/apmr.2001.25156
23. Tran KN, Nguyen NPK, Nguyen LTH, Shin HM, Yang IJ. Screening for neuroprotective and rapid antidepressant-like effects of 20 essential oils. *Biomedicines*. 2023;11(5):1248. doi:10.3390/biomedicines11051248
24. Battaglia MR, Di Fazio C, Battaglia S. Activated tryptophan-kynurenine metabolic system in the human brain is associated with learned fear. *Front Mol Neurosci*. 2023;16:1217090. doi:10.3389/fnmol.2023.1217090
25. Polyák H, Galla Z, Nánási N, et al. The tryptophan-kynurenine metabolic system is suppressed in cuprizone-induced model of demyelination simulating progressive multiple sclerosis. *Biomedicines*. 2023;11(3):945. doi:10.3390/biomedicines11030945
26. Battaglia S, Di Fazio C, Vicario CM, Avenanti A. Neuropharmacological modulation of N-methyl-D-aspartate, noradrenaline and endocannabinoid receptors in fear extinction learning: Synaptic transmission and plasticity. *Int J Mol Sci*. 2023;24(6):5926. doi:10.3390/ijms24065926
27. Westbrook KE, Nessel TA, Hohman MH, Varacallo M. Anatomy. Head and neck: Facial muscles. In: *StatPearls*. Treasure Island, USA: StatPearls Publishing; 2024:Bookshelf ID NBK493209. http://www.ncbi.nlm.nih.gov/books/NBK493209. Accessed May 23, 2024.
28. Asdaghi Mamaghani SM, Bode H, Ehmer U. Orofacial findings in conjunction with infantile cerebral paralysis in adults of two different age groups: A cross-sectional study. *J Orofac Orthop*. 2008;69(4):240–256. doi:10.1007/s00056-008-0745-2
29. Hohoff A, Rabe H, Ehmer U, Harms E. Palatal development of preterm and low birthweight infants compared to term infants: What do we know? Part 2: The palate of the preterm/low birthweight infant. *Head Face Med*. 2005;1(1):9. doi:10.1186/1746-160X-1-9
30. Tanaka M, Diano M, Battaglia S. Editorial: Insights into structural and functional organization of the brain: Evidence from neuroimaging and non-invasive brain stimulation techniques. *Front Psychiatry*. 2023;14:1225755. doi:10.3389/fpsyt.2023.1225755
31. Baliellas DEM, Barros MP, Vardaris CV, et al. Propentofylline improves thiol-based antioxidant defenses and limits lipid peroxidation following gliotoxic injury in the rat brainstem. *Biomedicines*. 2023;11(6):1652. doi:10.3390/biomedicines11061652
32. Tanaka M, Szabó Á, Vécsei L. Preclinical modeling in depression and anxiety: Current challenges and future research directions. *Adv Clin Exp Med*. 2023;32(5):505–509. doi:10.17219/acem/165944
33. Marpole R, Blackmore AM, Gibson N, Cooper MS, Langdon K, Wilson AC. Evaluation and management of respiratory illness in children with cerebral palsy. *Front Pediatr*. 2020;8:333. doi:10.3389/fped.2020.00333
34. Umay E, Eyigor S, Giray E, et al. Pediatric dysphagia overview: Best practice recommendation study by multidisciplinary experts. *World J Pediatr*. 2022;18(11):715–724. doi:10.1007/s12519-022-00584-8
35. Ramsey D, Smithard D, Donaldson N, Kalra L. Is the gag reflex useful in the management of swallowing problems in acute stroke? *Dysphagia*. 2005;20(2):105–107. doi:10.1007/s00455-004-0024-3
36. Tanaka N, Nohara K, Uota C, et al. Relationship between daily swallowing frequency and pneumonia in patients with severe cerebral palsy. *BMC Pediatr*. 2022;22(1):485. doi:10.1186/s12887-022-03547-0
37. Kumar S, Satheesh P, Savadi RC. Gagging. *N Y State Dent J*. 2011;77(4): 22–27. PMID:21894827.
38. Rosted P, Bundgaard M, Fiske J, Pedersen AML. The use of acupuncture in controlling the gag reflex in patients requiring an upper alginate impression: An audit. *Br Dent J*. 2006;201(11):721–725. doi:10.1038/sj.bdj.4814305
39. Hosseini SM, Jamshir M, Maleki A. The effect of gag reflex on cardiac sympatovagal tone. *Oman Med J*. 2012;27(3):249–250. doi:10.5001/omj.2012.57
40. Mohamed NE, Hussein AA, Sanad DAM. Temporal effects of two interferential current applications on peripheral circulation in children with hemiplegic cerebral palsy. *J Taibah Univ Med Sci*. 2023;18(1): 140–147. doi:10.1016/j.jtumed.2022.08.004
41. Sakamoto T, Fukuda K, Saita N, et al. Autonomic nervous activity of patients with gagging problems during dental mirror insertion. *Special Care Dentist*. 2016;36(2):80–84. doi:10.1111/scd.12148

42. Bejdová Š, Krajíček V, Peterka M, Trefný P, Velemínská J. Variability in palatal shape and size in patients with bilateral complete cleft lip and palate assessed using dense surface model construction and 3D geometric morphometrics. *J Craniomaxillofac Surg*. 2012;40(3): 201–208. doi:10.1016/j.jcms.2011.04.013
43. Malak R, Fechner B, Sikorska D, et al. Application of the neonatal behavioral assessment scale to evaluate the neurobehavior of preterm neonates. *Brain Sci*. 2021;11(10):1285. doi:10.3390/brainsci11101285
44. Malak R, Wiecheć K, Fechner B, et al. The influence of parent education on the neurobehavior and sucking reflexes of very preterm infants. *Brain Sci*. 2022;12(7):840. doi:10.3390/brainsci12070840
45. Dos Santos MTBR, Nogueira MLG. Infantile reflexes and their effects on dental caries and oral hygiene in cerebral palsy individuals. *J Oral Rehabil*. 2005;32(12):880–885. doi:10.1111/j.1365-2842.2005.01518.x
46. Bassi GS, Humphris GM, Longman LP. The etiology and management of gagging: A review of the literature. *J Prosthet Dent*. 2004;91(5): 459–467. doi:10.1016/j.prosdent.2004.02.018
47. Mu L, Chen J, Li J, et al. Innervation of human soft palate muscles. *Anat Rec (Hoboken)*. 2021;304(5):1054–1070. doi:10.1002/ar.24531
48. Mu L, Chen J, Li J, et al. Sensory innervation of the human soft palate. *Anat Rec (Hoboken)*. 2018;301(11):1861–1870. doi:10.1002/ar.23864
49. Schroeder MF, Daniels SK, McClain M, Corey DM, Foundas AL. Clinical and cognitive predictors of swallowing recovery in stroke. *J Rehabil Res Dev*. 2006;43(3):301. doi:10.1682/jrrd.2004.12.0154
50. Kondraciuk A, Manias S, Misiuk E, et al. Impact of the orofacial area reflexes on infant's speech development. *Prog Health Sci*. 2014;4(1): 188–194. https://www.umb.edu.pl/photo/pliki/progress-file/phs/phs_2014_1/188-194_kondraciuk.pdf. Accessed August 15, 2023.

Evaluation of the use of an antigravity device in leveling functional inequalities of the lower limbs and inhibiting the progression of idiopathic scoliosis

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Abstract

Background. The conservative treatment of idiopathic scoliosis (IS) may be enhanced through a combination of specialized physiotherapy, bracing, and the utilization of assistive devices.

Objectives. This study aims to evaluate the efficacy of the GraviSpine device in supporting the conservative treatment of IS in children.

Materials and methods. A cohort of 142 patients, aged 10–17 years with an average age of 12.76 ±1.75 years, undergoing treatment for IS with specific physiotherapy and bracing, received additional treatment with the GraviSpine device. The participants, selected based on inclusion and exclusion criteria, were divided into 2 age groups: group A (10–12 years) and group B (13–17 years). The mean follow-up period was 28.71 ±10.98 months. The assessment involved changes in post-treatment trunk rotation angles (ATR), Cobb angles, and functional lower limb length discrepancies (FLLDs) concerning age groups and scoliosis location.

Results. The proportion of patients showing improvement and stabilization was high in both groups A and B, at 71% and 90%, respectively. In group B, a significant reduction in the mean Cobb angle of –1.83° ±6.88°, $p < 0.002$, was observed. Furthermore, a significant decrease in the incidence of FLLDs was noted in thoracic and lumbar scoliosis locations, $p < 0.002$.

Conclusions. To enhance the effectiveness of conservative treatment for IS, the utilization of an assistive device such as GraviSpine may be considered, particularly when the child presents functional inequality of the lower limbs.

Key words: body posture, scoliosis, physiotherapeutic exercises specific to scoliosis, conservative treatment of scoliosis

Background

Idiopathic scoliosis (IS), characterized by a three-dimensional (3D) deformity of the spine, manifest in seemingly healthy children and can progress during periods of rapid growth.[1–3] The etiology of IS remains elusive, with reports suggesting that scoliosis formation and progression may stem from the combined influence of various factors.[2] Factors implicated in curvature progression include disturbances in central control of spinal posture[4–6] and pelvic asymmetries.[7–10] Research indicates that the body schema, a stable yet adaptable representation of the central nervous system, can be influenced by sensory experiences.[6] Children with IS may lack clear awareness of trunk misalignment, leading to gradual adaptation of their body pattern to the scoliotic state without recognizing the deformity.[11,12]

In the construction of a body diagram, particularly in children at risk for the development of IS, the pelvic aspect and functional length of the lower limbs are crucial.[13–20] Numerous studies underscore the impact of functional leg length discrepancies (FLLDs) during childhood on the internal stresses within pelvic structures, potentially resulting in structural adaptive changes and pelvic asymmetries.[21–23] Pelvic asymmetry, in turn, may contribute to spinal developmental disorders.[7–10,14,16] Grivas et al.[14] suggest that FLLD affects 3–15% of the population and may stem from muscle contractures, biomechanical issues of pelvic joints, and dysfunction in other lower limb joints. Therefore, early detection and correction of FLLDs are reported to not only benefit IS but also aid in correcting lower limb deformities.[24–26]

The aforementioned findings and years of clinical observations inspired Dr. Marek Kluszczyński, a co-author of this study, to develop the innovative GraviSpine device (Technomex Sp z o.o., Gliwice, Poland). This device resembles an inversion table on which the child lies on their back and is suspended upside down at an angle of approx. 20–30°, held in place by 1 or both lower limbs (Fig. 1).

Fig. 1. GraviSpine device developed by M. Kluszczyński

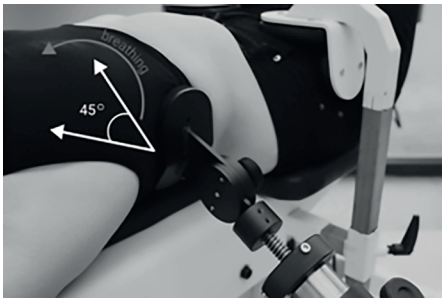

Fig. 2. Asymmetric breathing exercises combined with passive curvature correction by pads

GraviSpine features movable side pads mounted on rails at right angles to the child's torso. Upon placing the child on the device, the physiotherapist positions the corrective pad's arm on top of the curvature, applies a pad to the torso, and adjusts the sliding lever of the pad, resulting in compression of the torso from the back and side to the front and center, thus actively correcting the scoliotic curve. During the GraviSpine procedure, the patient concurrently performs derotational breathing exercises, which are facilitated by passive pad correction (Fig. 2).

Properly constructed rotating head pads enable simultaneous correction of the spine in the frontal plane and derotation of the spine in the transverse plane by applying pressure to the trunk at the correct angle (Fig. 2). GraviSpine leverages a reverse gravity phenomenon and passive 3D correction to stretch the contracted support structures, including the ligaments, tendons, muscle attachments, joint capsules, and intervertebral discs on the concave side of the curvature.

The arrangement of the articular surfaces in the facet joints of the spine in a standing position favors spine stabilization, posing challenges for correcting the deformed spine in scoliosis. Conversely, relieving spinal joints in the antigravity position causes the articular surfaces to move apart (Fig. 3A).

Relaxation of the intervertebral joints and supporting tissues reduces the pressure required from the corrective pad to correct the curvature[27] (Fig. 3B).

If a child with scoliosis exhibited FLLDs during the examination, they were positioned on the GraviSpine with 1 (the functionally shorter) lower limb behind, aiming to increase tensile forces on the pelvic connections of the lumbosacral spine and pelvic internal ligaments (Fig. 4). The distribution of force vectors on the GraviSpine is illustrated in Fig. 5.

When the patient's lower limbs are secured on an inclined plane, a frictional force (T) acts along the surface of the plane, preventing the body from sliding downward. Additionally, gravitational forces (Fg) and reaction forces (R) act on the body, along with the pressure exerted by the body on the plane. The contact force equals the component of the weight normal to the surface (F2).

The sliding force (F1) is the force that could potentially cause the person to slide off the plane, representing

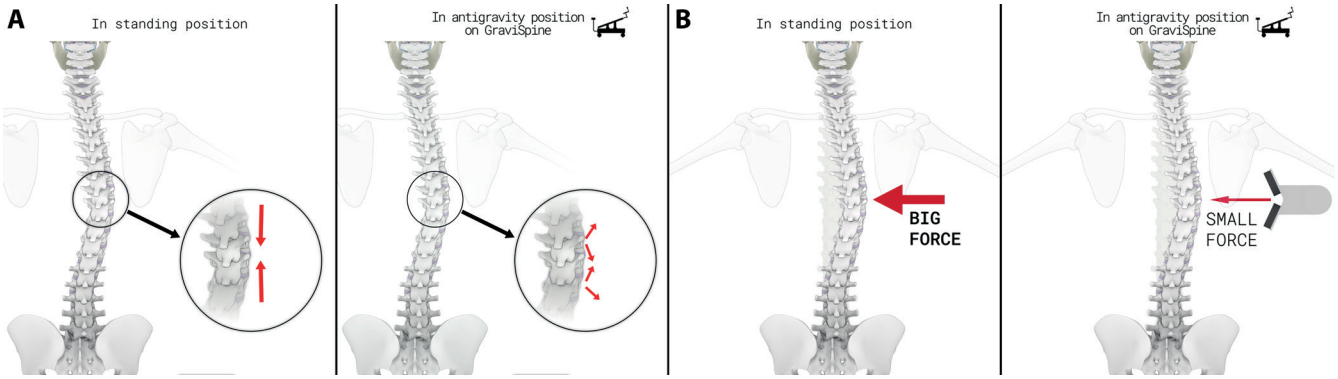

Fig. 3. A. Relaxing the facet joints during stretching; B. stretching the spine allows you to use less force to correct the curve

Fig. 4. Attaching the GraviSpine to the right (shortened) limb allows stretching of the pelvic ligaments and mobilization of the sacroiliac joints and contracted myofascial structures, which reduces or even eliminates FLLDs

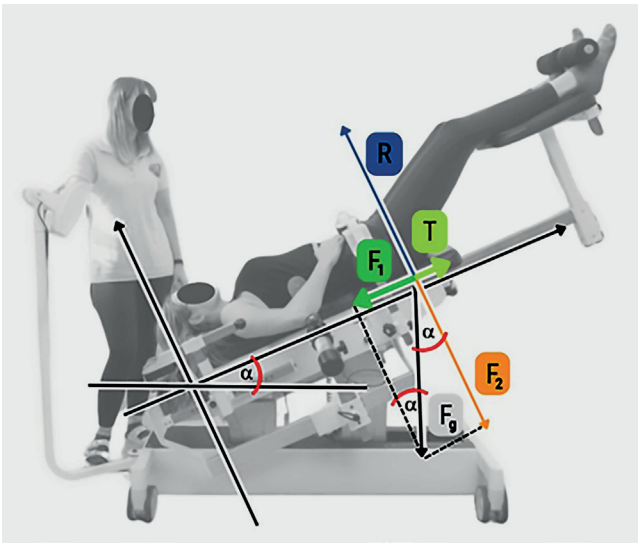

Fig. 5. Distribution of the force vectors acting on the patient using GraviSpine

the tensile force of the pelvic structures on the shortened side of the lower limb. It is a component of the gravitational force acting parallel to the plane, according to the formula:

$$F1=|Fg| \times \sin(\alpha),$$

where α – angle between the force and the direction in which the gravitational force acts.

The decision to utilize GraviSpine to influence the hip girdle system in compensating for FLLDs stemmed from the authors' prior experience with the inversion table, practical insights in manual therapy, and literature reports confirming the positive impact of FLLD alignment on scoliosis.[7,15]

Contraindications for the use of GraviSpine include:

- Post-surgical treatment of scoliosis;
- Infectious diseases affecting bodily functions;
- Congenital malformations of the osteoarticular system, especially of the spine and lower limbs, pose a risk of spinal cord injury during the procedure;
- Congenital osteogenesis imperfecta;
- Neurological conditions predisposing the child to procedural complications (e.g., epilepsy); and
- Cardiovascular disorders (after consultation with a cardiologist).

Other assistive devices are currently employed in the conservative treatment of IS globally. One such device utilizes the method referred to as fixation in space, elongation, derotation (FED), developed by Ferdinand Sastre. This device's structure and function resemble GraviSpine, but the child is positioned in the standing position (Fig. 6A).

The device operates by passively correcting curvatures using hydraulic pads with vertical traction of the spine fixed under the armpits.[28,29] The goal of FED is to equalize pressure on the facet joints of the vertebrae, which differs on the concave and convex sides of the curvature, a factor perceived by the author as contributing to IS development.

Another device, "SKOL-AS," devised by Andrzej Stolarz,[30] employs active-passive correction of curvatures in a seated position, coupled with asymmetrical breathing controlled by measuring pressure in cushions on the concave side of the curvature. To enhance efficacy, biofeedback, and visualization of pressure are utilized.

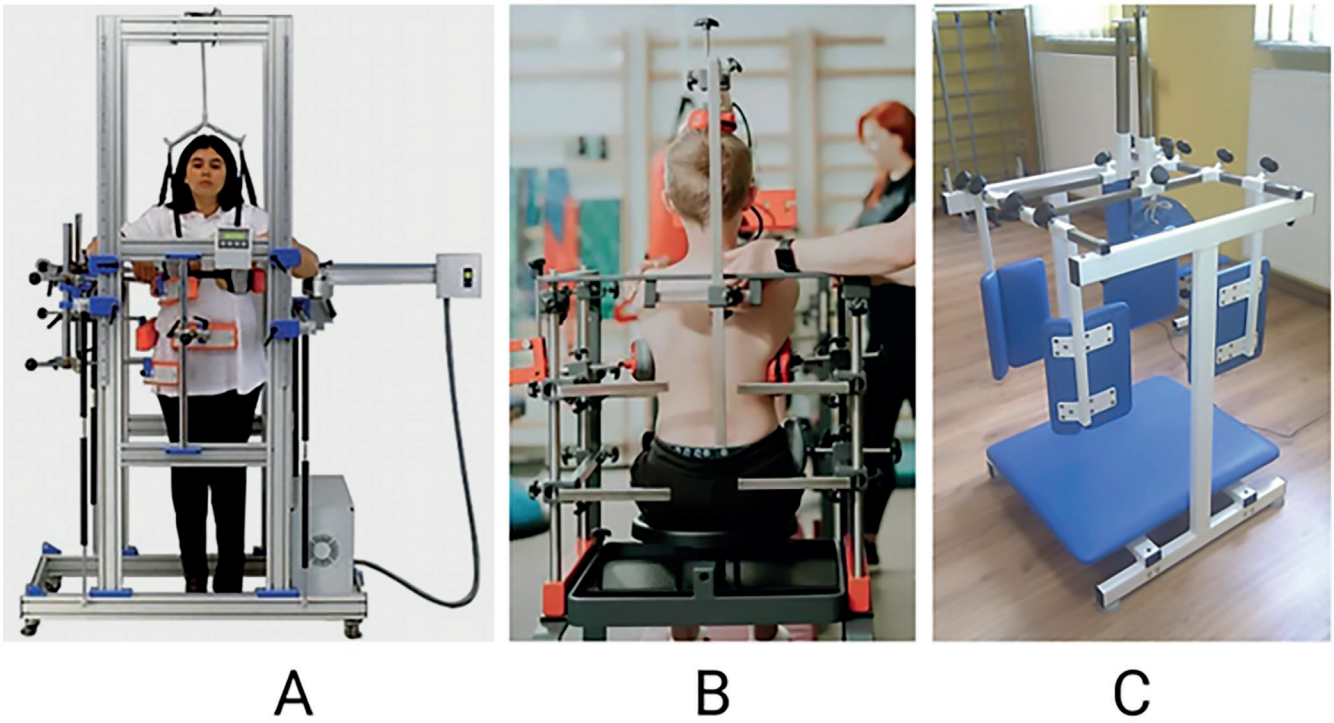

Fig. 6. A. FED device developed by Dr. F. Sastre; B. "SKOL-AS" device developed by A. Stolarz; C. "Delfin" device developed by T. Szurmik, J. Sitarz, and M. Segiet

According to the author, exercises on the SKOL-AS device serve as a form of postural re-education, combining 3D active-passive correction with proprioceptive stimulation techniques to program a new pattern of correct posture in the central nervous system (Fig. 6B).

Another assistive device, "Delfin," designed by Tomasz Szurmik, Józef Sitarz, and Marek Segiet, utilizes the Klapp position for passive-active self-correction of scoliosis by compressing the rib hump and lumbar shaft using properly positioned pads in an exercise known as "cat's back" (Fig. 6C).

The GraviSpine device utilized in this study is distinguished by its ability to not only provide active-passive curvature correction but also affect the hip girdle system using the antigravity phenomenon.

Objectives

This study aimed to assess the effectiveness of GraviSpine in supporting Physiotherapeutic Specific Scoliosis Exercises (PSSE) and bracing in the treatment of IS, as well as in eliminating functional inequalities in the lower limbs.

Materials and methods

The study received approval from the Jan Dlugosz University Ethical Committee under resolution KE-U/10/2021, dated September 28, 2021, and adhered to the principles of the Declaration of Helsinki. All parents of the participants were briefed on the study's objectives and procedures, and their written consent was obtained prior to commencement. Individuals depicted in photographs provided written consent for the publication of their images.

Conducted at the "Tronina" Medical Rehabilitation Center, the study utilized data from patients treated between 2017 and 2021. Patients and their parents/caregivers were informed by physicians about the methods and procedures involved and provided written consent for treatment. Qualifications for treatment were based on X-ray examinations in accordance with the criteria outlined by the Society on Scoliosis Orthopedic and Rehabilitation Treatment (SOSORT),[31] specifically Cobb scoliosis ≥10° combined with vertebral rotation.

The study group received outpatient treatment following a standardized protocol, comprising weekly sessions lasting 90 min each. Treatment included 40 min of individual exercises based on selected schemes from the PSSE methods by Dobomed and Schroth, 2 sessions of 20 min each of derotational breathing exercises on the GraviSpine device,[32] and 10 min devoted to learning correct posture during daily activities.

For each child, the physiotherapist recommended 2–3 exercises to be performed at home between sessions. If a FLLD was detected during clinical assessment, the GraviSpine suspension was adjusted to accommodate the shorter limb and correct the FLLD. A difference of 0.5 to 1.9 cm was considered indicative of a FLLD, while a difference exceeding 2 cm prompted additional examination with a tape measure to assess for structural limb shortening, which was an exclusion criterion. Although each child followed the same treatment model, exercises varied based on individual factors such as the type of IS, orthopedic

deficits (e.g., muscle contractures, valgus knees, or tarsus), and sensorimotor deficits (e.g., posture, balance, and coordination disorders).

In the study group, 32% of children underwent combined therapy with PSSE, GraviSpine, and a Cheneau brace, in accordance with SOSORT criteria. This combination was recommended when the Cobb angle of the greatest scoliosis curvature was ≥20° and a Risser stage between 0–3.[31] Despite the recommendation of wearing the brace for 23 h a day, actual usage, as reported by parents, ranged between 6 and 12 h daily. The duration of brace treatment varied from 6 to 38 months. Every 3 months, treatment progress was assessed by a physician through evaluation of the trunk rotation angle (ATR) using the Bunnell scoliometer, anteroposterior spinal curvature angles using a Saunders inclinometer, and brace fit. Functional lower limb length discrepancies were evaluated based on the level of heels when lying supine, following methods described by Cooperstein,[33] modified by Travella et al.,[34] and Friberg et al.[35] Cobb angles were analyzed from initial and final X-rays. X-rays were measured twice using standard computer radiography software (RSR2LITE) by a specialist radiologist with 21 years of experience, who was blinded to patient data, and the results were averaged. In cases involving brace treatment, correction was consulted with an orthopedic technician. The treatment was conducted by a team consisting of 2 doctors, 8 physiotherapists, and 2 orthopedic technicians, working closely together. The mean follow-up period was 28.71 ±10.98 months.

Participants

Inclusion criteria included newly diagnosed children meeting the SOSORT criteria for IS,[31] attending weekly visits for a minimum of 2 treatment cycles consisting of 10 sessions each, having a Risser stage between 0–4, and undergoing at least 1 year of follow-up. Exclusion criteria encompassed the presence of secondary scoliosis (congenital, neurological, metabolic, post-traumatic, etc.), mental retardation, respiratory disease, and prior treatment of IS using other methods.

The study group meeting these criteria included 142 children out of a total of 228 treated at the center, aged between 10 and 17 years, with an average age of 12.76 ±1.75 years. Women predominated, constituting 121 (85.2%) of the participants, with a mean age of 11.85 ±3.2 years, while 21 men (14.8%) with a mean age of 12.54 ±3.05 years were included. Patients were categorized into 2 age groups: group A (10–12 years old, n = 66) and group B (13–17 years old, n = 76), based on center protocols and established literature.[31]

Data analysis

Initial and final X-rays were used to analyze Cobb angles. Improvement in the curve (Cobb angle decrease ≥5°), stability (Cobb angle change ±5°), and progression (Cobb angle increase ≥5°) were compared.[31] Similarly, initial and final measurements were utilized to analyze ATR values, with improvements (ATR angle decrease ≥2°), stability (ATR angle change ±2°), and progression (ATR angle increase ≥2°) assessed.[31]

Statistics

Due to the non-parametric distribution of data (verified via Q–Q plots), differences between pre- and post-treatment results were assessed using the Wilcoxon signed-rank test. The discrepancy in the functional length of the lower limbs was evaluated based on heel level in the supine position, assuming a difference of ≥5 mm.[13] Fisher's test was employed to analyze relationships between categorical variables.

Results

The mean follow-up was 28.71 ±10.98 months. The Cobb angles ranged from 10° to 46° within the group, with an average of 24.36° ±11.82°. The range of ATR was from 3° to 16°, with an average of 6.99° ±2.95°. Double-curved scoliosis predominated, with over 33% of children exhibiting a FLLD. The mean Risser score values for groups A and B were 1.38° ±1.4° and 2.33° ±1.1°, respectively (Table 1).

In group B, a statistically significant mean reduction in the Cobb angles after treatment was observed, amounting to 2.33° ±1.1°, with p = 0.002. Conversely, in group A, the post-treatment mean Cobb angle also decreased, but not significantly, by 1.38° ±1.4°. The percentage of positive treatment effects was high and increased with age, with the most frequent occurrences of improvement and stabilization found in group B (25% and 65%, respectively), while worsening was observed in only 10% of patients. Group A exhibited slightly lower frequencies, with improvement and stabilization at 18% and 53%, respectively (Table 2,3).

Significant differences in changes in the Cobb angle were observed depending on the location of the curvature. In the thoracic spine, group B reported significant reductions of –2.2° ±7.54° (10.17%), with improvement and stabilization in 23% and 67% of cases, respectively, and deterioration in 10% of patients. Group A showed slightly poorer results, with the mean Cobb angle decreasing by –1.62° ±9.36°, predominantly exhibiting stabilization in 54% of cases, while improvement and deterioration were equally present in 23% (Fig. 7). In Fig. 7, the upper and lower whiskers represent scores outside the middle 50% (i.e., the lower 25% of scores and the upper 25% of scores). The median marks the midpoint of the data and is shown by the line that divides the box into 2 parts. Points represent outliers.

In the thoracolumbar spine, the most significant reduction in Cobb angles was observed in group B, where 87% exhibited stabilization and 13% showed progression, with a mean Cobb angle decrease of –2.2° ±6.58° (6.36%). Group A had slightly inferior outcomes, with

Table 1. Main characteristics of the patients

Variable	Parameter	Value (%)
Age	n	142 (100)
	average (SD)	12.81 ±2.27
	median (Q1–Q3)	13 (10.25–15)
	range	10–17
Gender	girls	121 (85.2)
	boys	21 (14.8)
ATR before treatment	n	139
	average (SD)	6.99 ±2.95
	median (Q1–Q3)	6 (5–9)
	range	3–16
Location of dominant curvature	Th	56 (39.4)
	Th-L	28 (19.7)
	L	58 (40.9)
Scoliosis type	single arch	34 (23.9)
	double arch	108 (76.1)
X-ray Cobb angle before treatment	average (SD)	24.36° ±11.82°
	median (Q1–Q3)	22 (15–30)
	range	10–75
FLLD before treatment	right	33 (32.2)
	left	14 (9.9)
	equal	95 (66.9)
Amount of shortening in millimeters, before treatment	n	47
	average (SD)	8.32 (3.43)
	median (Q1–Q3)	7 (6–10)
	range	3–20
Skeletal maturity according to Risser	group A	1.38° ±1.4°
	group B	2.33° ±1.1°

ATR – angle of trunk rotation; FLLD – functional lower limb length discrepancies; n – number; Th – thoracic; Th-L – thoraco-lumbar; L – lumbar; SD – standard deviation.

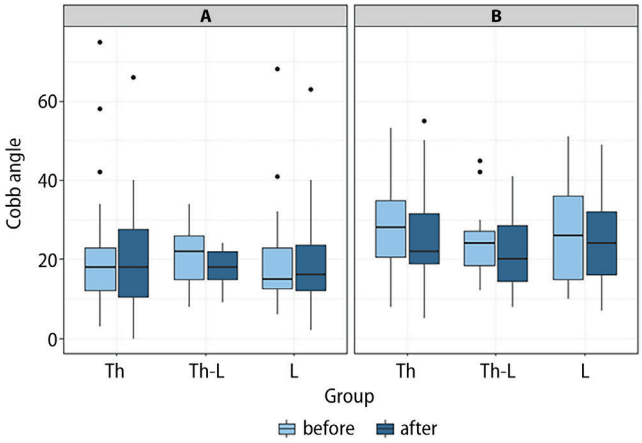

Fig. 7. Change in the Cobb angle relative to the location of curvature

62% experiencing stabilization, 15% improvement, and 23% deterioration, resulting in a mean Cobb angle reduction of –1.62° ±7.07° (–3.24%, Fig. 7). In the lumbar spine, group B also achieved better results, with a 39% improvement and 51% stabilization, compared to group A's 15% improvement and 45% stabilization (Fig. 7).

A statistically significant reduction in mean post-treatment ATR was noted for the entire group, decreasing from a baseline ATR of 6.99° ±2.95° to 6.14° ±2.71° ($p < 0.001$). The most significant reductions in ATR were observed in the lumbar section, decreasing from 6.52 ±2.51 to 5.45 ±2.19 ($p < 0.001$), and thoracolumbar section, decreasing from 8 ±2.96 to 6.29 ±2.4 ($p < 0.002$, Table 4).

Following treatment, a significant reduction in FLLD was observed in both the thoracic ($p = 0.002$) and lumbar ($p = 0.002$) sections. The pre-treatment rate of FLLD ranged from 14.3% to 24.4%, while post-treatment rates ranged from 1.8% to 7.1% (Table 5).

Table 2. Change of Cobb angle before and after treatment in groups

Parameter		Group (n = 142)	
		A	B
n		66	76
Gender: F/M		55/11	66/10
Age group [years]		10–12	13–17
mAge [years]		10.67 ±0.81	14.67 ±1.25
mRg		1.38 ±1.4	2.33 ±1.1
mCobb	before treatment	20.42 ±13.17; 17 (12–23)	26.15 ±10.6; 25 (19.5–34.25)
	after treatment	19.96 ±12.28; 18 (12.25–23.75)	24.32 ±11.54; 23 (16–32)
test/effect size		0.654 (U = 1074)/0.060	0.002 (V = 1901.5)/0.351
ACD%		0.46 ±8.95 (8.28%)	1.83 ±6.88 (6.31%)

n – number; F – female; M – male; mAge [years] – mean age ±SD [years]; mRg – mean Risser grade ±SD; mCobb – mean Cobb angle ±SD°; median (Q1–Q3); test/effect size-value and U statistic of Wilcoxon signed-rank test and effect size – value of r effect size; ACD% – angle correction in degrees ±SD (percentages).

Table 3. Numbers and percentage values of IS improvement, stabilization, and progression – total

Group n/%	Improvement		Stabilization		Progression	
	n	%	n	%	n	%
A: 66/33.2	12	18	35	53	19	29
B: 76/38.2	19	25	49	65	8	10

Table 4. Change in ATR relative to the location of curvaturet

Section of the spine/n	Parameter	Before treatment	After treatment	*Test/effect size
Total / 139	average (SD)	6.99 (2.95)	6.14 (2.71)	<0.001 (U = 4496)/0.327
	median (Q1–Q3)	6 (5–9)	6 (4–7.5)	
	range	3–31	3–16	
Thoracic (Th) / 55	average (SD)	6.95 (3.27)	6.78 (3.18)	0.575 (U = 520)/0.060
	median (Q1–Q3)	6 (4–9.5)	6 (5–8)	
	range	3–25	3–15	
Thoraco-lumbar (Th-L) / 28	average (SD)	8 (2.96)	6.29 (2.4)	0.002 (U = 258)/0.587
	median (Q1–Q3)	7.5 (5.75–9.25)	6 (4.75–7)	
	range	3–16	4–16	
Lumbar (L) / 56	average (SD)	6.52 (2.51)	5.45 (2.19)	0.001 (U = 806)/0.445
	median (Q1–Q3)	6 (5–8)	5 (4–7)	
	range	3–31	3–13	

*test/effect size – p-value and U statistic of Wilcoxon signed-rank test and effect size – value of r effect size.

Table 5. Changes in the shortening of the lower limb of patients before and after treatment

Parameters	Shortening side	Shortening of the lower limb before treatment			*Test/effect size
Shortening of the lower limb after treatment	thoracic (Th) n (56)				
	–	left n (5)	equal n (38)	right n (13)	–
	left n (1)	20% n (1)	0% n (0)	0% n (0)	0.002/0.431
	equal n (52)	80% n (4)	100% n (38)	76.9% n (10)	
	right n (3)	0% n (0)	0% n (0)	23.1% n (3)	
	thoraco-lumbar (Th-L) n (28)				
	–	left n (3)	equal n (19)	right n (6)	–
	equal n (27)	100% n (3)	100% n (19)	83.3% n (5)	0.321/0.367
	right n (1)	0% n (0)	0% n (0)	16.7% n (1)	
	lumbar (L) n (58)				
	–	left N (6)	equal n (38)	right n (14)	–
	left n (2)	16.7% n (1)	0% n (0)	0% n (0)	0.002/0.401
	equal n (53)	83.3% n (5)	100% n (38)	78.6% n (11)	
	right n (3)	0% n (0)	0% n (0)	24.4% n (3)	

*test/effect size – p-value of Fisher's exact test and effect size – value of V Cramer effect size.

Discussion

The study assessed the efficacy of GraviSpine-assisted PSSE and bracing therapy in children with IS, focusing on changes in Cobb, ATR, and FLLD angles post-treatment. While the use of devices in the conservative management of IS is well established, there are few reports evaluating their effectiveness. The treatment model employed in this study involved weekly treatment sessions and daily PSSE exercises at home under parental supervision, a regimen also recommended in other PSSE methods such as Schroth.[36,37]

The risk of IS progression in the study group ranged from 40% to 75% according to the Lonstein and Corlson curve,[38] prompting 32% of children to require orthopedic bracing in accordance with SOSORT guidelines.[31]

Results of the treatment model demonstrated high efficacy, particularly in group B, where a total of 90% exhibited improvement and stabilization, with a significant mean Cobb angle reduction of –2.33° ±1.1 ($p < 0.002$, Table 2,3). Slightly less favorable outcomes were observed in the younger group A, possibly attributed to the longer study duration encompassing puberty, typically associated with the risk of scoliosis progression.[39]

A significant decrease in mean ATR was observed across the entire group, declining from a baseline of 6.99° ±2.95° to 6.14° ±2.71° ($p < 0.001$), with the most notable reductions in the lumbar ($p < 0.001$) and thoracolumbar ($p < 0.002$) locations (Table 4). Comparisons with device-assisted IS treatment studies revealed a study by Trzcińska et al.,[40] where a significant reduction in the mean ATR angle was found in a group treated with FITS and the Dr. Sastre FED device.[28,29] However, the comparison was challenged by short follow-up times and a lack of X-ray evaluation. Another study by Kamelska-Sadowska et al.[41] reported reductions in mean ATR angles following treatment with PSSE assisted by the SKOL-AS device.[30] Nonetheless, this study also lacked an evaluation of Cobb angle changes.

Comparing the results of our study with reports evaluating the effectiveness of conservative IS treatment using PSSE and bracing without assistive devices, most publications focus on the Schroth method combined with bracing.[42,43]

The study by Kwan et al.[44] stands out for its longest follow-up period (18 ±6.2 months), where they achieved an improvement in 17% of patients, stabilization in 62%, and progression in 21% of patients. Similarly, Schreiber et al.[37] reported comparable results, albeit with a shorter follow-up period of 6 months, noting a decrease in the Cobb angle of –0.4° and a significant decrease in the Cobb angle of the largest curve from –3.5° to –5.9° ($p = 0.006$). In another study focusing on the Schroth method, Kuru et al.[36] obtained a reduction in the study group's Cobb angle by –2.53° ($p < 0.001$).

The presented results of treatment using the Schroth method are similar to those obtained in our study, however, direct comparison is difficult due to the wide variety of studies.

When comparing our study's results to those of the SEAS method, another longstanding approach in Europe, Negrini et al.[45] showed slightly less favorable outcomes, with improvement in 23.5%, stabilization in 64.7%, and deterioration in 11.8% of treated patients. The most promising results were seen in a randomized controlled trial (RCT) evaluating the effectiveness of the SEAS method presented by Monticone et al.,[46] demonstrating a reduction of the mean Cobb angle in the study group by –5.3° ($p = 0.001$). The majority of reports describing the effectiveness of PSSE methods highlight the significant benefits of combined therapy, namely PSSE with bracing.[39,42,45,46]

In a study by Weinstein et al.,[47] the success rate of brace treatment was 72%, compared to 48% after follow-up. In our study, 32% of the children were also treated with a brace, but the actual bracing time averaged only 6–12 h, which, according to Weinstein et al.,[47] may yield an efficacy of only 40–70%.

In our study, we observed a significantly higher efficacy (80–90% combined improvement and stabilization in both groups A and B) than anticipated, given the short bracing duration. We suspect that we achieved this noteworthy effectiveness through combination therapy using the GraviSpine assist device. It is worth emphasizing that GraviSpine, like no other method, has an innovative effect on the correction of scoliosis in conditions of reduced gravity and elimination of limb length disproportions. Regarding FLLDs, our study demonstrated the effectiveness of combined PSSE and GraviSpine therapy in compensating for functional lower limb shortening, evidenced by a significant reduction in FLLDs in the thoracic and lumbar scoliosis locations. Reports by Brady et al. and Landauer et al.[16,22] underscore the critical impact of FLLDs on spinal deformities when occurring in childhood. Conversely, studies by D'Amico et al.[7] and Moseley et al.[15] affirm that aligning FLLDs during IS treatment, along with the posterior superior iliac spine planes of SIPS, is a favorable aspect in scoliosis management.

Numerous studies emphasize the importance of interventions aimed at compensating for functional lower limb shortening in treatment protocols.[5,7–10] These interventions aim to enhance neuromotor postural control and spinal stability by achieving neutral sacrum alignment. Researchers suggest that improving neurological control of the postural pattern, particularly during scoliosis formative stages, can potentially mitigate scoliosis progression.[5,6,48–51] In scoliosis deformities, the constricted ligaments, muscle tendons, and joint capsules, due to their specific elasticity, can lead to secondary mutual interactions, exacerbating vertebral deformities. This imbalance contributes to a vicious cycle of force responses between the vertebrae and 3-dimensional anatomical abnormalities.[52,53] Meanwhile, during the GraviSpine treatment, the perivertebral structures on the concave side of the curve are stretched, which helps prevent the progression of deformation and breaks the vicious circle.

Antigravity alignment of the spine on the GraviSpine induces relaxation of connections between vertebrae, facilitating correction of the deformity. Studies by Torell et al.[27] and Little et al.[54] have demonstrated that relieving the spinal joints significantly reduces the corrective force required for scoliosis correction.

Derotational breathing exercises performed on the GraviSpine combine 2 corrective factors: internal, involving active derotational breathing by the child, and external, involving passive correction of curves in the frontal and transverse planes through pad action.[55] This beneficial combination enhances the efficacy of scoliosis correction during treatment.

Therefore, the GraviSpine assistive device presented in this study may yield beneficial effects in the combined therapy of IS in children and adolescents, influencing various factors in the pathologically altered spine. It is worth noting that the treatment was well tolerated by the children, with a thorough analysis of possible side effects revealing no early or late risks. Data analysis on potential adverse events did not reveal any serious incidents up to the time the manuscript was sent, aside from a few instances of mild, short-term dizziness, particularly in tall, thin girls. These symptoms were effectively addressed by introducing a 1-min adaptation in a sitting position after transitioning from the head-down position, followed by dismounting from the device.

Limitations

Limitations of the study include its single-center nature and the absence of a control group for the presented treatment model. Additionally, the assessment of treatment effectiveness was compared only with the risk of progression using the Lonstein–Corlson curve in the discussion. It is important to acknowledge that the inventor of the GraviSpine method and device (Dr. M. Kluszczyński) is also the coordinator for the "Tronina" Medical Rehabilitation Center, where the GraviSpine device was evaluated. Further research with larger sample sizes and controlled designs would provide additional insights into the efficacy and safety of GraviSpine-assisted therapy.

Conclusions

To enhance the effectiveness of conservative treatment of IS, consideration may be given to incorporating an assistive device like GraviSpine, especially in cases where functional inequality of the lower limbs is diagnosed.

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References

1. Rigo MD, Grivas TB. "Rehabilitation schools for scoliosis" thematic series: Describing the methods and results. *Scoliosis*. 2010;5(1):27. doi:10.1186/1748-7161-5-27
2. Weinstein SL, Dolan LA, Cheng JC, Danielsson A, Morcuende JA. Adolescent idiopathic scoliosis. *Lancet*. 2008;371(9623):1527–1537. doi:10.1016/S0140-6736(08)60658-3
3. Goldberg MS, Mayo NE, Poitras B, Scott S, Hanley J. The Ste-Justine Adolescent Idiopathic Scoliosis Cohort Study: Part I. *Spine (Phila Pa 1976)*. 1994;19(14):1551–1561. doi:10.1097/00007632-199407001-00003
4. Domenech J, García-Martí G, Martí-Bonmatí L, Barrios C, Tormos JM, Pascual-Leone A. Abnormal activation of the motor cortical network in idiopathic scoliosis demonstrated by functional MRI. *Eur Spine J*. 2011;20(7):1069–1078. doi:10.1007/s00586-011-1776-8
5. Hawes MC, O'Brien JP. The transformation of spinal curvature into spinal deformity: Pathological processes and implications for treatment. *Scoliosis*. 2006;1(1):3. doi:10.1186/1748-7161-1-3
6. Smania N, Picelli A, Romano M, Negrini S. Neurophysiological basis of rehabilitation of adolescent idiopathic scoliosis. *Disabil Rehabil*. 2008;30(10):763–771. doi:10.1080/17483100801921311
7. D'Amico M, Roncoletta P, Di Felice F, Porto D, Bellomo R, Saggini R. Leg length discrepancy in scoliotic patients. *Stud Health Technol Inform*. 2012;176:146–150.
8. Pasha S, Aubin CE, Sangole AP, Labelle H, Parent S, Mac-Thiong JM. Three-dimensional spinopelvic relative alignment in adolescent idiopathic scoliosis. *Spine (Phila Pa 1976)*. 2014;39(7):564–570. doi:10.1097/BRS.0000000000000193
9. Stylianides GA, Beaulieu M, Dalleau G, Rivard CH, Allard P. Iliac crest orientation and geometry in able-bodied and non-treated adolescent idiopathic scoliosis girls with moderate and severe spinal deformity. *Eur Spine J*. 2012;21(4):725–732. doi:10.1007/s00586-011-2070-5
10. Kluszczyński M. The incidence of dorsal and pelvis asymmetries in school-age children with hip dysplasia in the neonatal-infancy period. *Scoliosis*. 2007;2(Suppl 1):S33. doi:10.1186/1748-7161-2-S1-S33
11. Mooney V, Gulick J, Pozos R. A preliminary report on the effect of measured strength training in adolescent idiopathic scoliosis. *J Spinal Disord*. 2000;13(2):102–107. doi:10.1097/00002517-200004000-00002
12. Lateiner JE, Sainburg RL. Differential contributions of vision and proprioception to movement accuracy. *Exp Brain Res*. 2003;151(4):446–454. doi:10.1007/s00221-003-1503-1508.
13. Raczkowski JW, Daniszewska B, Zolynski K. Clinical research: Functional scoliosis caused by leg length discrepancy. *Arch Med Sci*. 2010;3:393–398. doi:10.5114/aoms.2010.14262
14. Grivas TB, Angouris K, Chandrinos M, Kechagias V. Truncal changes in children with mild limb length inequality: A surface topography study. *Scoliosis*. 2018;13(1):27. doi:10.1186/s13013-018-0173-z
15. Moseley CF. Leg-length discrepancy. *Pediatr Clin North Am*. 1986;33(6):1385–1394. doi:10.1016/S0031-3955(16)36149-1
16. Brady RJ, Dean JB, Skinner TM, Gross MT. Limb length inequality: Clinical implications for assessment and intervention. *J Orthop Sports Phys Ther*. 2003;33(5):221–234. doi:10.2519/jospt.2003.33.5.221
17. Sekiya T, Aota Y, Yamada K, Kaneko K, Ide M, Saito T. Evaluation of functional and structural leg length discrepancy in patients with adolescent idiopathic scoliosis using the EOS imaging system: A prospective comparative study. *Scoliosis Spinal Disord*. 2018;13(1):7. doi:10.1186/s13013-018-0152-4
18. Mannello DM. Leg length inequality. *J Manipulative Physiol Ther*. 1992;15(9):576–590.
19. Stricker S, Hunt T. Evaluation of leg length discrepancy in children. *Int Pediatr*. 2004;19(3):134–146.
20. Kotwicki T, Kubiak A, Szulc A. Static pelvic obliquity can influence clinical assessment of trunk rotation in idiopathic scoliosis. *Scoliosis*. 2009;4(Suppl 2):O2. doi:10.1186/1748-7161-4-S2-O2
21. Knutson GA. Anatomic and functional leg-length inequality: A review and recommendation for clinical decision-making. Part I, anatomic leg-length inequality: prevalence, magnitude, effects and clinical significance. *Chiropr Man Therap*. 2005;13(1):11. doi:10.1186/1746-1340-13-11
22. Landauer F. Diagnosis and treatment of leg-length discrepancy in scoliosis. *Scoliosis*. 2013;8(Suppl 2):O41. doi:10.1186/1748-7161-8-S2-O41

23. Cummings G, Scholz JP, Barnes K. The effect of imposed leg length difference on pelvic bone symmetry. *Spine (Phila Pa 1976)*. 1993; 18(3):368–373. doi:10.1097/00007632-199303000-00012
24. Gurney B. Leg length discrepancy. *Gait Posture*. 2002;15(2):195–206. doi:10.1016/S0966-6362(01)00148-5
25. Rannisto S, Okuloff A, Uitti J, et al. Leg-length discrepancy is associated with low back pain among those who must stand while working. *BMC Musculoskelet Disord*. 2015;16(1):110. doi:10.1186/s12891-015-0571-9
26. Betsch M, Rapp W, Przibylla A, et al. Determination of the amount of leg length inequality that alters spinal posture in healthy subjects using rasterstereography. *Eur Spine J*. 2013;22(6):1354–1361. doi:10.1007/s00586-013-2720-x
27. Torell G, Nachemson A, Haderspeck-Grib K, Schultz A. Standing and supine Cobb measures in girls with idiopathic scoliosis. *Spine (Phila Pa 1976)*. 1985;10(5):425–427. doi:10.1097/00007632-198506000-00004
28. Sastre Fernández S, Grzyborowska M, Śliwiński Z. *Metoda leczenia skolioz, kifoz i lordoz*. Ostrowiec Świętokrzyski, Poland: Wydawnictwo Markmed Rehabilitacja; 2008.
29. Trzcińska S, Koszela K. Retrospective analysis of FED method treatment results in 11–17-year-old children with idiopathic scoliosis. *Children*. 2022; 9(10):1513. https://doi.org/10.3390/children9101513
30. Stolarz A, Suchanowski A. *Korekcja Bocznych Skrzywień Kręgosłupa: Skojarzone Postępowanie Metodą S-E-T i Aparatem "SKOL-AS."* Gdańsk, Poland: Wydawnictwo Uczelniane Akademii Wychowania Fizycznego i Sportu; 2011.
31. Negrini S, Donzelli S, Aulisa AG, et al. 2016 SOSORT guidelines: Orthopaedic and rehabilitation treatment of idiopathic scoliosis during growth. *Scoliosis Spinal Disord*. 2018;13(1):3. doi:10.1186/s13013-017-0145-8
32. Kluszczyński M, Zaborowska-Sapeta K, Kowalski I, Karpiel I. The effectiveness of early rehabilitation in limiting the progression of idiopathic scoliosis. *J Clin Med*. 2024;13(5):1422. doi:10.3390/jcm13051422
33. Cooperstein R, Lucente M. Comparison of supine and prone methods of leg length inequality assessment. *J Chiropract Med*. 2017;16(2):103–110. doi:10.1016/j.jcm.2017.01.001
34. Donnelly JM, ed. *Travell, Simons & Simons' Myofascial Pain and Dysfunction: The Trigger Point Manual*. 3rd ed. Baltimore, USA: Wolters Kluwer; 2019.
35. Friberg O, Nurminen M, Korhonen K, Soininen E, Mänttäri T. Accuracy and precision of clinical estimation of leg length inequality and lumbar scoliosis: Comparison of clinical and radiological measurements. *Int Disabil Stud*. 1988;10(2):49–53. doi:10.3109/09638288809164098
36. Kuru T, Yeldan İ, Dereli EE, Özdinçler AR, Dikici F, Çolak İ. The efficacy of three-dimensional Schroth exercises in adolescent idiopathic scoliosis: a randomised controlled clinical trial. *Clin Rehabil*. 2016;30(2):181–190. doi:10.1177/0269215515575745
37. Schreiber S, Parent EC, Khodayari Moez E, et al. Schroth physiotherapeutic scoliosis-specific exercises added to the standard of care lead to better Cobb angle outcomes in adolescents with idiopathic scoliosis: An assessor and statistician blinded randomized controlled trial. *PLoS ONE*. 2016;11(12):e0168746. doi:10.1371/journal.pone.0168746
38. Lonstein JE, Carlson JM. The prediction of curve progression in untreated idiopathic scoliosis during growth. *J Bone Joint Surg*. 1984;66(7):1061–1071. doi:10.2106/00004623-198466070-00013
39. Park JH, Jeon HS, Park HW. Effects of the Schroth exercise on idiopathic scoliosis: A meta-analysis. *Eur J Phys Rehabil Med*. 2018;54(3):440–449. doi:10.23736/S1973-9087.17.04461-6
40. Trzcińska S, Koszela K, Kuszewski M. Effectiveness of the FED method in the treatment of idiopathic scoliosis of girls aged 11–15 years. *Int J Environ Res Public Health*. 2021;19(1):65. doi:10.3390/ijerph19010065
41. Kamelska-Sadowska AM, Protasiewicz-Fałdowska H, Zakrzewska L, Zaborowska-Sapeta K, Nowakowski JJ, Kowalski IM. The effect of an innovative biofeedback SKOL-AS® treatment on the body posture and trunk rotation in children with idiopathic scoliosis: Preliminary study. *Medicina (Kaunas)*. 2019;55(6):254. doi:10.3390/medicina55060254
42. Seleviciene V, Cesnaviciute A, Strukcinskiene B, Marcinowicz L, Strazdiene N, Genowska A. Physiotherapeutic scoliosis-specific exercise methodologies used for conservative treatment of adolescent idiopathic scoliosis, and their effectiveness: An extended literature review of current research and practice. *Int J Environ Res Public Health*. 2022;19(15):9240. doi:10.3390/ijerph19159240
43. Kocaman H, Bek N, Kaya MH, Büyükturan B, Yetiş M, Büyükturan Ö. The effectiveness of two different exercise approaches in adolescent idiopathic scoliosis: A single-blind, randomized-controlled trial. *PLoS ONE*. 2021;16(4):e0249492. doi:10.1371/journal.pone.0249492
44. Kwan KYH, Cheng ACS, Koh HY, Chiu AYY, Cheung KMC. Effectiveness of Schroth exercises during bracing in adolescent idiopathic scoliosis: Results from a preliminary study: SOSORT Award 2017 Winner. *Scoliosis Spinal Disord*. 2017;12(1):32. doi:10.1186/s13013-017-0139-6
45. Negrini S, Fusco C, Minozzi S, Atanasio S, Zaina F, Romano M. Exercises reduce the progression rate of adolescent idiopathic scoliosis: Results of a comprehensive systematic review of the literature. *Disabil Rehabil*. 2008;30(10):772–785. doi:10.1080/09638280801889568
46. Monticone M, Ambrosini E, Cazzaniga D, Rocca B, Ferrante S. Active self-correction and task-oriented exercises reduce spinal deformity and improve quality of life in subjects with mild adolescent idiopathic scoliosis: Results of a randomised controlled trial. *Eur Spine J*. 2014;23(6):1204–1214. doi:10.1007/s00586-014-3241-y
47. Weinstein SL, Dolan LA, Wright JG, Dobbs MB. Effects of bracing in adolescents with idiopathic scoliosis. *N Engl J Med*. 2013;369(16):1512–1521. doi:10.1056/NEJMoa1307337
48. Kluszczyński M, Pilis A, Czaprowski D. The importance of the size of the trunk inclination angle in the early detection of scoliosis in children. *BMC Musculoskelet Disord*. 2022;23(1):5. doi:10.1186/s12891-021-04965-4
49. Simoneau M, Mercier P, Blouin J, Allard P, Teasdale N. Altered sensory-weighting mechanisms is observed in adolescents with idiopathic scoliosis. *BMC Neurosci*. 2006;7(1):68. doi:10.1186/1471-2202-7-68
50. Cheung J, Veldhuizen AG, Halbertsma JPK, et al. The relation between electromyography and growth velocity of the spine in the evaluation of curve progression in idiopathic scoliosis. *Spine (Phila Pa 1976)*. 2004;29(9):1011–1016. doi:10.1097/00007632-200405010-00012
51. Staudenmann D, Roeleveld K, Stegeman DF, Van Dieën JH. Methodological aspects of SEMG recordings for force estimation: A tutorial and review. *J Electromyogr Kinesiol*. 2010;20(3):375–387. doi:10.1016/j.jelekin.2009.08.005
52. Canavese F, Dimeglio A, Granier M, et al. Arthrodesis of the first six dorsal vertebre in prepubertal New Zealand White rabbits and thoracic growth to skeletal maturity: The role of the "Rib-Vertebral-Sternal complex." *Minerva Ortop Traumatol*. 2007;58(5):369–377.
53. Stokes IA, Burwell RG, Dangerfield PH. Biomechanical spinal growth modulation and progressive adolescent scoliosis: A test of the "vicious cycle" pathogenetic hypothesis: Summary of an electronic focus group debate of the IBSE. *Scoliosis*. 2006;1(1):16. doi:10.1186/1748-7161-1-16
54. Little JP, Izatt MT, Labrom RD, Askin GN, Adam CJ. Investigating the change in three dimensional deformity for idiopathic scoliosis using axially loaded MRI. *Clin Biomech*. 2012;27(5):415–421. doi:10.1016/j.clinbiomech.2011.12.004
55. Curtin M, Lowery MM. Musculoskeletal modelling of muscle activation and applied external forces for the correction of scoliosis. *J Neuroeng Rehabil*. 2014;11(1):52. doi:10.1186/1743-0003-11-52

Hematologists' state of knowledge on practical aspects of hemophagocytic lymphohistiocytosis (HLH) in adult patients: A Young Hematologists' Club survey

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Abstract

Background. Hemophagocytic lymphohistiocytosis (HLH) is a life-threatening hyperinflammatory syndrome with various etiologies. Its treatment is complicated by several important but not immediately obvious issues (e.g., HLH-2004 criteria are the most commonly used for diagnosis, but the recommended therapeutic regimen is HLH-94).

Objectives. The study aimed to assess hematologists' practical knowledge of HLH.

Materials and methods. A survey was conducted among physicians treating adult hematological patients. A 10-question paper questionnaire was distributed to physicians from various hematology centers. A total of 126 questionnaires were analyzed.

Results. Most respondents had little-to-moderate experience in caring for HLH patients: 59% treated 0–2 patients and 36% treated between 3–5 patients. Among the respondents, the preferred diagnostic criteria were HLH-2004, either in its original form (5 out of 8 criteria) for 70 respondents or its modified version (4 out of 6 available criteria when ferritin exceeds 2,000 ng/mL) for 56 respondents. The preferred treatment regimen was HLH-2004, with a full dose of etoposide in 72 responses or reduced in 39 responses. Fifty percent of respondents incorrectly answered that meeting the HLH-2004 criteria necessitates the use of the HLH-94/2004 regimen in full doses and duration. Sixty-four percent of respondents correctly identified that hemophagocytosis is not necessary for the diagnosis.

Conclusions. This survey reveals that the majority of surveyed physicians adhere to international HLH recommendations. However, there are instances where these guidelines are not fully implemented, which underlines the need for further efforts to raise awareness and share clinical experiences about this patient group.

Key words: hemophagocytic lymphohistiocytosis, HLH, questionnaire, hemophagocytic syndrome

Background

Hemophagocytic lymphohistiocytosis (HLH) is a rare disorder characterized by extreme inflammation. Its pathogenesis is complex. Familial HLH in children is associated with mutations in *PRF1*, *STX11*, *STXBP2*, *UNC13D*, and other genes causing syndromes.[1] In adults, although some degree of genetic predisposition is required, the main role is attributed to the triggering factor, with the most important being malignancies (especially lymphoma), infections (notably Epstein–Barr virus (EBV)) and autoimmune diseases (in this context, hyperinflammation is often called macrophage activation syndrome).[2] Differential diagnosis can be challenging because HLH shares similarities with other conditions characterized by high inflammation. Symptoms may include fever, hepatosplenomegaly, cytopenia, hyperferritinemia, hypertriglyceridemia, and hypofibrinogenemia, which are part of the HLH-2004 criteria.[3] Additional symptoms may include jaundice, rash and hypertransaminasemia. Prognosis depends on multiple factors, such as the triggering factor, patient age and the intensity of hyperinflammation. If HLH is not diagnosed and treated promptly, it can be fatal.

Hemophagocytic lymphohistiocytosis went unrecognized for many years, but efforts were made to raise awareness – first in pediatric and subsequently in adult settings. In Poland, awareness of HLH grew significantly following publications and lectures by Prof. Wiesław Wiktor Jędrzejczak.[4] Today, HLH is well recognized among hematologists and is included in textbooks and articles aimed at specialists in internal medicine.[5,6] In recent years, the Histocyte Society has issued important recommendations, including the use of etoposide,[7] management approaches for adult HLH patients[8] and considerations for HLH in intensive care settings.[9]

Despite these efforts, HLH gained widespread recognition relatively late, particularly over the past decade. Additionally, HLH is heterogeneous, and some aspects may seem counterintuitive; e.g., diagnostic criteria are based on HLH-2004, but the treatment follows HLH-94 guidelines (with reduced etoposide dosing for adults). Understanding these elements is fundamental for optimal patient care, yet the overall experience level among hematologists remains relatively low. Moreover, there is a lack of studies evaluating the current knowledge of HLH among physicians.

Objectives

The objective of this study was to evaluate the knowledge of hematologists regarding the practical aspects of HLH diagnosis and treatment in adult patients.

Materials and methods

Study design

The study was held under the aegis of the Young Hematologists' Club. To assess physicians' knowledge of HLH, a quantitative method was adopted using a paper-based questionnaire. This cross-sectional study utilized a 1-page questionnaire comprising 10 closed questions (with an option to write any chosen answer as "other"). The original questionnaire is available in the Supplementary material (https://doi.org/10.5281/zenodo.11209475). The questionnaire was designed uniquely: Most answers were partially correct but tailored either to pediatric or adult HLH patients. Thus, differences in interpretation were attributed to the physician's patient population rather than the test itself. Given that respondents in this study exclusively treated adult patients, some of their responses were incorrect.

Data collection methods

Questionnaires were distributed during sessions at the 28th Congress of the Polish Society of Hematology and Transfusion Medicine (September 2019), as well as in courses for hematology specialists and various clinical centers (both academic and non-academic) until the end of 2021.

Study preparation and survey administration

The survey was anonymous, with participants instructed to participate only once. Interviewers were instructed to mix the received questionnaires with previously collected ones to ensure anonymity.

A total of 137 questionnaires were obtained, with 126 included in further analyses. Eleven questionnaires were excluded for the following reasons: respondents were from medical professions other than adult hematology physicians, or there was a complete lack of answers regarding HLH. Manual data entry was assisted by a custom-designed color-coded spreadsheet to minimize human error. Moreover, unique numbers were assigned to the anonymous questionnaires during data entry to facilitate reanalysis in potentially ambiguous cases.

Statistical analyses

The questionnaire included questions only about qualitative (not quantitative) parameters; therefore, the results are presented as numbers of answers and percentages of total responses. No imputation methods were used for missing data. Univariate analyses were performed using Fisher's exact test, with significance set at a p-value of less than 0.05. The Bonferroni correction was applied for multiple

comparisons (with a p-value <0.00625 considered significant for 8 comparisons). Analyses were performed using MedCalc v. 22.019 software (MedCalc Software, Ostend, Belgium).

Results

Respondents were categorized based on their overall work experience and expertise in treating HLH patients (Table 1). Thirty-seven (37.3%) physicians reported less than 10 years of experience. Practical involvement in treating HLH patients was generally low, with 73 (57.9%) respondents caring for fewer than 3 such patients and 45 (35.7%) caring for between 3 and 5.

Table 1. Respondents' characteristics (n = 126)

Years working as a physician [n, %]		
<10	47	37.9%
10–20	45	36.3%
21–30	22	17.7%
31–40	6	4.8%
>40	4	3.2%
Number of HLH patients taken care of (total) [n, %]		
0–2	73	58.9%
3–5	45	36.3%
5–10	3	2.4%
11–20	2	1.6%
>20	1	0.8%

HLH – hemophagocytic lymphohisiocytosis.

In the analyzed population, the preferred diagnostic criteria were HLH-2004, either in its original form (5 out of 8 criteria), chosen by 70 respondents, or its modified version (4 out of 6 available criteria when ferritin exceeds 2000 ng/mL), selected by 56 respondents (Fig. 1; multiple answers were possible). The preferred treatment regimen was HLH-2004, with a full dose of etoposide chosen in 72 responses, whereas in 39 responses reduced dosing was chosen. As multiple answers were possible, the result describes number of responses not respondents. One respondent could have chosen more than 1 response (Fig. 2).

The final part of the questionnaire evaluated responses (yes/no/do not know) to 4 statements that are false for adult HLH patients (Fig. 3). Overall, most of the questions were answered in accordance with guidelines: 54 (42.9%) physicians answered 3 out of 4 questions correctly, and 24 (19.0%) correctly answered all 4 questions in accordance with guideline recommendations. Only 4 participants (3.2%), who had limited experience in treating adult HLH patients, did not answer any questions correctly. The least frequently opposed false statement was that fulfillment of HLH-2004 criteria necessitates the use of the HLH-2004 protocol at full doses and duration (45 physicians, 35.7%). Univariate analysis did not reveal significant differences between physician subgroups based on their work experience (less or more than 10 years) or the number of HLH patients treated (2 or more), as summarized in Table 2.

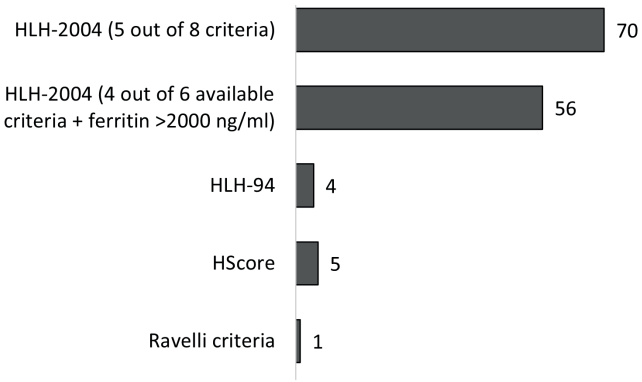

Fig. 1. Preferred diagnostic criteria (more than 1 answer possible)

HLH – hemophagocytic lymphohisiocytosis.

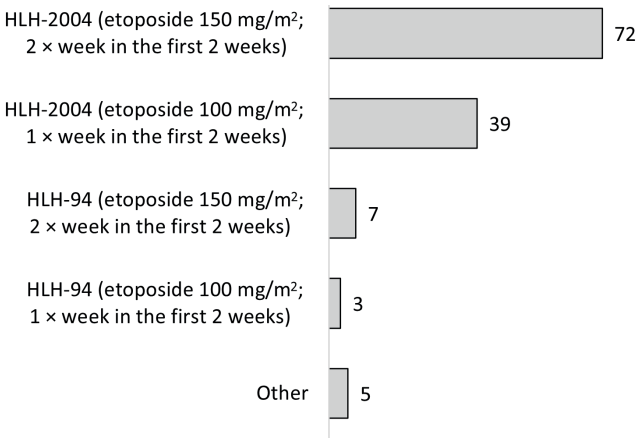

Fig. 2. Preferred treatment regimen

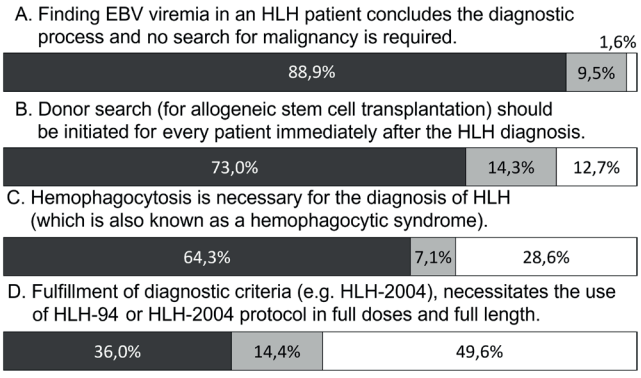

Fig. 3. Opinions about 4 statements that are not true for adult hemophagocytic lymphohistiocytosis (HLM) patients

EBV – Epstein–Barr virus.

Table 2. Number and percentage of correct (negative) opinions about 4 statements from Fig. 3, divided by physicians' work experience and number of HLH patients treated

Statement	Years working as a physician			Number of HLH patients taken care of (total)		
	<10	≥10	p-value (Fisher's exact test)	0–2	>2	p-value (Fisher's exact test)
A. Finding EBV viremia in an HLH patient concludes the diagnostic process and no search for malignancy is required.	45 (96%)	65 (84%)	0.078	63 (86%)	47 (92%)	0.394
B. Donor search (for allogeneic stem cell transplantation) should be initiated for every patient immediately after the HLH diagnosis.	31 (66%)	59 (77%)	0.218	48 (66%)	42 (82%)	0.065
C. Hemophagocytosis is necessary for the diagnosis of HLH (which is also known as a hemophagocytic syndrome).	30 (64%)	49 (64%)	1	45 (62%)	35 (69%)	0.452
D. Fulfillment of diagnostic criteria (e.g., HLH-2004), necessitates the use of HLH-94 or HLH-2004 protocol in full doses and full length.	22 (47%)	23 (30%)	0.083	19 (26%)	26 (51%)	0.0076

HLH – hemophagocytic lymphohisiocytosis; EBV – Epstein–Barr virus; Fisher's exact test, with Bonferroni correction for multiple comparisons, was used ($p < 0.00625$ was considered significant).

Discussion

The questionnaire findings indicate awareness of practical aspects related to HLH diagnosis and treatment; however, there is still considerable potential for improvement.

Results from the question regarding the preferred diagnostic system generally show a consensus that the HLH-2004 criteria are the most popular. However, the main limitation of HLH-2004 is that 2 out of 8 parameters (sCD25 concentration and NK-cell activity) are not widely accessible. Attempts to address this issue include modified criteria proposed by Prof. Wiesław Wiktor Jędrzejczak (requiring 4 out of 5 available criteria, when ferritin exceeds 2,000 ng/mL, with no other identifiable cause) or the HScore, which relies only on commonly used parameters.[10] Using these systems together can improve diagnostic certainty, given that initiating HLH treatment may result in significant toxicity.

Hemophagocytic lymphohistiocytosis treatment depends on the triggering factor. Immunosuppressive treatment, even for patients meeting HLH-2004 criteria, may not be indicated – e.g., in cases of "HLH disease mimics".[11] Many patients may benefit more from direct therapy targeting the underlying trigger, such as chemotherapy appropriate for a diagnosed malignancy, rather than etoposide with dexamethasone. Respondents were asked about their preferred HLH-oriented treatment regimen, with options including a combination of HLH-94 and HLH-2004 protocols with 2 doses of etoposide: full (etoposide 150 mg/m^2; 2 × week in the first 2 weeks) or reduced (etoposide 100 mg/m^2; 1 × week in the first 2 weeks). Full pediatric doses can be excessively high and toxic for adults because children are able to recover form higher doses of chemotherapy than adults. Professor Jan-Inge Henter proposed a regimen with reduced etoposide frequency for treatment of HLH-like severe avian influenza.[12] This reduced, less myelotoxic, regimen was then generally recommended for treating HLH in adults.[6,8]

HLH-94 and HLH-2004 refer to names of pediatric clinical trials.[3] In a single-arm design, these trials tested treatment regimens believed to be optimal. The HLH-2004 regimen is very similar to HLH-94, with the main difference being the timing of initiating cyclosporine A: after 8 weeks in HLH-94 or from the beginning of treatment in HLH-2004. This modification did not significantly affect the comparison of these 2 trials – despite HLH-94 patients being a historical control, HLH-2004 did not demonstrate superiority ($p = 0.064$; proportional hazards model using competing risks methodology, adjusted for age and sex).[13] Therefore, the recommended regimen for adults is HLH-94 with reduced doses of etoposide. It should be noted that diagnostic criteria also take names from these trials. While HLH-94 diagnostic criteria are no longer in use, the HLH-94 treatment regimen remains the basis for treatment, alongside the HLH-2004 diagnostic criteria.

The 4 statements presented in Fig. 3 are false for adult patients, but 2 may be true for the pediatric population. As discussed above, meeting the HLH-2004 criteria does not automatically require initiating the HLH-94 or HLH-2004 protocol at full doses and duration. The treatment regimen should be tailored to the specific triggering factor. If an HLH-oriented protocol is initiated in adults, it does not need to continue for a fixed duration; it can be tapered and stopped once remission is achieved. In children, treatment must be extended as a bridge to allogeneic hematopoietic stem cell transplantation (alloHSCT), which leads to different considerations about donor search between adults and children. For adults, a donor search does not need to begin immediately after diagnosis because only a small fraction of adult HLH patients require alloHSCT (87 patients in EBMT databases from 1995–2018).[14] The alloHSCT is indicated for adults with primary HLH and, on a clinical basis, for some other patients (relapsed/refractory).[8] In most adult patients, HLH is triggered by a significant factor (e.g., malignancy) with a lesser role of genetic predisposition. Therefore,

treatment focuses more on controlling the trigger (e.g., achieving malignancy remission) rather than replacing the immune system with a new one less prone to HLH. In children, a strong genetic predisposition causes HLH when exposed to seemingly minor triggers. Because it is impossible to protect such a child from all potential triggers (e.g., viral infections), alloHSCT is the only option for long-term remission. For asymptomatic siblings with certain predisposing mutations, undergoing upfront alloHSCT before developing HLH can be more beneficial than waiting for HLH to manifest[15]

Two statements that are false for all HLH patients, refer to EBV being accompanied by another trigger and to hemophagocytosis. Finding Epstein–Barr viremia does not conclude the diagnostic process; there remains a risk of underlying malignancy, such as lymphoma. Malignancy is less likely in infants, but teenagers are at higher risk. Hemophagocytosis is not necessary for HLH diagnosis. It is one of the criteria but cannot be considered pathognomonic for HLH. It can occur in various other conditions and may initially be absent, developing in later stages of HLH.[16]

Limitations

The main limitation of this research was due to the COVID-19 pandemic, which made distributing paper questionnaires much more difficult. Switching to an online questionnaire could have affected the homogeneity of the results, so with long intervals, a paper version was used. However, since the beginning of the questionnaire distribution, there was no change in recommendations that would affect the answers.

This is the first (to our knowledge) attempt to assess physicians' adherence to the diagnostic and treatment recommendations for HLH globally, especially among those treating adult patients. In 2019–2021, there were 555–585 hematologists and 235–272 hematologists in training in Poland, making the group of 126 respondents generally representative of this population.

As the first study of its kind, this research was innovative and exploratory. Therefore, it is worth continuing to monitor the state of knowledge regarding HLH. This will enable faster diagnosis of this rare disease in the future and the application of different treatments for children and adults.

Conclusions

The results of the Young Hematologists' Club survey reveal that most surveyed physicians adhere to international HLH recommendations. However, in some cases, these guidelines are still not being adequately implemented, highlighting the need for further actions to raise awareness and share clinical experiences about this patient group.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Zhang K, Astigarraga I, Bryceson Y, et al. Familial hemophagocytic lymphohistiocytosis. In: Adam MP, Feldman J, Mirzaa GM, et al., eds. *GeneReviews*. Seattle, USA: University of Washington; 2006:Bookshelf ID: NBK1444. http://www.ncbi.nlm.nih.gov/books/NBK1444. Accessed April 26, 2024.
2. La Rosée P, Machowicz R. HLH in adults. In: Abla O, Janka G, eds. *Histiocytic Disorders*. Cham, Switzerland: Springer International Publishing; 2018:275–290. doi:10.1007/978-3-319-59632-7_16
3. Henter J, Horne A, Aricó M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. 2007;48(2):124–131. doi:10.1002/pbc.21039
4. Machaczka M. Specyfika występowania limfohistiocytozy hemofagocytarnej w okresie wieku dorosłego. *Acta Haematol Pol*. 2013;44(3):307–313. doi:10.1016/j.achaem.2013.07.020
5. Jędrzejczak WW. Zespół hemofagocytowy. In: Augustynowicz-Kopeć E, Gajewski P, Szczeklik A, eds. *Interna Szczeklika: Podręcznik Chorób Wewnętrznych 2013*. Cracow, Poland: Medycyna Praktyczna; 2013:1728–1729.
6. Machowicz R, Basak G. How can an internal medicine specialist save a patient with hemophagocytic lymphohistiocytosis (HLH)? *Pol Arch Intern Med*. 2020;130(5):431–437. doi:10.20452/pamw.15226
7. Ehl S, Astigarraga I, Von Bahr Greenwood T, et al. Recommendations for the use of etoposide-based therapy and bone marrow transplantation for the treatment of HLH: Consensus Statements by the HLH Steering Committee of the Histiocyte Society. *J Allergy Clin Immunol Pract*. 2018;6(5):1508–1517. doi:10.1016/j.jaip.2018.05.031
8. La Rosée P, Horne A, Hines M, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood*. 2019;133(23):2465–2477. doi:10.1182/blood.2018894618
9. Hines MR, Von Bahr Greenwood T, Beutel G, et al. Consensus-based guidelines for the recognition, diagnosis, and management of hemophagocytic lymphohistiocytosis in critically ill children and adults. *Crit Care Med*. 2022;50(5):860–872. doi:10.1097/CCM.0000000000005361
10. Fardet L, Galicier L, Lambotte O, et al. Development and validation of the HScore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis Rheum*. 2014;66(9):2613–2620. doi:10.1002/art.38690
11. Jordan MB, Allen CE, Greenberg J, et al. Challenges in the diagnosis of hemophagocytic lymphohistiocytosis: Recommendations from the North American Consortium for Histiocytosis (NACHO). *Pediatr Blood Cancer*. 2019;66(11):e27929. doi:10.1002/pbc.27929
12. Henter JI, Chow CB, Leung CW, Lau YL. Cytotoxic therapy for severe avian influenza A (H5N1) infection. *Lancet*. 2006;367(9513):870–873. doi:10.1016/S0140-6736(06)68232-9

13. Bergsten E, Horne A, Aricó M, et al. Confirmed efficacy of etoposide and dexamethasone in HLH treatment: Long-term results of the cooperative HLH-2004 study. *Blood*. 2017;130(25):2728–2738. doi:10.1182/blood-2017-06-788349
14. Machowicz R, Suarez F, Wiktor-Jedrzejczak W, et al. Allogeneic hematopoietic stem cell transplantation for adult HLH: A retrospective study by the chronic malignancies and inborn errors working parties of EBMT. *Bone Marrow Transplant*. 2022;57(5):817–823. doi:10.1038/s41409-022-01634-5
15. Lucchini G, Marsh R, Gilmour K, et al. Treatment dilemmas in asymptomatic children with primary hemophagocytic lymphohistiocytosis. *Blood*. 2018;132(19):2088–2096. doi:10.1182/blood-2018-01-827485
16. Machowicz R, Janka G, Wiktor-Jedrzejczak W. Similar but not the same: Differential diagnosis of HLH and sepsis. *Crit Rev Oncol Hematol*. 2017;114:1–12. doi:10.1016/j.critrevonc.2017.03.023

Quality of education and mental health of pharmacy students in Poland

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Abstract

Background. Pharmacists in the healthcare system play an important role in providing safe, optimal pharmacotherapy and patient education. During their studies, in which they acquire the competencies to fulfill the pharmacist's future tasks, pharmacy students are exposed to significant stress and pressure.

Objectives. This study aims to demonstrate the extent to which the unique demands and obstacles of Polish pharmacy schools contribute to the deterioration of students' mental health and overall wellbeing.

Materials and methods. A cross-sectional study of 420 pharmacy students in Poland evaluates the quality of education at Polish universities and presents the impact of studying on students' mental health. The criteria for choosing the field of study, the particular major, the university itself, the quality of education, the academic work, and their impact on students' wellbeing were evaluated. The evaluation of the quality of education was influenced by mentoring and tutoring at the university. Pearson's χ^2 test and principal component analysis (PCA) were used in the statistical analyses.

Results. Unequal treatment of pharmacy students relative to students in other areas of medical study was marked by 90.2% of respondents, and opportunities for scientific development were indicated as good by 60.0% of pharmacy students. It was shown that 82.1% of the students rated studying as very stressful; the level of test difficulty and exams, as well as an inadequate level of knowledge imparted during classes contributed to this response. According to 75.2% of the respondents, the perceived stress had long-term effects in the form of anxiety and depression, with the need for pharmacotherapy.

Conclusions. It was shown that studies contributed to the onset and/or exacerbation of depressive and anxiety symptoms. The results indicate the need to support psychological care and extend it to to pharmacy students.

Key words: mental health, depression, education, pharmacy

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Background

Universities are an integral part of social development. They prepare the next generations to practice their profession and to benefit society.[1] Pharmaceutical science, one of the pillars of healthcare, is an exceptionally challenging field. The work of a pharmacist carries a responsibility that falls on the students as soon as they graduate. Medical studies are characterized by high level of stress, lots of study material and strong competition. It is not uncommon for students to be discriminated against, harassed or bullied; these might be perceived as a motivational approach for students to work more efficiently, but in practice, they often lead to demotivation and significant deterioration of mental health.[2,3] This carries long-term consequences not only for those directly affected but also for society. It makes it difficult and sometimes even impossible for students to function on a daily basis, and being forced to perform under severe stress obstructs their path to graduation and employment, which can contribute to a continuing decline in the number of people in the profession.[4]

Poland, among other countries, is struggling with the deterioration of students' mental health, especially those who have undertaken difficult and demanding studies. Anxiety disorders, depressive illnesses and other mental health issues are becoming increasingly common in the modern world.[5,6] Mental disorders represent 5 of the top 10 causes of disability. Their total treatment cost across Europe is estimated at more than 600 billion €.[7] This poses a problem for the national economy, which was exacerbated during the COVID-19 pandemic. At that time, exposure to various stress factors was increased due to uncertainty and health concerns.[8] The quarantines resulted in a lack of human interaction, thus leading to loneliness.[9] Additionally, remote learning in academic education worsened the problem of social isolation. The post-pandemic world has greatly changed. Digitalization has resulted in the weakening of social relationships and a decline in bonds within peer groups as a result of reduced opportunities for conversation during classes.[10]

Objectives

The aim of this study is to demonstrate to what extent pharmacy education in Poland, with its specific challenges and demands, contributes to the deterioration of students' mental health and wellbeing.

These findings are relevant not only to academic communities and other educational institutions but also to healthcare providers and students who are facing the problem of choosing their academic studies and career paths. Learning about the causes of the clinical problem of mental illness among the study group can contribute to more effective prevention of these disorders.

Materials and methods

Study design, setting and participants

A cross-sectional study assessing overall satisfaction with the quality of pharmacy student education in Poland and its impact on mental health was carried out.

The questionnaire consisted of 58 closed-ended multiple-choice single-answer questions. Our target group consisted of students and graduates of Polish universities (Table 1) who were studying or had completed a degree in pharmacy.

Students were required to complete an online survey using Google Forms in order to gather data. Universities and social networks were used to find study participants. Data were gathered between May 6 and July 6, 2023. A total of 420 students were included in the study, with 353 women (84.05%) and 67 men (15.95%). The study's participants were made aware that participation was optional and that responses would be kept confidential. Table 2 displays the study group's characteristics. Microsoft Excel (Microsoft Corp., Armonk, USA) was used to transfer responses from Google Forms (Google LLC, Mountain View, USA).

Table 1. Number of surveyed pharmacy students from each university

University	Number of respondents
Wroclaw Medical University	121
Medical University of Lodz	45
Poznan University of Medical Sciences	32
Pomeranian Medical University	21
Medical University of Silesia	56
Medical University of Bialystok	34
Medical University of Lublin	24
Collegium Medicum in Bydgoszcz	13
Medical University of Gdańsk	10
Jagiellonian University Medical College	13
Medical University of Warsaw	28
University of Opole	23

Table 2. Characteristics of the students group

Sex	Men	Women
Number of participants	67	353
Participants aged 18–25	51	277
Participants aged 26–30	14	58
Participants aged 30+	2	18
Students	47	254
Graduates	20	99

Variables

The data collected were divided into 3 main sections: "Introductory questions," "Study specifics and quality

of education" and "Mental health and academic issues." The "Introductory questions" section included information regarding the participant's age, sex, the university they took/had taken the pharmacy course at, and their current student status. The "Study specifics and quality of education" section contained inquiries about the main reason for choosing the particular university, the quality of education provided by the university, and the academic teachers. It also included information about the teachers' encouragement to ask questions, their attitude, their constructive evaluations of students' work, and the possibility of academic discussion with students, among other things. Participants were also asked if teachers established friendly contact with their students and contributed to the potential amicable atmosphere during classes. Questions regarding the defined grading criteria and fair grading process, along with adherence to the grading rules and standards, were also included. Participants' awareness of mentoring and tutoring opportunities at the university was examined as well. They were asked about the possibilities for scientific development and pursuing that career path at the university, their overall satisfaction with their chosen university, and whether they would recommend it to others.

The "Mental health and academic issues" section included questions regarding the amount of stress students faced before tests, exams and on a daily basis, as well as its consequences as a potentially harmful factor affecting their mental health. Attention was also drawn to the occurrence of depressive episodes and anxiety disorders, taking into account potential favoritism among teachers, harassment in verbal and non-verbal forms, sexist and chauvinistic comments, and acts of physical and psychological violence upon students. Access to psychological help offered by the university and respect for students' rights were also assessed. Participants were additionally asked about their amount of free time and whether pharmacy is equally treated and perceived by the academic authorities relative to other university courses.

Statistical analyses

Nominal scales, including dichotomous scales, were used for the variables subjected to statistical analysis. Pearson's non-parametric χ^2 test and principal component analysis (PCA), which is a method of visualizing the results for multiple comparisons of any variable to any variable and is based on the reduction of dimensions to a 2-dimensional scale – served as the basis for determining the statistical significance of the relationship between the analyzed variables. All variables in these analyses are treated the same and can, therefore, be used as dependent variables.

Regardless of the scale, PCA was used to assess overall correlations between the main study variables. It can be used to identify non-obvious and difficult-to-predict relationships between study variables with different scales of measurement. The Nonlinear Iterative Partial Least Squares (NIPALS) iterative technique was used to estimate the generated PCA model. The maximum number of iterations was set at 1,000, and the convergence threshold was set at 0.0001. The maximum predictive power of Q2 was calculated using the V-fold cross-validation method, assuming V_max = 7, and the number of components was calculated.

The results are presented in a graph (Fig. 1), along with information on the statistical significance of each component's contribution to the total percentage of explained variance and the reduction of the ideal PCA model to just 2 main components (PC 1 and PC 2).

A significance threshold of 0.05 was used in all statistical calculations. Statistica v. 13.3 PL (StatSoft Polska, Cracow, Poland) software was used to conduct the statistical tests and generate the presented graphs.

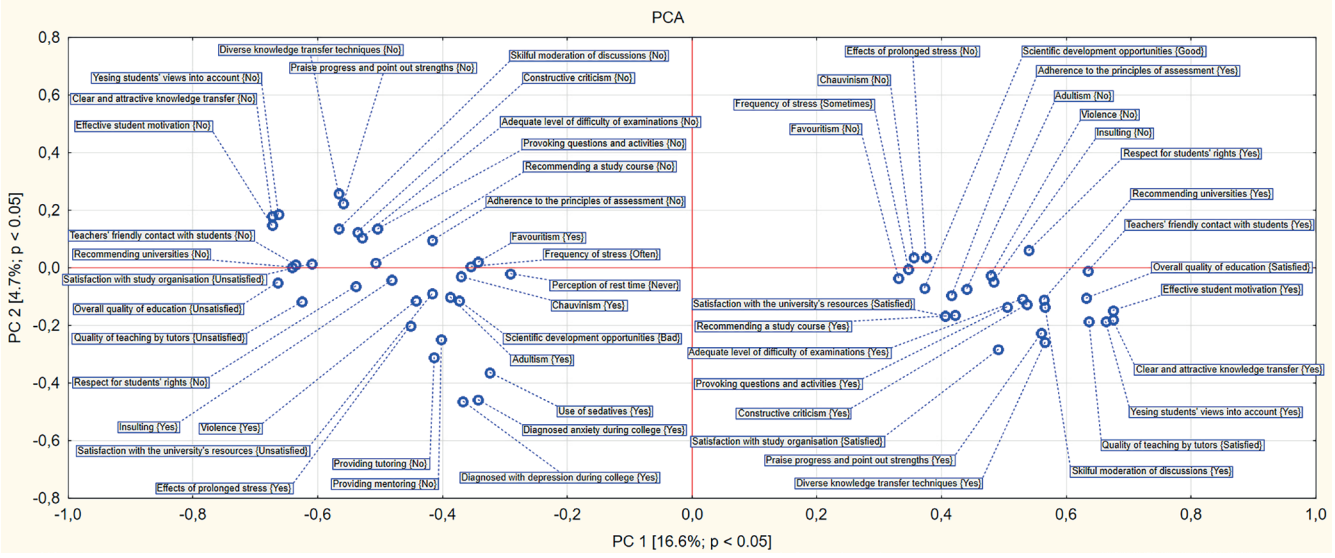

Fig. 1. The principal component analysis (PCA)

Results

Criteria for choosing the course and a university

Choosing pharmacy as a field of study was declared by 38.6% of respondents, with the reasoning that they wanted to work in a health-related field. For 18.6%, it was a second-choice due to not being accepted to their dream areas of study; for 16.7%, the criterion was finding employment after completing their education, and 16.0% said that pharmacy had always been their dream. In contrast, only 6.4% of respondents said that it was family, friends or the prestige of the profession that made them choose this field of study. The remaining 3.7% chose this major because of their desire to help others. When choosing a college, as many as 77.6% of respondents were guided by location, 11.0% said their choice was random, 7.1% said by rankings, and 4.3% said by advice from parents and/or friends.

Evaluation of education quality according to surveyed students

The survey showed that 36.4% of respondents were dissatisfied with the quality of education at pharmaceutical faculties, 34.1% were satisfied and the remaining 29.5% had no opinion. The correspondence analysis showed that students at the University of Opole were the most satisfied, while students at the Silesian Medical University in Sosnowiec were the least satisfied. Students' evaluation of their satisfaction with the quality of education at each university is shown in Fig. 2. The evaluation of the quality of education was influenced by mentoring ($p = 0.0008$, degrees of freedom (df) = 4) and tutoring ($p < 0.0001$, df = 4) at the university. When asked whether mentoring (a partnership between an academic teacher and a student to discover and develop a student's potential) was practiced at their university, respondents answered 5.0% in the affirmative, 48.6% in the negative and 46.4% had no knowledge of the subject. Only 2.14% of respondents had used mentoring opportunities at the university. It is worth noting that 53.81% said they were willing to use mentor support.

A similar relationship could be observed for tutoring (long-term, systematic and individual work, the purpose of which is to support the student in his development in accordance with his interests, aptitudes and abilities). When asked whether tutoring is conducted at their university, respondents answered 5.48% in the affirmative, 46.43% in the negative and 48.09% of them did not know. Only 3.33% of respondents used tutoring at the university, while 96.67% did not. A total of 50.95% of respondents would like to use the support of a tutor, 36.67% of respondents did not know about such an opportunity and 12.38% did not want to do so.

When asked whether the level of difficulty of tests and exams is adequate to the level of knowledge imparted in classes, as many as 53.6% of respondents answered negatively. Opportunities to gain practical experience in the field of pharmacy were negatively evaluated by 42.1% of respondents, positively by 26.4% and 31.4% gave a neutral answer, which could have an impact on the opinions and recommendation of the university ($p < 0.0001$, df = 4), as well as the faculty ($p < 0.0001$, df = 4), to future candidates. The majority of respondents (76.7%) believed that the pharmaceutical major is not treated equally compared to other majors at the medical school, which, according to 90.2% of respondents, declared it was associated with comparatively fewer privileges ($p = 0.0035$, df = 2), such as the ability to make up absences from classes.

In the evaluation of opportunities for scientific development at the university, for conducting research and for activities in student research groups, 60.0% said these opportunities were good, 18.3% said they were bad and 21.7% had no opinion. Assessment of student satisfaction with the overall organization and structure of the university

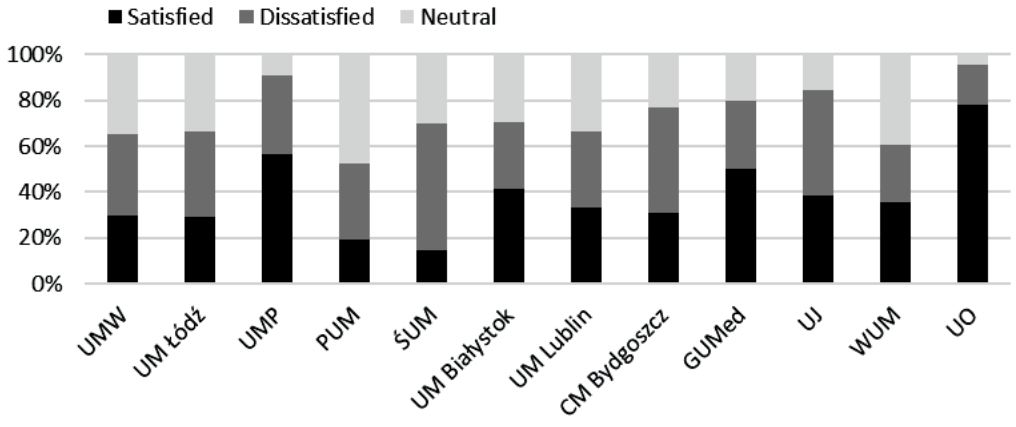

Fig. 2. The level of satisfaction with the overall quality of teaching at specific universities in Poland.

UMW – Wroclaw Medical University; UM Łódź – Medical University of Lodz; UMP – Poznan University of Medical Sciences; PUM – Pomeranian Medical University; ŚUM – Medical University of Silesia; UM – Białystok Medical University of Bialystok; UM – Lublin Medical University of Lublin; CM Bydgoszcz – Collegium Medicum in Bydgoszcz; GUMed – Medical University of Gdańsk; UJ – Jagiellonian University Medical College; WUM – Medical University of Warsaw; UO – University of Opole

showed that 55.7% of respondents were dissatisfied, 16.2% were satisfied and 28.1% declared neutral attitude. Among the factors negatively influencing this, respondents indicated, among other things, that students are belittled ($p < 0.0001$, df = 4), that pharmacy students are treated unequally to other medical majors ($p < 0.0001$, df = 4) and that students' rights are not respected ($p < 0.0001$, df = 2). A total of 78.8% of respondents felt that students' rights were respected, while 21.2% felt that they were not taken into account, which resulted in them not recommending to others their university ($p < 0.0001$, df = 2) or the pharmacy major they were studying ($p < 0.0001$, df = 2). Respondents whose rights were respected were less likely to experience long-term negative effects of stress ($p = 0.0016$, df = 1), to use tranquilizers ($p = 0.0029$, df = 1), and to experience depression ($p < 0.0001$, df = 1) and anxiety ($p = 0.0006$, df = 1).

A total of 50.0% of the respondents declared that they would not recommend the field of study to others, justifying this, among other things, by high stress ($p < 0.0001$, df = 4) and insufficient free time ($p = 0.0002$, df = 4), and also that they were ignored by the teaching staff due to their "lack of experience" both in life and work ($p = 0.0003$, df = 4) or due to the unequal treatment of pharmacy students relative to other medical school students ($p = 0.0014$, df = 4).

Students' opinion of academic teachers

Only 32.9% of the students surveyed said that academics effectively motivated them to work, while as many as 67.1% believed that they were ineffective in this regard. The effectiveness of motivating students to work was related to the year of study they were in ($p = 0.0371$, df = 5). Students in higher years felt less motivated compared to students in lower years, who experienced more effective motivation. The vast majority, representing 84.5% of respondents, answered that grading rules are consistently followed at the university. Praising progress and pointing out strengths by academics was reported by only 17.2% of respondents, while the vast majority of 82.8% said the opposite. Questions were provoked and activity encouraged according to 47.8% of respondents, 52.2% did not share this opinion and 64.3% of respondents felt that they were not listened to, while 35.7% indicated that their opinions were taken into account. In the case of the majority of respondents, 74.3% of faculty did not use varied techniques to impart knowledge. Comprehensible and attractive transfer of knowledge took place for 40.2% of respondents, while 59.8% rated the didactic process as unstructured and unattractive to receive.

According to 68.6% of the students, the academic staff did not moderate the group discussion well. This translated into opinions regarding opportunities for scientific development at the university ($p = 0.0222$, df = 2). A correlation was observed between the opportunities for scientific development perceived by students and the quality of discussion skills demonstrated by academic teachers. Overall, 47.9% of respondents declared that academics pointed out mistakes and addressed them using constructive criticism, while 52.1% did not share this position. The majority of respondents (64.3%) answered that academics established friendly contact with students. The survey found that the universities with the highest level of friendly contact with students were the University of Opole and the Warsaw Medical University, with Medical University of Silesia being the least friendly.

The majority of respondents (65.0%) experienced the phenomenon of some students being favored by university staff. Ignoring students' opinions due to their "inexperience" both in life and work was reported by 36.9% of respondents. As many as 60.5% of students experienced insults during classes. Slightly fewer, 56.2% of respondents, reported chauvinistic/sexist remarks from instructors. Some students, accounting for 29.8%, witnessed physical/psychological violence by academics during class. According to 84.6% of respondents, this occurred sometimes, and according to 15.4%, often.

When asked whether surveys evaluating academics are conducted at their university, 96.7% of respondents answered affirmatively. The survey showed that 27.6% of students do not fill them out; within this group, 32.7% of respondents explained their decision as due to the belief that their opinion does not count and will not change anything, 28.2% of respondents feared the consequences of giving a negative evaluation, and 28.2% of respondents forgot to fill out the survey. In contrast, 12.0% said it was too time-consuming. Students' opinions on the actions of university teachers are shown in Fig. 3.

Differences in the treatment of students of different sexes

Survey data showed that academics' willingness to establish friendly contact with students depended on the sex of the student ($p = 0.0275$, df = 1). More friendly attitudes were experienced by men. The level of stress ($p = 0.0017$, df = 2), experiencing its long-term effects ($p = 0.0003$, df = 2) and taking tranquilizers ($p = 0.001$, df = 1) were also dependent on sex. It was shown that women were more prone to stress. A relationship was also shown for the incidence of depression ($p = 0.0253$, df = 1), anxiety ($p = 0.0006$, df = 1) and exacerbation of symptoms of both disorders during the course of the study ($p = 0.0026$, df = 2). More women than men reported mood-related health problems. In addition, the declared amount of leisure time was dependent on the sex of the respondents ($p = 0.0173$, df = 2). Women experienced less leisure time during their studies than men. The effect of respondents' sex on their mental wellbeing was examined during the meta-analysis and is shown in Fig. 4.

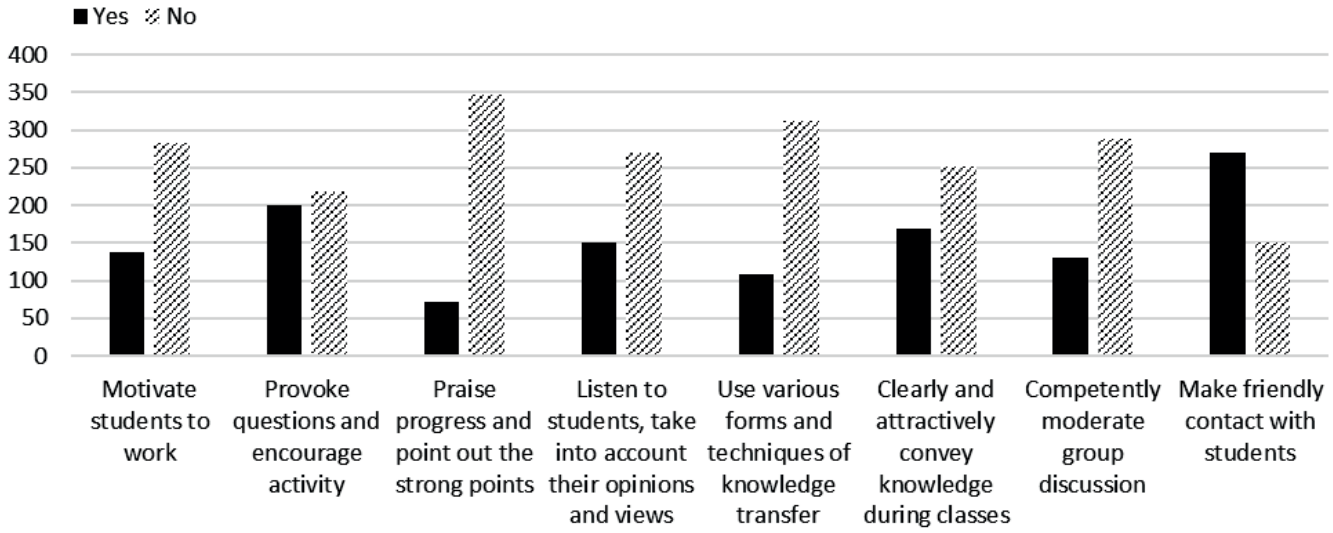

Fig. 3. Students' opinions on the actions taken by academicians

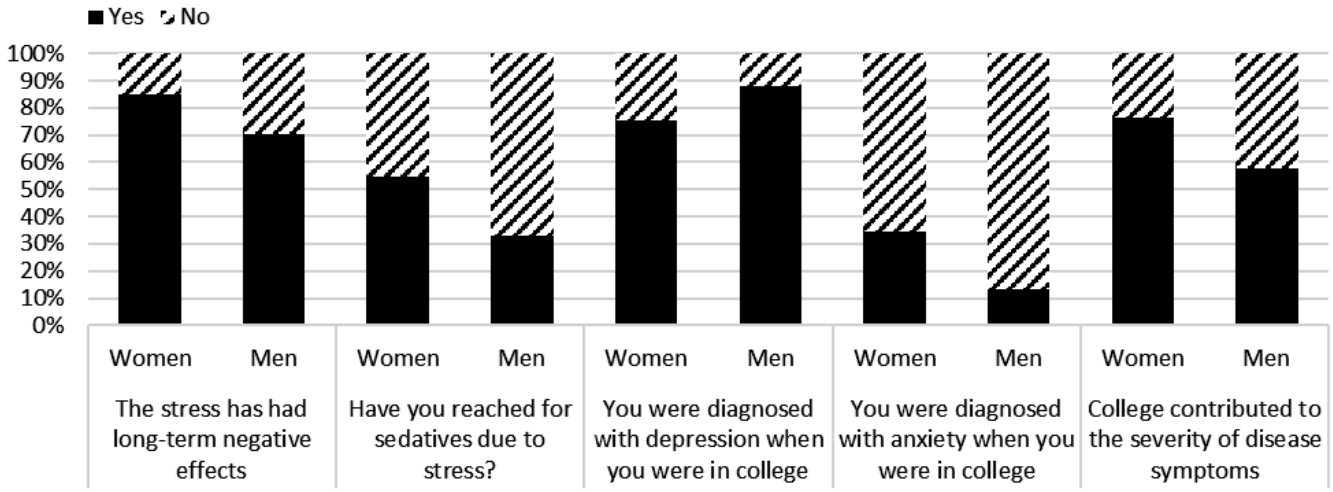

Fig. 4. Impact of sex on mental state

The impact of the teaching process on students' mental health

When asked how often they felt stressed because of classes and program requirements, 82.1% answered often, 16.7% answered sometimes and 1.2% answered never. One of the factors contributing to the level of perceived stress was the incongruency of test and exam difficulty relative to the level of knowledge imparted in class ($p < 0.0001$, $df = 1$). This phenomenon significantly contributed to the occurrence of anxiety ($p = 0.0068$, $df = 1$), depression ($p = 0.0125$, $df = 1$) as well as taking tranquilizers ($p = 0.0020$, $df = 1$). According to 75.2% of respondents, the perceived stress had long-term effects on them; for 15.7%, it had no long-term consequences and 9.1% had no opinion on the matter. The long-term consequences of perceived stress manifested themselves in anxiety ($p < 0.0001$, $df = 2$), as well as exacerbation of these symptoms ($p < 0.0001$, $df = 4$) and the need to take sedatives ($p < 0.0001$, $df = 2$). Also contributing to this phenomenon were insults to students by academics ($p = 0.0011$, $df = 4$), chauvinism ($p = 0.0339$, $df = 4$) and ignoring of student opinions due to "lack of experience" ($p = 0.0017$, $df = 4$). When asked if they were forced to turn to tranquilizers due to the stress of studying, 51.2% of the participants said yes, while 48.8% denied it. This was directly related to the previously mentioned stress, lack of free time and the overload of didactic content ($p = 0.0004$, $df = 2$). It was found that 22.4% of the students were diagnosed with depression during their studies and 31.2% with anxiety.

According to the respondents, the incidence of psychological and physical violence ($p = 0.0007$, $df = 2$), discrediting and belittling of students ($p = 0.0219$, $df = 2$), chauvinism ($p = 0.0388$, $df = 2$), as well as favoritism towards individual students ($p = 0.0024$, $df = 2$) were the main reasons for depression in students. Insulting students ($p = 0.0188$, $df = 2$) and psychological/physical violence by academics ($p = 0.0141$, $df = 2$) were shown to be the main contributors to the aforementioned conditions.

When asked about the impact of studying on exacerbation of the previously mentioned disorder symptoms, 22.5% of respondents answered negatively and 13.0% were unable to determine this. For 64.5% of respondents, studying led to an exacerbation of depressive and anxiety symptoms, which was significantly influenced by lack of free time to pursue activities outside of studying ($p = 0.0056$, $df = 4$), favoritism of students by university staff ($p = 0.0013$, $df = 4$), ignoring their opinions due to "lack of experience" ($p = 0.0031$, $df = 4$), the phenomenon of belittling ($p = 0.0076$, $df = 4$), chauvinism ($p = 0.0030$, $df = 4$), and physical as well as psychological violence during classes ($p < 0.0001$, $df = 4$). Only 15.5% of respondents said they often have plenty of free time to carry out activities outside of teaching, 70.5% said they sometimes find time and 14.0% responded never.

Assessment of the students' knowledge of the possibility of psychological help showed that 35.2% did not know about it, 58.3% were aware that they had access to such help and 6.5% claimed that they did not have it. Among the students who had access to psychological assistance, only 7.6% of the respondents used it.

Career path

The surveyed students had various plans for career path development. The largest group of respondents, accounting for 39.5%, were students who planned to start working in a community pharmacy after graduation; 15.2% said they would work in the pharmaceutical industry, 13.8% in the field of clinical research, 5.7% abroad in the pharmaceutical industry, 3.8% at university, and 3.4% planned to start working outside the field. Those with no specific plans accounted for 18.6% of respondents.

Of the surveyed pharmacy graduates, 83.7% were working in the profession. Among them, 76.2% worked in a community pharmacy, 16.2% in the pharmaceutical industry, 3.8% in a hospital pharmacy, 2.9% did research and teaching work at a university, and 1.0% found employment in a clinical pharmacy.

Discussion

In recent years, there has been an alarming global increase in the prevalence of mental health problems, regardless of age, occupation and socioeconomic status. This growing trend prompted the study and analysis of the academic communities training future pharmacists in Poland. This is important because mental health issues affect not only private but also professional life.

The aim of this study was to assess the mental health of pharmacy students at Polish universities and the quality of education. Attention was also paid to the atmosphere at the university, systemic inadequacies declared by students at individual universities, and the didactic skills and attitudes of academic staff towards students. The collected data made it possible to analyze and evaluate the impact of the above factors on mental health and satisfaction with the course of study at pharmaceutical faculties in different academic centers in Poland.

Other researchers have indicated that medical faculties, including pharmacy, place high demands on the amount of knowledge acquired, its enforcement and the practical skills necessary while working in the profession since human health and life are directly at stake. Students have reported high levels of stress, which are described as up to twice that of the general adult population.[11] Several studies have shown that this is especially true for students in medical professions (e.g., medicine, pharmacy).[12–14] Students experience high stress due to their study load, the large number of exams or the high difficulty of tests.[15] According to our results, women reported higher levels of stress than men.[16,17] As in our study, high levels of stress, anxiety and emotional distress were shown to lead to the development of depression and anxiety states,[18] of which were also more frequently experienced by women.[19,20] Our study found a correlation between sex and stress response, incidence of depression and experience of anxiety in the academic community. An increased incidence of the aforementioned conditions was observed in women.

The COVID-19 pandemic also had an impact on mental health and was a triggering factor for stress and anxiety among students. One of the effects of the pandemic was reduced physical contact with other people and decreased interaction with peers, which fostered depression.[21] A study conducted in Saudi Arabia found that medical students taking classes on a classroom basis with the onset of the COVID-19 pandemic began to feel less anxious after the introduction of classes conducted remotely. This may have been due to a greater sense of security and a lower risk of virus infection facilitated by frequent physical contact with people.[22] Also, a study published in 2022 showed a direct link between greater feelings of stress and poorer wellbeing among students. The effects of the mental burden caused by academic problems and the additional stressor of a newfound illness worsened the overall wellbeing of respondents.[23]

The COVID-19 pandemic has exacerbated the symptoms of depression in those diagnosed but also increased the number of patients suffering from the disorder. Experiencing stress due to the pandemic and online learning is one of the risk factors for depression. It has been shown that people with hobbies experienced less stress during the pandemic and thus had a lower risk of developing depression.[24,25] A solution to the problem of lack of hobbies could be the organization of extracurricular activities by the university, such as canoeing, team games and movie nights.

Lack of time to develop one's passions or engage in non-curricular activities, as indicated by participants in our study, can significantly affect wellbeing. Our results suggest that students who did not find time for non-curricular activities suffered the negative effects of prolonged stress in the form of depression and anxiety, which were often exacerbated and

required sedatives. A cross-sectional study conducted in Jordan with medical students also supports our hypothesis that hobbies have an impact on wellbeing and mental health.[26] A study conducted in the UK involving dental students also found that the opportunity to develop hobbies contributed to less stress and better wellbeing among students and had a beneficial effect on study–life balance.[27]

Two studies conducted in Southern Africa, which focused on satisfaction with studies, the psychological needs of pharmacy students and challenges along the path of study, indicated the importance of support directed to students by university lecturers. It was noted that students who reported being overwhelmed by a large amount of material perceived less support from lecturers.[28,29] A Nigerian study found little effect of mentoring on improving and meeting students' psychological needs despite its advantages in enhancing work motivation, study habits and academic performance.[30] The data show that there is a need to support students both in terms of professional orientation, increasing performance in completing academic activities, as well as supporting students' mental status, relieving the mental burden of academic pressure, and developing soft skills and social skills. The establishment of friendly contact by academics with students, as demonstrated in our study, can build an attitude of openness among students and overcome the internal barrier against asking questions of instructors, as well as enable them to draw on guidance and information from their professional experience.

The survey showed that a large percentage of students are unaware of the possibility of mentoring and tutoring at their university. This may be due to a lack of awareness among academics about the possibility of such initiatives and a general failure to disseminate information on the subject. The majority of respondents would be willing to take advantage of such an initiative, which could have a positive impact on talent and skill development, as well as the development of appropriate individual standards for self-improvement. This in turn would enable them to learn about areas of future professional work in which they could specialize and feel confident. The 2020 review and the 2023 analysis proved that the impact of positive mentor–student relationships can benefit mentees in their professional development, reducing differences in their experiences, but also in social aspects. It has also been emphasized that mentoring itself can have an impact on students' wellbeing and should be considered by programs and academia, as it is an important tool to enhance the development of professional identity.[31,32]

Receiving feedback is one of the most important aspects of quality education. It has been identified as a key component of clinical education programs and one of the most important tools affecting the ability to develop skills and engage in learning. Feedback can also play a part in communicating the progress made by the student.[33] Our research indicates that only 17.2% of students received feedback from academics in the form of praise for progress and strengths. It can have a significant impact on student motivation, but also, in particular, on improving the quality of the tasks performed – drawing attention to activities performed correctly, as well as pointing out elements that need to be improved or changed to avoid mistakes in the future. In another Polish study evaluating students' practical preparation for the profession, students pointed out the problem of insufficient practical preparation in the provision of pharmaceutical care, despite the fact that the students had adequate theoretical knowledge and that the competence of their academic teachers and the level of knowledge the teachers imparted was rated high. It was also pointed out that greater use of a variety of active teaching methods would improve the quality of student preparation in this area.[34] Assessment is an important aspect of educational quality and is a key function affecting the educational process, as it can be used to improve subsequent learning.[35] As an important component, it should be used in a standardized and unbiased manner. Our survey results show a positive trend, with 84.5% of university teachers consistently following established grading rules.

The quality of education, according to research reports, varies from university to university. A cross-sectional survey of Kuwait University's pharmacy students found that most students were satisfied with the quality of education. A total of 78.5% of respondents indicated that the study program had developed their problem-solving skills, and 66.4% also admitted that it had improved their communication skills. Unfortunately, in the same survey, 88% felt that the workload put a heavy physical as well as mental strain on them, which was also confirmed by the results of our survey.[36] In Brazil, 467 pharmacy schools were analyzed, most of which scored excellent in the category of quality of education.[37] Student achievement was considered in terms of factors of educational quality, but unfortunately, student opinions on educational quality were not included. This poses a problem in a reliable evaluation, as it does not provide information on the quality of education but only on educational outcomes, which may be, e.g., due to students' abilities.

A problem faced by students is the phenomenon of ageism, i.e., ignoring the opinions of young people due to age and inexperience as perceived by the academic faculty. In the field of healthcare, it has been shown that the older the patient is, the less biased the assessment of their clinical problem is. Thus, more unfairly, younger people face more subjective evaluation.[38] This phenomenon is the cause of a decrease in the quality of healthcare for young people due to ignoring their symptoms and opinions about their own health. The effect of experiencing ageism can also result in a lack of desire to help the elderly in the future due to fear of being judged.[39] The feeling that a younger person's opinion does not count also contributes to greater stress and, thus, the development of mental illness.[40] Our results confirm that students ignored due to "inexperience" do not feel motivated to learn ($p < 0.0001$, $df = 2$) and are at risk of long-term stress effects ($p = 0.0016$, $df = 4$).

Data from our survey show that the desire to work in a health-related field was the main motive for choosing

a degree program. Many students chose this major because they were not admitted to their other dream studies. For 40.5% of pharmacy students in Kuwait, not being accepted to a university was the main reason for choosing this major.[36] In the UAE, encouragement from family (84.5%), the desire to obtain a medical degree, e.g., the title of pharmacist (79.0%), and personal interests (71.0%) were the deciding factors in choosing to study pharmacy.[41] A study conducted in the USA identified anticipatory socialization, career orientation and the desire to help others as the main motivations of students.[42] A significant proportion of pharmacy students in the UK do not plan to work in the profession in which they were trained.[43] A survey conducted in Saudi Arabia identified the following as the main factors in choosing pharmacy: the desire to work in a respected profession (83.7%), the desire to work in a popular and sought-after profession (81.7%) and encouragement from family members (66.0%).[44]

The career plans of Polish students are another important finding of our survey. Various career paths are available to them, including work in a general pharmacy, a hospital pharmacy, the pharmaceutical industry (laboratories, pharmacovigilance departments, drug registration), in the field of clinical research, and in academia. Respondents to this survey mainly declared a desire to work in community pharmacies. In contrast, almost half of the students in Kuwait and the majority of students in the UAE and Saudi Arabia expressed a desire to work in a hospital pharmacy.[45] Swedish pharmacy students, when asked where they would like to work after graduation, also indicated a community pharmacy. It also represented the first place of work for most of the country's graduates.[46]

Limitations

Although the purpose of our survey was to assess teaching standards, mental health and student–teacher relationships among pharmacy students in Poland, it is important to be aware of certain limitations that may affect the interpretation of the results. The difficulty of reaching all respondents and the risk of over-representation (too much variation in the number of respondents from different universities) were limitations of this study. In addition, our cross-sectional methodology, cultural differences between different regions of Poland and the lack of additional relevant variables were elements that may have affected the results. It is important to understand that the survey covers a specific group of students, and a cause-and-effect relationship cannot be established in every area. Finally, in assessing the accuracy of our survey, it is important to consider the risk of misunderstanding the questions and the lack of supplementary information, such as accurate descriptions of particular situations. Despite these limitations, the survey provides reliable information on the perceptions and experiences of pharmacy students in Poland. In presenting the results, these limitations have been taken into account.

Conclusions

Assessing the impact of higher education on students' mental state can help understand psychological conditions in relation to education, academic achievement, perception, and impact on future professional life. Given the evolving role of the pharmacist in patient care, it is important to take a closer look at students' mental resilience and potentially implement appropriate solutions. This action could prevent the deteriorating mental status of students preparing for the profession.

The results of our study may be helpful in implementing preventive measures to prevent the development of depression and anxiety among students and in developing therapeutic strategies for those affected by these disease symptoms. They also draw attention to the proper psychological preparation of students for entry into the healthcare system, which is the key to a rewarding and sustainable career. Medical school authorities should take the lead in destigmatizing mental illness and promoting help-seeking behavior when students are stressed and anxious. Many universities offer psychological help to students, but as our survey indicates, few students take advantage of it.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Chankseliani M, McCowan T. Higher education and the Sustainable Development Goals. *High Educ*. 2021;81(1):1–8. doi:10.1007/s10734-020-00652-w
2. Hall S. A mental-health crisis is gripping science: Toxic research culture is to blame. *Nature*. 2023;617(7962):666–668. doi:10.1038/d41586-023-01708-4
3. Lattie EG, Adkins EC, Winquist N, Stiles-Shields C, Wafford QE, Graham AK. Digital mental health interventions for depression, anxiety, and enhancement of psychological well-being among college students: Systematic review. *J Med Internet Res*. 2019;21(7):e12869. doi:10.2196/12869
4. Sheldon E, Simmonds-Buckley M, Bone C, et al. Prevalence and risk factors for mental health problems in university undergraduate students: A systematic review with meta-analysis. *J Affect Disord*. 2021;287:282–292. doi:10.1016/j.jad.2021.03.054
5. Eisenberg D, Gollust SE, Golberstein E, Hefner JL. Prevalence and correlates of depression, anxiety, and suicidality among university students. *Am J Orthopsychiatry*. 2007;77(4):534–542. doi:10.1037/0002-9432.77.4.534

6. Lei X, Liu C, Jiang H. Mental health of college students and associated factors in Hubei of China. *PLoS One*. 2021;16(7):e0254183. doi:10.1371/journal.pone.0254183
7. Laidi C, Blampain-Segar L, Godin O, De Danne A, Leboyer M, Durand-Zaleski I. The cost of mental health: Where do we stand in France? *Eur Neuropsychopharmacol*. 2023;69:87–95. doi:10.1016/j.euroneuro.2023.02.004
8. Ornell F, Schuch JB, Sordi AO, Kessler FHP. "Pandemic fear" and COVID-19: Mental health burden and strategies. *Braz J Psychiatry*. 2020;42(3):232–235. doi:10.1590/1516-4446-2020-0008
9. Jones EAK, Mitra AK, Bhuiyan AR. Impact of COVID-19 on mental health in adolescents: A systematic review. *Int J Environ Res Public Health*. 2021;18(5):2470. doi:10.3390/ijerph18052470
10. Lee J, Solomon M, Stead T, Kwon B, Ganti L. Impact of COVID-19 on the mental health of US college students. *BMC Psychol*. 2021;9(1):95. doi:10.1186/s40359-021-00598-3
11. Silva RG, Figueiredo-Braga M. Evaluation of the relationships among happiness, stress, anxiety, and depression in pharmacy students. *Curr Pharm Teach Learn*. 2018;10(7):903–910. doi:10.1016/j.cptl.2018.04.002
12. Fischbein R, Bonfine N. Pharmacy and medical students' mental health symptoms, experiences, attitudes and help-seeking behaviors. *Am J Pharm Educ*. 2019;83(10):7558. doi:10.5688/ajpe7558
13. Garber MC. Exercise as a stress coping mechanism in a pharmacy student population. *Am J Pharm Educ*. 2017;81(3):50. doi:10.5688/ajpe81350
14. Ibrahim MB, Abdelreheem MH. Prevalence of anxiety and depression among medical and pharmaceutical students in Alexandria University. *Alexandria J Med*. 2015;51(2):167–173. doi:10.1016/j.ajme.2014.06.002
15. Samreen S, Siddiqui NA, Mothana RA. Prevalence of anxiety and associated factors among pharmacy students in Saudi Arabia: A cross-sectional study. *Biomed Res Int*. 2020;2020:2436538. doi:10.1155/2020/2436538
16. Votta RJ, Benau EM. Predictors of stress in doctor of pharmacy students: Results from a nationwide survey. *Curr Pharm Teach Learn*. 2013;5(5):365–372. doi:10.1016/j.cptl.2013.06.014
17. Rincón-Cortés M, Herman JP, Lupien S, Maguire J, Shansky RM. Stress: Influence of sex, reproductive status and gender. *Neurobiol Stress*. 2019;10:100155. doi:10.1016/j.ynstr.2019.100155
18. Aktekin M, Karaman T, Senol YY, Erdem S, Erengin H, Akaydin M. Anxiety, depression and stressful life events among medical students: A prospective study in Antalya, Turkey. *Med Educ*. 2001;35(1):12–17. doi:10.1046/j.1365-2923.2001.00726.x
19. Puthran R, Zhang MWB, Tam WW, Ho RC. Prevalence of depression amongst medical students: A meta-analysis. *Med Educ*. 2016;50(4):456–468. doi:10.1111/medu.12962
20. Venkatarao E, Iqbal S, Gupta S. Stress, anxiety & depression among medical undergraduate students & their socio-demographic correlates. *Indian J Med Res*. 2015;141(3):354. doi:10.4103/0971-5916.156571
21. Sauer N, Sałek A, Szlasa W, et al. The impact of COVID-19 on the mental well-being of college students. *Int J Environ Res Public Health*. 2022;19(9):5089. doi:10.3390/ijerph19095089
22. Saddik B, Hussein A, Sharif-Askari FS, et al. Increased levels of anxiety among medical and non-medical university students during the COVID-19 pandemic in the United Arab Emirates. *Risk Manag Healthc Policy*. 2020;13:2395–2406. doi:10.2147/RMHP.S273333
23. Barbayannis G, Bandari M, Zheng X, Baquerizo H, Pecor KW, Ming X. Academic stress and mental well-being in college students: Correlations, affected groups, and COVID-19. *Front Psychol*. 2022;13:886344. doi:10.3389/fpsyg.2022.886344
24. Gawrych M, Cichoń E, Kiejna A. Depression among young adults: Risks and protective factors in the COVID-19 pandemic. *Postep Psychiatr Neurol*. 2022;31(2):52–61. doi:10.5114/ppn.2022.118265
25. İlhan B, Küpeli İ. Secondary traumatic stress, anxiety, and depression among emergency healthcare workers in the middle of the COVID-19 outbreak: A cross-sectional study. *Am J Emerg Med*. 2022;52:99–104. doi:10.1016/j.ajem.2021.11.051
26. Seetan K, Al-Zubi M, Rubbai Y, Athamneh M, Khamees A, Radaideh T. Impact of COVID-19 on medical students' mental wellbeing in Jordan. *PLoS One*. 2021;16(6):e0253295. doi:10.1371/journal.pone.0253295
27. Jenkins S, Johnson I, Ginley J. Work, Stress and Play: Students' perceptions of factors impacting on their studies and well-being. *Eur J Dent Educ*. 2019;23(3):349–354. doi:10.1111/eje.12436
28. Basson MJ, Rothmann S. Antecedents of basic psychological need satisfaction of pharmacy students: The role of peers, family, lecturers and workload. *Res Soc Admin Pharm*. 2018;14(4):372–381. doi:10.1016/j.sapharm.2017.04.015
29. Basson MJ, Rothmann S. Pathways to flourishing among pharmacy students: The role of study demands and lecturer support. *J Psychol Afr*. 2019;29(4):338–345. doi:10.1080/14330237.2019.1647953
30. Jegede AO, Erhun WO, Oyinlola IO. Mentorship in pharmacy schools: Students' perspective. *Indian J Pharm Educ Res*. 2023;57(3):669–677. doi:10.5530/ijper.57.3.81
31. Park SK, Chen AMH, Daugherty KK, Frankart LM, Koenig RA. A scoping review of the hidden curriculum in pharmacy education. *Am J Pharm Educ*. 2023;87(3):ajpe8999. doi:10.5688/ajpe8999
32. Howard ML, Yuet WC, Isaacs AN. A review of development initiatives for pharmacy student and resident preceptors. *Am J Pharm Educ*. 2020;84(10):ajpe7991. doi:10.5688/ajpe7991
33. Nelson NR, Carlson RB, Corbett AH, Williams DM, Rhoney DH. Feedback for learning in pharmacy education: A scoping review. *Pharmacy (Basel)*. 2021;9(2):91. doi:10.3390/pharmacy9020091
34. Plewka B, Waszyk-Nowaczyk M, Cerbin-Koczorowska M, et al. Polish pharmacy students' attitudes toward undergraduate teaching and practical implementation of pharmaceutical care: A cross sectional study. *Int J Environ Res Public Health*. 2022;19(12):7358. doi:10.3390/ijerph19127358
35. Croft H, Gilligan C, Rasiah R, Levett-Jones T, Schneider J. Current trends and opportunities for competency assessment in pharmacy education: A literature review. *Pharmacy (Basel)*. 2019;7(2):67. doi:10.3390/pharmacy7020067
36. Awad A, Al-Haqan A, Moreau P. Motivations, career aspiration, and learning experience of students in the pharmacy program at Kuwait University: A tool to guide curriculum development. *Curr Pharm Teach Learn*. 2017;9(2):332–338. doi:10.1016/j.cptl.2016.11.018
37. Mara De Sousa Lopes N, Soares Gondim AP, Silva Soares AC, Barbosa Dos Santos D, Ribeiro De Sales Neto M, Pinto DM. A quantitative analysis of the quality of pharmacy education in Brazil. *Am J Pharm Educ*. 2019;83(3):6543. doi:10.5688/ajpe6543
38. Tomko JK, Munley PH. Predicting counseling psychologists attitudes and clinical judgments with respect to older adults. *Aging Ment Health*. 2013;17(2):233–241. doi:10.1080/13607863.2012.715141
39. Graham KL, King KD. Evaluating the working with older adults scale with clinical psychology doctoral students. *Gerontologist*. 2022;62(8):1217–1225. doi:10.1093/geront/gnac044
40. Teixeira S, Augsberger A, Richards-Schuster K, Sprague Martinez L. Participatory research approaches with youth: Ethics, engagement, and meaningful action. *Am J Commun Psychol*. 2021;68(1–2):142–153. doi:10.1002/ajcp.12501
41. Abduelkarem A, Hamrouni. The choice of pharmacy profession as a career: UAE experience. *Asian J Pharm Clin Res*. 2016;9(4):220–226. https://journals.innovareacademics.in/index.php/ajpcr/article/view/12014. Accessed February 2, 2024.
42. Keshishian F. Factors influencing pharmacy students' choice of major and its relationship to anticipatory socialization. *Am J Pharm Educ*. 2010;74(4):75. doi:10.5688/aj740475
43. Willis S, Hassell K, Noyce P. Career intentions of pharmacy students. *J Health Serv Res Policy*. 2008;13(2 Suppl):45–51. doi:10.1258/jhsrp.2007.007112
44. Alhaddad MS. Undergraduate pharmacy students' motivations, satisfaction levels, and future career plans. *J Taibah Univ Med Sci*. 2018;13(3):247–253. doi:10.1016/j.jtumed.2018.03.004
45. Mukhalalati B, Ashour M, Al Noami AE. Examining the motivations and future career aspirations of Qatari pharmacy students and alumni: A case study. *Curr Pharm Teach Learn*. 2020;12(11):1329–1339. doi:10.1016/j.cptl.2020.06.003
46. Gustafsson M, Mattsson S. Swedish pharmacy students' expectations and perceptions of their education and future pharmacy profession. *Pharmacy (Basel)*. 2019;7(4):139. doi:10.3390/pharmacy7040139

An innovative method for three-dimensional bone reconstruction of the anterior mandible with preserved dentition using an allogeneic bone block: A 6-month follow-up

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Abstract

Background. Bone defects around the teeth affect a large portion of the population. Bone regeneration in the area of existing teeth is completely different from that in an edentulous area. To date, no method has been developed for three-dimensional (3D) bone reconstruction in regions with preserved teeth.

Objectives. This study aimed to radiologically evaluate the results of the new method of 3D mandibular bone reconstruction in preserved dentition using a custom-made allogeneic bone block with a 6-month follow-up.

Materials and methods. Alveolar ridge dimensions were radiographically assessed before and 6 months after reconstruction using cone beam computed tomography (CBCT) scans in 32 patients (192 teeth). Reconstruction used a bone block that had been previously planned and prepared using CAD/CAM technology.

Results. The observed changes in alveolar bone dimensions were highly significant in most cases ($p < 0.001$). The closer to the tooth root apex, the lower the bone growth in the sagittal dimension (average of the mean values for each tooth examined in the measured heights): CEJ2: 2.9 mm, ½ CEJ2: 2.7 mm, ¼ CEJ2: 1.9 mm, and API: 1.4 mm. The maximum bone growth in the vertical dimension was observed on tooth 43 (9.9 mm), followed by 32 (9.8 mm), 33 (8.5 mm), 31 (8.4 mm), 42 (8 mm), and 41 (7 mm). The degree of decrease in vestibular dehiscence of the bone was greater the closer the tooth was to the midline (average of –3.8 mm and –3.4 mm for the central incisors; average of –2.8 mm and –2.6 mm for the lateral incisors; average of –2.6 mm and –2.5 mm for the canines).

Conclusions. The results prove that it is possible to prevent bone dehiscence in patients undergoing orthodontic treatment, increasing the ability and effectiveness of covering recessions and improving the morphology of the lower part of the face.

Key words: allograft, mandibular reconstruction, allogeneic bone block, bone reconstruction, orthodontics

Background

A variety of techniques and materials can be found in the available literature, and their continuous improvement is aimed at increasing the effectiveness of alveolar bone regeneration procedures. The methods reported use bone granules, chips, wedges, bone rings, plates, and blocks, including individualized ones, among other things. The effects of using autogenous and allogeneic bone, as well as xenogenous and alloplastic materials, are constantly being compared.[1–5]

However, these procedures are based on achieving the effect of bone growth in edentulous sections of the alveolar process, mainly as preimplantation preparation. To date, many cases have been evaluated and described that bone regeneration in edentulous sections is currently considered a predictable procedure, provided that certain rules are followed.[2] The situation of bone regeneration in areas with existing teeth is completely different.

Dental reports on the regeneration of periodontal vertical bone defects (intrabony) confirm the possibility of achieving satisfactory results.[6–8] Nevertheless, the three-dimensional (3D) regeneration of the alveolar ridge in the dental area poses a problem, especially when it comes to regeneration in the vertical dimension.

It is important to address this issue and look for solutions because the problem of bone defects around the teeth affects a large part of the population.[9,10] This problem becomes especially important when orthodontic treatment is required or when advanced gingival recessions need to be covered.[11–13]

It turns out that the procedures for adequate bone regeneration require careful planning and consideration not only of the type of graft material and its ready-to-use form but also of the surrounding anatomy, with special attention to the quality and quantity of the soft tissues and the function of the surrounding muscles.[14,15]

In this article, we present a novel and innovative method for 3D bone reconstruction of the anterior mandible with preserved dentition using an allogeneic bone block (ABB), focusing on the method of appropriate patient qualification, treatment planning, and the necessary preparatory steps for the basic bone regeneration procedure.

Objectives

This prospective, nonrandomized study aimed to radiologically evaluate the results of a 3D bone reconstruction method for the anterior mandible with preserved dentition with a 6-month follow-up. The main goal was to measure changes in bone dimensions in the anterior mandible based on preoperative cone beam computed tomography (CBCT) analysis and after 6 months.

Materials and methods

Study group

The analysis included data from CBCT scans and the medical records of 32 patients who had undergone anterior mandibular reconstruction surgery using a 3D ABB (as below) and appeared for a 6-month follow-up and CBCT scan. Patients were treated in a private dental practice in Wrocław, Poland, between 2018 and 2021.

Participants were initially referred for surgical consultation for the following reasons:

1. Clinically confirmed gingival recession and consecutive radiographically visible bone defects in the alveolar part of the mandible – both in patients who have never received orthodontic treatment and in those undergoing and following orthodontic treatment. 2. Radiologically identified bone defects without concomitant gingival recession in patients who had a CBCT prior to orthodontic treatment, taking into account the movements of the anterior mandibular teeth.

The analysis included adult (non-growing) patients with preserved dentition, at least in the anterior mandible (teeth 33–43), who had signed an informed consent form for the procedure and participation in the study. This study included patients with bone defects covering the anterior region of the mandible in the area of teeth 33–43 (from the right canine to the left canine) with various configurations and the advancement of dehiscence and/or fenestration.

Patients undergoing orthodontic treatment were also included. In these patients, tooth movements in the analyzed area were suspended for the duration of surgical treatment (passive treatment for 6 months). Smoking patients were advised to give up smoking and were informed about its possible negative effects on healing. Diabetics were also not excluded from the study, provided the disease was stable. There was no upper age limit.

The exclusion criteria included systemic diseases and drug treatments that could affect bone tissue (e.g., Paget's disease, osteoporosis, use of bisphosphonates, or denosumab), previous surgical and periodontal treatments in the anterior mandible, craniofacial anomalies, and previous trauma to the mandible. Patients who did not report for the control CBCT 6 months after reconstruction (4 patients) were also not evaluated.

The primary goal was to quantify bone growth in a population that had a similar procedure performed in a standardized way – values that could be reliably compared.

Research components

The clinical examination mainly included periodontal assessment, identification of possible periodontal pockets (probing depth >4 mm), presence and advancement of the gingival recession, biotype, presence of calculus

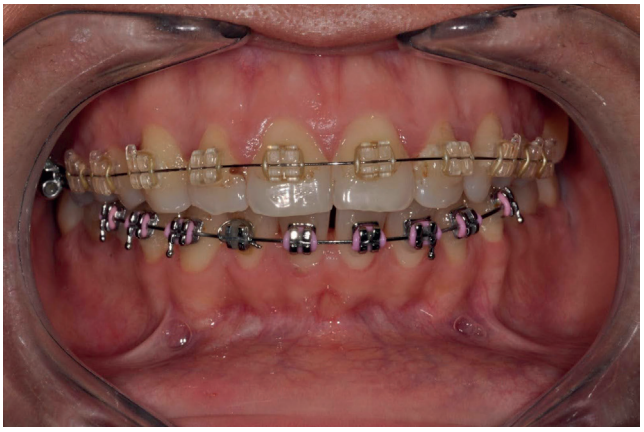

Fig. 1. Initial clinical situation of the example patient. Prominent alveolar yokes are visible

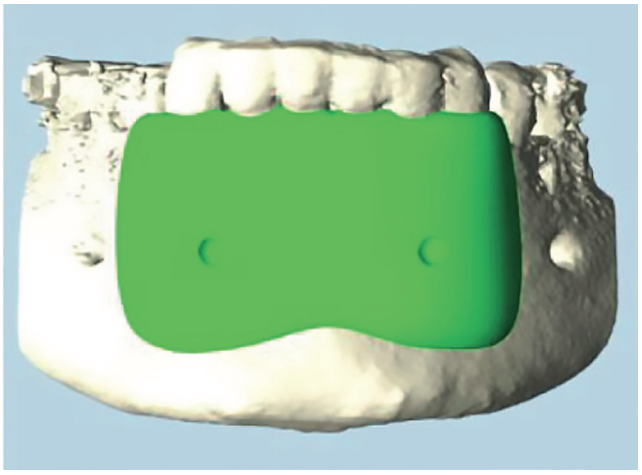

Fig. 2. Bone block planning based on cone beam computed tomography (CBCT). The defect planning is divided into 5 regions (4 external and 1 internal). The position of the screws was taken into account in the planning

and plaque, abnormal frenal attachments (especially on the lower lip), and mentalis muscle tension. It was also determined if the patient had received orthodontic treatment and what type of malocclusion it was, as well as if a gingival graft was required (connective tissue graft (CTG) or free gingival graft (FGG) or both) (Fig. 1).

To adequately prepare and manage the patient, the FLOS concept was developed. This approach consists of physiotherapy, speech therapy, osteopathy, and dental procedures if it's required, due to:

1. a lot of tension of facial muscles and the floor of the mouth from a short lingual frenulum,[16,17]
2. infantile types of swallowing and other similar,
3. wrong posture,[18] and
4. non-carious lesions such as wedge defects or abfraction defects.

The ABB was prepared and obtained in the patented manner presented in the previous article.[19] Briefly, precise cephalometric measurements were first performed with the calculation of the ANB angle and the determination of the face type, since the value of the ANB angle after reconstruction should not exceed 4–5°. The position of the incisors and mandible and the inclination of the mandibular incisors were assessed. The relationship between the maxillary and mandibular incisors was also considered. The crestal bone level was the decisive parameter. In cases undergoing or planning to undergo orthodontic treatment, bone loss should also be predicted in relation to tooth movement. Therefore, the ALi-CEJ2-B angle was measured. Three points were marked: the deepest concavity on the anterior surface of the mandibular symphysis (point B), the apical point of the anteriormost mandibular central incisor (Iia), and the point 2 mm apical to the cementoenamel junction (CEJ) of the incisor, which reflects the sulcus depth.

The CBCT scans played a special role in planning the bone block. The area of the bone defect was divided into 4 external regions and 1 internal region, and the reference points were set on the recipient bed. The point is that the bone graft must be very suitable. Therefore, the internal surface of the block was assessed to make the contact area with the underlying bone as wide as possible (Fig. 2).

Target values were updated to include actual reference point values, and a new range of target values was added to the diagnostic defect area. The bone block body was connected to the first analyzed plane of the mandibular segments with the bone block used. The shape of the chin and the design of the block were also determined. It was important to keep the thickness of the block similar to the thickness of the possible physiological bone regeneration. In addition, the longitudinal axes of the basal symphysis and alveolar symphysis were positioned as parallel to each other as possible, and the total angle created between them did not exceed 10°. The shape of the chin and the design of the block were also checked.

The size of the ABB depended on the size of the bone defect. The assessment was made by the surgeon based on the analysis of the horizontal cross-section of the alveolar process and objectively by orthodontic analysis. The width was assessed from the right to the left canine to ensure that it matched the shape of the mandible in all dimensions. The incisor crowns were measured, and the value determined the arch shape between the canines. The position of the teeth and the mandibular alveolar region were drawn on CBCT scans, and the desired size of the arch was added.

The height of the bone block was determined using 2 measurements in the direction of the crown and the apex, while the level of the crestal bone was positioned 2–3 mm below the CEJ level, which corresponds to the biological width of teeth with healthy periodontium.

The final step was computer-aided design and fabrication (CAD/CAM). A virtual model of the mandible was created by converting CBCT scans and intraoral scans into digital models. A bone block design was placed on the model, and the actual and nominal points were combined to form

Table 1. Clinical steps for preparing the bone block recipient bed

Protocol for 3D bone reconstruction	
1. With recessions	
a. FLOS technique and botox injection in mental muscle – big tension	12–2 weeks before surgery
b. cutting of high attachment of frenum – lingual, buccal, central	4 weeks before surgery
1.1 free gingival graft – is required when are II–IV Miller class of recessions, shallow vestibule	12 weeks to next ST or bone procedure
1.2. soft tissue augmentation	12 weeks to bone surgery
a. thin biotype – superficial connective tissue graft (CTG)	
b. thick biotype – subepithelial CTG	
1.3. 3D bone block- severe loss of the bone	6 months to ortho treatment
2. Without recessions	
a. FLOS technique and botox injection in mental muscle – big tension	12–2 weeks before surgery
b. cutting of high attachment of frenum – lingual, buccal, central	weeks before surgery
1.1. deepening of vestibule – is required when shallow vestibule is present – FGG	4 weeks to next ST or bone procedure
1.2. soft tissue augmentation	12 weeks to bone surgery
a. thin biotype – superficial CTG	
1.3. 3D bone block – severe loss of the bone	months to ortho treatment

ST – soft tissue; CTG – connective tissue graft.

a single unit. A suitable living donor was selected based on adequate cancellous and/or cortical bone volume with the correct bone density. After milling, the block was cleaned, packaged, sterilized, and sent to the surgeon.

Each patient was operated on in the same way and by the same surgeon. Before bone reconstruction was started, it was determined whether the condition of the soft tissues was sufficient. If the biotype was too thin and gingival recession was advanced, FGG and/or CTG were used first (3 months before bone reconstruction). Excessive tension of the mentalis muscle with a specific "orange peel" sign was reduced with an intramuscular injection of botulinum toxin (Table 1).

Bone reconstruction was performed under local anesthesia and with an antibiotic (0.6 g clindamycin) administered orally 1 h before the procedure. First, an intrasulcular incision was made that was 2 spaces wider mesially and distally than the planned reconstruction area, and the mucoperiosteal flap was elevated above the mental eminence to create a catch bed without excessive tension. The bone dehiscences and concavities of the mandibular alveolar region were then exposed (Fig. 3). The root surfaces were then mechanically cleaned of debris, and a surgical drill was used to decorticate the compact bone in the interdental spaces to improve vascularization. The advanced platelet-rich fibrin (PRF) membranes (A-PRF)[20] were prepared using the patient's peripheral blood and placed on the surface. Allogeneic bone particles were placed in the deepest bone defects, followed by the insertion of an individualized 3D block of allogeneic bone. The ABB was stabilized in the desired position with 2 titanium screws (Fig. 4), which were removed after 6–8 weeks. Screws approx. 8 mm in length were used. The A-PRF membranes were placed on the outer surface of the block, and sometimes a pure resorbable collagen

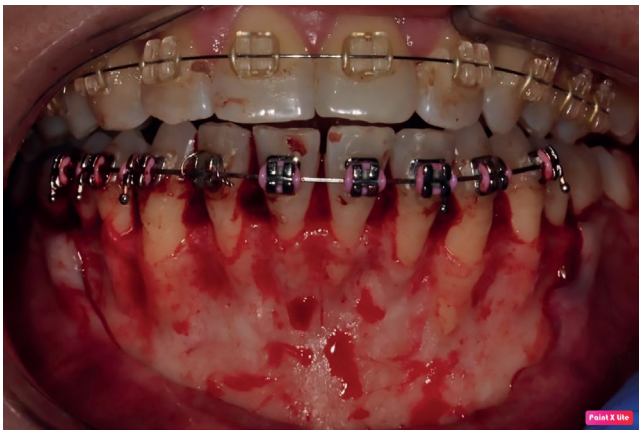

Fig. 3. View after mucoperiosteal flap elevation. Advanced bone dehiscences, fenestrations and exposed tooth roots are visible

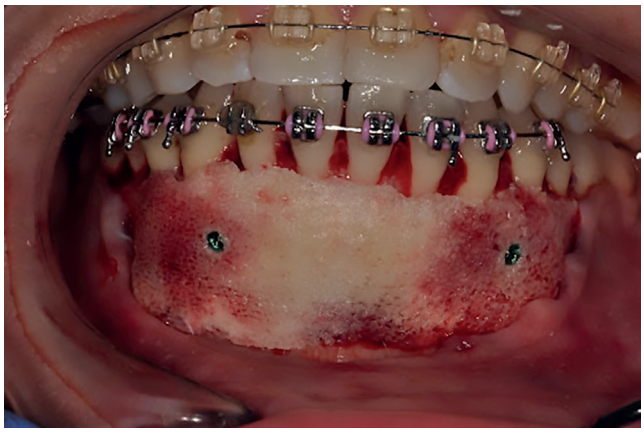

Fig. 4. View of the block after fixation with titanium screws to the bone base

barrier membrane previously soaked with a liquid fraction of PRF (i-PRF) was placed apically. The final stage was suturing non-resorbable 6-0 sutures using the suspended and mattress suturing technique, which prevents excessive flap tension. An antibiotic, 0.6 g of clindamycin (Clindamycin MIP; MIP Pharma GmbH, Blieskastel, Germany) twice daily and painkillers for 3 days were prescribed. The patient was instructed to perform proper oral hygiene with an antiseptic mouth rinse (Alfa; ATOS, Warsaw, Poland) and to use a soft toothbrush (Elgydium Clinic 7/100; Elgydium Pierre Fabre, Paris, France) for postoperative care. Immediately after the procedure, biostimulation using a Nd:YAG laser (10 Hz, 0.5 W) was performed (TwinLight®; Fotona, Ljubljana, Slovenia). The sutures were removed 2 weeks after the procedure. The whole procedure was performed according to the method patented by Dominiak M. and Gedrange T. (EP3287097B1 , European Patent Office, Munich, Germany, 2016).

All CBCT scans (before and 6 months after surgery) were acquired with the Pax Flex3D Vatech computed tomography system (Vatech Europe, Warsaw, Poland). The mandible center image: field of view (FOV): 80 mm width and 50 mm height. The voxel size was 0.200. All images were analyzed using EzDent-i software (Vatech). Defined parameters were measured in the sagittal plane at the center of the central incisors (teeth 31 and 41), lateral incisors (teeth 32 and 42) and canines (teeth 33 and 43). Only the buccolingual bone dimension was measured in the axial plane. The reference lines were perpendicular to each other, and the vertical line was aligned with the long axis of the tooth. The tooth inclination was not determined.

The width of the alveolar ridge was measured at 4 points on the tooth root: at the CEJ-2 mm level (the crestal bone level of the healthy periodontium), at the root apex, at half-length from CEJ-2 to the apex, and at the quarter-length from CEJ-2 to the apex. It was determined if there was dehiscence (the marginal bone level was below the CEJ-2 mm level). The height was measured from the vestibular (HDV) and/or lingual (HDL) side. It was determined whether vestibular and lingual fenestration were present (FV and FL, respectively). If fenestration was present, its height was measured (HFV and HFL). When the vestibular and lingual bone layers were extremely thin, this was noted separately. The value of this dimension was then determined (HVCL – the height of the vestibular cortical layer and HLCP – the height of the lingual cortical layer). If the level of marginal bone on both sides was below the CEJ-2 (bilateral dehiscence), then the measured width at this level corresponded to the width of the tooth; therefore, the value for the alveolar width was considered to be 0. The buccolingual dimension halfway from CEJ-2 to the apex was measured. This value was determined on the axial section, mesial and distal to the teeth (WAM – width of alveolar bone medial and WAD – width of alveolar bone distal) (Fig. 5). A detailed description of the measurement in such cases has already been described.[9]

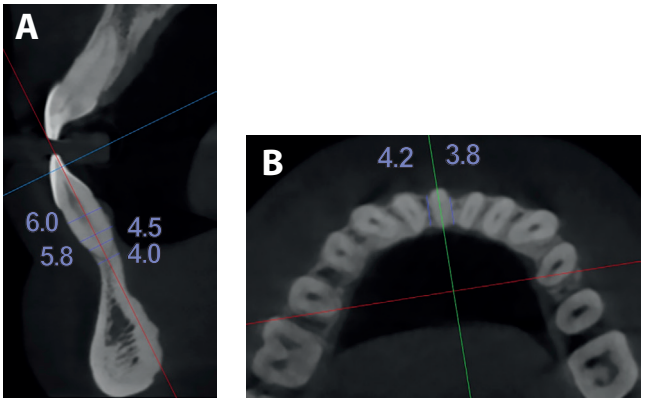

Fig. 5. Scheme of the performance of radiological measurements of the alveolar width – sagittal (A) and axial (B) views

The types and advancement of bone defects were classified according to the classification by Yang et al.[21] and the classification we made (DM classification) as described in another paper.[9] (Fig. 6).

In the radiological assessment, existing dehiscences and lingual fenestrations were also noted, although they were not subject to surgical treatment. The lack of changes after reconstruction is related only to the post-surgical observation of the type of changes in a given area (Fig. 7,8).

Statistical analyses

Bone growth was defined as the difference between the measurements taken after 6 months and before treatment in a given patient (repeated measurements). An additional condition of the study was the interdependence of the given variables within the set of all 6 teeth coming from 1 patient. Furthermore, the distribution of differences between the pre- and post-treatment measurements deviated statistically significantly from the normal distribution in 81% of the tests (Supplementary Table 1). Considering the circumstances described above, the pattern of tooth changes was analyzed using a model for all teeth, for 1 of the 12 variables studied (CEJ2, 1/2CEJ2, etc.), using the R package nparLD,[22] which is a tool for nonparametric analysis of repeated measures data. We used the command 'nparLD': nparLD(data=dane, CEJ2 ~ Time*Tooth, alpha = 0.05, …). The effect size in this test is the "relative treatment effect" (RTE). The model evaluated the statistical significance of Treatment, Tooth, and the Treatment × Tooth effects interactions. The results of running this model for the twelve variables included (1) a table with the statistical significance of the effects (listed above), (2) figures visualizing the effects, and (3) a table with the RTE and its 95% confidence intervals (95% CIs). Since the nparLD does not provide a post hoc test for such a model, the last table above served as the basis for detecting statistically significant differences between before and after treatment measurements.

Due to non-normal data distribution in many of the variables, Spearman's rho rank correlation coefficients were used

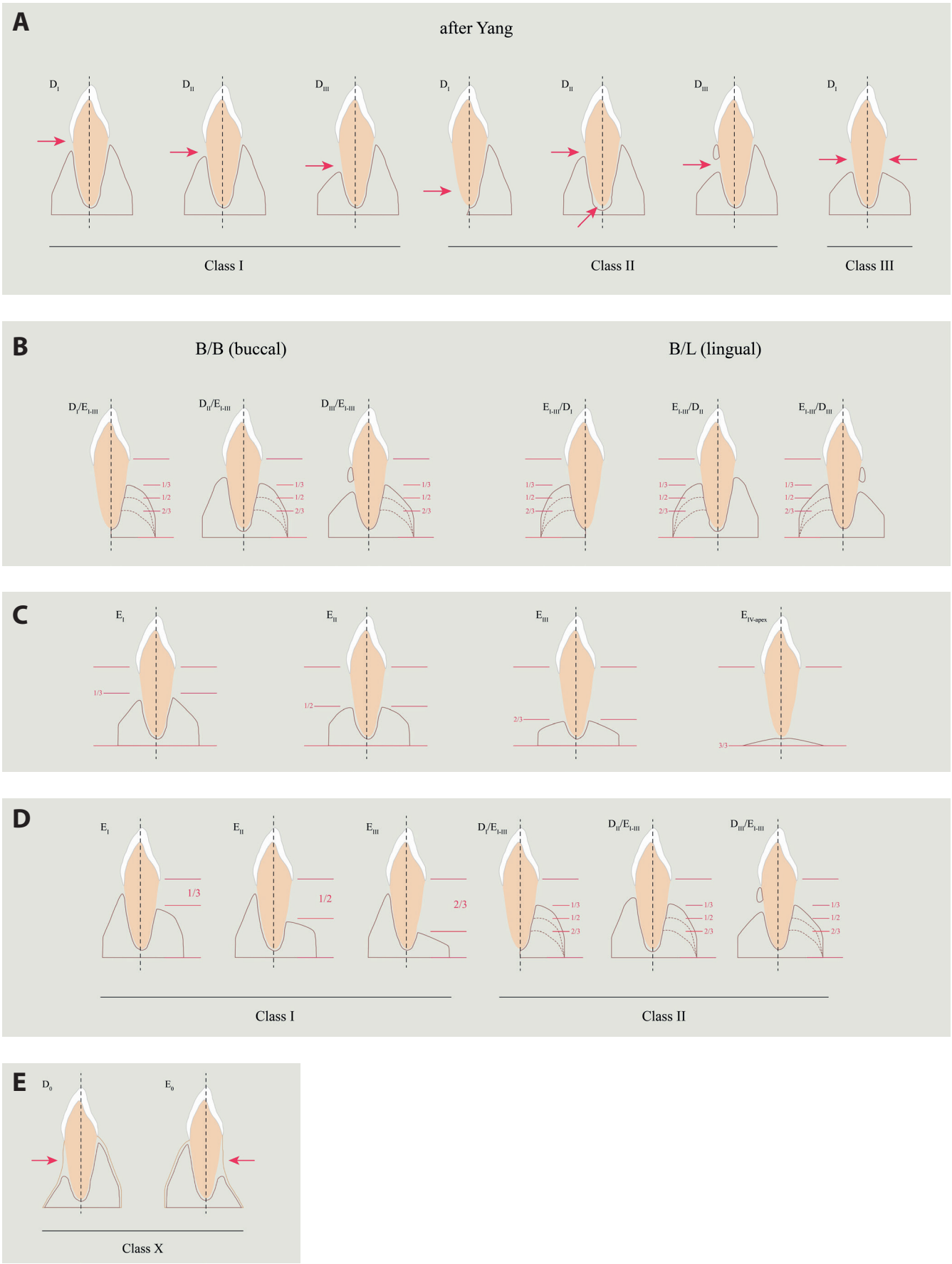

Fig. 6. Yang et al.[21] (A) and DM[9] classifications – class I (B), class II (C), class III (D), and class X (E)

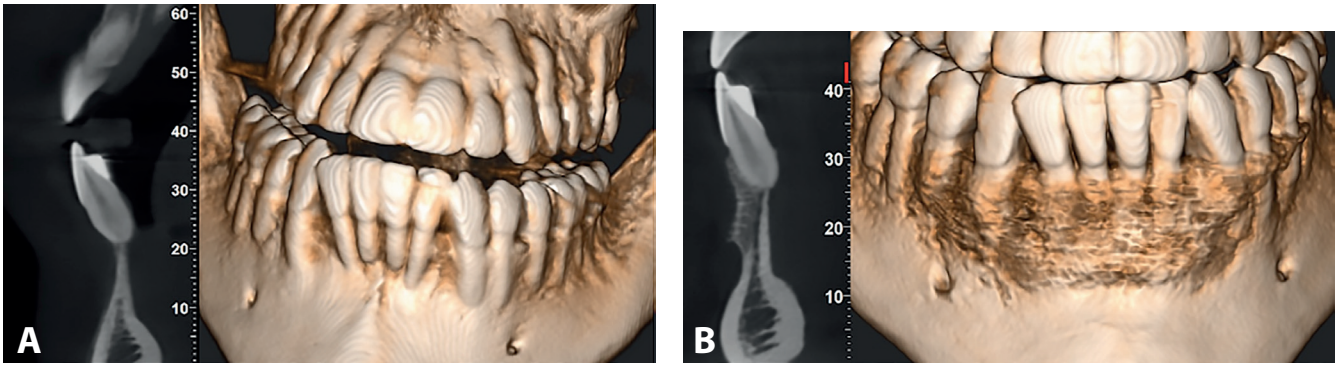

Fig. 7. Cone beam computed tomography (CBCT) scans before (A) and 6 months after bone reconstruction (B) – example patient No. 1

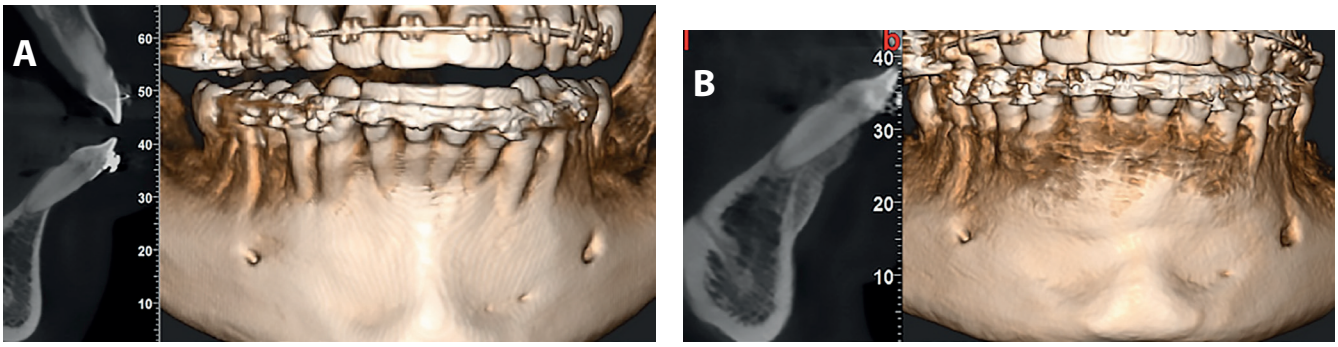

Fig. 8. Cone beam computed tomography (CBCT) scans before (A) and 6 months after bone reconstruction (B) – example patient No. 2

to evaluate the relationship between patient age and changes in bone dimensions. The Shapiro–Wilk test was used to check data distribution normality. Spearman's rho rank correlation coefficients were used to evaluate the monotonic component of the correlation between patient age and changes in bone dimensions. Although the nonparametric test was used in the analysis of the tooth changes, all variables are presented as means and standard deviation (SD), which are more precise than medians and quartiles when tiny changes have occurred. Additionally, the means and median were strongly correlated (Spearman's correlation: n = 72, R = 0.93, p < 0.001), which justifies using the means for presenting bone growth patterns. The statistical description of bone growth parameters is presented in Supplementary Table 2.

The Fisher's exact test was used for the contingency table analysis. As the comparison of bone growth variables between the sexes was considered an exploratory approach in the analysis, no correction for the controlling of Type-I errors was used.

When estimating the required sample size, a postoperative bone growth of at least 1.0 mm, a SD of 1.5 mm and a test power of at least 0.8 were assumed. With these assumptions and an assumed significance level of $\alpha = 0.05$, the minimum sample size was n = 20. The selection of patients for the research sample was done successively, and the adequacy of the sample size was continuously checked by estimating the power of the statistical tests used. Patients were included in the research group until the main objective of the study, i.e., bone growth after surgery measured at CEJ2, 1/2CEJ2 and 1/4CEJ2 levels, was achieved with a test power of $1 - \beta = 0.80$.

The lowest growth was observed in tooth 43. For the measured bone growth at the CEJ2 level (1.6 ±3.0), the significance of the test with a sample size of n = 32 was 0.832 and thus above the assumed minimum value of 0.8. For the same tooth and an increment of 1/4CEJ2 of 1.1 ±1.3, the significance of the test was 0.996.

Statistical analysis was performed using the R environment (R Foundation for Statistical Computing, Vienna, Austria, https://www.r-project.org) and Statistica v. 13.3 software (TIBCO Software Inc., Palo Alto, USA).

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and an approval of the Bioethics Committee of Wroclaw Medical University was obtained (No. KB-284/2023N). The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) checklist was completed.

Results

Overall

The analysis included 32 patients – 25 women (78.1%) and 7 men (21.9%) with a F:M ratio = 1:3.6. Patients' ages

Table 2. The changes in tooth variables as the difference between after and before treatment (means and standard deviation; SD). Statistically significant differences are in bold. Statistical significance was calculated using the Relative Treatment Effect and R-package "nparLD" (see Supplementary Table 3 for details)

Variable	Tooth						Average
	43	42	41	31	32	33	
ΔCEJ2	1.6 ±3.0	**3.3 ±3.5**	**3.9 ±3.2**	**3.0 ±3.3**	**3.2 ±3.7**	2.0 ±3.5	2.9 ±2.1
Δ½CEJ2	**1.1 ±1.8**	**3.3 ±2.5**	**3.4 ±2.4**	**3.4 ±2.7**	**3.2 ±2.8**	**2.8 ±2.0**	2.7 ±1.6
Δ¼CEJ2	**1.1 ±1.3**	**2.4 ±1.9**	**2.4 ±1.8**	**2.4 ±2.1**	**2.1 ±1.5**	**1.2 ±1.3**	1.9 ±1.3
ΔAPI	0.7 ±0.9	**1.8 ±1.3**	**1.8 ±1.1**	**1.7 ±1.1**	**1.6 ±1.2**	0.8 ±0.9	1.4 ±0.8
ΔHD1	**–2.6 ±3.1**	**–2.6 ±2.9**	**–3.4 ±2.3**	**–3.8 ±2.3**	**–2.8 ±2.8**	**–2.5 ±3.0**	–2.9 ±1.8
ΔHD2	–0.0 ±0.1	–0.1 ±0.2	0.1 ±0.7	–0.1 ±0.2	–0.0 ±0.1	0.0 ±0.1	–0.0 ±0.1
ΔHBP	**–0.1 ±1.7**	**–0.4 ±1.9**	**–0.5 ±1.3**	**–0.3 ±1.5**	–0.8 ±1.6	–1.0 ±2.1	–0.5 ±0.8
ΔHBJ	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	–0.1 ±0.5	0.0 ±0.0	–0.2 ±0.9	–0.0 ±0.2
ΔHF1	**–0.9 ±2.1**	**–0.8 ±1.6**	–0.2 ±1.5	0.0 ±0.2	–0.8 ±1.6	–0.4 ±2.1	–0.5 ±1.1
ΔHF2	0.0 ±0.0	–0.0 ±0.0	–0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	0.0 ±0.0	–0.0 ±0.0
ΔWAM	**1.6 ±1.1**	**2.4 ±1.4**	**2.4 ±1.3**	**2.3 ±1.5**	**2.2 ±1.6**	**1.9 ±1.4**	2.1 ±1.0
ΔWAD	**1.2 ±1.0**	**2.1 ±1.1**	**2.7 ±1.6**	**2.1 ±1.2**	**2.1 ±1.3**	**1.4 ±1.1**	1.9 ±0.9

ranged from 18 to 50 years at the time of the procedure; the mean was 32.1 ±9.2 years.

The largest study group consisted of patients after orthodontic treatment (n = 10; 31.25%; mean age of 35.5 years), followed by study participants before planned orthodontic treatment (n = 9; 28.1%; mean age of 33.5 years) and during orthodontic treatment (n = 9; 28.1%; mean age of 31.6 years). On the other hand, orthodontic treatment was not planned in only 4 study participants (12.5%, mean age of 34 years) who had not been treated previously.

The results of the procedures are shown in Fig. 7,8 using the example of selected patients from the studied group.

The observed changes in alveolar bone dimensions (Table 2) were statistically significant in most variables except for the level of lingual dehiscence (ΔHD2), lingual cortical bone (ΔHBJ) and FL (ΔHF2) (Table 3). The analysis was performed using the R-package nparLD. Among the 9 variables statistically significantly affected by Time (i.e., treatment), no interactions with teeth occurred in 2 variables only (CEJ2 and HBP). In the case of the other variables, such interactions were statistically significant, which means that the changes differed between some teeth (Fig. 9, Supplementary Table 3).

The closer to the tooth root apex, the lower the bone growth in the sagittal dimension. Average of the mean values for each analyzed tooth in the measured heights: CEJ2: 2.9 mm, ½ CEJ2: 2.7 mm, ¼ CEJ2: 1.9 mm, and API: 1.4 mm.

In the sagittal dimension, the level at the:

– CEJ2 had the highest average bone growth at tooth 41 (3.9 mm) and the lowest at 43 (1.6 mm),

– ½ CEJ2 had the highest average bone growth at 31 and 41 (3.4 mm) and the lowest at 43 (1.1 mm),

– ¼ CEJ2 had the highest average bone growth at teeth 31, 41, and 42 (2.4 mm) and the lowest at 43 (1.1 mm), and

– API found the highest average bone growth at 41 and 42 (1.8 mm) and the lowest at 43 (0.7 mm).

Maximum bone growth in the vertical dimension was found at tooth 43 (9.9 mm), followed by 32 (9.8 mm), 33 (8.5 mm), 31 (8.4 mm), 42 (8 mm), and 41 (7 mm).

The degree of decrease in vestibular dehiscence of the bone was greater the closer the tooth was to the midline (average –3.8 mm and –3.4 mm for central incisors, 31 and 41, respectively; average –2.8 mm and –2.6 mm for lateral incisors, 32 and 42, respectively; and average –2.6 mm and –2.5 mm for canines 43 and 33, respectively).

The presence of an extremely thin cortical plate before reconstruction was noted in 26 of 192 teeth examined (13.5%) and FV in 28 cases (14.6%). The average height of the vestibular plate was 4.3 mm, while the height of the fenestration plate was 4.1 mm.

Due to the lack of surgical intervention on the lingual side and the elimination of possible orthodontic movements in patients with braces, no differences in the dimensions of bone dehiscences and fenestrations were observed (only values of ±max 0.2 mm, mainly due to measurement errors).

Tangential to the mesial surface of the tooth at the level of ½ CEJ2, the average bone increment in the sagittal dimension (WAM) was 2.1 mm and was greatest at teeth 41 and 42, while distal (WAD) averaged 1.9 mm and was also greatest at tooth 41.

Analysis of the influence of age and sex

There was no statistically significant correlation between patient age and bone growth in any of the variables studied (Supplementary Table 4). There was also no statistically significant difference between men and women in terms of bone growth (Supplementary Table 5).

Table 3. Statistical significance of time ("after" vs "before"), tooth and interaction time × tooth for treatment effects measured with the use of variables CEJ2, 1/2CEJ2, etc. The analysis was performed using the R-package nparLD (see the section "Statistical analyses" for details)

Explained variable	Effect	Statistic	df	p-value
CEJ2	time	62.09	1	<0.001
	tooth	21.49	3.20	<0.001
	time:tooth	1.8	3.98	0.127
1/2CEJ2	time	149.81	1	<0.001
	tooth	27.3	2.69	<0.001
	time:tooth	17.3	3.32	<0.001
1/4CEJ2	time	147.93	1	<0.001
	tooth	20.11	3.48	<0.001
	time:tooth	11.35	3.31	<0.001
API	time	106.77	1	<0.001
	tooth	7.98	3.08	<0.001
	time:tooth	11.63	3.67	<0.001
HD1	time	92.17	1	<0.001
	tooth	3.72	3.32	0.008
	time:tooth	2.41	3.87	0.049
HD2	time	0.45	1	0.504
	tooth	14.66	3.44	<0.001
	time:tooth	0.7	1.69	0.472
HBP	time	12.13	1	<0.001
	tooth	0.36	4.51	0.858
	time:tooth	1.27	4.11	0.280
HBJ	time	1.84	1	0.175
	tooth	0.88	1.89	0.411
	time:tooth	1.65	1.41	0.198
HF1	time	6.81	1	0.009
	tooth	2.78	3.99	0.026
	time:tooth	2.56	3.88	0.038
HF2	time	2.07	1	0.151
	tooth	0.68	1.72	0.484
	time:tooth	0.79	1.90	0.446
WAM	time	160.27	1	<0.001
	tooth	25.2	3.64	<0.001
	time:tooth	5.09	4.27	<0.001
WAD	time	159.63	1	<0.001
	tooth	22.39	3.40	<0.001
	time:tooth	12.72	4.24	<0.001

df – degrees of freedom.

The average bone growth in the group of patients who underwent orthodontic treatment with a passive archwire and in the group of patients without ongoing orthodontic treatment did not differ significantly except for HBJ and HF2, in which small and marginally statistically significant differences occurred (Supplementary Table 6).

Analysis of the impact of adverse features (gingival recessions, thin biotype, excessive function of the mentalis muscle)

Before treatment, the presence of the above factors that might affect the final treatment effect was determined.

In 22 (68.75%) of the cases, the gingival biotype was thin at baseline. The same number of participants (n = 22; 66.75%) were diagnosed with significant gingival recession. Almost half of the patients (n = 14; 43.75%) showed mentalis muscle hyperactivity.

The chance of achieving an optimal therapeutic effect was 4 times greater in the group of patients without recessions than in the group with recessions (OR = 4.20), but the 95% CI was (0.44–39.9) at a p = 0.38 using Fisher's exact test, which should be interpreted as equal chances of an optimal outcome in both groups (not significantly dependent on the presence of recessions). There was no statistically significant difference between patients differing in the occurrence of recessions in relation to bone growth except for marginal significance for HF2 (Supplementary Table 7). A similar lack of dependence was seen in the patient groups with thick and thin biotypes (except for the marginal significance of HBJ) (Supplementary Table 8) and excessive mentalis muscle tension (Supplementary Table 9). Considering the above presentation of the method of preparing the patient for the procedure, the absence of these effects proves the proper implementation of the adopted algorithm – among other recession coverages and elimination of tension.

Predicting optimal treatment effect

The ideal therapeutic effect is evidenced by the ADI class according to DM after treatment. The assessment of treatment outcomes did not take into account the condition of the tissues on the lingual side. This effect was obtained in 24 (75%) patients. In the remaining patients, the ADI class was not definitively achieved, but the therapeutic effect was satisfactory.

The "gold standard" for optimal therapeutic effect was the DM classification, based on which patients were divided into 1 of 2 groups with ideal or satisfactory effects. Continuous variables such as age and changes in bone dimensions after 6 months of treatment were transformed into dichotomous (binary) variables, with cutoff values based on the analysis of receiver operating characteristic (ROC) curves. Patient age is a destimulating (reducing) variable for the chance of achieving an optimal treatment effect. As age increases, the probability of achieving an optimal therapeutic effect decreases. For an age cutoff ≥34 years, Sensitivity (Sens.) = 79.2%, Specificity (Spec.) = 75.0%, Accuracy (Acc.) = 78.1%, PPV = 90.5; Likelihood Ratio (+) = 3.17.

The ideal treatment effect was more frequent in the case of an increase in the:

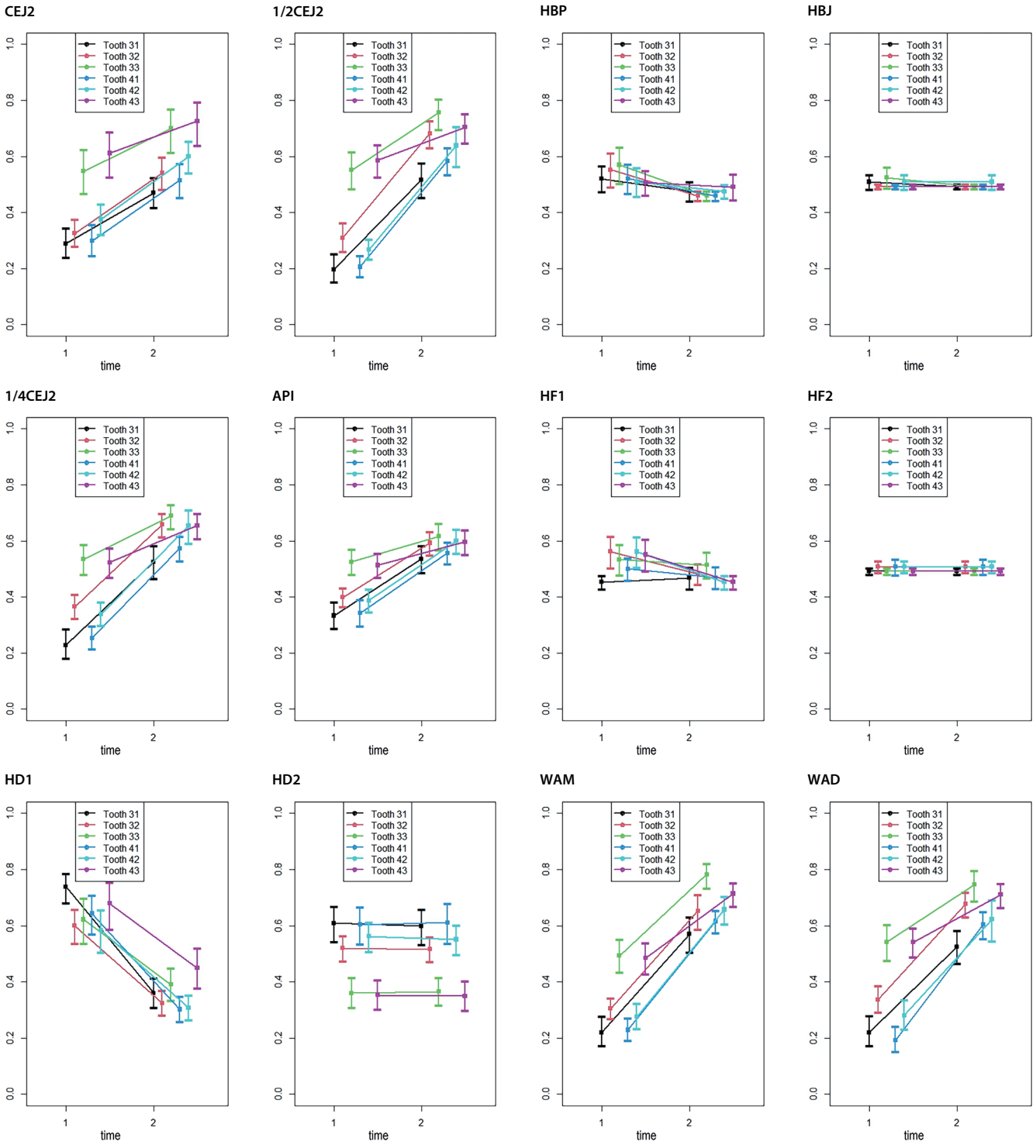

Fig. 9. Relative treatment effect (1 – measurements before treatment; 2 – measurements after treatment) with 95% confidence intervals (95% CIs), calculated with the use of R-package nparLD (see Supplementary Table 2 for details and statistical significance of the differences)

– ΔCEJ2 of tooth 31 by at least 0.5 mm, tooth 41 by 0.4 mm and tooth 42 by 0.1 mm;
– ½ CEJ2 of tooth 41 by at least 5.9 mm and of tooth 42 by 3.0 mm;
– ¼ CEJ2 of tooth 32 by at least 1.6 mm; and
– WAM tooth 33 by at least 2.1 mm.

Discussion

Orthodontic treatment is currently very common in a significant percentage of dental patients, regardless of age. It is well known that proper and effective tooth movement requires the presence of an adequate amount

and quality of bone. Induced tooth movement should only be carried out at the alveolar bone trabeculae.[12] Unfortunately, the occurrence of a gingival recession is still a common side effect. One of the basic assumptions is that the thickness of the anterior part of the alveolar bone should be considered as a limiting factor for orthodontic treatment. Exceeding these anatomical limits is associated with an increased risk of bone loss and the formation of alveolar defects – dehiscences and fenestrations. Anterior teeth in the mandible are found to be most susceptible to these problems, and it has also been observed that the greatest treatment-related bone loss occurred on the side to which a tooth was moved.[23] In addition, pre-existing bone defects often act as "predisposing factors" for gingival recession.[12]

An exposed root surface due to gingival recession is often associated with dentin hypersensitivity, root caries, noncarious cervical lesions, impaired plaque control, and unaesthetic appearances. In addition, untreated gingival recession can lead to further apical displacement over time if the patient does not behave correctly.[24] It would be much better to prevent a recession as much as possible.

In the absence of similar methods developed for the purpose described, the analysis of the effectiveness evaluation focuses on the results obtained. First, it is necessary to consider why the ABB and CAD/CAM technology were used and what conditions must be met to achieve a satisfactory effect.

Allografts are a commonly used graft material nowadays. They come from a donor of the same species, which can be fresh frozen, freeze-dried or demineralized freeze-dried bone. This material may not only serve as an osteoconductive scaffold for new bone formation but may also have some osteoinductive potential due to the presence of proteins such as bone morphogenetic proteins.[25] No donor site morbidity, less postoperative discomfort, a much larger bone availability, and less bone resorption than autologous bone are leading surgeons to increasingly choose this graft material.[26] It is produced and used in various forms, ranging from tiny granules to large 3D blocks.

Brugnami et al. showed that the combination of corticotomy and guided bone regeneration (GBR) in orthodontically treated patients allows for an increase in the dimensions of the "bone envelope" so that the possible deleterious effects of orthodontic movements on periodontal tissues can be overcome, even when the movements are outside the original alveolar anatomy. However, the use of granules with membranes is associated with the movement of the material, the lack of a significantly stable 3D space and a relatively low regeneration potential. Such treatment leads to the formation of a conglomerate of augmentation material so that no new layer of cortical bone is formed with a new point B.[27]

Knowing the excellent properties of allogeneic bone as a graft material, the search for a better and more effective method leads to modifications in the shape and structure of the graft.

As early as the end of the 20th century, the idea of using CAD/CAM technology for the fabrication of onlay blocks in augmentation procedures was presented.[28] This technology allows for a custom fabrication of allogeneic bone blocks for a variety of alveolar ridge augmentation procedures. Many successful cases have been described, highlighting in particular the accuracy, precision and perfect fit of the bone blocks fabricated using CAD/CAM technology.[26]

In our cases, the block was placed directly out of the sterile packaging onto the donor bone with a passive fit. Since no shaping or multiple adjustments were required, the open wound time and overall surgical time were significantly reduced.

The ideal therapeutic effect, as defined by the ADI class according to the DM classification after treatment, was achieved in 24 (75%) patients. In others, the ADI class was not fully reached, but the therapeutic effect was satisfactory. Very satisfactory results of maximum bone growth in the vertical dimensions were obtained because, in some cases, even more than 9 mm were reached.

Before performing the basic bone reconstruction procedure, possible complications and their causes should be considered, and an attempt should be made to eliminate them at the preparatory stage.

Common problems with allogeneic bone blocks include wound dehiscence with exposure of the membrane, opening of the incision and exposure of the bone block. These problems are largely due to poor oral hygiene, pre-existing disease, a thin biotype, and thus poor soft tissue management rather than the allogeneic blocks themselves.[26,29] Therefore, proper soft tissue management should not be a way to treat the above complications but should be an appropriate preparatory phase for advanced bone reconstructions.

Only after the soft tissues (labial side) achieved a stable condition did reconstruction begin. Too thin a biotype, too little keratinized tissue, a shallow vestibule combined with a high and strong frenal attachment, and strong tensions from the mentalis muscle can lead to recession relapse, flap retraction and exposure of the bone block, which could result in a negative outcome, especially during the early phases of healing.[14]

In the group of patients we analyzed, there was no statistically significant difference between the patients who differed in the occurrence of recession in relation to bone growth. A similar lack of dependence was seen in the patient groups with thick and thin biotypes and excessive mentalis muscle tension. However, these results were the result of adequate patient preparation. The above factors, which could have a significant negative impact on the final effect of the procedure, were eliminated by gingival augmentation (CTG, FGG or both), ensuring the correct quantity and quality of soft tissue, the depth of the oral

vestibule and the performance of a frenectomy or frenuloplasty, especially in cases of pull syndrome and injections of botulinum toxin into the mentalis muscle. The analysis of the direct influence of the above factors on the effect of reconstruction would have to be based on the division of patients into groups – one group in which the respective factor was eliminated and another in which bone reconstruction was performed with the factor retained. However, this would deliberately expose patients to a worse outcome or to complete failure. The lack of differences is a confirmation of the effectiveness of such preparations of the patient for the procedure.

For the first time, we presented this method as an example of treating a patient with a 6-month follow-up. Finally, the radiographic images revealed the formation of a new layer of cortical bone on the vestibular side and a certain volume of cancellous bone, noting that the block was prepared only from the spongy bone. This is probably related to the way bone blocks are remodeled, which depends on the force and physiological loads acting on them.[30] Similar radiological observations were presented by other authors who also showed the formation of a compact bone layer after a 10-month observation.[31] Hence, the functional adaptation of the bone block to the current morphological and functional conditions is visible. The formation of new cortical bone makes it possible to determine new cephalometric points in this area, especially point D,[32] which is important because it determines the directions of possible future orthodontic treatment.

Limitations

A limitation but also further perspectives of this study would include a longer observation period, an analysis of cases in which the methods were applied in other jaw sections, and a separate analysis of changes in bone dimensions during orthodontic treatment, taking into account the inclination of the teeth as we consider different moments of orthodontics treatment (despite suspension of orthodontic movement for the duration of surgical treatment).

Conclusions

This is the first developed and proven method of 3D bone reconstruction in areas with existing teeth. It creates the possibility of safe and predictable reconstruction of vertical and horizontal alveolar bone in the toothed area above the tooth, increases the long-term results of covering gingival recessions through buccal bone reconstruction, enables the prevention of bone dehiscence in orthodontically treated patients, and improves the morphology of the lower part of the face. It can be successfully performed under local anesthesia. A similar method is worth considering for other areas of the oral cavity.

Supplementary files

The Supplementary materials are available at https://doi.org/10.5281/zenodo.11609828. The package includes the following files:

Supplementary Table 1. The Shapiro –Wilk's normality test (n = 32 for each variable and each tooth) for the differences between before and after the treatment.

Supplementary Table 2. Descriptive statistics of the studied bone growth parameters.

Supplementary Table 3. Relative Treatment Effects for the studied teeth and the statistical significance of the differences between before and after treatment.

Supplementary Table 4. Spearman's correlations of the bone growth parameters (averaged data for all teeth).

Supplementary Table 5. The differences in bone growth parameters (averaged data for all teeth) between sexes.

Supplementary Table 6. The differences in bone growth parameters (averaged data for all teeth) between patients who underwent orthodontic treatment with a passive wire (Wire) and patients without ongoing treatment (Without wire).

Supplementary Table 7. The differences in bone growth parameters (averaged data for all teeth) between patients with recessions (Yes) and patients without recession (No).

Supplementary Table 8. The differences in bone growth parameters (averaged data for all teeth) between patients with thin biotype (Thin) and patients with thick biotype (Thick).

Supplementary Table 9. The differences in bone growth parameters (averaged data for all teeth) between patients with excessive mentalis muscle tension (Yes) and patients without such the feature (No).

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Jun CM, Yun JH. Three-dimensional bone regeneration of alveolar ridge defects using corticocancellous allogeneic block grafts: Histologic and immunohistochemical analysis. *Int J Periodontics Restorative Dent*. 2016;36(1):75–81. doi:10.11607/prd.1950
2. Sanz M, Vignoletti F. Key aspects on the use of bone substitutes for bone regeneration of edentulous ridges. *Dent Mater*. 2015;31(6):640–647. doi:10.1016/j.dental.2015.03.005

3. Canullo L, Del Fabbro M, Khijmatgar S, et al. Dimensional and histomorphometric evaluation of biomaterials used for alveolar ridge preservation: A systematic review and network meta-analysis. *Clin Oral Invest*. 2022;26(1):141–158. doi:10.1007/s00784-021-04248-1
4. Chavda S, Levin L. Human studies of vertical and horizontal alveolar ridge augmentation comparing different types of bone graft materials: A systematic review. *J Oral Implantol*. 2018;44(1):74–84. doi:10.1563/aaid-joi-D-17-00053
5. Motamedian S, Khojaste M, Khojasteh A. Success rate of implants placed in autogenous bone blocks versus allogenic bone blocks: A systematic literature review. *Ann Maxillofac Surg*. 2016;6(1):78. doi:10.4103/2231-0746.186143
6. Dominiak M, Łysiak-Drwal K, Solski L, Żywicka B, Rybak Z, Gedrange T. Evaluation of healing processes of intraosseous defects with and without guided bone regeneration and platelet rich plasma: An animal study. *Ann Anat*. 2012;194(6):549–555. doi:10.1016/j.aanat.2012.07.007
7. Gupta A, Arora K, Aggarwal P, Kaur K, Mohapatra S, Pareek S. Evaluation of biphasic hydroxapatite and β-tricalcium phosphate as a bone graft material in the treatment of periodontal vertical bony defects: A clinical and digital radiological measurement study. *Indian J Dent Res*. 2022;33(2):152. doi:10.4103/ijdr.IJDR_234_19
8. Kao RT, Nares S, Reynolds MA. Periodontal regeneration – intrabony defects: A systematic review from the AAP Regeneration Workshop. *J Periodontol*. 2015;86(2 Suppl):S77–S104. doi:10.1902/jop.2015.130685
9. Dominiak M, Hnitecka S, Olchowy C, Olchowy A, Gedrange T. Analysis of alveolar ridge width in an area of central lower incisor using cone-beam computed tomography in vivo. *Ann Anat*. 2021;236:151699. doi:10.1016/j.aanat.2021.151699
10. Nimigean VR, Nimigean V, Bencze MA, Dimcevici-Poesina N, Cergan R, Moraru S. Alveolar bone dehiscences and fenestrations: An anatomical study and review. *Rom J Morphol Embryol*. 2009;50(3):391–397.
11. Antoun JS, Mei L, Gibbs K, Farella M. Effect of orthodontic treatment on the periodontal tissues. *Periodontol 2000*. 2017;74(1):140–157. doi:10.1111/prd.12194
12. Jati AS, Furquim LZ, Consolaro A. Gingival recession: Its causes and types, and the importance of orthodontic treatment. *Dental Press J Orthod*. 2016;21(3):18–29. doi:10.1590/2177-6709.21.3.018-029.oin
13. Saminsky M, Meir H, Sebaoun A, Tal H. Positive relationship between the dimensions of clinical gingival recessions and associated alveolar bone dehiscences: A biometric study. *Quintessence Int*. 2022;53(4):308–313. doi:10.3290/j.qi.b2644803
14. Stimmelmayr M, Allen EP, Gernet W, et al. Treatment of gingival recession in the anterior mandible using the tunnel technique and a combination epithelialized-subepithelial connective tissue graft: A case series. *Int J Periodontics Restorative Dent*. 2011;31(2):165–173. PMID:21491016.
15. Melo LGN, Almeida ALPF, Lopes JFS, et al. A modified approach for vestibuloplasty in severely resorbed mandible using an implant-retained postoperative stent: A case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2008;106(4):e7–e14. doi:10.1016/j.tripleo.2008.05.029
16. Murias I, Grzech-Leśniak K, Murias A, et al. Efficacy of various laser wavelengths in the surgical treatment of ankyloglossia: A systematic review. *Life*. 2022;12(4):558. doi:10.3390/life12040558
17. Olivi G, Signore A, Olivi M, Genovese MD. Lingual frenectomy: Functional evaluation and new therapeutical approach. *Eur J Paediatr Dent*. 2012;13(2):101–106. PMID:22762170.
18. Kalecińska E, Dominiak M, Dominiak P, Anwejler J. Postural disturbances in the presence of the dysfunction of the masticatory system: Case report. *Dent Med Probl*. 2004;41:305–312.
19. Dominiak M, Dominiak S, Targonska S, Gedrange T. Three-dimensional bone block planning for mandibular sagittal bone defect reconstruction. *J Healthcare Eng*. 2020;2020:8829288. doi:10.1155/2020/8829288
20. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2006;101(3):e37–e44. doi:10.1016/j.tripleo.2005.07.008
21. Yang Y, Yang H, Pan H, Xu J, Hu T. Evaluation and new classification of alveolar bone dehiscences using cone-beam computed tomography in vivo. *Int J Morphol*. 2015;33(1):361–368. doi:10.4067/S0717-95022015000100057
22. Noguchi K, Gel YR, Brunner E, Konietschke F. nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments. *J Stat Soft*. 2012;50(12):1–23. doi:10.18637/jss.v050.i12
23. Jäger F, Mah JK, Bumann A. Peridental bone changes after orthodontic tooth movement with fixed appliances: A cone-beam computed tomographic study. *Angle Orthod*. 2017;87(5):672–680. doi:10.2319/102716-774.1
24. Imber JC, Kasaj A. Treatment of gingival recession: When and how? *Int Dent J*. 2021;71(3):178–187. doi:10.1111/idj.12617
25. Sheikh Z, Hamdan N, Ikeda Y, Grynpas M, Ganss B, Glogauer M. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: A review. *Biomater Res*. 2017;21(1):9. doi:10.1186/s40824-017-0095-5
26. Pfaffeneder-Mantai F, Meller O, Schneider B, et al. Specially designed and CAD/CAM manufactured allogeneic bone blocks using for augmentation of a highly atrophic maxilla show a stable base for an all-on-six treatment concept: A case report. *Maxillofac Plast Reconstr Surg*. 2022;44(1):21. doi:10.1186/s40902-022-00351-9
27. Brugnami F, Caiazzo A, Mehra P. Can corticotomy (with or without bone grafting) expand the limits of safe orthodontic therapy? *J Oral Biol Craniofac Res*. 2018;8(1):1–6. doi:10.1016/j.jobcr.2017.11.001
28. Eufinger H. Individual augmentation of the atrophic mandible based on CAD/CAM-manipulated computed tomography data: In vitro results. *Int J Oral Maxillofac Surg*. 1994;23(6):399–402. doi:10.1016/S0901-5027(05)80029-3
29. Chaushu G, Mardinger O, Peleg M, Ghelfan O, Nissan J. Analysis of complications following augmentation with cancellous block allografts. *J Periodontol*. 2010;81(12):1759–1764. doi:10.1902/jop.2010.100235
30. Dominiak M, Hnitecka S, Olchowy C, Dominiak S, Gedrange T. Possible treatment of severe bone dehiscences based on 3D bone reconstruction: A description of treatment methodology. *Appl Sci*. 2021;11(21):10299. doi:10.3390/app112110299
31. Blume O, Donkiewicz P, Back M, Born T. Bilateral maxillary augmentation using CAD/CAM manufactured allogenic bone blocks for restoration of congenitally missing teeth: A case report. *J Esthet Restor Dent*. 2019;31(3):171–178. doi:10.1111/jerd.12454
32. Dominiak M. Własna metoda oceny prognozowania recesji przyzębia [habilitation thesis]. Wrocław, Poland: Wroclaw Medical University; 2009.

Impact of magnesium on intraperitoneal adhesion in an experimental rat model

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Abstract

Background. Intraperitoneal adhesions are fibrous bands that form between tissues and organs in the abdominal cavity, which can result from the body's healing process after surgery, leading to pain, bowel obstruction, and infertility in severe cases. Magnesium (Mg), known for its anti-inflammatory and anticoagulant properties, has been hypothesized to influence adhesion formation.

Objectives. This study is designed to explore the hypothesized benefits of Mg, known for its anti-inflammatory and anticoagulant properties, on the prevention of intraperitoneal adhesions that commonly occur following abdominal surgeries. It seeks to provide a comprehensive understanding of Mg's potential role in mitigating adhesion formation, aiming to contribute valuable insights into postoperative recovery processes and outcomes.

Materials and methods. We employed an experimental model of intestinal abrasion in male Wistar rats. The rats were categorized into control and treatment groups, with the latter receiving varying doses of Mg sulfate. Intraperitoneal adhesions were induced using a multi-abrasion model.

Results. Based on both the Evans model and histopathological evaluations, it was observed that there were significant differences in adhesion scores between the groups. Magnesium-treated groups showed significantly fewer adhesions than the control group. Histopathological analyses indicated variations in adhesion characteristics and inflammatory responses among the groups.

Conclusions. Preliminary results indicated the potential role of Mg in mitigating postoperative intraperitoneal adhesions. These findings suggest the need for further research to confirm the efficacy of Mg and to explore its mechanisms of action in clinical settings.

Key words: intraperitoneal adhesion, anti-inflammatory agents, magnesium sulfate, adhesion prevention

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Background

Postoperative adhesions are fibrovascular bands that form between peritoneal surfaces, usually occurring after abdominal or pelvic surgeries, with a reported incidence of up to 54%.[1,2] These adhesions develop as part of the natural healing process, involving factors such as coagulation, inflammation and fibrinolysis, and their clinical significance varies.[3] Despite advances in surgical techniques, peritoneal adhesions remain a significant clinical challenge, often necessitating further intervention. While not classified as complications, adhesions can lead to various issues in approx. 19% of patients, including acute/chronic abdominal pain, bowel obstruction, infertility, and iatrogenic intestinal injury during adhesiolysis.[2,4]

Magnesium (Mg), the 4th most abundant mineral in the human body, serves as a cofactor in more than 300 enzymatic reactions and influences energy metabolism, protein synthesis and nucleic acid synthesis.[5–7] Moreover, its anti-inflammatory,[8–14] antioxidant,[11,15] bronchodilator,[16] vasodilator,[17,18] antiaggregant,[19] and neuroprotective[20,21] properties have been demonstrated. These properties are linked to reduced anesthesia requirements during surgery[22] and are effective at controlling neuropathic pain.[23]

The inflammatory process and fibrin matrix formation following peritoneal injury are major factors responsible for adhesion formation,[3] and the anti-inflammatory and coagulation-related effects of Mg have been demonstrated in many studies.

Objectives

Given Mg's well-known effects on the inflammatory and coagulation cascades, this study aimed to investigate the effect of Mg on intra-abdominal adhesions in an experimental intestinal abrasion rat model.

Materials and methods

Study design

Twenty-six male Wistar albino rats, aged 9–10 weeks and weighing 300–400 g, were obtained from the Experimental Animals Research Unit of the Bülent Ecevit University, Zonguldak, Turkey. The rats were allowed to acclimate under standard laboratory conditions, namely, 23 ±2°C, 50% humidity and a 12-h artificial light cycle, for 1 week. Throughout this period, the animals had ad libitum access to food and water.

Magnesium sulfate ($MgSO_4$) (15%; Biofarma, Istanbul, Turkey) was utilized in the experimental procedures.

This study was conducted in line with the ethical standards set by the National Institutes of Health Guidelines for the care and use of laboratory animals. Approval for the study was obtained from the Ethics Review Board of the Zonguldak Bulent Ecevit University (Zonguldak, Turkey; protocol No. 2021-20-02/09).

Multi-abrasion model

The intra-abdominal adhesion model described by Öncel et al. was used in this study.[24] A 12-mm incision was made in the midline to reveal the cecum and small intestine. The anterior wall of the cecum was gently abraded with 20 strokes using a brush. During this procedure, the soft motion of the brush carefully abraded the surface of the organ wall. Additionally, 5 abrasions were induced on the small intestine at intervals of 3 cm, starting 5 cm from the ileocecal valve, as illustrated in Fig. 1. Following the creation of the abrasions, the cecum and small intestine were repositioned. The abdominal incision was closed in 2 layers using 3-0 polyglactin and 3-0 polypropylene sutures.

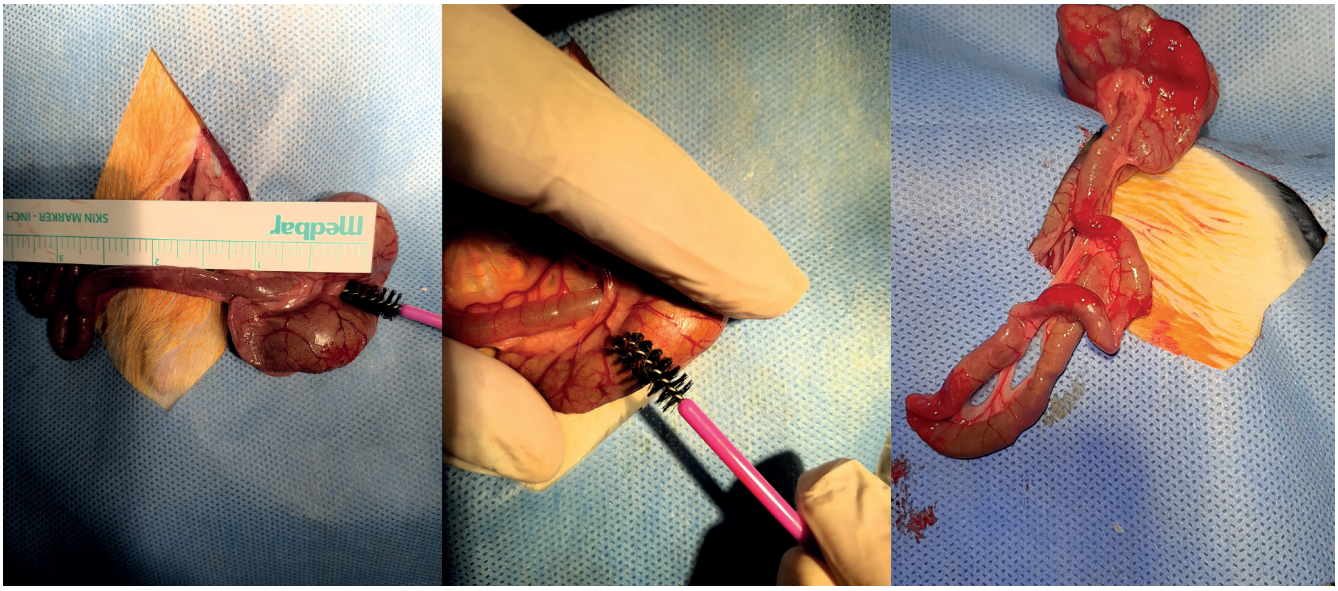

Fig. 1. Illustration of the intra-abdominal adhesion model as implemented by Öncel et al.[24]

Surgical procedure

The rats were randomly assigned to 1 of the following 4 groups: group 1 (sham group, n = 2), group 2 (control group, n = 8), group 3 (300 mg/kg $MgSO_4$ treatment group, n = 8), and group 4 (500 mg/kg $MgSO_4$ treatment group, n = 8).

The rats were weighed and then anesthetized using an intramuscular injection of 100 mg/kg ketamine (Ketalar; Parke Davis Eczacıbaşı, Istanbul, Turkey). The lower abdomen was shaved and cleaned with alcohol and povidone-iodine. All surgical procedures were performed in a semi-sterile environment by the same surgeon.

Rats in group 1 did not undergo surgery. Rats in groups 2, 3 and 4 were subjected to the multi-abrasion model, as detailed by Öncel et al.[24] For groups 3 and 4, before the abdominal cavity was closed, $MgSO_4$ in proportion to their body weight was intraperitoneally administered. After the surgery, each animal was placed in an individual cage. Rats were provided standard food and had adequate access to water; they were euthanized on post-operative day 7. Laparotomy was conducted to evaluate adhesion formation using a validated adhesion scoring system. Adhesions were assessed and graded according to the Evans model[25] (Table 1). An observer blinded to the study design performed the scoring process, as shown in Fig. 2,3.

Table 1. Adhesion severity score (Evans model)

Adhesion grade	Definition
0	no adhesions
1	filmy adhesions separate spontaneously
2	firm adhesions separated by traction
3	dense adhesions requiring sharp dissection

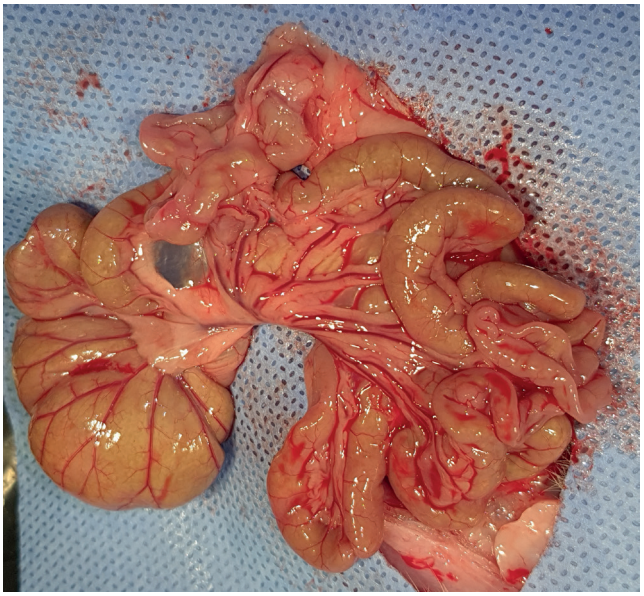

Fig. 2. Adhesion formations observed in rats following laparotomy on the 7th postoperative day. Adhesion scoring using the Evans model (grade 0: no adhesions)

The terminal ileum and cecum were dissected for histological analysis without separating the adhesions.

Histology

Tissue specimens from the terminal ileum and cecum were fixed in 10% neutral formalin and embedded in paraffin. Sections were cut from the paraffin blocks of each tissue sample using a microtome at a thickness of 4–5 µm. The specimens were then deparaffinized and stained with hematoxylin and eosin (H&E) or with Perls Prussian blue, a histochemical method for detecting hemosiderin pigments. A light microscope (Leica DM3000 LED; Leica Camera AG, Jena, Germany) was used for evaluation. The presence of adhesions and the intensity of inflammation around the terminal ileum and cecum were evaluated histopathologically (Fig. 4). These findings were observed during the microscopic evaluation of the H&E-stained sections and graded on a scale of 0 (absence of the characteristic) to 3 (intensive presence of the characteristic). Sections stained with Perls Prussian blue were evaluated for the presence of hemosiderin. The same pathologist examined all tissue sections collected for light microscopy examinations without any knowledge of the group assignments.

Statistical analyses

The data were analyzed using IBM SPSS v. 23 software (IBM Corp., Armonk, USA). The Fisher–Freeman–Halton

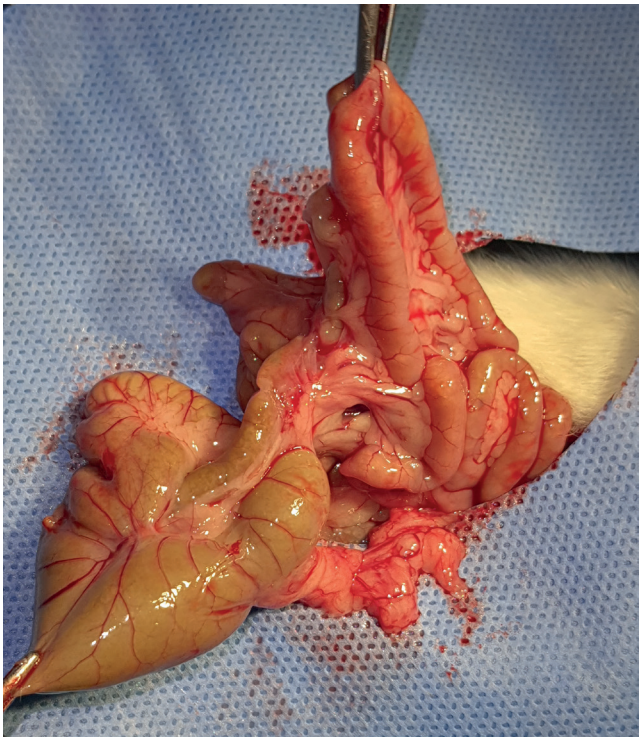

Fig. 3. Adhesion formations observed in rats following laparotomy on the 7th postoperative day. Adhesion scoring using the Evans model (grade 3: dense adhesions requiring sharp dissection)

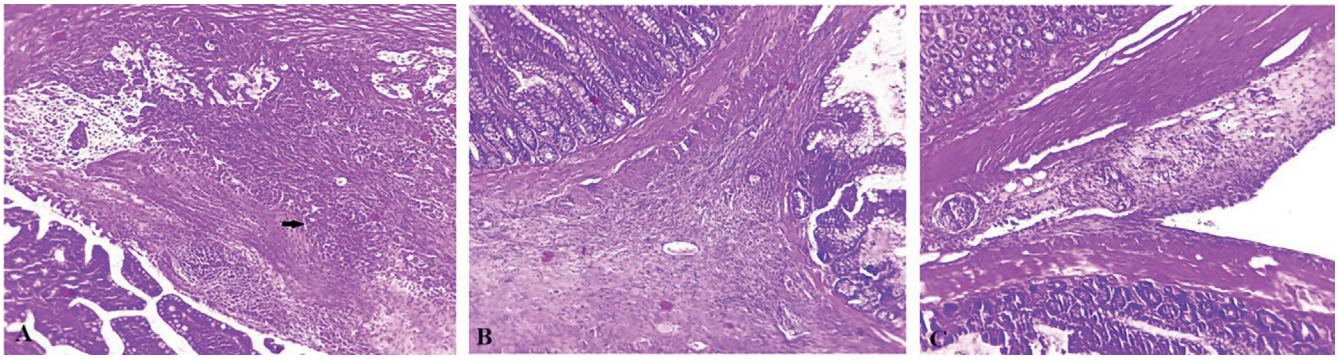

Fig. 4. Histopathological examination of adhesions and inflammation intensity around the terminal ileum-cecum. A. Photomicrograph of the control group. Serosal thickening, edema, dense inflammatory cell reaction, micro abscess focus (arrow), and granulation tissue formation in the serosal surfaces (hematoxylin & eosin (H&E) staining, ×100 magnification); B. Photomicrograph of the magnesium-treated (300 mg) group. Edema, moderate inflammatory cell reactions and granulation tissue formation on serosal surfaces (H&E, ×100 magnification); C. Photomicrograph of the magnesium-treated (500 mg) group. Edema, mild inflammatory cell reactions and granulation tissue formation on serosal surfaces (H&E, ×100 magnification)

test was used to compare categorical variables between groups since the minimum expected value was less than 5, and the data were not arranged in a 2×2 contingency table format. Additionally, multiple comparisons of proportions were examined using the Bonferroni-adjusted Z test. Categorical variables were expressed as a frequency (percentage). A p-value < 0.05 was considered statistically significant.

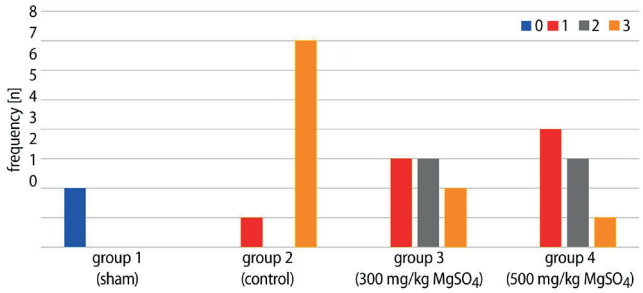

Fig. 5. Comparative analysis of adhesion severity scores across different groups using the Evans model

Results

Analysis of adhesion scores using the Evans model

Evaluation of adhesion scores according to the Evans model revealed distinct variations among the groups. In group 1, no adhesions were observed, whereas in group 2, 12.5% of cases showed filmy adhesions that separated spontaneously, and a significant majority (87.5%) of cases exhibited dense adhesions necessitating sharp dissection. In group 3, filmy adhesions that separated spontaneously and firm adhesions separable by traction were observed in 37.5% of the cases, and dense adhesions requiring sharp dissection were observed in 25% of the cases. In group 4, 50% of the cases had filmy adhesions that separated spontaneously, 37.5% had firm adhesions separable by traction, and 12.5% had dense adhesions necessitating sharp dissection. Upon evaluation, a statistically significant difference in adhesion scores across the groups was noted (p = 0.001). These results underscore the variability in adhesion characteristics and the effectiveness of the interventions in the different groups. The adhesion scores determined using the Evans model are detailed in Table 2 and illustrated in Fig. 5.

Histopathological analysis of adhesions

An analysis of adhesion scores evaluated histopathologically revealed significant differences among the groups. Histopathological findings were categorized as "none" (0), "weak"

Table 2. Comparative analysis of adhesion scores among groups based on the Evans model

Group	Adhesion score				Test statistic	p-value*
	0 n (%)	1 n (%)	2 n (%)	3 n (%)		
Group 1 (sham)	2 (100)	0 (0)	0 (0)	0 (0)	19.412	0.001
Group 2 (control)	0 (0)	1 (12.5)	0 (0)	7 (87.5)		
Group 3 (300 mg/kg $MgSO_4$)	0 (0)	3 (37.5)	3 (37.5)	2 (25)		
Group 4 (500 mg/kg $MgSO_4$)	0 (0)	4 (50)	3 (37.5)	1 (12.5)		

*Fisher–Freeman–Halton test. Adhesion severity score according to the Evans model: 0 = no adhesions, 1 = filmy adhesions separating spontaneously, 2 = firm adhesions separated by traction, 3 = dense adhesions requiring sharp dissection.

Table 3. Analysis of histopathological characteristics in tissue samples from the terminal ileum and cecum

Score	Sham				Group 2 (control, n = 8)				Group 3 (300 mg/kg $MgSO_4$, n = 8)				Group 4 (500 mg/kg $MgSO_4$, n = 8)				Test statistic	p-value*
	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3		
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)		
Mucosal inflammation	1 (50)	1 (50)	0	0	7 (87.5)	1 (12.5)	0	0	3 (37.5)	3 (37.5)	0	2 (25)	2 (25)	2 (25)	1 (12.5)	3 (37.5)	10.915	0.215
Submucosal inflammation	2 (100)	0	0	0	5 (62.5)	3 (37.5)	0	0	2 (25)	2 (25)	3 (37.5)	1 (12.5)	2 (25)	1 (12.5)	3 (37.5)	2 (25)	10.157	0.288
Serosal inflammation	2 (100)	0	0	0	0	1 (12.5)	3 (37.5)	4 (50)	2 (25)	1 (12.5)	3 (37.5)	2 (25)	1 (12.5)	3 (37.5)	2 (25)	2 (25)	9.382	0.380
Fat necrosis	2 (100)	0	0	0	8 (100)	0	0	0	4 (50)	2 (25)	2 (25)	0	4 (50)	3 (37.5)	1 (12.5)	0	7.378	0.192
Hemosiderin-laden macrophages	2 (100)	0	0	0	8 (100)	0	0	0	1 (12.5)	3 (37.5)	2 (25)	2 (25)	0	2 (25)	2 (25)	4 (50)	24.012	<0.001
Adhesion	2 (100)	0	0	0	0	8 (100)	0	0	4 (50)	4 (50)	0	0	5 (62.5)	3 (37.5)	0	0	10.216	0.016

*Fisher–Freeman–Halton test. Histopathologic findings are scored as 0 = none, 1 = weak, 2 = moderate, and 3 = intense. Adhesion is classified as 0 = absent and 1 = present.

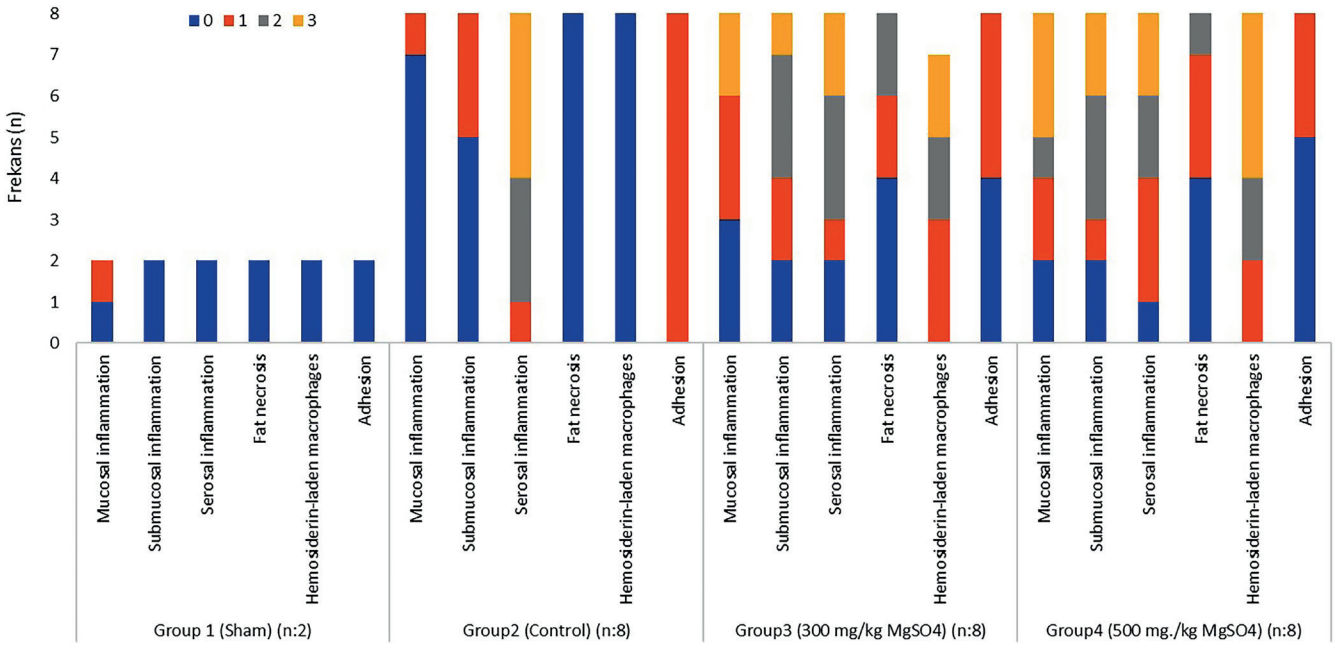

Fig. 6. Histopathological evaluation of adhesion scores in different groups. Comparison of mucosal inflammation, submucosal inflammation, fatty necrosis, macrophage infiltration, and adhesion presence

(1), "moderate" (2), or "intense" (3). Adhesions were classified as "absent" (0) or "present" (1). These histopathological findings are detailed in Table 3 and illustrated in Fig. 6.

Distribution of hemosiderin-laden macrophage scores across groups

In groups 1 and 2, hemosiderin-laden macrophages were categorized as "none" in all cases (100%), and no weak, moderate or intense instances were observed. In group 3, the distribution was found to be 12.5% "none", 37.5% "weak", 25% "moderate", and 25% "intense". In group 4, the distribution was 0% "none", 25% "weak", 25% "moderate", and 50% "intense". Based on the scores from the different groups, there were no statistically significant differences in mucosal inflammation, submucosal inflammation, serosal inflammation, or fat necrosis between the groups ($p > 0.05$). However, a statistically significant difference was noted in the scoring of hemosiderin-laden macrophages between the groups ($p < 0.001$).

Adhesion scores across groups

Significant variation was observed in the distribution of adhesion scores among the groups ($p < 0.05$). In group 1, adhesions were absent in all cases (100%). In group 2, adhesions were present in all cases (100%). In group 3, adhesions were absent in 50% of the cases and present in the remaining 50%. In group 4, adhesions were absent in 62.5% of the cases and present in 37.5% of cases.

Discussion

Our study revealed significant differences in adhesion formation among the groups ($p < 0.05$). Specifically, the Mg-treated groups (groups 3 and 4) showed significantly fewer adhesions than the control group (group 2). Our histopathological analysis revealed the detailed characteristics of these adhesions. We observed variations in the number of hemosiderin-laden macrophages among the groups. This, coupled with the differences in serosal inflammation, suggests a potential role for Mg in managing inflammation and reducing adhesion formation.

Adhesions account for 3% of all laparotomy cases and approx. 1% of general surgery admissions.[26] Various factors are implicated in adhesion formation, including rough surgical techniques, tissue drying, infections, peritoneal endometriosis, suture materials, foreign bodies, and the presence of blood or clots in the peritoneal cavity.[27] The following strategies aim to prevent adhesions by intervening in their pathophysiological mechanisms: surgical techniques, drugs, materials (such as barrier methods), and advanced technologies (such as nanoparticle and gene therapy).[28] Despite advances in modern medicine, postoperative adhesions remain an unsolved problem.[29] Adhesions can develop in response to nonsurgical or surgical injuries and are typically associated with the disruption of the balance between inflammation, fibrin formation and fibrinolysis.[27,30,31]

Local inflammation activates fibrin coagulation pathways, resulting in infiltration of inflammatory cells and fibrinogen deposition.[31,32] Fibrinogen is converted into fibrin, and if there is an imbalance between fibrin formation and fibrinolysis, adhesive bonds form.[33] As a result, abnormal intraperitoneal fibrous bands connected to the surface form abdominal adhesions.[34]

It has been observed that the levels of pro-inflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) increase in the peritoneal fluid during acute inflammation.[35,36] Cytokines may contribute to the remodeling of the extracellular matrix (ECM) by interacting with the fibrinolytic pathway,[37,38] promoting inflammation and coagulation, reducing fibrinolytic capacity by stimulating the release of plasminogen activator inhibitors,[39] and suppressing the production of tissue plasminogen activators.[40,41] These mechanisms suggest that the interruption of processes during the early stages of the inflammatory cascade may reduce adhesion formation.[42] The anti-inflammatory and antioxidant properties of vitamin C and vitamin E have shown potential in significantly reducing postoperative adhesions. These treatments modulate inflammatory responses and reduce oxidative stress in the affected tissues.[43] Similarly, the administration of intraperitoneal surfactant has been demonstrated to be effective in mitigating the formation of postoperative intra-abdominal adhesions, primarily by decreasing inflammation and fibrosis at the surgical site.[44]

The inhibitory effect of Mg on thrombus formation is dose-dependent and may also delay the formation of arterial blood clots by inhibiting platelet activity.[45] Calcium (Ca) ions, known as clotting factor IV, are involved in all 3 routes of clot formation,[46] and the antagonism between Ca and Mg is well known.[47] The effect of Mg on coagulation involves the displacement of Ca from the structure of procoagulant proteins. These procoagulant proteins (prothrombin, FII; blood coagulation factors FXIII, FX, FXI, FVII, FVIII, and FIX; and protein C) are Ca-dependent. However, their activity levels may decline because of excessive Mg.[45] In addition, Mg can affect coagulation through proteolysis of von Willebrand factor; thus, Mg is a natural disaggregant and anticoagulant.

The most likely mechanism of action of the anti-inflammatory effects of Mg on the arachidonic acid cascade is the direct inhibition of phospholipase.[45] According to Liu et al., hypomagnesemia induces inflammation via various signaling pathways, including the induction of cellular oxidative stress, the opening of the Ca channel, activation of the renin–angiotensin–aldosterone system and phagocytic cells, nuclear factor-kB signaling, a reduction in the levels of anti-inflammatory mediators, and the release of overactive N-methyl-D-aspartate receptors and substance P.[47] Magnesium plays a significant role in regulating intracellular pH and osmotic balance.[48] The influence of Mg on intracellular signaling pathways can modulate intracellular pH levels. This interaction has critical implications, considering the effects of intracellular pH on cellular metabolism and function. Specifically, the role of Mg in the regulation of intracellular pH is crucial for determining cellular energy metabolism and vulnerability to stress, as evidenced in mitochondrial Mg homeostasis studies.[49] The potential of Mg to reduce cell adhesion can be linked to its role in modulating the intracellular pH and relevant signaling pathways. Changes in pH can influence the conformation and function of cell adhesion molecules. Additionally, Mg may affect the signaling pathways that regulate the expression and activation of these molecules, leading to reduced adhesions.[50] This reported theoretical basis suggests a significant area for future research to fully elucidate this effect. In contrast, the effects of Mg on integrin binding affinity may affect the migration of inflammatory cells to the abraded site and their subsequent adhesion-forming function. Integrins are

cell surface receptors that play significant roles in the interaction of cells with the ECM. The activity of these receptors is crucial for processes such as cell migration and adhesion. Magnesium modulates intracellular signaling pathways and influences the binding affinity of integrins. This suggests that Mg plays a direct role in cell behavior. In particular, increases in Mg concentrations have been shown to affect the activation state of integrins, thereby altering cell migration and adhesion capacities. This interaction is supported by detailed nuclear magnetic resonance spectroscopy studies on the integrin α1 I domain, demonstrating that Mg regulates integrin-collagen recognition and binding through microsecond dynamics.[51] These interactions may have significant implications for the migration of inflammatory cells. By influencing the activity of integrins, Mg can modulate the ability of inflammatory cells to migrate to damaged tissues and form adhesions. This concept is further supported by studies on synovial stem cells, wherein Mg has been shown to enhance adhesions to collagen. This effect was inhibited by neutralizing antibodies against integrin α3 and β1, indicating the role of Mg in promoting integrin-mediated adhesion and early-phase cartilage matrix synthesis.[52]

We hypothesized that Mg would reduce adhesion development by altering thromboxane and prostaglandin synthesis via arachidonic acid metabolism, lowering vascular permeability, plasmin inhibitors, platelet aggregation, and coagulation, and modulating intracellular pH and signaling pathways. Magnesium is readily available, cost-effective and safe. Notably, this is the first study to examine the effects of Mg on intra-abdominal adhesions. To clinically evaluate adhesions, we utilized the Evans grading model and found that adhesions were noticeably reduced in the Mg groups compared to controls. Hemosiderin-laden macrophages are commonly associated with tissue repair and inflammation.[53] We found that Mg potentially accelerated the inflammation resolution phase, which may explain the observed increase in hemosiderin-laden macrophages.[54,55] Specifically, the Mg-treated groups demonstrated a significantly higher number of these macrophages than the control group ($p < 0.001$). Additionally, although not statistically significant, we noted a discernible reduction in serosal inflammation rates in the Mg-treated groups relative to the control group based on histopathological analyses. Brochhausen et al. pointed out that initial localized ischemia, followed by inflammation in the injured tissues, plays a role in the development of peritoneal adhesions.[56] There is a robust link between adhesions and serosal surface inflammation. Given the anti-inflammatory properties of Mg, the reduced inflammation observed in the Mg groups compared to the controls in the histological assessments may underscore one of the mechanisms by which Mg curbs adhesion growth. Further studies are warranted to elucidate the cellular and molecular mechanisms by which Mg inhibits adhesion development.

Limitations

This study has some limitations. First, we were uncertain about the effective intraperitoneal dose of the administered substance. Another limitation arises from the scoring of the adhesions. Although a blinded researcher performed the adhesion scoring process, the potential subjectivity and insensitivity of the observer's scoring technique may have compromised its repeatability and consistency.

Conclusions

Our findings show that Mg may be an effective agent in preventing intra-abdominal adhesions, laying the groundwork for future studies involving more detailed cellular analyses and various dosages and administration methods. Clarifying the effects of Mg on the formation of abdominal adhesions will enrich our knowledge in this area and may lead to significant changes in clinical practice.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Awonuga AO, Saed GM, Diamond MP. Laparoscopy in gynecologic surgery: Adhesion development, prevention, and use of adjunctive therapies. *Clin Obstet Gynecol*. 2009;52(3):412–422. doi:10.1097/GRF.0b013e3181b0c121
2. Okabayashi K, Ashrafian H, Zacharakis E, et al. Adhesions after abdominal surgery: A systematic review of the incidence, distribution and severity. *Surg Today*. 2014;44(3):405–420. doi:10.1007/s00595-013-0591-8
3. Tang J, Xiang Z, Bernards MT, Chen S. Peritoneal adhesions: Occurrence, prevention and experimental models. *Acta Biomater*. 2020;116:84–104. doi:10.1016/j.actbio.2020.08.036
4. Van Der Krabben AA, Dijkstra FR, Nieuwenhuijzen M, Reijnen MMPJ, Schaapveld M, Van Goor H. Morbidity and mortality of inadvertent enterotomy during adhesiotomy. *Br J Surg*. 2002;87(4):467–471. doi:10.1046/j.1365-2168.2000.01394.x
5. Gröber U, Schmidt J, Kisters K. Magnesium in prevention and therapy. *Nutrients*. 2015;7(9):8199–8226. doi:10.3390/nu7095388
6. Ohyama T. New aspects of magnesium function: A key regulator in nucleosome self-assembly, chromatin folding and phase separation. *Int J Mol Sci*. 2019;20(17):4232. doi:10.3390/ijms20174232
7. Sissi C, Palumbo M. Effects of magnesium and related divalent metal ions in topoisomerase structure and function. *Nucl Acids Res*. 2009;37(3):702–711. doi:10.1093/nar/gkp024

8. Abiri B, Vafa M. Effects of vitamin D and/or magnesium supplementation on mood, serum levels of BDNF, inflammatory biomarkers, and SIRT1 in obese women: A study protocol for a double-blind, randomized, placebo-controlled trial. *Trials*. 2020;21(1):225. doi:10.1186/s13063-020-4122-9
9. Eshraghi T, Eidi A, Mortazavi P, Asghari A, Tavangar SM. Magnesium protects against bile duct ligation-induced liver injury in male Wistar rats. *Magnesium Res*. 2015;28(1):32–45. doi:10.1684/mrh.2015.0380
10. Han F, Xu L, Huang Y, Chen T, Zhou T, Yang L. Magnesium sulphate can alleviate oxidative stress and reduce inflammatory cytokines in rat placenta of intrahepatic cholestasis of pregnancy model. *Arch Gynecol Obstet*. 2018;298(3):631–638. doi:10.1007/s00404-018-4850-1
11. Kao MC, Jan WC, Tsai PS, Wang TY, Huang CJ. Magnesium sulfate mitigates lung injury induced by bilateral lower limb ischemia–reperfusion in rats. *J Surg Res*. 2011;171(1):e97–e106. doi:10.1016/j.jss.2011.03.028
12. Ozen M, Xie H, Shin N, et al. Magnesium sulfate inhibits inflammation through P2X7 receptors in human umbilical vein endothelial cells. *Pediatr Res*. 2020;87(3):463–471. doi:10.1038/s41390-019-0557-7
13. Rochelson B, Dowling O, Schwartz N, Metz CN. Magnesium sulfate suppresses inflammatory responses by human umbilical vein endothelial cells (HuVECs) through the NFκB pathway. *J Reprod Immunol*. 2007;73(2):101–107. doi:10.1016/j.jri.2006.06.004
14. Turner DL, Ford WR, Kidd EJ, Broadley KJ, Powell C. Effects of nebulised magnesium sulphate on inflammation and function of the guinea-pig airway. *Eur J Pharmacol*. 2017;801:79–85. doi:10.1016/j.ejphar.2017.03.004
15. Güzel A, Doğan E, Türkçü G, et al. Dexmedetomidine and magnesium sulfate: A good combination treatment for acute lung injury? *J Invest Surg*. 2019;32(4):331–342. doi:10.1080/08941939.2017.1422575
16. Rowe BH, Camargo CA. The role of magnesium sulfate in the acute and chronic management of asthma. *Curr Opin Pulm Med*. 2008;14(1):70–76. doi:10.1097/MCP.0b013e3282f19867
17. Bachnas MA, Akbar MIA, Dachlan EG, Dekker G. The role of magnesium sulfate ($MgSO_4$) in fetal neuroprotection. *J Matern Fetal Neonatal Med*. 2021;34(6):966–978. doi:10.1080/14767058.2019.1619688
18. Jameson RA, Bernstein HB. Magnesium sulfate and novel therapies to promote neuroprotection. *Clin Perinatol*. 2019;46(2):187–201. doi:10.1016/j.clp.2019.02.008
19. Ravn HB, Vissinger H, Kristensen SD, Husted SE. Magnesium inhibits platelet activity: An in vitro study. *Thromb Haemost*. 1996;76(1):88–93. PMID:8819258.
20. Shimosawa T, Takano K, Ando K, Fujita T. Magnesium inhibits norepinephrine release by blocking N-type calcium channels at peripheral sympathetic nerve endings. *Hypertension*. 2004;44(6):897–902. doi:10.1161/01.HYP.0000146536.68208.84
21. Wang Y, Zhang X, Han Y, Yan F, Wu R. Efficacy of combined medication of nifedipine and magnesium sulfate on gestational hypertension and the effect on PAPP-A, VEGF, NO, Hcy and vWF. *Saudi J Biol Sci*. 2019;26(8):2043–2047. doi:10.1016/j.sjbs.2019.08.012
22. Telci L, Esen F, Akcora D, Erden T, Canbolat AT, Akpir K. Evaluation of effects of magnesium sulphate in reducing intraoperative anaesthetic requirements. *Br J Anaesth*. 2002;89(4):594–598. doi:10.1093/bja/aef238
23. Brill S, Sedgwick PM, Hamann W, Di Vadi PP. Efficacy of intravenous magnesium in neuropathic pain. *Br J Anaesth*. 2002;89(5):711–714. PMID:12393768.
24. Oncel M, Remzi FH, Connor J, Fazio VW. Comparison of cecal abrasion and multiple-abrasion models in generating intra-abdominal adhesions for animal studies. *Tech Coloproctol*. 2005;9(1):29–33. doi:10.1007/s10151-005-0189-2
25. Evans DM, McAree K, Guyton DP, Hawkins N, Stakleff K. Dose dependency and wound healing aspects of the use of tissue plasminogen activator in the prevention of intra-abdominal adhesions. *Am J Surg*. 1993;165(2):229–232. doi:10.1016/S0002-9610(05)80516-1
26. Welle NJ, Sajjad H, Adkins A, Burns B. Bowel adhesions. In: *StatPearls*. Treasure Island, USA: StatPearls Publishing; 2024:Bookshelf ID NBK470544. http://www.ncbi.nlm.nih.gov/books/NBK470544. Accessed April 23, 2024.
27. Kamel RM. Prevention of postoperative peritoneal adhesions. *Eur J Obstet Gynecol Reprod Biol*. 2010;150(2):111–118. doi:10.1016/j.ejogrb.2010.02.003
28. Flutur IM, Păduraru DN, Bolocan A, Palcău AC, Ion D, Andronic O. Postsurgical adhesions: Is there any prophylactic strategy really working? *J Clin Med*. 2023;12(12):3931. doi:10.3390/jcm12123931
29. Ten Broek RPG, Stommel MWJ, Strik C, Van Laarhoven CJHM, Keus F, Van Goor H. Benefits and harms of adhesion barriers for abdominal surgery: A systematic review and meta-analysis. *Lancet*. 2014;383(9911):48–59. doi:10.1016/S0140-6736(13)61687-6
30. Hu Q, Xia X, Kang X, et al. A review of physiological and cellular mechanisms underlying fibrotic postoperative adhesion. *Int J Biol Sci*. 2021;17(1):298–306. doi:10.7150/ijbs.54403
31. Hellebrekers BWJ, Kooistra T. Pathogenesis of postoperative adhesion formation. *Br J Surg*. 2011;98(11):1503–1516. doi:10.1002/bjs.7657
32. Mutsaers SE, Birnie K, Lansley S, Herrick SE, Lim CB, Prêle CM. Mesothelial cells in tissue repair and fibrosis. *Front Pharmacol*. 2015;6:113. doi:10.3389/fphar.2015.00113
33. Arung W, Meurisse M, Detry O. Pathophysiology and prevention of postoperative peritoneal adhesions. *World J Gastroenterol*. 2011;17(41):4545. doi:10.3748/wjg.v17.i41.4545
34. Rami Reddy SR, Cappell MS. A systematic review of the clinical presentation, diagnosis, and treatment of small bowel obstruction. *Curr Gastroenterol Rep*. 2017;19(6):28. doi:10.1007/s11894-017-0566-9
35. Bauer J, Ganter U, Geiger T, et al. Regulation of interleukin-6 expression in cultured human blood monocytes and monocyte-derived macrophages. *Blood*. 1988;72(4):1134–1140. PMID:3262381.
36. Zemel D, Koomen GC, Hart AA, ten Berge IJ, Struijk DG, Krediet RT. Relationship of TNF-alpha, interleukin-6, and prostaglandins to peritoneal permeability for macromolecules during longitudinal follow-up of peritonitis in continuous ambulatory peritoneal dialysis. *J Lab Clin Med*. 1993;122(6):686–696. PMID:8245688.
37. Cheong YC, Laird SM, Li TC, Shelton JB, Ledger WL, Cooke ID. Peritoneal healing and adhesion formation/reformation. *Hum Reprod Update*. 2001;7(6):556–566. doi:10.1093/humupd/7.6.556
38. Whawell SA, Scott-Coombes DM, Vipond MN, Tebbutt SJ, Thompson JN. Tumour necrosis factor-mediated release of plasminogen activator inhibitor 1 by human peritoneal mesothelial cells. *Br J Surg*. 2005;81(2):214–216. doi:10.1002/bjs.1800810218
39. Whawell SA, Thompson JN. Cytokine-induced release of plasminogen activator inhibitor-1 by human mesothelial cells. *Eur J Surg*. 1995;161(5):315–318. PMID:7662773.
40. Sitter T, Gödde M, Spannagl M, Fricke H, Kooistra T. Intraperitoneal coagulation and fibrinolysis during inflammation: In vivo and in vitro observations. *Fibrinolysis*. 1996;10:99–104. doi:10.1016/S0268-9499(96)80060-9
41. Tietze L, Elbrecht A, Schauerte C, et al. Modulation of pro- and antifibrinolytic properties of human peritoneal mesothelial cells by transforming growth factor beta1 (TGF-beta1), tumor necrosis factor alpha (TNF-alpha) and interleukin 1beta (IL-1beta). *Thromb Haemost*. 1998;79(2):362–370. PMID:9493592.
42. Cahill RA, Redmond HP. Cytokine orchestration in post-operative peritoneal adhesion formation. *World J Gastroenterol*. 2008;14(31):4861. doi:10.3748/wjg.14.4861
43. Atilgan R, Kuloglu T, Ozkan ZS, et al. Evaluation of vitamin C and vitamin E for prevention of postoperative adhesion: A rat uterine horn model study. *J Obstet Gynaecol*. 2015;41(3):418–423. doi:10.1111/jog.12544
44. Pala Ş, Kuloğlu T, Atilgan R, Özkan ZS, Hançer S. What is the impact of intraperitoneal surfactant administration against postoperative intraabdominal adhesion formation? An experimental study. *Turk J Med Sci*. 2023;53(6):1817–1824. doi:10.55730/1300-0144.5752
45. Torshin IY, Gromova OR. *Magnesium and Pyridoxine Fundamental Studies and Clinical Practice*. New York, USA: Nova Science Publishers; 2011. ISBN:978-1-60741-704-0.
46. Barmore W, Bajwa T, Burns B. Biochemistry. Clotting factors. In: *StatPearls*. Treasure Island, USA: StatPearls Publishing; 2024: Bookshelf ID NBK507850. http://www.ncbi.nlm.nih.gov/books/NBK507850. Accessed April 23, 2024.
47. Liu M, Dudley SC. Magnesium, oxidative stress, inflammation, and cardiovascular disease. *Antioxidants*. 2020;9(10):907. doi:10.3390/antiox9100907
48. Ashique S, Kumar S, Hussain A, et al. A narrative review on the role of magnesium in immune regulation, inflammation, infectious diseases, and cancer. *J Health Popul Nutr*. 2023;42(1):74. doi:10.1186/s41043-023-00423-0

49. Yamanaka R, Tabata S, Shindo Y, et al. Mitochondrial Mg^{2+} homeostasis decides cellular energy metabolism and vulnerability to stress. *Sci Rep*. 2016;6(1):30027. doi:10.1038/srep30027
50. Rotondi S, Mazzaferro S. Magnesium: Extracellular, intracellular or total magnesium status? *Nephrol Dial Transplant*. 2023;38(6):1349–1351. doi:10.1093/ndt/gfad059
51. Nunes AM, Minetti CASA, Remeta DP, Baum J. Magnesium activates microsecond dynamics to regulate integrin-collagen recognition. *Structure*. 2018;26(8):1080–1090.e5. doi:10.1016/j.str.2018.05.010
52. Shimaya M, Muneta T, Ichinose S, Tsuji K, Sekiya I. Magnesium enhances adherence and cartilage formation of synovial mesenchymal stem cells through integrins. *Osteoarthritis Cartilage*. 2010;18(10):1300–1309. doi:10.1016/j.joca.2010.06.005
53. Watanabe S, Alexander M, Misharin AV, Budinger GRS. The role of macrophages in the resolution of inflammation. *J Clin Invest*. 2019; 129(7):2619–2628. doi:10.1172/JCI124615
54. Mazur A, Maier JAM, Rock E, Gueux E, Nowacki W, Rayssiguier Y. Magnesium and the inflammatory response: Potential physiopathological implications. *Arch Biochem Biophys*. 2007;458(1):48–56. doi:10.1016/j.abb.2006.03.031
55. Shahi A, Aslani S, Ataollahi M, Mahmoudi M. The role of magnesium in different inflammatory diseases. *Inflammopharmacology*. 2019;27(4):649–661. doi:10.1007/s10787-019-00603-7
56. Brochhausen C, Schmitt VH, Rajab TK, et al. Intraperitoneal adhesions: An ongoing challenge between biomedical engineering and the life sciences. *J Biomed Mater Res A*. 2011;98A(1):143–156. doi:10.1002/jbm.a.33083

An analysis of the clinical significance of the TKI-resistant gene *ZNF687* for hepatocellular carcinoma patients

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Conflict of interest
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Abstract

Background. Novel treatments such as monotherapy and combined immunotherapy significantly extend overall survival (OS) for hepatocellular carcinoma (HCC) patients, but HCC is susceptible to treatment resistance during long-term therapy. The resistance mechanism to targeted drugs in HCC remains ambiguous, making research on HCC drug resistance targets crucial for the development of precision medicine.

Objectives. To investigate the transcriptional features, biological functions and potential clinical value of the tyrosine kinase inhibitor (TKI)-resistant gene *ZNF687* in HCC.

Materials and methods. The TKI-resistant genes of HCC were identified using clustered regularly interspaced short palindromic repeats (CRISPR) in vitro screening. Then, the dependence of HCC cell lines on *ZNF687* was investigated in silico. We collected global mRNA datasets of HCC tissue, integrated the mRNA expression characteristics of *ZNF687* in HCC and explored the impact of *ZNF687* on HCC patient prognoses using the Kaplan–Meier method (in silico). The Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyses were then conducted, and a connectivity map and molecular docking technology were applied to find the underlying agent opposing ZNF687.

Results. In vitro, the guide RNA corresponding to *ZNF687* was weakly detected in HCC cells, and *ZNF687* deficiency was found to inhibit growth in HCC cell lines. *ZNF687* mRNA was overexpressed and had a high discriminatory ability for HCC in 2,975 HCC samples, contrasting with 2,340 non-HCC samples. Moreover, an excessive *ZNF687* transcript level was related to a worse overall survival (OS) prognosis. Histone modification, spliceosome, transcription coregulator activity, and nucleocytoplasmic transport were the most significant pathways for *ZNF687* differential-related gene enrichment. Chaetocin was found to be a candidate compound and presented a strong affinity to ZNF687.

Conclusions. *ZNF687* represents a TKI-resistant and growth-dependent gene for HCC, the overexpression of which indicates poor OS for HCC patients. Additionally, ZNF687 is expected to be a druggable target for overcoming TKI resistance, and chaetocin may be a candidate therapeutic compound for ZNF687.

Key words: tyrosine kinase inhibitor, resistance, mRNA, hepatocellular carcinoma, *ZNF687*

Background

According to statistics from the American Cancer Society (ACC), the mortality rate of liver cancer has fallen compared to previous decades. However, the burden of liver cancer is still heavy, with the estimated number of deaths ranking 5th among all cancer deaths in men and 7th in women.[1] Generally, hepatocellular carcinoma (HCC) dominates liver cancer cases (75–85%),[2] and the risk factors include hepatitis virus infection, alcohol and aflatoxin, as well as water contamination.[3,4] A lack of specific clinical signs in the early stages of HCC causes many diagnoses to be delayed until an advanced stage. With early diagnosis, liver transplantation and surgical resection are the recommended therapy choices. Because the complete resection of pathological tissue is difficult, patients are at risk of tumor recurrence, metastasis, hemorrhage, infection, and abdominal wall hernia.[5,6] Advanced HCC is common in clinical practice and has a poor prognosis for many complications, such as serious ascites, jaundice, hemorrhage, and hepatic encephalopathy. For instance, the prognosis of advanced HCC combined with obstructive jaundice is poor, and although endoscopic biliary drainage may improve patient outcomes, the risk of cholangitis increases.[7,8] Currently, systematic therapy, especially tyrosine kinase inhibitors (TKI), is suggested for advanced HCC patients who are classed as A class using Child–Pugh score for hepatic function.[9] Due to the disorder of protein kinase activity in many malignancies, targeting protein kinases has become a significant anti-cancer strategy. Tyrosine kinase inhibitor is one of the U.S. Food and Drug Administration (FDA)-approved protein kinase inhibitors, which occupy an important position in targeted therapy.[10] Monotherapy or combined immunotherapy significantly extends HCC patients' survival times. Nevertheless, HCC is susceptible to treatment resistance during long-term medication therapy,[11] leading to relapse and disease progression.[12] The problem is that TKI resistance occurs in the late stage of treatment, which limits long-term therapeutic benefits.[13] Regrettably, the resistance mechanisms of targeted drugs in HCC have not been completely elucidated except for epithelial–mesenchymal transition, ATP-binding cassette transporters, hypoxia, autophagy, and angiogenesis.[14] Therefore, research on the mechanism and therapeutic target of HCC drug resistance is crucial for improving treatment response, reducing complications, and thus enhancing long-term efficacy.

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) is an RNA-guided and Cas nuclease-cleaved gene-editing technique that can modify a single gene and reveal its function.[15,16] There are 3 groups of CRISPR/Cas systems, with CRISPR/Cas9 belonging to the 2nd category.[17] CRISPR/Cas9 comprises the Cas9 nuclease and CRISPR RNA or gRNA, which can lead Cas9 to specific sites and cut DNA for gene knockout.[18] Genome-wide CRISPR knockout screening (GeCKO) technology offers an effective method for observing genomic alterations under certain conditions. During the CRISPR loss-of-function screen, gRNAs are randomly carried into various cells by lentiviral vectors containing Cas9 and puromycin resistance. Cells that are not effectively transfected by the lentivirus are eliminated by puromycin, and a collection of genome-wide mutant HCC cells is created. The genomic expression profile of mutant cells changes under a particular intervention, and high-throughput sequencing is utilized to detect variations between treatment and control groups.[19,20] In this work, we conducted an in vitro CRISPR screen experiment for TKI resistance in HCC cells and gathered genes instead of TKIs. *ZNF687* was identified as a TKI-resistant gene and is anticipated to be a therapeutic target for HCC.

Located at chromosome 1q21.3,[21] *ZNF687* is a recently discovered C_2H_2-type zinc finger protein that has been reported to be overexpressed in the kidneys, spleen and other hemopoietic organs and to be associated with the proliferation and differentiation of hematopoietic cells.[22] Aberrant *ZNF687* expression is a driver of some cancers. For instance, *ZNF687* mutations are implicated in Paget's disease of bone, and their overexpression is linked to giant cell tumors associated with Paget's disease of bone.[21,23,24] It is now suspected that *ZNF687* may induce HCC cells to produce stem cell-like characteristics by upregulating *BMI1*, *NANOG* and *OCT4*, which then contributes to HCC progression. In vitro experiments have demonstrated that *ZNF687* knockdown increases the susceptibility of HCC cells to cisplatin; thus, *ZNF687* may be engaged in the development of HCC chemoresistance.[22] However, no studies have discovered the mechanism of *ZNF687* that results in tumor-targeted drug resistance.

Consequently, this is the first comprehensive research to explore the expression status, TKI therapy response, biology, and clinical implications of *ZNF687* in HCC. We hypothesized that *ZNF687* may be a candidate gene for TKI resistance in HCC, estimated the mean expression level of the *ZNF687* gene using global HCC cohorts, and analyzed its capacity to distinguish HCC tissue from controls. Survival curves and clinicopathological characteristics analysis were applied to investigate the association between *ZNF687* transcriptional-level expression and patient prognosis. Moreover, we identified the potential molecular mechanism and predicted the underlying therapeutic component based on the biological abnormalities resulting from increased *ZNF687* expression.

Objectives

This study was designed to investigate the transcriptional expression features, biological functions and potential clinical value of the TKI-resistant gene *ZNF687* in HCC, exploring the probability of overcoming the TKI-resistance problem.

Materials and methods

In vitro genome-wide CRISPR/Cas9 knockout library

Experimental material

The experiment followed the protocol described in the research of Joung et al.[25] The human HCC cell line Huh7 was acquired from the cell bank of the Chinese Academy of Sciences (Beijing, China). The genome-wide CRISPR knockout v2 (GeCKO v2) library and gRNA were obtained from the Addgene Corporation (Watertown, USA; https://www.addgene.org/pooled-library/zhang-human-gecko-v2; 1000000048).

Lentivirus transfection

When cell growth reached 70–90% confluence, the adherent cells were separated with trypsin and counted. In total, 1.10×10^8 cells were transfected with the GeCKO v2 library containing 65,386 specific gRNAs. The multiplicity of infection was controlled to be <0.3.

TKI intervention and DNA extraction

Hepatocellular carcinoma cells successfully infected with lentivirus were dosed with the TKI drug for 21 days. The TKI component we adopted, anlotinib, was sourced from the Jiangsu Zheng Da Tian Qing company (Nanjing, China). Afterward, we used a Quick-DNA Midiprep Plus Kit (D4075) developed by Zymo Research (Orange, USA) to extract the DNA of the surviving HCC cells for library construction and high-throughput sequencing.

Library construction

The library construction was completed by the Beijing Nuo He Zhi Yuan Technology Company (Beijing, China), according to the following process. The extracted DNA was randomly digested into 350 bp fragments using a Covaris breaker (Covaris, Woburn, USA). The library was then prepared after conducting terminal repair and adding a poly-A-tail, and index connectors sequentially to the DNA fragments.

Analysis of sequencing results and negative screening strategies

Two treated samples and 2 control samples were subjected to high-throughput sequencing by the Beijing Nuo He Zhi Yuan Technology Company. After connector data, unknown data and low-quality test data were removed and quality control was conducted using FastQC software, high-quality gene counts were included. Model-based analysis of genome-wide CRISPR/Cas9 knockout software was employed for positive and negative screening. Both analysis tools were employed with default parameters, and genes corresponding to significantly reduced gRNAs were considered potential TKI-resistant genes.[19]

Gene effect

The gene effect can reflect the necessity of a specific gene in various cell lines. We downloaded the data for "CRISPR (DepMap 22Q2 Public + Score Chronos)" from the Cancer Dependency Map website (DepMap; https://depmap.org/portal/). The "gene effect" values of each cancer cell line after *ZNF687* knockout or inhibition were extracted, and a scatterplot was drawn using the ggplot2 package. The effects of *ZNF687* deficiency on the growth vitality of 20 HCC cell lines were then investigated.

Collection strategy of mRNA datasets

Microarray and RNA sequencing datasets were retrieved and screened from multiple databases, including the Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/), ArrayExpress (https://www.ebi.ac.uk/arrayexpress/), Oncomine (https://www.oncomine.com/), The Cancer Genome Atlas (TCGA) (https://portal.gdc.cancer.gov/), and Genotype-Tissue Expression (https://www.gtexportal.org/) on March 1, 2023, using the following search terms: (malignancy OR cancer OR tumor OR neoplasia OR carcinoma OR carcinomatosis) AND (hepatocellular OR liver OR hepatic OR HCC). The datasets were included if they met the following criteria, namely: 1) the species was homo sapiens; 2) there were tissue samples; and 3) there were at least n = 3 HCC and noncancerous tissue samples. Simultaneously, samples that had been genetically modified or treated with drugs were excluded. The screened data were then further processed, and datasets from the same platform were merged and eliminated using batch effect, and unstandardized datasets were normalized with the $\log_2(x + 1)$ method.

Combined analysis in silico

We integrated the 35 public datasets and computed the standardized mean difference (SMD) to compare the *ZNF687* mRNA expression differences between HCC tissue and noncancerous tissue. For each dataset, the sample number, average expression level and standard deviation (SD) of *ZNF687* in HCC tissue and noncancerous tissue were listed. The I^2 index and Q test were used to examine the overall heterogeneity of the data, with $I^2 > 50\%$ or $p < 0.10$ illustrating obvious heterogeneity, for which the random-effect model was chosen. Otherwise, the fixed-effect model was used to combine the SMDs. To appraise the differential diagnostic significance of *ZNF687* for HCC, a summary receiver operating characteristic (SROC) curve

was created. Sensitivity, specificity and likelihood ratios were used to assess the effectiveness of the diagnostic test, and publication bias was detected using the Egger's test.

Prognostic analysis in silico

The clinicopathological information of 333 HCC patients was downloaded from TCGA, and the clinical value of *ZNF687* mRNA expression levels on HCC patient outcomes was investigated using univariate Cox regression with clinicopathological features. A Schoenfeld residual was analyzed for proportional hazards (PH) assumption, and a Martingale residual was analyzed to detect whether the log-hazard function was linearly related to the continuous variable *ZNF687* expression. McFadden's pseudo-R^2 was employed to determine the goodnes-of-fit, with a McFadden's pseudo-R^2 closer to 1 indicating better goodness-of-fit. During data cleaning, clinical parameters with more uncertain information (e.g., "Unknown," "unreport," "Tx," "Nx," and "Mx") were eliminated. For the identification of overall survival (OS), if a patient's survival status was "alive", the survival time was selected as the last follow-up time. In the event that a patient's survival status was "dead", the OS was selected as the time of death. Finally, the impact of each clinicopathological parameter on HCC patient outcomes was assessed by independently calculating the hazard ratios (HRs) of *ZNF687* expression, age, sex, race, primary T (pT) stage, and American Joint Committee on Cancer (AJCC) stage. Additionally, the survminer R package was utilized to identify the optimum cutoff value, which divided the 333 samples into a high-expression *ZNF687* group and a low-expression *ZNF687* group, and the relationship between *ZNF687* mRNA status and HCC patient prognosis was explored through the Kaplan–Meier method. The computational formula was as follows:

$$\text{McFadden's } R^2 = \\ = [(\text{likelihood (null)} - \text{likelihood (model)})/ \\ /\text{likelihood (null)}]$$

Biological pathway exploration

An expression profile of 371 HCC tissue samples and 50 control samples (data source: TCGA) was executed using the limma package in R 4.1.1 (R Foundation for Statistical Computing, Vienna, Austria). The genes with a log fold change (logFC) of >1 were defined as differentially high expression genes of HCC. Genes with a strongly positive relationship to *ZNF687* (Pearson's correlation coefficient >0.75) were acquired using Pearson's analysis.[26] *ZNF687*-related differential genes were obtained by intersecting differential highly expressed genes and *ZNF687* strongly positively correlated genes, which were used for enrichment analysis of the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. The STRING tool v. 11.5 (https://cn.string-db.org/) was employed to calculate the connection score between *ZNF687*-related differential genes, and the potential protein complex subnetworks were analyzed using the molecular complex detection (MCODE) algorithm with Cytoscape software (https://cytoscape.org).

Candidate drugs prediction and evaluation in silico

A connectivity map (CMAP) is a query tool for predicting candidate drugs by comparing similarities or dissimilarities between the reference perturbation signatures and the input gene set.[27] In this study, due to the limitation of no more than 150 genes in the CMAP tool for drug prediction, the *ZNF687* genes positively related (Pearson's coefficient >0.80) and simultaneously differentially upregulated in HCC were inputted to CMAP (https://clue.io/query) to predict the compounds opposing *ZNF687*. The two-dimensional (2D) structure of each molecular drug was downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/), and energy was minimized via Chem3D software (PerkinElmer Informatics, Inc, Waltham, USA). The AlphaFold structure of ZNF687 (ID: Q8N1G0) was downloaded from the UniProt database (https://www.uniprot.org/). Subsequently, PYMOL 2.5.2 (https://pymol.org) and Autodock Vina 1.1.2 (https://autodock.scripps.edu) were used to simulate the docking of each candidate compound, and the molecular structure of ZNF687.[28] Discovery Studio v. 4.5 software (https://www.3ds.com/products/biovia/discovery-studio) was used to visualize the docking results.

Statistical analyses

R 4.1.1, Stata v. 17 (StataCorp LLC., College Station, USA) and IBM SPSS v. 26 (IBM Corp., Armonk, USA) were used for statistical analyses. Standardized mean difference was calculated as an effect indicator to reveal the mRNA expression status of *ZNF687* in HCC tissue. The I^2 index and Q test were used to examine the overall heterogeneity of the data, and sensitivity analysis and meta-regression analysis were used to explore the sources of heterogeneity. The summary receiver operating characteristic (SROC) curve, sensitivity, specificity, and positive and negative likelihood ratios were analyzed to evaluate the discrimination efficiency of *ZNF687* for HCC. For the area under the SROC curve (AUC), the criteria for verifying its efficacy were as follows: 0.50–0.70 indicated low estimated capacity, 0.70–0.80 indicated moderate estimated capacity, 0.80–0.90 indicated good, estimated capacity, and an AUC > 0.90 indicated strong estimated capacity. The Egger's test was utilized for detecting publication bias. The univariate Cox regression and Kaplan–Meier methods were applied to explore the prognostic risk factors. The analysis was considered statistically significant at a value of $p < 0.05$.

Results

CRISPR positive and negative screening in vitro

Under the positive and negative screening results (Fig. 1), 904 lowly enriched gRNAs (logFC < 0) and 949 gRNAs highly enriched gRNAs (logFC > 0) were identified (t-test with a $p < 0.05$). Compared with the control group, gRNA corresponding to *ZNF687* was weakly detected in the whole-genome mutant HCC cells treated with TKI (logFC = −0.56, t-test with a $p = 0.048$), which suggested that for *ZNF687*-defective HCC cells, they were more sensitive to TKI intervention and more likely to die.

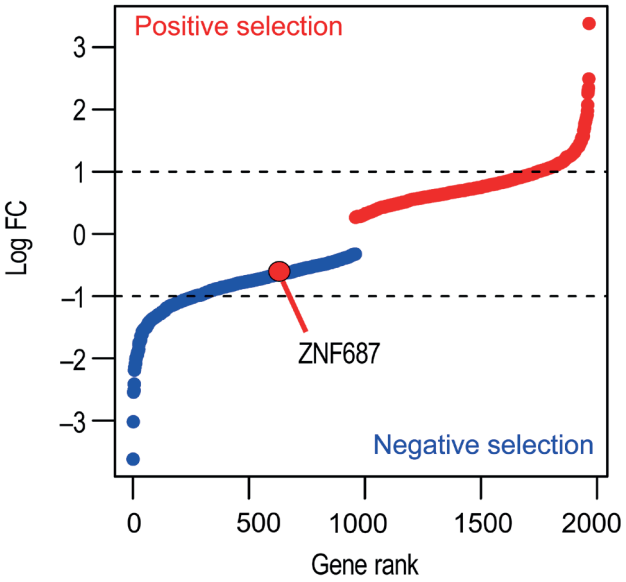


Fig. 1. Positive and negative selections of the clustered regularly interspaced short palindromic repeats screening experiment

Gene rank: The order of all genes after ranking according to the logFC value. The genes with logFC <0 were considered the potential resistant genes, and the genes with logFC >0 were considered the potential sensitive genes.

Carcinogenic effect of *ZNF687*

The gene effect of *ZNF687* was less than 0 in 19 HCC cell lines (SNU182, PLCPRF5, JHH2, JHH4, JHH6, SNU387, HLF, SKHEP1, SNU423, JHH5, SNU761, SNU398, JHH7, SNU475, JHH1, HUH7, SNU886, HUH1, and SNU449) and greater than 0 only in the HEPG2 cell line (Fig. 2, Supplementary Table 1). Thus, the high expression of *ZNF687* likely promoted HCC cell growth.

Overexpression of *ZNF687* and its discriminatory efficacy against HCC

In total, 35 mRNA datasets were collected (Fig. 3), including 2,975 HCC tissue samples and 2,340 noncancerous tissue samples. The *ZNF687* transcriptional expression in each dataset, displayed as mean and SD, is shown in Supplementary

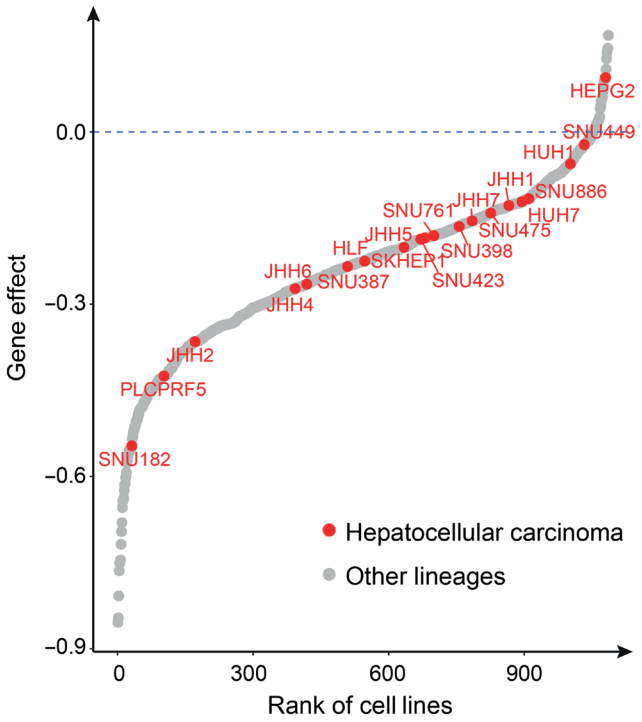


Fig. 2. The zinc finger protein 687 gene dependency distribution for 20 cell lines of hepatocellular carcinoma

Rank of cell lines: The order of all cell lines after ranking according to the gene effect scores. A score <0 indicates cell inhibition after knocking out the gene.

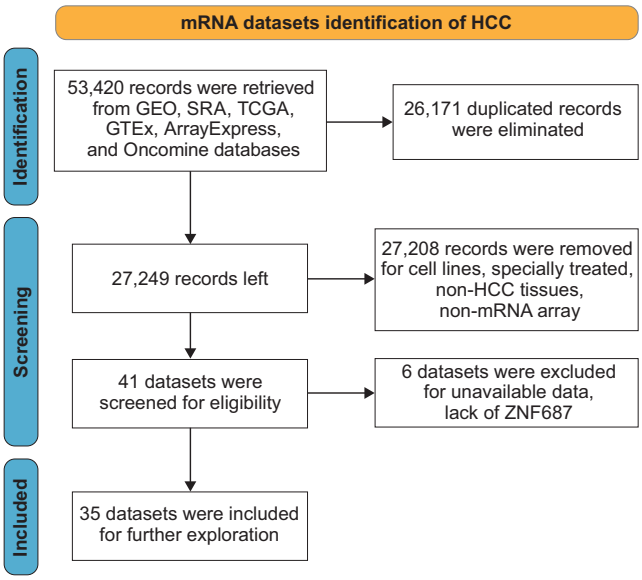


Fig. 3. Flowchart of hepatocellular carcinoma datasets collection

Table 2. The SMD was greater than 0 in most separate datasets, and the combined SMD was 1.10 (95% confidence interval (95% CI): 0.87–1.33), indicating that *ZNF687* was upregulated at the transcriptional level in large HCC samples (Fig. 4A). The results of the I^2 index and Q test suggested high heterogeneity ($I^2 = 90.4\%$, Q test with a $p < 0.01$), so the random-effect model was applied. For the investigation of heterogeneity, sensitivity analysis indicated that

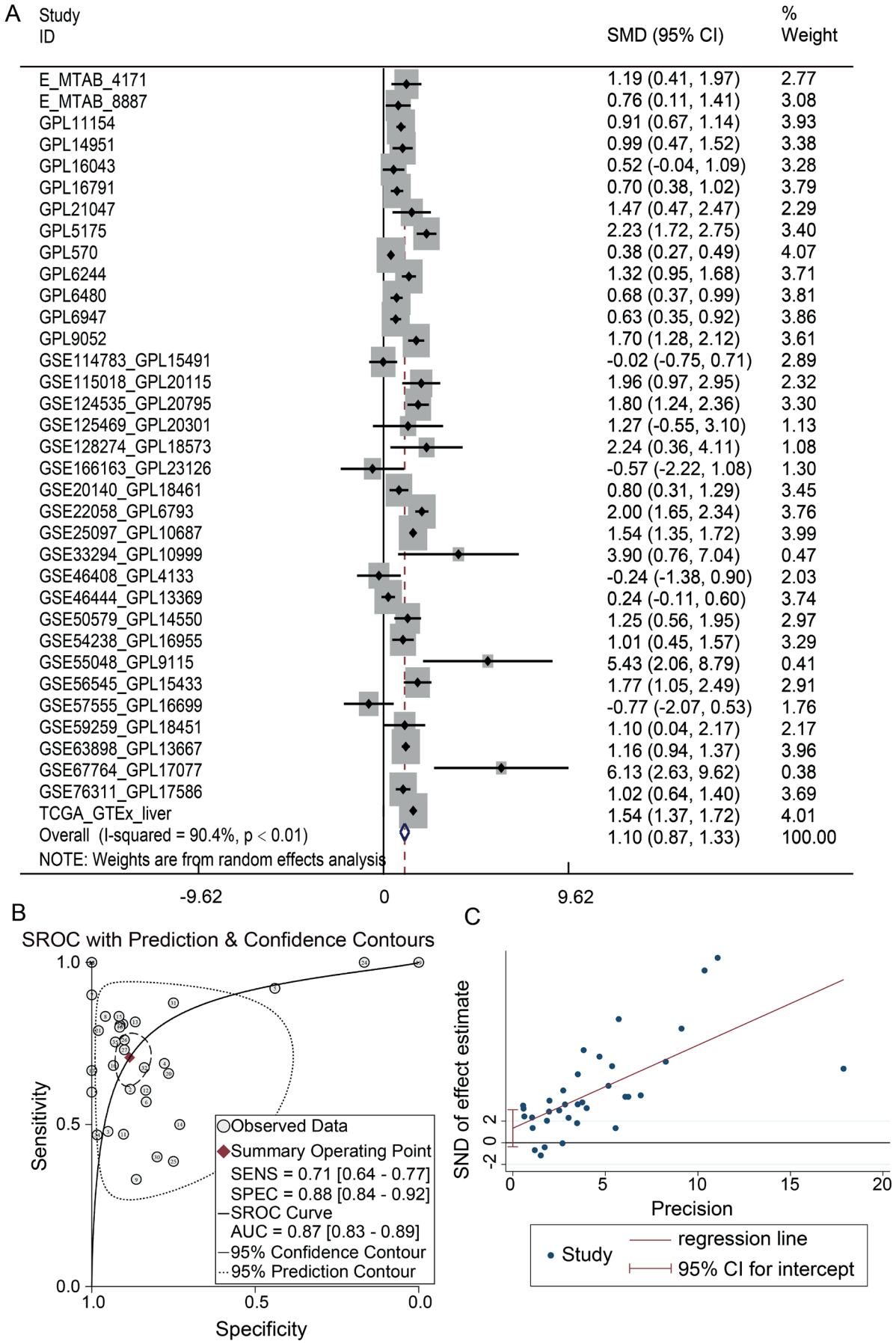


Fig. 4. mRNA expression status of zinc finger protein 687 in hepatocellular carcinoma tissue. A. Forest map of the standardized mean difference; B. Summary receiver operating characteristic (ROC) curve; C. Egger funnel plot (p = 0.12)

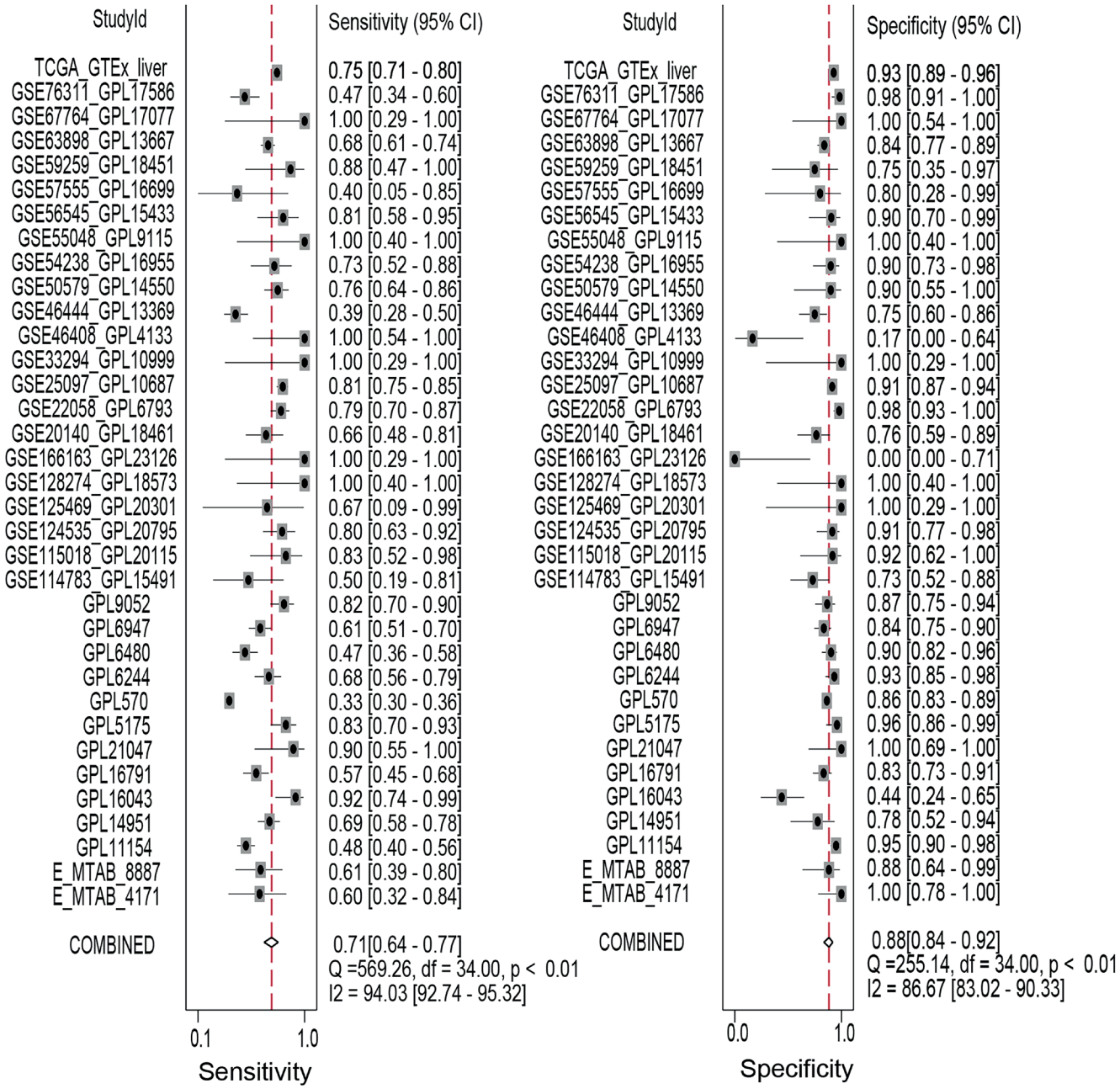


Fig. 5. Sensitivity and specificity of diagnostic test

the pooled SMD was greater than 0 and stable after removing any of the datasets (Supplementary Fig. 1). Based on the result of the meta-regression analysis, the sequencing technique contributed to the heterogeneity ($p < 0.01$), but neither the merged datasets nor the sample size demonstrated any relationship to the heterogeneity (Supplementary Table 3). The AUC was 0.87 (95% CI: 0.83–0.89) (Fig. 4B), the sensitivity was 0.71 (95% CI: 0.64–0.77) (Fig. 4B, Fig. 5) and the specificity was 0.88 (95% CI: 0.84–0.89) (Fig. 4B, Fig. 5). The positive and negative likelihood ratios were respectively 6.09 (95% CI: 4.25–8.71) (Fig. 6) and 0.33 (95% CI: 0.27–0.41) (Fig. 6), indicating a high discrimination capacity of *ZNF687* for HCC. Finally, no compelling publication bias was found according to the Egger's test ($p = 0.12$) (Fig. 4C).

Clinical significance

Based on the Kaplan–Meier curves, OS time appeared more depressed in the *ZNF687*-overexpression group (288 samples) than in the *ZNF687* underexpression group (45 samples), with an HR of 2.00 (95% CI: 1.09–3.69, log-rank test with a $p < 0.05$) (Fig. 7). This illustrates that elevated mRNA expression of *ZNF687* may be connected with poor prognosis in HCC. According to the Supplementary Fig. 2, the Schoenfeld residuals of "*ZNF687* (continuous)," "Age," "Gender," "pT stage," and "AJCC stage" show no relationship with time and comply with the PH assumption (PH test, $p > 0.05$), but the covariate "Race" does not comply with the PH assumption (PH test, $p < 0.05$). The predictor

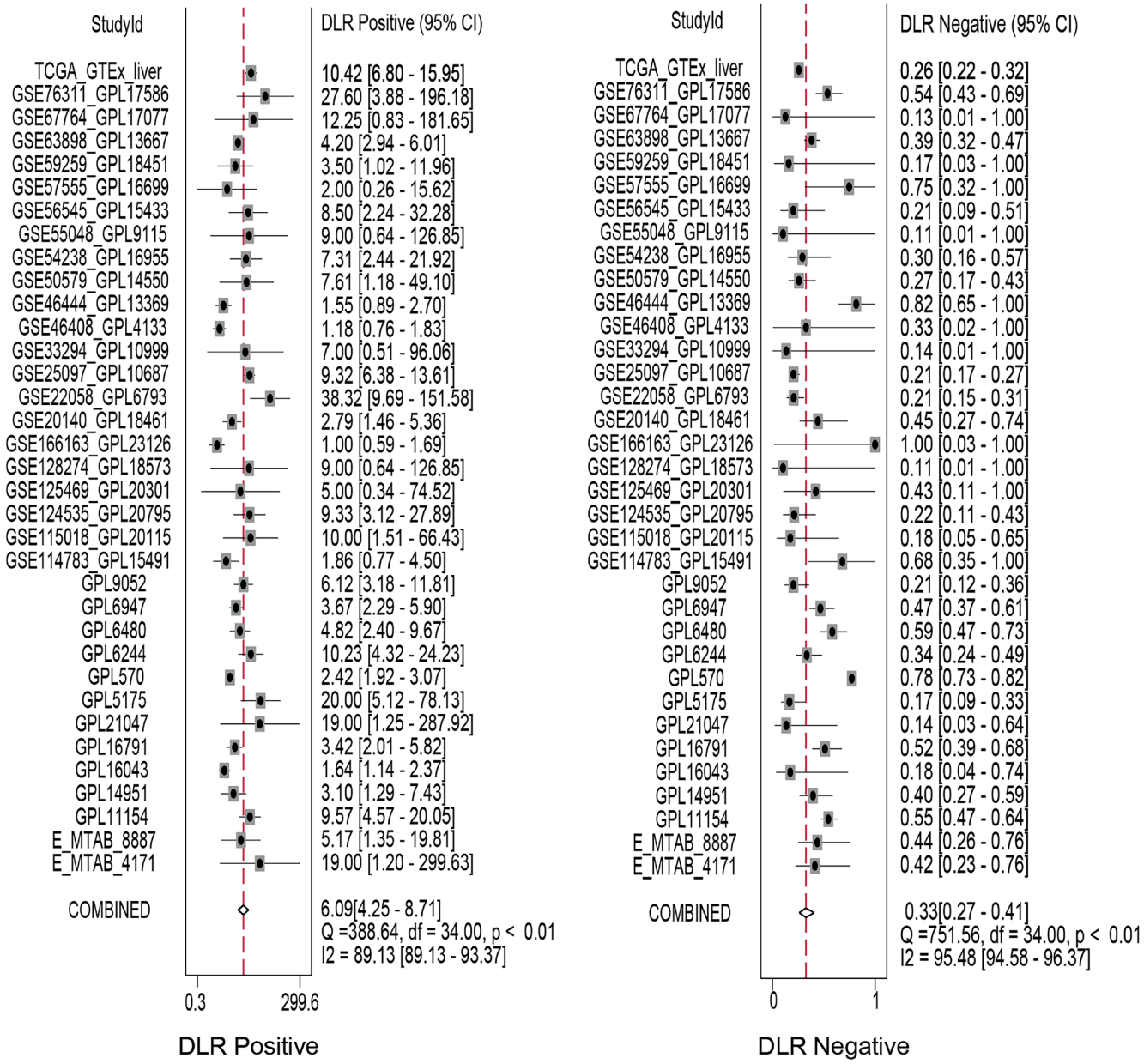


Fig. 6. Double likelihood ratios of a diagnostic test

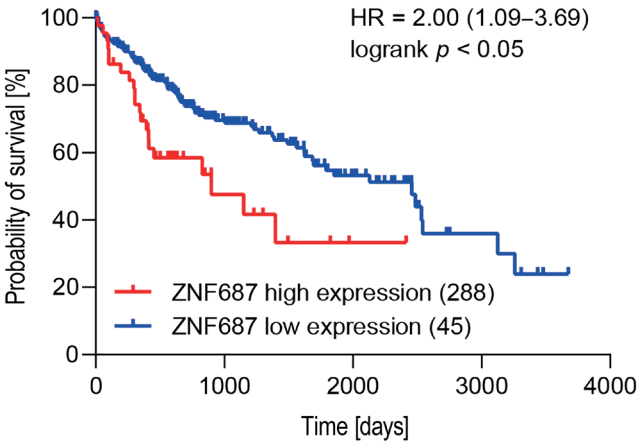


Fig. 7. Association between zinc finger protein 687 transcriptional level and the overall survival (OS) prognosis of hepatocellular carcinoma (hazard ratio (HR) = 2.00, 95% confidence interval (95% CI): 1.09–3.69, log-rank test with a $p < 0.05$)

Table 1. Clinicopathologic features associated with hepatocellular carcinoma death using univariate Cox analysis

Variables	HR (95% CI)	Wald test p-value	McFadden's pseudo R^2
ZNF687 (continuous)	1.01 (1.00–1.02)	0.04	<0.01
Age (>65 vs ≤65 years)	1.14 (0.78–1.67)	0.49	<0.01
Gender (male vs female)	0.76 (0.52–1.12)	0.17	<0.01
Race (Asian vs non-Asian)	–	–	–
T (T3 + T4 vs T1 + T2) pT stage (pT3+pT4 vs pT1+pT2)	2.47 (1.68–3.61)	<0.01	0.02
AJCC stage (stage III+IV vs stage I+II)	2.45 (1.67–3.58)	<0.01	0.02

HR – hazard ratio; 95% CI – 95% confidence interval; AJCC – American Joint Committee on Cancer..

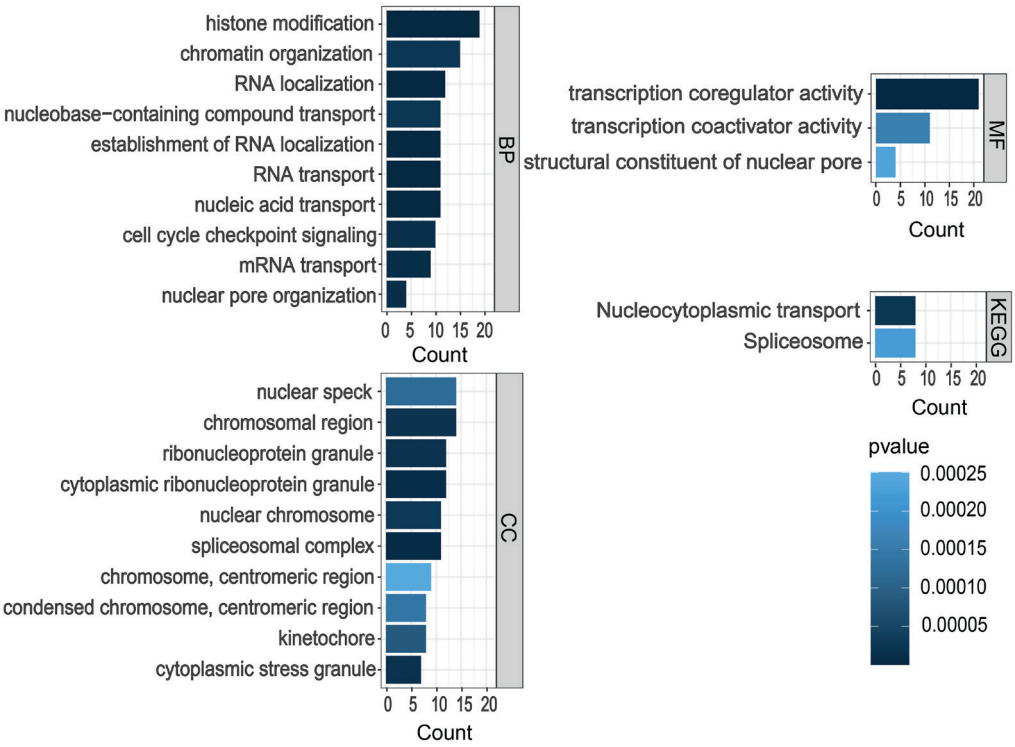


Fig. 8. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) functional pathways

ZNF687 (continuous) shows no linearity to the log hazard (Supplementary Fig. 3). In line with univariate Cox analysis (Table 1), individuals with a high *ZNF687* expression level had a higher risk of HCC death (HR = 1.01, 95% CI: 1.00–1.02, Wald test with a p = 0.04, McFadden's pseudo $R^2 < 0.01$). The probability of death in "T3 + T4" and "III + IV" HCC patients was higher than that in "T1 + T2" and "I + II" patients, with HRs of 2.47 (95% CI: 1.68–3.61, Wald test with a p < 0.01, McFadden's pseudo $R^2 = 0.02$) and 2.45 (95% CI: 1.67–3.58, Wald test with a p < 0.01, McFadden's pseudo $R^2 = 0.02$), respectively. Age and gender were not statistically significant regarding the HCC outcome (Wald test with a p > 0.05, McFadden's pseudo $R^2 < 0.01$).

GO and KEGG functional pathways

We intersected 2,705 differentially expressed HCC genes and 401 strongly positive *ZNF687*-related genes, of which 214 *ZNF687*-related differential genes were obtained and considered to have an oncogenic effect in HCC cooperating with *ZNF687*. The GO analysis showed that the 214 *ZNF687*-related differentially expressed genes were significantly enriched for "histone modification" of the biological process (BP) and "nuclear speak" of the cellular component (CC), and had "transcription coregulator activity" of molecular function (MF). For the KEGG pathway analysis, "nucleocytoplasmic transport" and "spliceosome" predominated (Fig. 8). Six protein-protein interaction networks were listed through the MCODE algorithm (Table 2). The GO-BP analysis was performed on the network with the highest score, and it was noted that the gene within

Table 2. Protein-protein interaction network analysis based on the MCODE algorithm

Cluster	Score	Nodes	Edges	Node IDs
1	9.333	10	42	ILF2, PTBP1, NONO, RBMX, PSME3, U2AF2, HNRNPU, ILF3, CPSF6, CHTOP
2	6.75	17	54	NUP205, MSH2, MCM2, PARP1, NUP133, MDC1, AHCTF1, TIMELESS, XPO1, CKAP5, NCOA5, CASC3, POLD1, PRIM1, TPR, TOPBP1, INCENP
3	4	4	6	PRPF3, DHX16, BUD13, SF3B4
4	3	3	3	SNAPIN, AP3B1, OCRL
5	3	5	6	USP21, UBQLN4, USP14, PTPN23, HGS
6	3	3	3	MED12, MED20, MED24

MCODE – molecular complex detection.

MCODE-Cluster1 was significantly related to the regulation of mRNA processing (Fig. 9).

Therapeutic compound opposing ZNF687

In total, 84 genes were input into CMAP, and the top 3 compounds (amiloride, chaetocin and phloretin) were identified (Table 3). As depicted in Fig. 10, the minimum binding energies of amiloride (PubChem CID: 16231), chaetocin (PubChem CID: 11657687) and phloretin (PubChem CID: 4788) docking ZNF687 protein were correspondingly –5.9 kcal/mol, –8.9 kcal/mol and –6.5 kcal/mol, of which chaetocin exhibited the highest affinity to the ZNF687

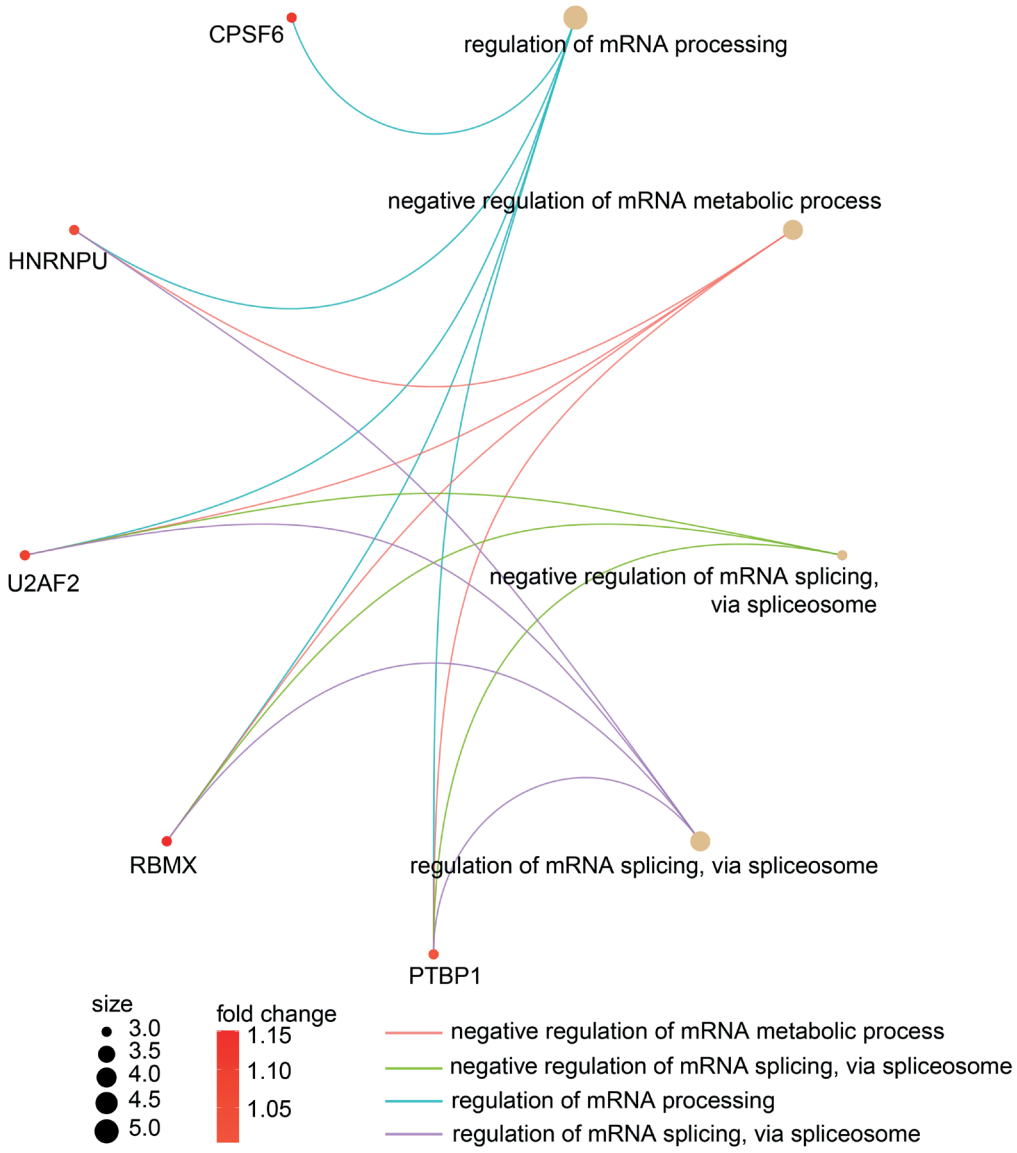


Fig. 9. The Gene Ontology (GO) – biological process pathway related to the genes of MCODE-Cluster1

MCODE – molecular complex detection.

protein. The enlarged three-dimensional (3D) structure and the 2D interactions of the binding site are presented in Fig. 10B. Based on the docking result of chaetocin and the ZNF687 protein, the small compound was predicted to form 8 hydrogen bonds with the amino acid residues SER704, ASN705, ALA702, ALA699, GLY701, LEU714, and PRO715, 3 hydrophobic bonds with amino acid residues MET713, PRO698 and LEU695, and an unfavorable acceptor–acceptor interaction with the ANA699 residue.

Discussion

Since 2007, TKI has dramatically improved the treatment of HCC,[29] yet according to a study from 2008, acquired resistance to TKI occurs within 6 months after using TKI drug.[30,31] It is reported that patients with sorafenib resistance had worse OS.[32] Recently, 2nd- and 3rd-generation TKI have been developed to treat TKI-resistant patients,[33,34] but they are inadequate for overcoming the difficulty of TKI resistance. The CRISPR screening is a genome-wide editing technology extensively applied in tumor drug-resistance research. CRISPR knockout, CRISPR inhibition and CRISPR activation screens are 3 common methods to explore the drug-resistance mechanism and identify responsible genes.[35] In this study, we conducted an in vitro CRISPR knockout screen and confirmed a potential TKI-resistant gene, *ZNF687*. Because *ZNF687* has been reported as overexpressed in HCC, we also collected global cohorts to demonstrate it is an oncogene and is expected to be a druggable target opposing TKI resistance.

In the in vitro study, the gRNA of *ZNF687* was enriched only to a low level, indicating that *ZNF687* knockout likely diminished TKI resistance for HCC cells. This is not the first time a zinc finger protein has been revealed as participating in TKI resistance. In 2020, zinc finger protein 703 was found to induce sorafenib resistance via transactivating CLDN4 expression.[36] However, the underlying mechanism of TKI resistance is unclear, except for presently known processes, including epithelial–mesenchymal

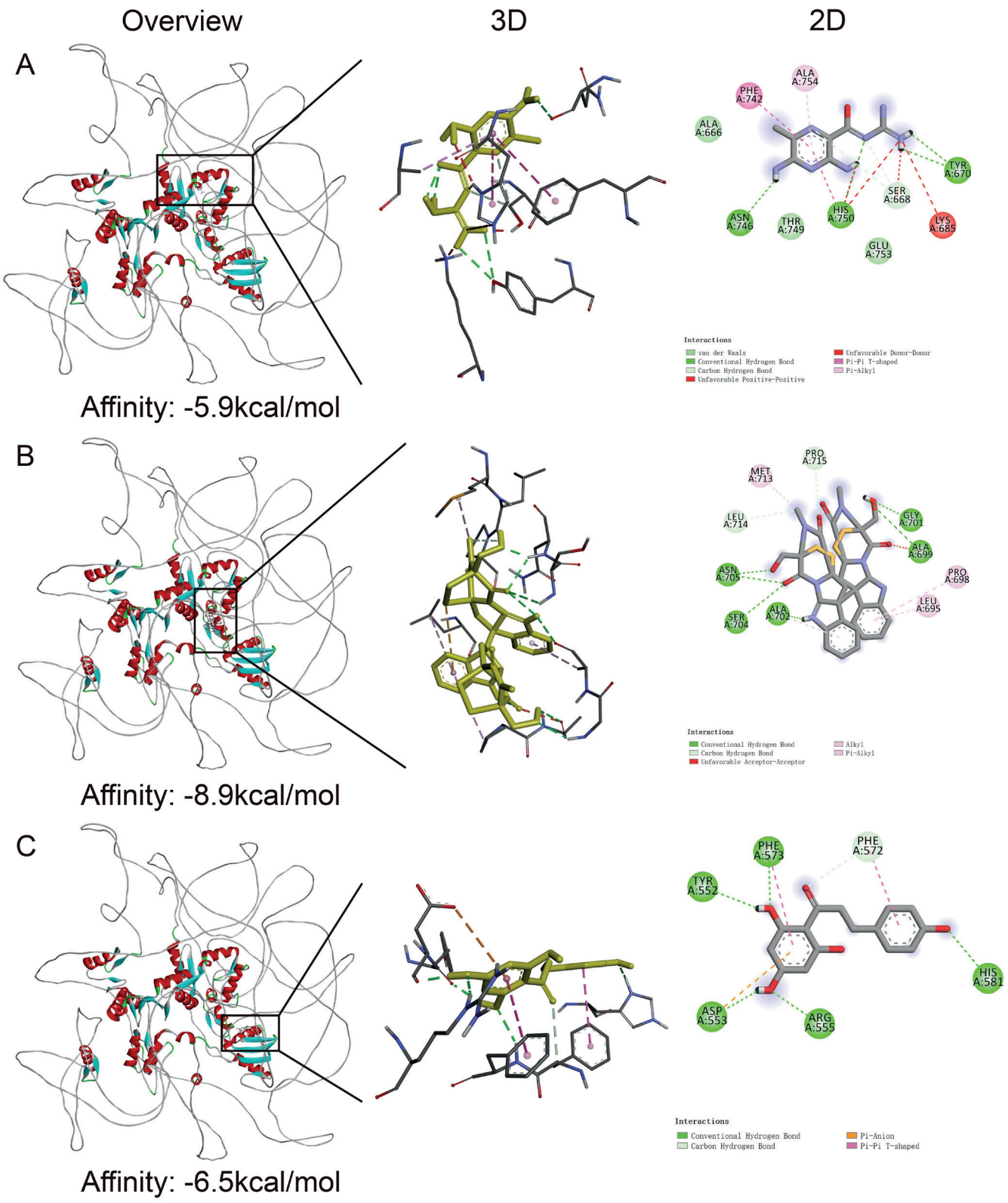
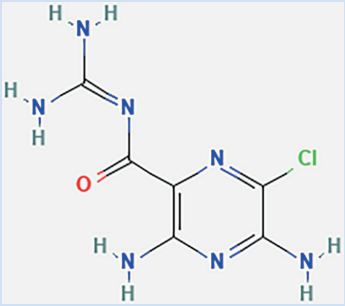
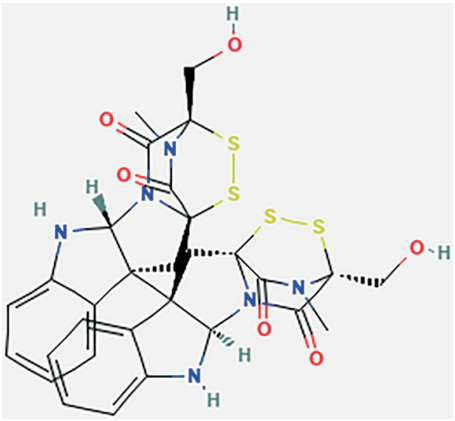
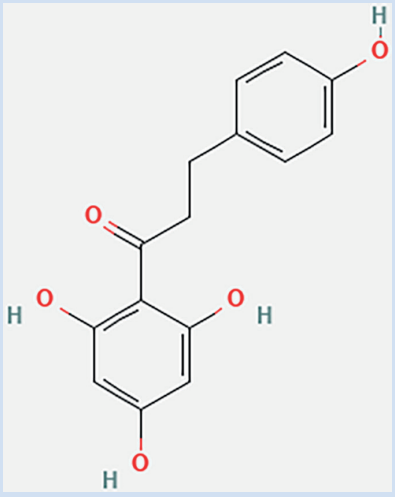


Fig. 10. The overview, enlarged 3D and 2D structures of molecular docking. A. Amiloride and ZNF687; B. Chaetocin and ZNF687; C. Phloretin and ZNF687 Affinity – the binding energy of compound and protein receptor. A lower affinity score indicates the combination is more stable.

transition, ATP-binding cassette transporters, hypoxia, autophagy, and angiogenesis.[14] Considering that no directly relevant evidence followed, we further explored the potential molecular mechanism of *ZNF687* in HCC. *ZNF687*-related differentially expressed genes were enriched for histone modification, nucleocytoplasmic transport, spliceosome, and transcription coregulator activity pathways. Histone modification is essential to resistance,

Table 3. The predicted compounds opposing ZNF687

Compound	Description	Tau	Structure (2D)
Amiloride	sodium channel blocker	–100	
Chaetocin	histone lysine methyltransferase inhibitor	–100	
Phloretin	sodium/glucose cotransporter inhibitor	–100	

Tau – the connectivity score ranging from –100 to 100. When the connectivity score is closer to 100, the gene list is more similar to the gene perturbation record treated with a compound. Conversely, when the connectivity score is closer to –100, the gene list is more opposite to the gene perturbation record treated with a compound.

with histone modification inhibition, such as histone methylase inhibitors, being demonstrated to reverse tumor drug resistance.[37] Additionally, for nucleocytoplasmic transport, it is well known that cancer cells can escape antitumor attacks through the normal nuclear-cytoplasmic transport process or nuclear pore complex. For example, the transport receptor protein CRM1 can mediate drug-target proteins exported from the nucleus. Consequently, antitumor pharmaceuticals cannot take effect in the nucleus, facilitating resistance.[38] Transcriptional coregulator activity and spliceosome pathways are also implicated in the gene transcription and transcript modification processes. Using the MCODE algorithm and GO enrichment analysis, we showed that *ZNF687*-related differential genes were significantly enriched for the regulation of mRNA processing and splicing. In recent decades, it has been shown that, apart from gene mutations, mRNA alterations are crucial for the occurrence and progression of tumors. Aberrant splicing and polyadenylation of mRNAs are connected with resistance to antitumor therapy, and certain

tumors are highly sensitive to components that inhibit splicing.[39] To overcome tumorigenesis and drug resistance caused by abnormal RNA splicing, research on and improvement of splice variant-specific siRNAs, splice-switching antisense oligonucleotides, and small molecule inhibitors aimed at splicing factors, splicing factor kinases, and aberrant carcinogenic protein isoforms recently been proposed.[40] Among the 5 genes found to be participating in the regulation of the mRNA processing pathway in this study, *PTBP1*, *RBMX*, *HNRNPU*, and *CPSF6* have been previously shown to enhance HCC development.[41–44] In particular, *RBMX* benefits sorafenib resistance in HCC cells. An obvious reduction in cell viability with increasing sorafenib concentration was observed in *RBMX*-deficient Huh7/Hch7-SR cells.[42] Moreover, *HNRNPU* can encourage cisplatin resistance in bladder cancer.[45]

We performed in silico analysis from tissue and at the cell level and revealed the carcinogenicity of *ZNF687*. Regarding biological functions, zinc finger proteins are involved in transcriptional regulation, protein interactions and post-transcriptional regulation.[46] Nevertheless, aberrant expression or dysfunction of zinc finger proteins causes hepatocarcinogenesis.[46,47] For instance, *ZNF687* was previously discovered to promote downstream target gene transcription through binding to the enhancer, thus contributing to HCC.[22] Compared with former research, we adopted an increased number of samples and public CRISPR/Cas9 gene-editing data, concluding that *ZNF687* is a prognostic factor for HCC patients and is significant for HCC cell growth.

We determined that *ZNF687* is TKI-resistant and oncogenic, and we subsequently supposed it to be a therapeutic target for overcoming TKI resistance. After further exploration with drug prediction and molecular docking, a small compound chaetocin exhibited a potential to resist ZNF687 in this study. Chaetocin is a natural metabolite from *Chaetomium* and has been reported to have an antitumor effect on various malignancies, including HCC.[48,49] Notably, chaetocin was previously claimed to overcome TKI resistance for chronic myelogenous leukemia.[50] Consequently, we propose that chaetocin can oppose TKI resistance to HCC through targeting ZNF687.

The highlights of the present study include the execution of in vitro CRISPR screening to identify the TKI-resistant gene *ZNF687*, integrated global sequencing datasets and public CRISPR/Cas9 knockout data to attest its carcinogenicity, and implemented a molecular simulation to declare ZNF687 a druggable target. We comprehensively illuminated the possible functions of *ZNF687* and the biological mechanism. The underlying molecular mechanism of *ZNF687* may be related to histone modification, spliceosome, transcription coregulator activity, and nucleocytoplasmic transport.

Limitations

During the collection of mRNA datasets of HCC tissue and calculating the demonstrated transcriptional expression status of the *ZNF687* gene, the authors detected high heterogeneity. However, random-effects analysis was adopted to make up for the deficiency. Conversely, the biological pathways that *ZNF687* may be involved in were predicted in this study, but they have not been verified through in vivo or in vitro experiments. Thus, robust experimental verification should be conducted for an intensive understanding of *ZNF687*.

Conclusions

ZNF687 was shown to be a TKI-resistant and growth-dependent gene for HCC, and overexpression of *ZNF687* indicates poor OS for HCC patients. Additionally, ZNF687 is expected to be a druggable target for overcoming TKI resistance, and chaetocin may be a candidate therapeutic compound for ZNF687.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.11075935. The package includes the following files:

Supplementary Table 1. Gene effects of zinc finger protein 687 deletion on the growth of hepatocellular carcinoma cell lines.

Supplementary Table 2. Statistical description and the true positive, false positive, true negative and false negative values of zinc finger protein 687 mRNA in datasets.

Supplementary Table 3. Exploring the sources of high heterogeneity using univariable meta-regression analysis

Supplementary Fig. 1. Sensitivity analysis using the one-by-one removal method.

Supplementary Fig. 2. Proportional hazards assumption test by Schoenfeld residual method. A. *ZNF687*-continuous ($p = 0.16$); B. Age ($p = 0.97$); C. Gender ($p = 0.15$); D. Race ($p < 0.05$); E. pT stage ($p = 0.66$); F. AJCC stage ($p = 0.53$).

Supplementary Fig. 3. Log-linear hypothesis test by Martingale residual method.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17–48. doi:10.3322/caac.21763
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
3. Shen C, Jiang X, Li M, Luo Y. Hepatitis virus and hepatocellular carcinoma: Recent advances. *Cancers (Basel)*. 2023;15(2):533. doi:10.3390/cancers15020533
4. Huang M, Liu J, Fan Y, et al. Development of curcumin-loaded galactosylated chitosan-coated nanoparticles for targeted delivery of hepatocellular carcinoma. *Int J Biol Macromol*. 2023;253:127219. doi:10.1016/j.ijbiomac.2023.127219
5. Köckerling F, Sheen AJ, Berrevoet F, et al. The reality of general surgery training and increased complexity of abdominal wall hernia surgery. *Hernia*. 2019;23(6):1081–1091. doi:10.1007/s10029-019-02062-z
6. Işık A, Fırat D. Letter to the editor concerning "Most cited 100 articles from Turkey on abdominal wall hernias: A bibliometric study." *Turk J Surg*. 2021;37(2):193–194. doi:10.47717/turkjsurg.2021.4973
7. Woo HY, Han SY, Heo J, et al. Role of endoscopic biliary drainage in advanced hepatocellular carcinoma with jaundice. *PLoS One*. 2017;12(11):e0187469. doi:10.1371/journal.pone.0187469
8. Isik A, Poyanli A, Tekant Y, et al. Incomplete or inappropriate endoscopic and radiologic interventions as leading causes of cholangitis. *Pol Przegl Chir*. 2021;93(6):47–52. doi:10.5604/01.3001.0015.0423
9. Chakraborty E, Sarkar D. Emerging therapies for hepatocellular carcinoma (HCC). *Cancers (Basel)*. 2022;14(11):2798. doi:10.3390/cancers14112798
10. Roskoski R. Properties of FDA-approved small molecule protein kinase inhibitors: A 2023 update. *Pharmacol Res*. 2023;187:106552. doi:10.1016/j.phrs.2022.106552
11. Xing R, Gao J, Cui Q, Wang Q. Strategies to improve the antitumor effect of immunotherapy for hepatocellular carcinoma. *Front Immunol*. 2021;12:783236. doi:10.3389/fimmu.2021.783236
12. Wang D, Zheng X, Fu B, et al. Hepatectomy promotes recurrence of liver cancer by enhancing IL-11-STAT3 signaling. *eBioMedicine*. 2019;46:119–132. doi:10.1016/j.ebiom.2019.07.058
13. Man KF, Ma S. Mechanisms of resistance to tyrosine kinase inhibitors in liver cancer stem cells and potential therapeutic approaches. *Essays Biochem*. 2022;66(4):371–386. doi:10.1042/EBC20220001
14. Tian Y, Lei Y, Fu Y, Sun H, Wang J, Xia F. Molecular mechanisms of resistance to tyrosine kinase inhibitors associated with hepatocellular carcinoma. *Curr Cancer Drug Targets*. 2022;22(6):454–462. doi:10.2174/1568009622666220330151725
15. Ma Y, Zhang L, Huang X. Genome modification by CRISPR/Cas9. *FEBS J*. 2014;281(23):5186–5193. doi:10.1111/febs.13110
16. Lander ES. The heroes of CRISPR. *Cell*. 2016;164(1–2):18–28. doi:10.1016/j.cell.2015.12.041
17. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science*. 2012;337(6096):816–821. doi:10.1126/science.1225829
18. Zhang F, Wen Y, Guo X. CRISPR/Cas9 for genome editing: Progress, implications and challenges. *Hum Mol Genet*. 2014;23(R1):R40–R46. doi:10.1093/hmg/ddu125
19. Shalem O, Sanjana NE, Hartenian E, et al. Genome-scale CRISPR-Cas9 knockout screening in human cells. *Science*. 2014;343(6166):84–87. doi:10.1126/science.1247005
20. Burton HW, Carlson BM, Faulkner JA. Microcirculatory adaptation to skeletal muscle transplantation. *Annu Rev Physiol*. 1987;49(1):439–451. doi:10.1146/annurev.ph.49.030187.002255
21. Divisato G, Formicola D, Esposito T, et al. ZNF687 mutations in severe Paget disease of bone associated with giant cell tumor. *Am J Hum Genet*. 2016;98(2):275–286. doi:10.1016/j.ajhg.2015.12.016
22. Zhang T, Huang Y, Liu W, et al. Overexpression of zinc finger protein 687 enhances tumorigenic capability and promotes recurrence of hepatocellular carcinoma. *Oncogenesis*. 2017;6(7):e363. doi:10.1038/oncsis.2017.63
23. Gennari L, Rendina D, Falchetti A, Merlotti D. Paget's disease of bone. *Calcif Tissue Int*. 2019;104(5):483–500. doi:10.1007/s00223-019-00522-3
24. Scotto Di Carlo F, Whyte MP, Gianfrancesco F. The two faces of giant cell tumor of bone. *Cancer Lett*. 2020;489:1–8. doi:10.1016/j.canlet.2020.05.031
25. Joung J, Konermann S, Gootenberg JS, et al. Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. *Nat Protoc*. 2017;12(4):828–863. doi:10.1038/nprot.2017.016
26. Iversen GR, Gergen MM. *Statistics: The Conceptual Approach*. New York, USA: Springer; 1997. ISBN:978-0-387-94610-8
27. Subramanian A, Narayan R, Corsello SM, et al. A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. *Cell*. 2017;171(6):1437–1452.e17. doi:10.1016/j.cell.2017.10.049
28. Trott O, Olson AJ. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem*. 2010;31(2):455–461. doi:10.1002/jcc.21334
29. Llovet JM, Ricci S, Mazzaferro V, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med*. 2008;359(4):378–390. doi:10.1056/NEJMoa0708857
30. Sui Z, Xue H, Jing F, Leng P. Sorafenib plus capecitabine for patients with advanced hepatocellular carcinoma. *China Pharmacy*. 2008;(11):848–849 [in Chinese].
31. Tang W, Chen Z, Zhang W, et al. The mechanisms of sorafenib resistance in hepatocellular carcinoma: Theoretical basis and therapeutic aspects. *Sig Transduct Target Ther*. 2020;5(1):87. doi:10.1038/s41392-020-0187-x
32. Ogasawara S, Chiba T, Ooka Y, et al. Post-progression survival in patients with advanced hepatocellular carcinoma resistant to sorafenib. *Invest New Drugs*. 2016;34(2):255–260. doi:10.1007/s10637-016-0323-1
33. Han Z, He Z, Wang C, Wang Q. The effect of apatinib in the treatment of sorafenib resistant metastatic hepatocellular carcinoma: A case report. *Medicine (Baltimore)*. 2018;97(49):e13388. doi:10.1097/MD.0000000000013388
34. Tan CS, Kumarakulasinghe NB, Huang YQ, et al. Third generation EGFR TKIs: Current data and future directions. *Mol Cancer*. 2018;17(1):29. doi:10.1186/s12943-018-0778-0
35. Alyateem G, Wade HM, Bickert AA, et al. Use of CRISPR-based screens to identify mechanisms of chemotherapy resistance. *Cancer Gene Ther*. 2023;30(8):1043–1050. doi:10.1038/s41417-023-00608-z
36. Wang H, Xu H, Ma F, et al. Zinc finger protein 703 induces EMT and sorafenib resistance in hepatocellular carcinoma by transactivating CLDN4 expression. *Cell Death Dis*. 2020;11(4):225. doi:10.1038/s41419-020-2422-3
37. Yang C, Zhang J, Ma Y, Wu C, Cui W, Wang L. Histone methyltransferase and drug resistance in cancers. *J Exp Clin Cancer Res*. 2020;39(1):173. doi:10.1186/s13046-020-01682-z
38. Turner JG, Dawson J, Sullivan DM. Nuclear export of proteins and drug resistance in cancer. *Biochem Pharmacol*. 2012;83(8):1021–1032. doi:10.1016/j.bcp.2011.12.016
39. Desterro J, Bak-Gordon P, Carmo-Fonseca M. Targeting mRNA processing as an anticancer strategy. *Nat Rev Drug Discov*. 2020;19(2):112–129. doi:10.1038/s41573-019-0042-3
40. Wang BD, Lee N. Aberrant RNA splicing in cancer and drug resistance. *Cancers (Basel)*. 2018;10(11):458. doi:10.3390/cancers10110458
41. Kang H, Heo S, Shin JJ, et al. A miR-194/PTBP1/CCND3 axis regulates tumor growth in human hepatocellular carcinoma. *J Pathol*. 2019;249(3):395–408. doi:10.1002/path.5325

42. Song Y, He S, Ma X, et al. RBMX contributes to hepatocellular carcinoma progression and sorafenib resistance by specifically binding and stabilizing BLACAT1. *Am J Cancer Res*. 2020;10(11):3644–3665. PMID:33294259. PMCID:PMC7716158.
43. Liang Y, Fan Y, Liu Y, Fan H. HNRNPU promotes the progression of hepatocellular carcinoma by enhancing CDK2 transcription. *Exp Cell Res*. 2021;409(1):112898. doi:10.1016/j.yexcr.2021.112898
44. Tan S, Zhang M, Shi X, et al. CPSF6 links alternative polyadenylation to metabolism adaption in hepatocellular carcinoma progression. *J Exp Clin Cancer Res*. 2021;40(1):85. doi:10.1186/s13046-021-01884-z
45. Shi ZD, Hao L, Han XX, et al. Targeting HNRNPU to overcome cisplatin resistance in bladder cancer. *Mol Cancer*. 2022;21(1):37. doi:10.1186/s12943-022-01517-9
46. Li X, Han M, Zhang H, et al. Structures and biological functions of zinc finger proteins and their roles in hepatocellular carcinoma. *Biomark Res*. 2022;10(1):2. doi:10.1186/s40364-021-00345-1
47. Li Q, Tan G, Wu F. The functions and roles of C2H2 zinc finger proteins in hepatocellular carcinoma. *Front Physiol*. 2023;14:1129889. doi:10.3389/fphys.2023.1129889
48. Zhang Q, Ruan F, Yang M, Wen Q. Natural compound chaetocin induced DNA damage and apoptosis through reactive oxygen species-dependent pathways in A549 lung cancer cells and in vitro evaluations. *IET Nanobiotechnol*. 2023;17(5):465–475. doi:10.1049/nbt2.12144
49. Hu P, Hu L, Chen Y, et al. Chaetocochin J exhibits anti-hepatocellular carcinoma effect independent of hypoxia. *Bioorg Chem*. 2023;139:106701. doi:10.1016/j.bioorg.2023.106701
50. Truitt L, Hutchinson C, DeCoteau JF, Geyer CR. Chaetocin antileukemia activity against chronic myelogenous leukemia cells is potentiated by bone marrow stromal factors and overcomes innate imatinib resistance. *Oncogenesis*. 2014;3(10):e122. doi:10.1038/oncsis.2014.37

Effect of gedunin on cell proliferation and apoptosis in skin melanoma cells A431 via the PI3K/JNK signaling pathway

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Abstract

Background. Melanoma is an aggressive skin malignancy with rapid metastasis and high morbidity. Gedunin (GN) is a tetranortriterpenoid belonging to the *Meliaceae* family, described for its anticancer, antiproliferative and apoptotic properties.

Objectives. In the present study, we investigated the effect of GN on A431 melanoma cell proliferation and apoptosis. The inflammatory proteins (tumor necrosis factor alpha (TNF-α), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), cycloxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), and interleukin 6 (IL-6)) apoptosis-related proteins, such as Bax, Bcl-2 and caspase-3, and alterations in the PI3K/JNK and p38 pathways in A431 cells after GN treatment were examined.

Materials and methods. The cytotoxicity assay and cell apoptosis of GN activity on A431 cells were assessed using MTT assay, acridine orange/ethidium bromide (AO/EB), DAPI (4′,6-diamidino-2-phenylindole), propidium iodide (PI), enzyme-linked immunosorbent assay (ELISA), reverse transcription polymerase chain reaction (RT-PCR) and western blot analyses.

Results. The findings demonstrated that GN (10 and 15 μM/mL) inhibits the growth of melanoma cells, triggers apoptosis by enhancing Bax and caspase, and reduces Bcl-2, cyclin-D1, c-myc, and survivin in a concentration-reliant manner. Additionally, GN attenuated the protein expression of inflammatory proteins (TNF-α, NF-κB, COX-2, iNOS, and IL-6) and the cell proliferative PI3K/JNK/p38 signaling pathway. Due to the imbalance in the Bax/Bcl-2 ratio, apoptosis is promoted, and the caspase cascade and Cyt-c are activated. This led us to conclude that GN treatment inhibited Bcl-2, cyclin-D1, c-myc, and survivin activity through the TNF-α/NF-κB and PI3K/JNK/p38 signaling pathways, further preventing the proliferation and stimulation of apoptosis, which contributes to growth arrest in melanoma cells.

Conclusions. Gedunin has been shown to promote melanoma cell death in vitro, suggesting that it could be used as a future treatment for malignant melanoma. Our findings suggested that GN might be applied as a preventative measure in the management of skin melanoma cells.

Key words: skin cancer, gedunin, apoptosis, AROS, P13K/JNK/p38 signaling

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Background

Skin cancer has an elevated incidence and mortality worldwide due to chemical carcinogens producing reactive oxygen species (ROS) and augmented UV radiation due to ozone diminution at the earth's surface.[1] Melanocyte-derived malignant melanoma (MM) causes the greater part of deaths ascribed to skin cancer. It is most commonly observed at the interface between the dermis and epidermis, as well as in melanocyte-rich regions of the skin, the ciliary body of the eye, the mucosa, the iris, the choroid, and the meninges. It is less frequently found in lymph nodes and internal organs.[2] Melanoma, which accounts for approx. 70% of skin cancer demises, is the most lethal form of skin cancer, featured by speedy degradation, premature metastasis and elevated mortality.[3] Melanoma has increased drastically over the past 20 years.[4] The most recent statement by the World Health Organization (WHO) indicated that there were 288,000 cases of melanoma and 61,000 deaths globally in 2018.[5] Melanoma can be treated with surgical excision, chemotherapy or immunotherapy,[6] but their efficacies are variable and the adverse effects are not negligible.[7] As a result, novel approaches to prevent melanoma formation and provide efficient protection are immediately needed.

Gedunin (GN) is one of the major tetranortriterpenoids found in the neem tree.[8] Contemporary studies have revealed that GN can reduce ovary, prostate and colon cancer cell proliferation.[9,10] Gedunin has been described to have potential anticancer activity by impeding breast cancer cell proliferation through the modulation of the assured heat shock proteins.[11] Also, the antiproliferative effects have been noticed in ovarian cancer cells in response to GN treatment via central signaling pathway regulation.[12] Furthermore, a current in silico study has shown a drug-likeness of GN for the β-catenin chain in cancer stem cells.[13] However, the apoptosis and antiproliferative effects of GN on melanoma cancer cells have not yet been investigated. The current study was conducted to evaluate anticancer and apoptotic effects of GN on A431 human melanoma cells.

Apoptosis is crucial for cellular homeostasis; hence, it became a central target for developing new anticancer remedies, influences the responsiveness to treatment, and is involved in the regulation of tumor development.[14,15] It is triggered by extrinsic and intrinsic ligands, which are regulated by various intracellular signaling pathways and coordinated by gene complexes.[16] During these processes, the instigation of the caspase cascade directs the cleavage of essential proteins that are involved in anti-apoptotic pathways, like members of the Bcl-2 protein family.[17] Bax and Bcl-2 are pro- and anti-apoptotic proteins belonging to the Bcl-2 family. These are key intermediates in mitochondrial outer membrane permeabilization supplemented by apoptosis.[18] They also play a vital role in controlling cytochrome-c discharge from the mitochondria.[19] Numerous downstream substrates of the PI3K/JNK signaling pathway, such as Bax and Bad, added to the chemotherapeutic resistance of tumor cells and reduced apoptosis.[20] Kishore et al.[21] documented that GN could suppress hamster buccal pouch carcinogenesis through the PI3K/JNK/p38 and tumor necrosis factor alpha (TNF-α) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), pathways. Likewise, Tanagala et al.[22] reported that in SCC131 oral cancer cells, GN downregulated PI3K/Akt/mTOR/ERK/NF-κB signaling. While recent studies have focused on GN as an effective anti-melanogenic agent, there are still not enough papers conducted on the effects of GN in cell proliferative and its impact on apoptosis in A431 MM cells via the PI3K/JNK signaling pathway. In the current hypothesis examine that GN affected the apoptotic and proliferation of A431 melanoma cells. After GN treatment, A431 cells were treated with inflammatory proteins (tumor necrosis factor alpha (TNF-α), nuclear factor kappa-light chain-enhancer of activated B cells (NF-κB), cycloxygenase 2 (COX-2), inducible nitric oxide synthase (iNOS), and interleukin 6 (IL-6), as well as apoptosis-related proteins (Bax, Bcl-2, and caspase-3), and changes in the PI3K/JNK and p38 pathways.

Objectives

We aimed to assess the anticancer impact of GN on A431 melanoma cells and clarify the molecular mechanisms underlying the PI3K/AKT/p38 apoptotic signaling pathway.

Materials and methods

Chemicals

Gedunin, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), antibiotics, phosphate-buffered saline (PBS), MTT, acridine orange/ethidium bromide (AO/EB), 4',6-diamidino-2-phenylindole (DAPI), propidium iodide (PI), sodium dodecyl sulphate (SDS), and dimethyl sulfoxide (DMSO) were provided by Merck (Darmstadt, Germany). JNK and p38 antibodies, the phosphorylated form and total PI3K, and horseradish peroxidase (HRP)-conjugated β-actin were acquired from Labome (Cambridge, USA).

Cell culture

Shanghai Aiyan Biotechnology Co., Ltd. (Shanghai, China) provided the A431 melanoma cell lines, which were obtained in Shanghai, China. The cells were cultivated in DMEM medium supplemented with 10% FBS and 1% antibiotics (penicillin/streptomycin) at 37°C in an environment of 5% CO_2 and less than 95% humidity.

MTT cell viability assay

The vitality of human melanoma cells was determined using the MTT assay.[23] After seeding A431 cells into 96 wells

(1×10^5 cells/well), they were grown at 37°C in a moist incubator with 5% CO_2. After overnight incubation, cells were dipped in PBS and exposed to GN at various doses (5, 10, 15, and 20 μM/mL) for a day. The cells were treated with a 10 μL MTT solution and left for 4 h to allow mitochondrial dehydrogenase to convert into insoluble formazan crystals. The formazan crystals were then dissolved using 150 μL of DMSO. The optical density (OD) at 490 nm was determined using an enzyme-linked immunosorbent assay (ELISA) plate Reader (Bio-Tek Instruments, USA). The IC_{50} values for 4-parameter logistic function dose-response curves were calculated using Sigma PlotTM software (Systat, San Jose, USA; GN concentrations that caused a 50% reduction in MTT assay). The concentrations selected for further investigation were used.

Apoptosis evaluation by AO/EB staining

The study used AO/EB staining and labeling to observe apoptotic morphological differentiation in melanoma cells exposed to GN at 10 and 15 μM doses.[24] For a full day, A431 cells were treated to 10 and 15 μM/mL of GN. The treated and control cells received the same amount of AO/EB dye mixture (100 g/mL). Following the incubation, the cells were examined using an Olympus BX51 fluorescence microscope (Olympus Corp., Tokyo, Japan) and left at room temperature in the dark for 20 min to ensure that all unbound dye had been removed by PBS washing.

Apoptosis assessed by DAPI staining

Six well plates with 1×10^5 human melanoma cells each were seeded with A431, and GN (10 and 15 μM/mL) was added as a supplement. These treated cells were labeled with DAPI in accordance with the previously described methodology to study the nuclear changes associated with apoptosis.[25] After that, the materials were placed on a glass slide and examined using an Olympus BX51 fluorescence microscope (Olympus Corp.).

Evaluation of apoptosis by PI staining

The PI staining test was used to identify apoptotic nuclei in A431 melanoma cells. The cells were treated to various GN concentrations (10 and 15 μM/mL) and stored for 48 h. The samples were subsequently extracted and stained with PI according to the described procedure.[26] A microscope (Olympus BX51) was used to look at the red fluorescence emitted by the nuclei.

Measurement of caspase-9 and -8

Caspase-9 and caspase-8 activity in GN-treated A431 cells was measured using the caspase-9 and -8 colorimetric assay kit (Biovision, Exton, USA) according to the manufacturer's instructions. The cells were homogenized in a lytic buffer (ethanesulfonic acid (HEPES) Ph 7.4; 5 mm 3-((3-cholamidopropyl) dimethylammonio)-1-propanesulfonate (CHAPS) and 5 mm dithiothreitol (DTT)) and kept in ice for 10 min. The homogenate was centrifuged in a microcentrifuge (10,000 × g) for 1 min. The supernatant (cytosolic extract) was transferred to a fresh tube and kept on ice for immediate assay. Fifty microliters of each sample containing 50–200 μg of protein were placed into microtiter plate wells. Fifty microliters of 2X Reaction Buffer (Biovision) (containing 10 mm DTT) were added to each well. Five microliters of 4 mm LEHD-pNA (caspase-9 substrate) substrate were added to each well and incubated at 37˚C for 1–2 h. After incubation, the samples on the microtiter plate were read at 400 or 405 nm in a microtiter plate reader. The concentration of pNA released from the substrate was calculated from the absorbance value. Fold increases were determined by comparing the results with the levels of the control samples. Caspase-9 and -8 activity was expressed as μmol of pNA formed/min/mL of cell lysate.

mRNA expressions detected by qRT-PCR

Total RNA was extracted from A431 human melanoma cells using the TRIzol® reagent (Invitrogen, Thermo Fisher Scientific, Waltham, USA) following the manufacturer's instructions. To reverse transcribe the isolated RNA into cDNA, a High-capacity cDNA Reverse Transcription kit (Bio-Rad, Hercules, USA) was used, following the manufacturer's instructions. Lastly, the cDNAs were evaluated in accordance with the company's instructions using a FastStart SYBR Green master mix (Invitrogen, Thermo Fisher Scientific). The band intensity was measured on 1.5% agarose gels using electrophoresis with ImageJ v. 1.48 software (National Institutes of Health (NIH), Bethesda, USA).

Western blot analysis

Human melanoma cells A431 were treated with 10 and 15 μM/mL of GN and grown for 1 day. Protease inhibitors were added, and the cell lysates were generated using an ice-cold lysis solution. Subsequently, a western blot analysis was conducted. The protein content was determined with the BCA Protein Assay Kit (Thermo FIsher Scientific). To summarize, the proteins were electrophoretically dispersed and then transferred to a polyvinyl difluoride (PVDF) film. After blocking the film for 1 h at room temperature with the probe, primary antibodies (TNF-α, NF-κB, COX-2, iNOS, and interleukin 6 (IL-6)) PI3K, JNK, and p38) were added at a dilution of 1:1,000 and the film was left overnight at 4°C. After that, secondary antibodies were added. To detect proteins, protein bands were colored and shown. Densitometry was used to quantify the protein bands using ImageJ software.

Statistical analyses

The statistical analysis of the data from each group was conducted using GraphPad Prism v. 8.0.2 (GraphPad Software, San Diego, USA) and IBM SPSS v. 25 (IBM Corp., Armonk, USA). Measurement data were presented as the median (min and max). As the sample size was too small to verify normal data distribution, the differences between the groups were analyzed with the non-parametric Kruskal–Wallis test and Dunn's post hoc test. Subsequently, significant differences among multiple groups were examined using the Kruskal–Wallis test and Dunn's post hoc test was employed for multiple comparisons. A statistically notable data divergence was considered at a p-value < 0.05. All tests in this study were bilateral.

Results

Cytotoxicity effect of GN on A431 melanoma cells

The cytotoxicity of GN (5, 10, 15, and 20 μM/mL) on human melanoma cells A431 was assessed using the MTT test. A431 proliferation could not be significantly affected by GN administration at concentrations less than 10 μM. However, GN doses of 5, 10, 15, and 20 μM/mL inhibited A431 cell survival. The findings revealed that GN had dose-dependent cytotoxic and antiproliferative effects on A431 melanoma cells. The IC_{50} value of GN was 15 μM, hence, 10 and 15 μM were chosen for further research (Fig. 1, Table 1).

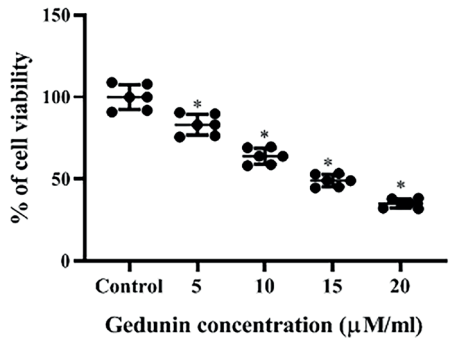

Fig. 1. Gedunin inhibits A431 human melanoma cell proliferation. Human A431 melanoma cells for a full day were exposed to varying concentrations of gedunin (GN) (5–20 μM/mL). Cell viability was assessed using the MTT test. A * p-value < 0.05 indicates significance compared to melanoma control cells that have not received treatment. The figure presents data (black dots) and the medians (horizontal lines). A * p-value < 0.05 compared to the control group (Table 1)

Assessment of apoptosis using AO/EB staining

Dual acridine orange/ethidium bromide staining revealed apoptotic changes in A431 melanoma cells (Fig. 2A). The untreated A431 melanoma cells were consistently labeled green and alive. In a concentration-dependent manner, GN (10 and 15 μM/mL) treated A431 cells revealed more apoptotic alterations than controls. Compressed chromatin, membrane blebbing, and early apoptotic cells were observed in A431 melanoma cells treated with 10 μM GN. The treatment of 15 μM GN caused late apoptotic alterations in A431 cells, including chromatin condensation, fragmented nuclei and orange-red membrane blebbing.

Influence of GN on A431 melanoma cell apoptosis using DAPI staining

Normal, live cells can be seen in the DAPI-stained human melanoma A431 cells (Fig. 2B). Gedunin-induced apoptosis-treated A431 melanoma cells were shown to have improved nuclei shape and nuclear body fragmentation compared to untreated control cells. Chromatin condensation, membrane blebbing, nuclear envelope damage, and cellular collapse were observed in A431 cells treated with GN (10 and 15 μM/mL). These results point out GN-induced involuntary cell demise in a concentration-dependent manner.

Influence of GN-induced apoptosis in A431 melanoma cells evinced using PI staining

In A431 cells, PI labeling was used to detect apoptotic nuclei (Fig. 2C). Propidium iodide (PI) stain enters the cells when membrane integrity is lost, which is associated with membrane polarity damage, guiding apoptosis. The findings demonstrate that GN caused dose-dependent apoptosis in A431 cells. Apoptotic activity was elevated by GN treatments (10 and 15 M/mL) compared to untreated A431 control cells. Hence, GN-prompted apoptosis could be one mechanism for averting melanoma cell proliferation.

Measurement of caspase-9 and -8 activity using ELISA

Treatment with GN of A431 melanoma cells exhibited augmented activity of caspase-9 and -8 compared to untreated control cells (Fig. 3, Table 2,3). The amount

Table 1. Compare groups with each other

Variables	Control	5 μM	10 μM	15 μM	20 μM	Test value (H)**	p-value*
MTT	100.03 (90.98–108.98)	83.15 (75.63–90.59)	63.93 (58.15–69.65)	49.07 (44.64–53.46)	35.12 (31.94–38.26)	27.87	<0.001

Data were presented as median (min and max); * p-value was generated from Kruskal–Wallis test with Dunn's post hoc test; ** degrees of freedom value is equal to 4.

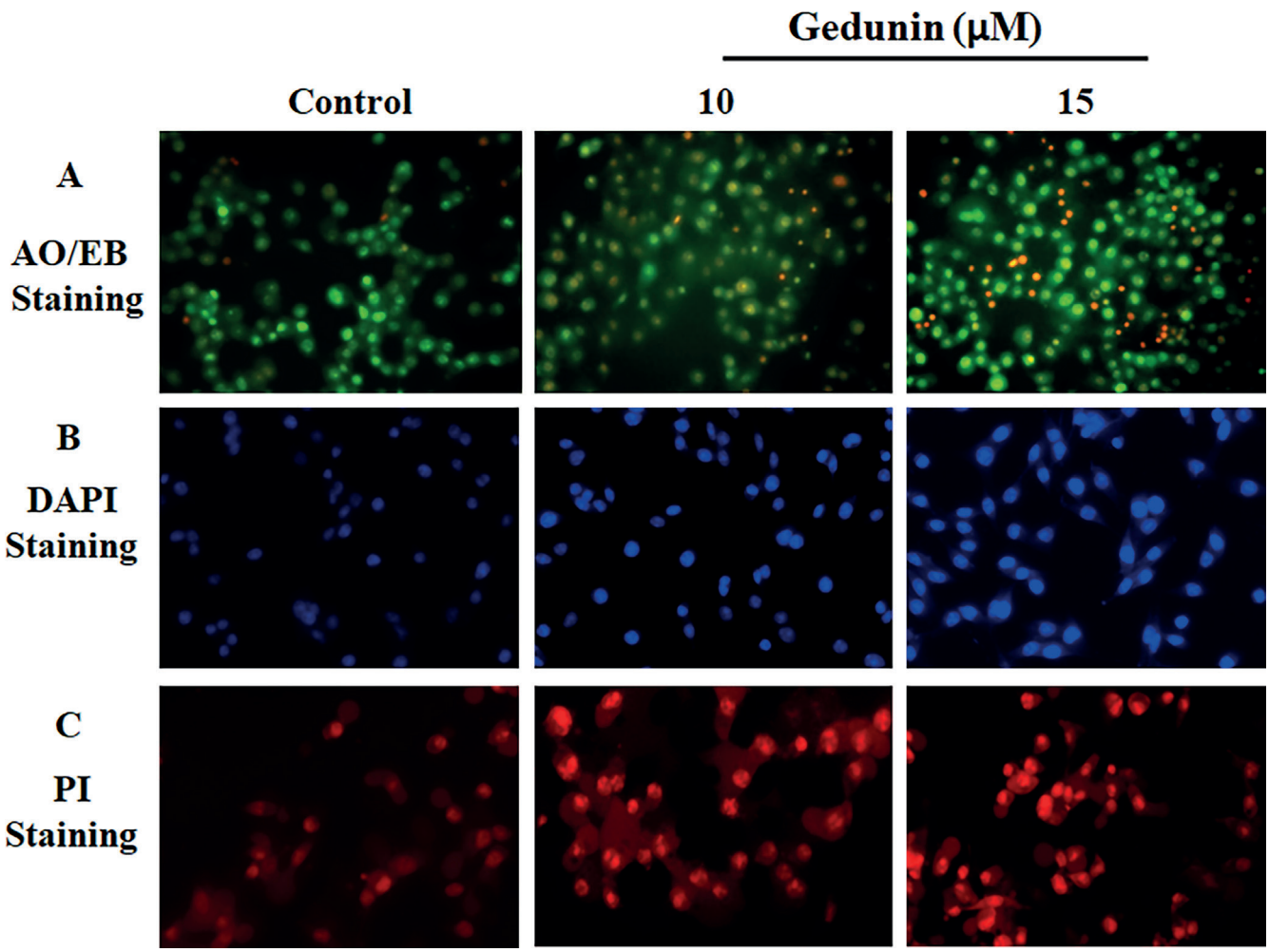

Fig. 2. The effect of gedunin (GN) on the apoptosis of A431 human melanoma cells. A. acridine orange/ethidium bromide (AO/EB); B. DAPI (4',6-diamidino-2-phenylindole), and C. Propidium iodide (PI) staining. Gedunin (10 and 15 μM/mL) treated with A431 melanoma cells for 24 h. Using AO/EB, DAPI and PI staining, apoptosis in melanoma cells was investigated and observed under a fluorescence microscope

Table 2. Comparisons of measured parameters between groups

Variables	Control (n = 6)	15 μM (n = 6)	20 μM (n = 6)	Test value (H)**	p-value*
Caspase-9	100.05 (91–109)	158.18 (143.87–172.33)	183.29 (166.71–199.69)	13.66	0.001
Caspase-8	100.05 (91–109)	116.14 (105.63–126.53)	130.20 (118.43–141.85)	11.97	0.003
Cyclin-D1	1.00 (0.91–1.09)	0.75 (0.68–0.82)	0.52 (0.47–0.57)	15.20	<0.001
Bcl-2	1.00 (0.91–1.09)	0.80 (0.73–0.87)	0.48 (0.44–0.52)	15.23	<0.001
Bax	1.00 (0.91–1.09)	2.15 (1.96–2.34)	3.50 (3.19–3.82)	15.20	<0.001
Caspase-3	1.00 (0.91–1.09)	2.02 (1.00–2.30)	3.08 (2.80–3.36)	14.07	<0.001
C-Myc	1.00 (0.91–1.09)	0.58 (0.53–0.63)	0.45 (0.41–0.49)	15.26	<0.001
Survivin	1.00 (0.91–1.09)	0.53 (0.48–0.58)	0.40 (0.36–0.44)	15.20	<0.001
TNF-α	1.00 (0.91–1.09)	0.71 (0.65–0.77)	0.48 (0.44–0.52)	15.26	<0.001
NF-κB	1.00 (0.91–1.09)	0.68 (0.62–0.74)	0.46 (0.42–0.50)	15.23	<0.001
COX-2	1.00 (0.91–1.09)	0.79 (0.72–0.86)	0.53 (0.48–0.58)	15.20	<0.001
iNOS	1.00 (0.91–1.09)	0.61 (0.56–0.66)	0.38 (0.35–0.41)	15.26	<0.001
IL-6	1.00 (0.91–1.09)	0.70 (0.64–0.76)	0.42 (0.38–0.46)	15.23	<0.001
p-PI3K/PI3K	1.00 (0.91–1.09)	0.80 (0.73–0.87)	0.53 (0.48–0.58)	15.20	<0.001
p-JNK/JNK	1.00 (0.91–1.09)	1.43 (1.30–1.56)	2.51 (2.28–2.74)	15.20	<0.001
p-P38/P38	1.00 (0.91–1.09)	1.52 (1.38–1.66)	2.68 (2.44–2.92)	15.20	<0.001

Data were presented as median (min and max); * p-value was generated from Kruskal–Wallis test with Dunn's post hoc test; ** degrees of freedom is equal to 2. TNF-α – tumor necrosis factor alpha; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; iNOS – inducible nitric oxide synthase; IL-6 – interleukin 6.

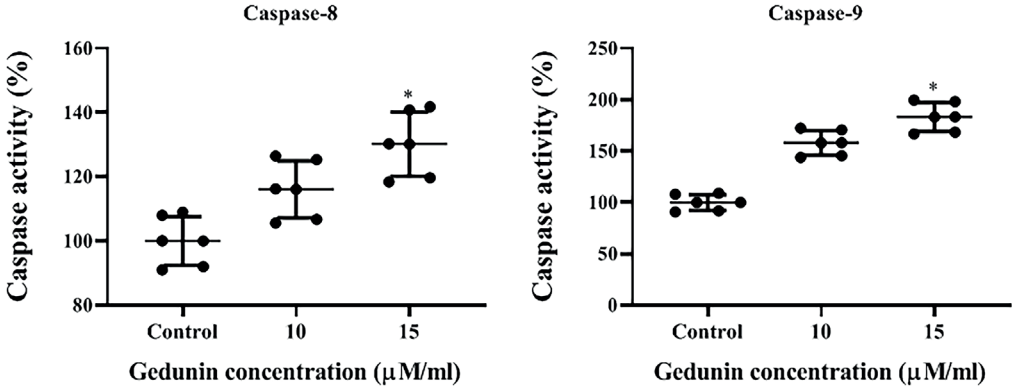

Fig. 3. Measurement of caspase-9 and -8 in A431 human melanoma cells treated with gedunin (GN). For a whole day, control, 10 and 15 μM/mL GN doses were administered to human melanoma A431 cells. Using enzyme-linked immunosorbent assay (ELISA), the activity of caspase-9 and -8 was quantified. The figure presents data (black dots) and the medians (horizontal lines); * $p < 0.05$ compared to the control group (Table 2,3)

Table 3. The results of the Dunn's post hoc test

Figure	Variable	C vs 15 μM	C vs 20 μM	15 μM vs 20 μM
Fig. 3	caspase-9	0.092	p < 0.001	0.390
Fig. 3	caspase-8	0.251	p < 0.002	0.251
Fig. 4	cyclin-d1	0.154	p < 0.001	0.154
Fig. 4	Bcl-2	0.153	p < 0.001	0.153
Fig. 4	Bax	0.154	p < 0.001	0.154
Fig. 4	caspase-3	0.312	p < 0.001	0.103
Fig. 4	C-Myc	0.152	p < 0.001	0.152
Fig. 4	survivin	0.154	p < 0.001	0.154
Fig. 5	TNF-α	0.152	p < 0.001	0.152
Fig. 5	NF-κB	0.153	p < 0.001	0.153
Fig. 5	COX-2	0.154	p < 0.001	0.154
Fig. 5	iNOS	0.152	p < 0.001	0.152
Fig. 5	IL-6	0.153	p<0.001	0.153
Fig. 6	p-PI3K/PI3K	0.154	p < 0.001	0.154
Fig. 6	p-JNK/JNK	0.154	p < 0.001	0.154
Fig. 6	p-P38/P38	0.154	p < 0.001	0.154

TNF-α – tumor necrosis factor alpha; NF-κB – nuclear factor kappa-light-chain-enhancer of activated B cells; AO/EB –acridine orange/ethidium bromide; iNOS – inducible nitric oxide synthase; IL-6 – interleukin 6.

of caspase-9 and -8 was significantly ($p < 0.05$) increased by GN at a dose of 15 μM compared to 10 μM GN treatment. Gedunin improved caspase activities in a concentration-dependent way.

Impact of GN on mRNA expression of cyclin-D1, Bcl-2, Bax, caspase-3, c-myc, and survivin in A431 melanoma cells

A431 cells were treated with GN (10 and 15 μM/mL) to measure the mRNA expression of proteins involved in apoptosis (Fig. 4, Table 2,3). While Bax and caspase-3 showed decreased mRNA expression, the untreated A431 control cells showed increased mRNA expression of cyclin-D1, Bcl-2, c-Myc, and survivin. When compared to untreated control melanoma cells, GN significantly decreased the expression of mRNAs for cyclin-D1, Bcl-2, c-Myc, and survivin, while increasing the expression of Bax and caspase-3 in a concentration-dependent manner.

Gedunin suppressed the NF-κB signaling pathway analyzed using western blot

The NF-κB/TNF-α signaling has a substantial role in cancer cell growth and development. Figure 5 depicts increased protein expression of TNF-α, NF-κB, COX-2, iNOS, and IL-6 in human A431 melanoma cells (Table 2,3). Treatment with GN (10 and 15 μM/mL) in the A431 cells decreased TNF-α, NF-κB, COX-2, iNOS, and IL-6 levels. In human melanoma cells, GN reduced these protein expressions in a concentration-dependent manner.

Gedunin suppressed the P13K/JNK/p38 signaling pathway analyzed using western blot

p-PI3K, PI3K, p-JNK, JNK, p38, and p38 signaling have a substantial role in cancer cell growth and development. Human A431 melanoma cells indicated intensified protein expression of P13K, JNK, and p38 (Fig. 6, Table 2,3). Treatment with GN (10 and 15 μM/mL) in the A431 cells decreased P13K/JNK/p38 levels. Gedunin reduced the expression of these proteins in the human melanoma cells in a concentration-dependent manner.

Discussion

Herbal plant-based natural products are still one of the greatest reservoirs for unique compounds with pharmacological activities.[27] Research is enduring to recognize a natural agent with the selective ability to block or impede cancer initiation and converse promotional stages by promoting apoptosis and arresting cancer cell growth deprived of typical cell cytotoxic effects.[28] The contemporary data indicate that several tumours are caused by anti-apoptotic protein genes

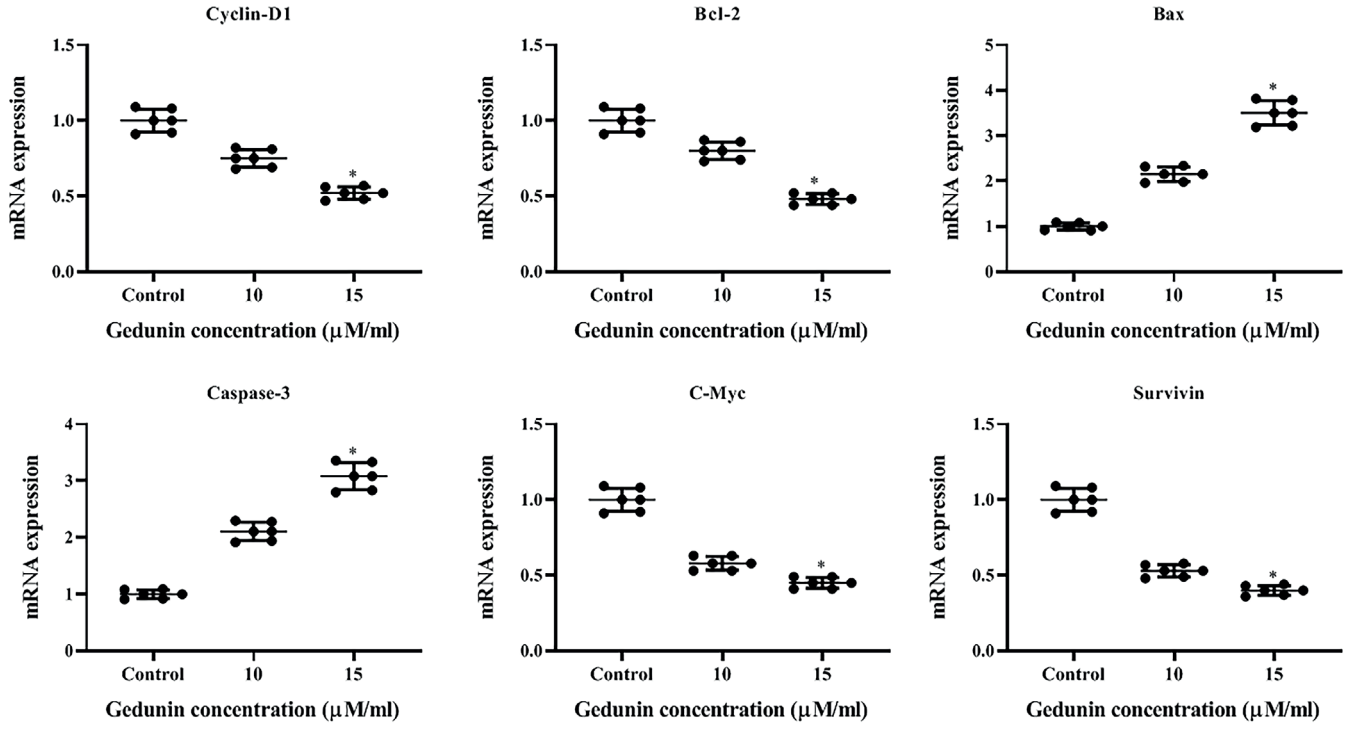

Fig. 4. Influence of gedunin (GN) on the mRNA expression of A431 human melanoma cells, including cyclin-D1, Bcl-2, Bax, caspase-3, c-Myc, and survivin. For a full day, A431 melanoma cells were exposed to control and 10 and 15 μM/mL doses of GN. The mRNA expression of cyclin-D1, Bcl-2, Bax, caspase-3, c-Myc, and survivin in A431 cells was determined using reverse transcription polymerase chain reaction (RT-PCR). The figure presents data (black dots) and the medians (horizontal lines); * $p < 0.05$ compared to the control group (Table 2,3)

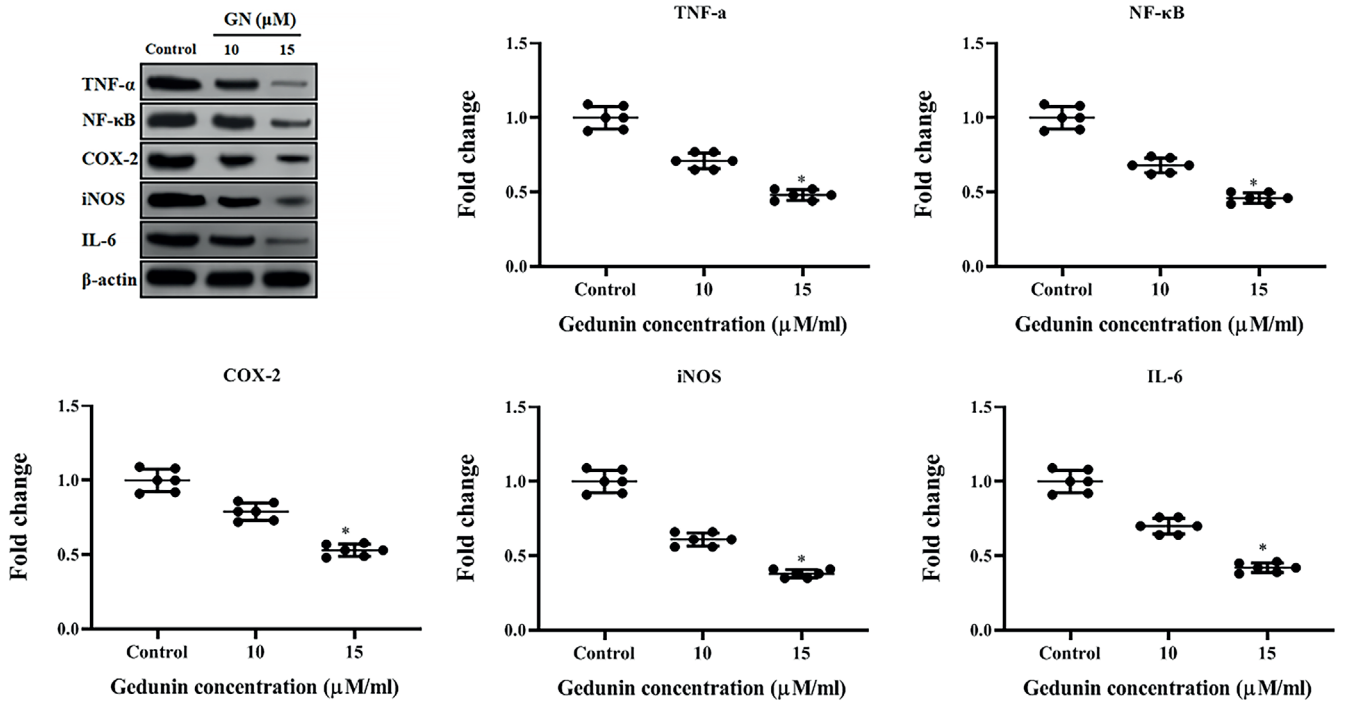

Fig. 5. Impact of gedunin (GN)-treated A431 human melanoma cells on tumor necrosis factor alpha (TNF-α), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), cyclooxygenase-2 (COX2), inducible nitric oxide synthase (iNOS), and interleukin 6 (IL-6) inflammatory markers. For 1 day, melanoma A431 cells were exposed to 10 and 15 μM/mL of GN. Using western blot analysis, the protein expression of TNF-α, NF-κB, COX-2, iNOS, and β-actin was determined. The figure presents data (black dots) and the medians (horizontal lines); * $p < 0.05$ compared to the control group (Table 2,3)

that code for dysfunction, growth receptors, growth factors, tumour suppressors, and transcription factors. These genes constitute cancer treatment targets.[29,30] In the current work, we assessed the capability of GN to constrain A431 cell proliferation as well as its apoptosis effects.

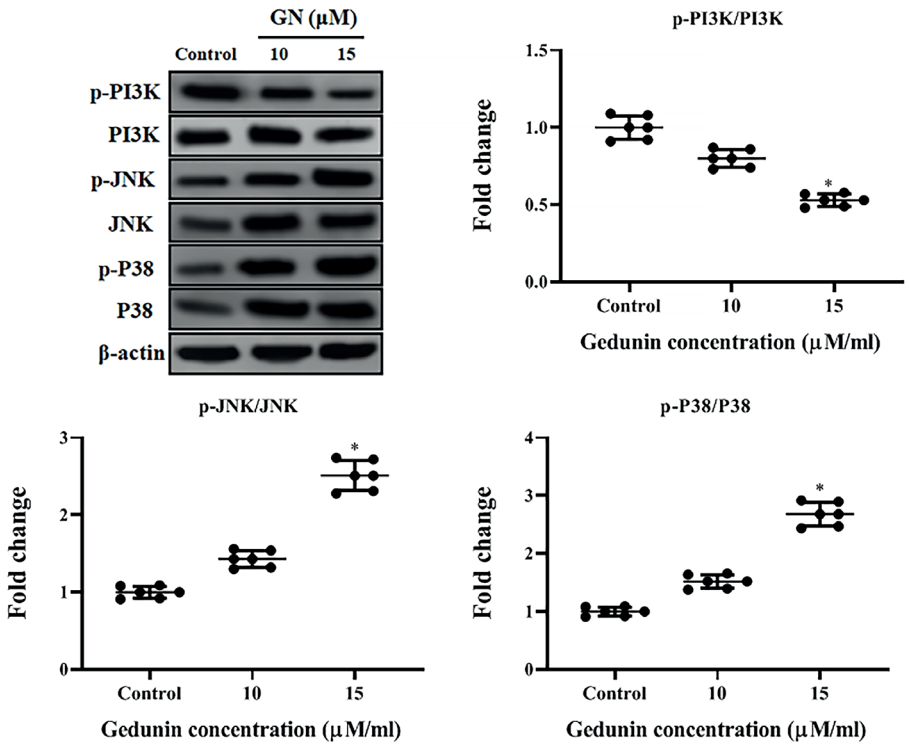

Fig. 6. Impact of gedunin (GN)-treated A431 cells on the P13K/AKT/p38 signaling pathway. For a whole day, melanoma A431 cells were exposed to 10 and 15 μM/mL of GN. Using western blot analysis, the protein expression of p-P13K, P13K, p-AKT, AKT, p-p38, p38, and β-actin was determined. The figure presents data (black dots) and the medians (horizontal lines); * $p < 0.05$ compared to the control group (Table 2,3)

Natural compounds are reported to have the competencies for reticence proliferation and triggering apoptosis in numerous malignant cells.[31] Gedunin is a bioactive limonoid, a derivative of the neem tree that seems to be an active natural chemotherapeutic agent due to its powerful anticancer efficacy on various kinds of malignancies, including oral, prostate, ovarian, and colon cancers.[9,10] It has been documented that GN is an effective anticancer drug.[32] Brandt et al.[33] claimed that GN has an antiproliferative impact on the breast cancer cell lines MCF-7 and SkBr3. Lamb et al.[34] demonstrated that GN modulated Hsp90 to demonstrate antiproliferative action. Our current study found that GN is an effective anticancer and antiproliferative agent against A431 human melanoma cells, evidenced by the MTT cytotoxicity assay. These results imply that GN may be a viable chemotherapeutic medication for the treatment of melanoma skin cancer.

Apoptosis is a gene-delimited spectacle triggered by many chemotherapeutic mediators.[14,15] It has been well established that both the intrinsic and extrinsic pathways are involved in apoptosis.[16] We observed augmented mRNA expressions of Bax and caspase-3 and levels of caspase-8, and -9, while cyclin-D1, c-Myc, survivin, and Bcl-2 expressions were reduced in GN treated melanoma cancer cells. The Bcl-2 family is generally elaborate in controlling the intrinsic apoptosis pathway. This depends on Bcl-2 and Bax expression as the main apoptotic molecules. Alternatively, an elevated expression points out the stimulation of the apoptotic extrinsic pathway.[35] Apoptosis can trigger cell cycle arrest. Proteins that regulate the cell cycle and cell cycle checkpoint transitions are regulated by cyclin-dependent kinases, which are regulated by cyclins. A cyclin involved in the G1 to S phase cell cycle transition is cyclin-D1. Numerous tumors have been linked to cyclin-D1 hyperactivity, and it has been shown that cyclin-D1 expression inhibition may help treat cancer.[36–38] Previously, Johnson et al.[39] have shown that, in comparison to ovarian cancer cells that were not treated, GN-treated cells had reduced the inhibitory phosphorylation (Y15) of CDK1 and increased levels of cyclin-B1. They also created double-strand breaks, increased the ratio of Bcl-2 to Bax proteins, and ultimately caused the release of cytochrome-c from the mitochondria. Research has shown a connection between apoptosis and cyclin-D1 attenuation.[40] The melanoma skin cancer cells in our study had high expression levels of cyclin-D1, C-Myc and survivin. Furthermore, GN administration significantly inhibited survivin, C-Myc and cyclin-D1 mRNA expression as well as cell proliferation. The expression of cyclin-D1, C-Myc and survivin were attenuated, which may have facilitated GN-mediated antiproliferative and apoptotic actions.

The regulation of essential elements accountable for an inflammatory response in skin cancer necessitates the activation of NF-κB.[41] The purpose of the current investigation was to ascertain how GN affects NF-κB transcription. As expected from the western blot analysis, the results demonstrated that NF-κB/p65 was activated and translocated into the nucleus upon TNF-α stimulation. However, this impact could be countered by GN pretreatment. These results unequivocally demonstrated that GN specifically blocked TNF-α-induced NF-κB translocation, which is believed to be the main mechanism triggering the inflammatory response.

Strong evidence suggests that MAPK has a role in GN treatments. JNK, p38 and the other 3 members of the MAPK family are frequently associated with GN processes in humans and mice, and their signaling pathways play a major role in the initiation of inflammation and death of a variety of cell types.[42] The findings were in line with the western blot analysis and demonstrated that NF-κB/p65 was activated and translocated into the nucleus following TNF-α stimulation. Gedunin pretreatment, however, had the ability to reverse this impact. These results unequivocally demonstrate that GN specifically blocked TNF-α-induced NF-κB translocation, which is believed to be the main mechanism causing the inflammatory response. Despite sharing 60–70% of their structural similarities, PI3K, JNK and p38 have distinct biological effects because of variations in their sizes and activation loop sequences.[43] Our results showed that TNF-α-induced endothelial cell injuries were accompanied by an increase in the phosphorylation levels of all MAPK family components, which is in line with previous findings. However, the only activities that were significantly lowered by GN pre-incubation were JNK and p38. Notably, anisomycin (AM), an activator of JNK and p38, totally undid the protective effects of GN treatment. These results unequivocally demonstrate that GN inhibits the p38, MAPK, and JNK signaling pathways, which mediates its protective properties.

Limitations

Within the current hypothesis, we examined the anticancer potential of GN on A431 melanoma cells and clarified the PI3K/AKT/p38 signaling pathway only, but more studies are required apoptotic and cell adhesive pathway molecular marker levels.

Conclusions

In vitro studies have demonstrated that GN has the capacity to avert the inflammatory markers TNF-α, NF-κB, COX-2, iNOS, and IL-6, to inhibit cell proliferation, and to trigger apoptosis of A431 human melanoma cells. This is achieved via the upregulation of the expression of Bcl-2, cyclin-D1, c-Myc, and survivin via the PI3K/JNK/p38 signaling pathway, and the subsequent decrease in the expression of Bax and caspases. Our findings point out that GN can be reflected as a promising anticancer remedy for treating human melanoma skin cancer. The results data from this section succinctly summarized the key findings of the study. This study effectively communicates that GN inhibited melanoma cell growth and induced apoptosis in a concentration-dependent manner. Additionally, it highlights the modulation of various proteins and signaling pathways involved in inflammation, cell proliferation and apoptosis by GN treatment. Based on our findings, we recommended GN as a therapeutic drug for carrying out an in vivo study in the future.

Supplementary data

The supplementary materials are available at https://doi.org/10.5281/zenodo.11392666. The package includes the following files:

Supplementary Fig. 1. Results of Kruskal–Wallis test as presented in Fig. 3.

Supplementary Fig. 2. Results of Kruskal–Wallis test as presented in Fig. 4.

Supplementary Fig. 3. Results of Kruskal–Wallis test as presented in Fig. 5.

Supplementary Fig. 4. Results of Kruskal–Wallis test as presented in Fig. 6.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Jaffri J. Reactive oxygen species and antioxidant system in selected skin disorders. *Malays J Med Sci*. 2023;30(1):7–20. doi:10.21315/mjms2023.30.1.2
2. Liu Y, Sheikh MS. Melanoma: Molecular pathogenesis and therapeutic management. *Mol Cell Pharmaco*l. 2014;6(3):228. PMID: 25745537
3. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. *CA Cancer J Clin*. 2021;71(1):7–33. doi:10.3322/caac.21654
4. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014;64(1):9–29. doi:10.3322/caac.21208
5. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424. doi:10.3322/caac.21492
6. Luke JJ, Flaherty KT, Ribas A, Long GV. Targeted agents and immunotherapies: Optimizing outcomes in melanoma. *Nat Rev Clin Oncol*. 2017;14(8):463–482. doi:10.1038/nrclinonc.2017.43
7. Rodríguez-Cerdeira C, Carnero Gregorio M, López-Barcenas A, et al. Advances in immunotherapy for melanoma: A comprehensive review. *Mediators Inflamm*. 2017;2017:3264217. doi:10.1155/2017/3264217
8. Nagini S, Palrasu M, Bishayee A. Limonoids from neem (*Azadirachta indica* A. Juss.) are potential anticancer drug candidates. *Med Res Rev*. 2024;44(2):457–496. doi:10.1002/med.21988
9. Uddin SJ, Nahar L, Shilpi JA, et al. Gedunin, a limonoid from *Xylocarpus granatum*, inhibits the growth of CaCo-2 colon cancer cell line in vitro. *Phytother Res*. 2007;21(8):757–761. doi:10.1002/ptr.2159
10. Sahai R, Bhattacharjee A, Shukla VN, et al. Gedunin isolated from the mangrove plant *Xylocarpus granatum* exerts its anti-proliferative activity in ovarian cancer cells through G2/M-phase arrest and oxidative stress-mediated intrinsic apoptosis. *Apoptosis*. 2020;25(7–8):481–499. doi:10.1007/s10495-020-01605-5

11. Hasan A, Haque E, Hameed R, et al. Hsp90 inhibitor gedunin causes apoptosis in A549 lung cancer cells by disrupting Hsp90:Beclin-1:Bcl-2 interaction and downregulating autophagy. *Life Sci*. 2020;256:118000. doi:10.1016/j.lfs.2020.118000
12. Kamath SG, Chen N, Xiong Y, et al. Gedunin, a novel natural substance, inhibits ovarian cancer cell proliferation. *Int J Gynecol Cancer*. 2009;19(9):1564–1569. doi:10.1111/IGC.0b013e3181a83135
13. Wanandi SI, Limanto A, Yunita E, et al. In silico and in vitro studies on the anti-cancer activity of andrographolide targeting survivin in human breast cancer stem cells. *PLoS One*. 2020;15(11):e0240020. doi:10.1371/journal.pone.0240020
14. Hanahan D. Hallmarks of cancer: New dimensions. *Cancer Discov*. 2022;12(1):31–46. doi:10.1158/2159-8290.CD-21-1059
15. Fulda S, Debatin KM. Extrinsic versus intrinsic apoptosis pathways in anticancer chemotherapy. *Oncogene*. 2006;25(34):4798–4811. doi:10.1038/sj.onc.1209608
16. Obeng E. Apoptosis (programmed cell death) and its signals: A review. *Braz J Biol*. 2021;81(4):1133–1143. doi:10.1590/1519-6984.228437
17. Fischer U, Jänicke RU, Schulze-Osthoff K. Many cuts to ruin: A comprehensive update of caspase substrates. *Cell Death Differ*. 2003;10(1):76–100. doi:10.1038/sj.cdd.4401160
18. Ola MS, Nawaz M, Ahsan H. Role of Bcl-2 family proteins and caspases in the regulation of apoptosis. *Mol Cell Biochem*. 2011;351(1–2):41–58. doi:10.1007/s11010-010-0709-x
19. Martinou JC, Youle RJ. Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Dev Cell*. 2011;21(1):92–101. doi:10.1016/j.devcel.2011.06.017
20. Badaboina S, Bai HW, Park CH, Jang DM, Choi BY, Chung BY. Molecular mechanism of apoptosis induction in skin cancer cells by the centipedegrass extract. *BMC Complement Altern Med*. 2013;13(1):350. doi:10.1186/1472-6882-13-350
21. Kishore TKK, Ganugula R, Gade DR, Reddy GB, Nagini S. Gedunin abrogates aldose reductase, PI3K/Akt/mToR, and NF-κB signaling pathways to inhibit angiogenesis in a hamster model of oral carcinogenesis. *Tumor Biol*. 2016;37(2):2083–2093. doi:10.1007/s13277-015-4003-0
22. Tanagala KKK, Baba AB, Kowshik J, Reddy GB, Nagini S. Gedunin, a neem limonoid in combination with epalrestat inhibits cancer hallmarks by attenuating aldose reductase-driven oncogenic signaling in SCC131 oral cancer cells. *Anticancer Agents Med Chem*. 2019;18(14):2042–2052. doi:10.2174/1871520618666180731093433
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1–2):55–63. doi:10.1016/0022-1759(83)90303-4
24. Kasibhatla S, Amarante-Mendes GP, Finucane D, Brunner T, Bossy-Wetzel E, Green DR. Acridine orange/ethidium bromide (AO/EB) staining to detect apoptosis. *Cold Spring Harb Protoc*. 2006;2006(3):pdb.prot4493. doi:10.1101/pdb.prot4493
25. Grimm D, Bauer J, Kossmehl P, et al. Simulated microgravity alters differentiation and increases apoptosis in human follicular thyroid carcinoma cells. *FASEB J*. 2002;16(6):604–606. doi:10.1096/fj.01-0673fje
26. Unuma K, Aki T, Funakoshi T, Hashimoto K, Uemura K. Extrusion of mitochondrial contents from lipopolysaccharide-stimulated cells: Involvement of autophagy. *Autophagy*. 2015;11(9):1520–1536. doi:10.1080/15548627.2015.1063765
27. Dehelean CA, Marcovici I, Soica C, et al. Plant-derived anticancer compounds as new perspectives in drug discovery and alternative therapy. *Molecules*. 2021;26(4):1109. doi:10.3390/molecules26041109
28. Boretti A. Natural products as cancer chemopreventive agents: Where we stand. *Nat Prod Commun*. 2022;17(12):1934578X2211445. doi:10.1177/1934578X221144579
29. Colotta F, Allavena P, Sica A, Garlanda C, Mantovani A. Cancer-related inflammation, the seventh hallmark of cancer: Links to genetic instability. *Carcinogenesis*. 2009;30(7):1073–1081. doi:10.1093/carcin/bgp127
30. Igney FH, Krammer PH. Death and anti-death: Tumour resistance to apoptosis. *Nat Rev Cancer*. 2002;2(4):277–288. doi:10.1038/nrc776
31. Stoletov K, Beatty PH, Lewis JD. Novel therapeutic targets for cancer metastasis. *Exp Rev Anticancer Ther*. 2020;20(2):97–109. doi:10.1080/14737140.2020.1718496
32. Patwardhan CA, Fauq A, Peterson LB, Miller C, Blagg BSJ, Chadli A. Gedunin inactivates the co-chaperone p23 protein causing cancer cell death by apoptosis. *J Biol Chem*. 2013;288(10):7313–7325. doi:10.1074/jbc.M112.427328
33. Brandt GEL, Schmidt MD, Prisinzano TE, Blagg BSJ. Gedunin, a novel Hsp90 inhibitor: Semisynthesis of derivatives and preliminary structure–activity relationships. *J Med Chem*. 2008;51(20):6495–6502. doi:10.1021/jm8007486
34. Lamb J, Crawford ED, Peck D, et al. The connectivity map: Using gene-expression signatures to connect small molecules, genes, and disease. *Science*. 2006;313(5795):1929–1935. doi:10.1126/science.1132939
35. Fitzgerald MC, O'Halloran PJ, Connolly NMC, Murphy BM. Targeting the apoptosis pathway to treat tumours of the paediatric nervous system. *Cell Death Dis*. 2022;13(5):460. doi:10.1038/s41419-022-04900-y
36. Barbieri F, Lorenzi P, Ragni N, et al. Overexpression of cyclin D1 is associated with poor survival in epithelial ovarian cancer. *Oncology*. 2004;66(4):310–315. doi:10.1159/000078332
37. Wang J, Su W, Zhang T, et al. Aberrant Cyclin D1 splicing in cancer: From molecular mechanism to therapeutic modulation. *Cell Death Dis*. 2023;14(4):244. doi:10.1038/s41419-023-05763-7
38. Chen S, Li L. Degradation strategy of cyclin D1 in cancer cells and the potential clinical application. *Front Oncol*. 2022;12:949688. doi:10.3389/fonc.2022.949688
39. Johnson J, Venugopal A, Kwatra D, Roby K, Godwin A, Anant S. Abstract 4553: Gedunin, a novel HSP-90 inhibitor, synergizes with cisplatin and paclitaxel to inhibit growth of chemoresistant ovarian cancer cell lines. *Cancer Res*. 2014;74(19 Suppl):4553–4553. doi:10.1158/1538-7445.AM2014-4553
40. Vitale I, Pietrocola F, Guilbaud E, et al. Apoptotic cell death in disease: Current understanding of the NCCD 2023. *Cell Death Differ*. 2023;30(5):1097–1154. doi:10.1038/s41418-023-01153-w
41. Yu H, Lin L, Zhang Z, Zhang H, Hu H. Targeting NF-κB pathway for the therapy of diseases: Mechanism and clinical study. *Sig Transduct Target Ther*. 2020;5(1):209. doi:10.1038/s41392-020-00312-6
42. García-Hernández L, García-Ortega MB, Ruiz-Alcalá G, Carrillo E, Marchal JA, García MÁ. The p38 MAPK components and modulators as biomarkers and molecular targets in cancer. *Int J Mol Sci*. 2021;23(1):370. doi:10.3390/ijms23010370
43. Pua LJW, Mai CW, Chung FFL, et al. Functional roles of JNK and p38 MAPK signaling in nasopharyngeal carcinoma. *Int J Mol Sci*. 2022;23(3):1108. doi:10.3390/ijms23031108

Evaluation of optical and mechanical properties of crown materials produced by 3D printing

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D – writing the article; E – critical revision of the article; F – final approval of the article

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Conflict of interest
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Abstract

Background. The various advantages of crown materials produced using three-dimensional (3D) printers have increased their use in restorative and prosthetic dentistry in recent years. Accordingly, their optical and mechanical properties have become more important.

Objectives. To evaluate the mechanical, surface and optical properties of crown materials produced with 3D printing and computer-aided design (CAD)/computer-aided manufacturing (CAM), which has recently been used frequently in the clinic.

Materials and methods. The 3-point bending test was used to evaluate the mechanical properties of 2 different crown materials produced with 3D printing (Permanent Crown and VarseoSmile Crown Plus) and a crown material produced using CAD/CAM (Vita Enamic). After the initial color and surface roughness measurements were made, the specimens were immersed in 4 different solutions.

Results. The most translucent material was VarseoSmile Crown Plus ($p < 0.05$). In all specimens, coffee caused the most discoloration ($p < 0.05$). The effects of the solutions on the roughness were mostly observed in Permanent Crown specimens ($p < 0.05$). Vita Enamic showed the highest statistically significant values in terms of flexural strength ($p < 0.05$).

Conclusions. The stereolithographic technique among the materials produced by 3D printing can be recommended for use in restorations due to its higher flexural strength.

Key words: roughness, 3-point bending test, color, CAD/CAM, 3-dimensional printing

Cite as
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Background

Currently, the use of computer-animated design (CAD)/computer-aided manufacturing (CAM) devices is becoming increasingly widespread clinically in dental technology. Computer-animated design/CAM blocks have revolutionized the construction of indirect restorations, resulting in industrially manufacturable, high-performance materials.[1] Initially, ceramics were the only material option for making a CAD/CAM restoration. However, today, in addition to various dental ceramics, temporary and permanent restorations can also be fabricated using resin composite materials with CAD/CAM technology.[2]

Resin composite block materials for dental CAD/CAM applications are produced by compressing and polymerizing a filler material and a monomer. The mechanical properties of the new resin composite blocks, such as flexural strength, have been improved compared to conventional resin composite blocks.[3]

Current CAD/CAM resin composite materials are available for subtractive manufacturing procedures with a CAD/CAM milling machine, mostly in the form of industrially homogeneously produced blocks. These blocks have been shown to have superior properties compared to direct resin composite materials. These materials are used for permanent single restorations. Resin composite-based CAD/CAM materials consist of a resin composite polymer matrix and embedded ceramic-based filler particles.[4] Computer-animated design/CAM technologies, as well as rapid prototyping techniques (additive manufacturing and 3-dimensional (3D) printing), have a wide range of applications in many areas of dentistry. One of the most frequently employed areas of these systems is prosthetic applications. Currently, 3D printers play an effective role in facilitating and shortening the challenging clinical phases in prosthodontic applications and constitute an important part of the digital workflow.[5] Unlike conventional manufacturing, 3D printers offer faster and more cost-effective production. 3D printers have been successful in the production of temporary crown bridge prostheses. However, recently, manufacturers have launched products produced with 3D printers as permanent crown material.

Physical and mechanical properties play a major role in the long-term clinical success of restorative materials used in dentistry. However, the aesthetic success of a restoration depends on its capacity to mimic the appearance of natural teeth – in other words, its optical properties. The color of natural teeth is due to a combination of the optical properties of enamel and dentin. Due to the complex optical properties of natural teeth affecting their color, such as light reflection, diffusion, absorption, and light transmission, it is very difficult to achieve aesthetic restorations. Many studies have examined the optical scattering properties of newly developed aesthetic materials, such as color, opalescence, hue angle, color saturation (chroma), surface gloss, and light transmittance.[6,7] However, the number of studies evaluating the color stability of 3D printer-fabricated restorations remains limited.

Objectives

There are only a few studies[8–10] that evaluate the mechanical and optical properties of newly released crown materials produced with 3D printers, and the crown material has been on the market for a long time. Expected results from this study will be used to compare the 3D printer production method with CAD/CAM production in terms of mechanical and optical properties. Moreover, to ensure that the clinical success of the restoration is increased, its life is extended and costs resulting from repetitive restorations are avoided, we will identify the material that has ideal properties according to the results of the study. This study aimed to evaluate the mechanical, surface and optical properties of crown materials produced using 3D printers, which have recently started to be used frequently in clinics due to their significant advantages, such as advanced chemical and mechanical properties and ease of application. The null hypothesis of the study was that staining solutions would not cause any change in any optical and surface properties of the materials and that there is no difference between the materials in terms of optical, surface and mechanical properties.

Materials and methods

Sample size calculations were performed using the package program G*Power (v. 3.1.9.6.; Franz Faul, Kiel University, Germany). Based on a 40% effect size, 80% power, 5% tolerance, and 25% possible data loss, each group comprised 14 specimens (n = 14).

This study evaluated the mechanical, surface and optical properties of 2 different crown materials produced with a 3D printer (Permanent Crown (PC) and VarseoSmile Crown Plus (VSCP)), and a crown material produced using CAD/CAM (Vita Enamic (VE)) (Table 1, Fig. 1,2).

Surface roughness and color stability tests

To evaluate the optical and surface properties of the materials, disc-shaped specimens (n = 14) with a diameter of 5 mm and a thickness of 2 mm were prepared from all 3 materials. Color stability measurements were performed using a spectrophotometer (Vita Easyshade V Spectrophotometer; Vita Zahnfabrik, Bad Sackingen, Germany) to evaluate the optical properties of the materials. To evaluate the surface roughness of the materials, a profilometer (Surftest SJ-301-Mitutoyo; Mitutoyo, Kawasaki, Japan) was used; an area of 100×100 μm^2 was determined and surface roughness values were measured

Table 1. Properties of the materials used in this study

Material	Contents	Translucency/ color	Manufacturer
Vita Enamic	hybrid ceramic porous structure – sintered ceramic matrix infiltrated with polymer material inorganic ceramic 86% by weight: Fine feldspar ceramic enriched with aluminum oxide (58–63% silicon dioxide, 20–23% aluminum oxide, 9–11% sodium oxide, 4–6% potassium oxide, 0.5–2% boron trioxide), zirconia <1%, calcium oxide <1% organic polymer 14% by weight (urethane dimethacrylate, triethylene glycol dimethacrylate)	T/A1	Vita Zahnfabrik, Bad Sackingen, Germany
Permanent Crown	polymethylmethacrylate esterification products of 4,4'-isopropylphenol, ethoxylated and 2-methylprop-2enoic acid, silanized dental glass, methyl benzoylformate, diphenyl (2,4,6 trimethyl benzoyl) phosphine oxide; 30–50% by weight of inorganic filler (particle size 0.7 µm).	T/A1	Formlabs, Somerville, USA
VarseoSmile Crown Plus	hybrid ceramic esterification products of 4,40 isopropylidiphenol, ethoxylated and 2-methylprop-2enoic acid, silanized dental glass, methyl benzoylformate, diphenyl (2,4,6 trimethyl-benzoyl) phosphine oxide; 30–50% total filler by weight.	T/A1	BEGO, Bremen, Germany

T – translucent.

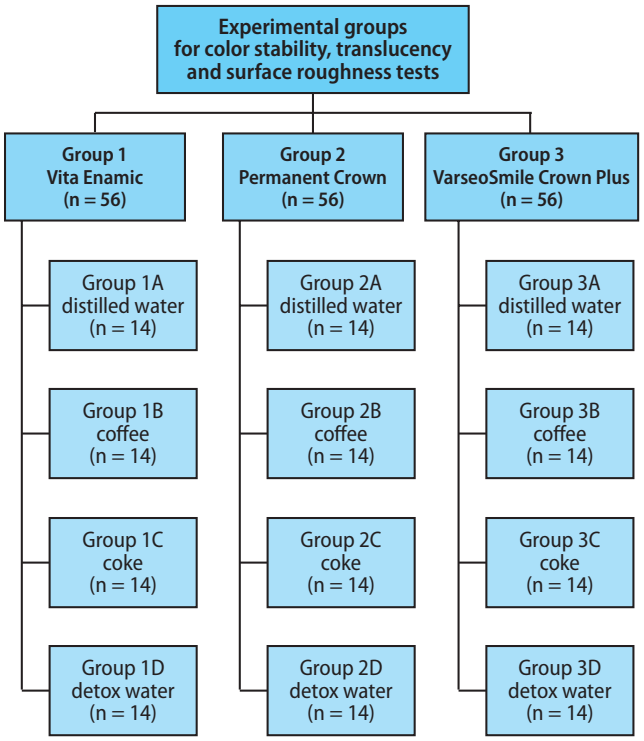

Fig. 1. Experimental groups for color stability, translucency, and surface roughness tests

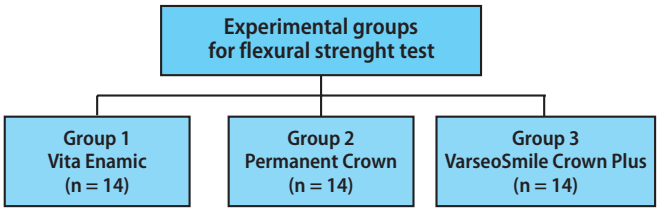

Fig. 2. Experimental groups for the flexural strength test

from 3 different planes. The mean of the measurement values obtained was recorded as the greatest surface roughness value of that specimen (ISO 4287).

After the initial color and surface roughness measurements, the specimens were kept in 4 different solutions (distilled water, coffee, coke, and detox water) for 15 min each day for a total of 14 days (Table 2). On the 7th day, color stability and surface roughness measurements were made and then placed in the solutions again. On the 14th day, the final color stability and surface roughness measurements were performed.

The color of the specimens was measured in a standard environment, under a standard light source, and on a white background with a spectrophotometer according

Table 2. Properties of the solutions used in the study

Solution	Manufacturer	Contents	Preparation	pH
Distilled water	–	–	–	6.74
Coffee	Nescafe Classic; Nestle, Vevey, Switzerland	instant coffee, sugar, flavoring, thickener, caffeine	2 g of instant coffee was dissolved in 200 mL of hot water	5.66
Coke	Coca-Cola, Atlanta, USA	water, sugar, caramel, phosphoric acid, natural sweeteners, caffeine	–	2.90
Detox water	Organik Smoothie Passion Red; Elite Naturel, Ankara, Turkey	organic watermelon juice (20%), organic strawberry puree (20%), organic banana puree (15%), organic apple puree (15%), organic pear puree (12%), organic black mulberry puree (12%), organic red beet juice (3%), organic black carrot juice (3%)	–	4.04

g – gram; mL – milliliter.

to the CIE-Lab scale, where L* represents the brightness of the material on a scale from 0 (black) to 100 (white); a* represents hue and chroma on the red–green axis; and b* represents hue and chroma on the yellow–blue axis. The spectrophotometer was properly calibrated before each measurement, following the manufacturer's instructions. The difference between 2 colored specimens or 2 time periods is represented as ΔE* (ISO/TR standard 28642:1999). L*, a* and b* values obtained from each specimen were recorded 24 h after specimen preparation, on day 7 and day 14. The color stability (ΔE) between day 7–24 h, day 14–24 h and day 7–day 14 for each specimen was calculated using the following equation:

$$\Delta E^* = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

$$\Delta E^* = [(L^*_1 - L^*_0)^2 + (a^*_1 - a^*_0)^2 + (b^*_1 - b^*_0)^2]^{1/2}$$

Three-point bending test

The study evaluated the mechanical properties, surface roughness, and optical properties of 2 different crown materials, PC (Formlabs, Somerville, USA) and VSCP (BEGO, Bremen, Germany) were produced with a 3D printer, and VE (Vita Zahnfabrik) was produced using CAD/CAM. The 3-point bending test was used to evaluate the mechanical properties of the materials. For this test, specimens 14 mm long, 4 mm wide and 1.2 mm thick were prepared. For each material group, 14 specimens were prepared (n = 14). CAD/CAM blocks were cut with a low-speed water-cooled diamond saw (Miracut 151; Metcon, Bursa, Turkey) to obtain bar-shaped specimens of these dimensions. Specimens were prepared with wet silicon carbide until the desired dimensions were achieved. Measurements were made using a micrometer (Mitutoyo Digimatic IP65; Mitutoyo). Specimens were stored dry at room temperature. Flexural strength values of the materials were measured using a universal testing machine (Shimadzu IG-IS; Shimadzu Corp., Kyoto, Japan) using a 3-point bending test with a support opening of 55 mm and a cross-speed of 1 mm/min (ISO 4049).

Flexural strength values of the specimens were calculated using the formula below:

$$\dot{e} = 3 \times F \times L/2b \times d^2$$

ė: Flexural strength of the material [kgf/cm^2]
F: Load causing breakage [kgf]
L: Distance of test specimen between supports [cm]
b: Width of test specimen [cm]
d: Thickness of the test specimen [cm]

Statistical analyses

Data analysis was performed using IBM SPSS v. 27.0 (IBM Corp., Armonk, USA) and was studied with a 95% confidence level. Frequency (n) and percentage (%) statistics are given for categorical (qualitative) variables, and mean (mean) and standard deviation (SD) statistics are given for numerical (quantitative) variables. Two-way and one-way analysis of variance (ANOVA) tests, which are parametric testing techniques, and Kruskal–Wallis and Mann–Whitney tests, which are non-parametric testing techniques, were used in the study. Additionally, Tukey's (homogeneous variance) and Games–Howell (inhomogeneous variance) tests were used for intragroup comparisons. Bonferroni correction (p/k = 4) was used when applying the Mann–Whitney test. The one-way ANOVA test is a testing technique used to compare k-independent groups (k > 2) in terms of a quantitative variable. Moreover, the one-way ANOVA test was used to compare variables with independent groups and their interaction in terms of a quantitative variable.

Results

Translucency

Material, solution and number of days were statistically significant in discoloration ($p < 0.05$). Each material showed a statistically significant change in translucency independent of solution type and holding time ($p < 0.05$). The most translucent material was determined as VSCP ($p < 0.05$). There was a statistically significant difference compared to other materials. Considering the duration, there was a difference between the initial translucency values and the 14th-day translucency values, but this value was not statistically significant ($p > 0.05$) (Table 3).

Color stability (ΔE)

Material, solution and time had a statistically significant effect on discoloration in all specimens ($p < 0.05$). When the solutions were compared with each other, the amount of discoloration was different between the solutions ($p < 0.05$). Coke and detox water were not statistically different from each other ($p > 0.05$), while the other solutions showed significant differences when compared with each other ($p < 0.05$). Coffee caused the most discoloration in all specimens ($p < 0.05$). Time also had a statistically significant effect on discoloration ($p < 0.05$) (Table 4).

Surface roughness

The effect of solutions on roughness was observed mostly on PC specimens. Specimens kept in detox water and coke showed higher roughness compared to the initial value ($p < 0.05$). The roughness values of 3 different specimen groups were statistically different from each other ($p < 0.05$). Vita Enamic specimens showed the least amount of roughness ($p < 0.05$) (Table 5).

Table 3. Means and standard deviations (SDs) of the translucency parameter (TP) of the experimental groups

Measurement periods by groups	Distilled water	Coffee	Coke	Detox water
VE-initially	5.45 ±1.05	5.27 ±1.77	5.72 ±0.99	5.7 ±2.10
PC-initially	9.44 ±2.44	10.09 ±2.74	7.85 ±2.81	8.6 ±3.16
VSCP-initially	17.25 ±3.47	18.18 ±5.10	17.74 ±5.23	15.29 ±4.04
VE-7th day	5.21 ±0.84	4.42 ±1.05	6.17 ±1.57	5.8 ±1.38
PC-7th day	9.46 ±2.34	6.66 ±2.60	9.73 ±2.43	7.5 ±2.09
VSCP-7th day	16.58 ±3.54	15.09 ±3.52	17.56 ±3.19	15.17 ±2.98
VE-14th day	4.77 ±0.65	4.56 ±1.12	5.25 ±1.04	5.59 ±1.03
PC-14th day	8.17 ±1.79	6.66 ±0.84	8.13 ±1.74	8.38 ±1.41
VSCP-14th day	14.82 ±4.50	13.86 ±2.83	15.85 ±4.64	13.48 ±3.12

VE – Vita Enamic; PC – Permanent Crown; VSCP – VarseoSmile Crown Plus.

Table 4. ΔE means and standard deviations (SDs) of the experimental groups

Measurement periods by groups	Distilled water	Coffee	Coke	Detox water
VE-7th day	0.74 ±0.28	6.69 ±0.69	3.84 ±0.40	3.74 ±0.42
PC-7th day	1.18 ±0.33	10.9 ±1.15	5.21 ±0.45	5.08 ±0.43
VSCP-7th day	1.17 ±0.31	15.61 ±2.10	8.01 ±0.62	7.86 ±0.93
VE-14th day	1.08 ±0.30	7.86 ±0.55	4.52 ±0.54	4.37 ±0.61
PC-14th day	1.66 ±0.35	13.91 ±1.71	7.23 ±0.48	6.99 ±0.37
VSCP-14th day	1.85 ±0.32	19.54 ±1.30	10.6 ±1.31	9.73 ±1.12

VE – Vita Enamic; PC – Permanent Crown; VSCP – VarseoSmile Crown Plus.

Table 5. Surface roughness means and standard deviations (SDs) of the experimental groups

Measurement periods by groups	Distilled water	Coffee	Coke	Detox water
VE-initially	0.19 ±0.01	0.19 ±0.01	0.18 ±0.03	0.19 ±0.02
PC-initially	0.17 ±0.08	0.16 ±0.07	0.17 ±0.06	0.17 ±0.03
VSCP-initially	0.33 ±0.20	0.33 ±0.1	0.33 ±0.14	0.33 ±0.18
VE-7th day	0.21 ±0.03	0.22 ±0.04	0.21 ±0.03	0.21 ±0.03
PC-7th day	0.20 ±0.08	0.19 ±0.09	0.30 ±0.11	0.26 ±0.06
VSCP-7th day	0.34 ±0.11	0.33 ±0.11	0.39 ±0.19	0.30 ±0.13
VE-14th day	0.23 ±0.06	0.23 ±0.05	0.25 ±0.05	0.23 ±0.04
PC-14th day	0.20 ±0.09	0.18 ±0.08	0.30 ±0.16	0.26 ±0.09
VSCP-14th day	0.35 ±0.11	0.35 ±0.10	0.35 ±0.11	0.38 ±0.18

VE – Vita Enamic; PC – Permanent Crown; VSCP – VarseoSmile Crown Plus.

Table 6. Flexural strength means and standard deviations (SDs) of experimental groups

Measurement periods by groups	VE	PC	VSCP
Flexural strength	386.20 ±50.55	220.20 ±38.28	39.60 ±4.00

VE – Vita Enamic; PC – Permanent Crown; VSCP – VarseoSmile Crown Plus.

Flexural strength

A statistically significant difference was found between the groups in the 3-point bending test ($p < 0.05$). Vita Enamic was the material that showed the highest statistically significant values in terms of flexural strength among the materials ($p < 0.05$), whereas VSCP exhibited the lowest statistically significant values ($p < 0.05$). The flexural strength values of the PC material were statistically significantly higher than the VSCP material ($p < 0.05$) (Table 6).

Discussion

Resin-ceramic hybrid materials are materials whose physical properties are very close to those of natural teeth,

show less wear than composite resin and cause less wear to the antagonist's tooth. However, they are more prone to discoloration, which limits the longevity and quality of aesthetic restorations.[11] In this study, the effect of exposing hybrid ceramic materials produced by different methods to different staining solutions on the optical and surface properties of the materials was evaluated. The flexural strength of the materials was also evaluated. Coffee, coke and detox water were chosen as staining solutions due to their frequent consumption. The specimens in the control group were kept in distilled water. According to coffee producers, it takes an average of 15 min to consume a cup of coffee.[12] Considering this time, the materials were kept in the solutions for 15 min every day for a total of 14 days.

Considering the data obtained in the case of the study, staining solutions were observed to cause changes in the optical and surface properties of the materials. However, there was a statistically significant difference between the materials in terms of optical, surface and mechanical properties, and the null hypothesis was rejected. According to our results, the most translucent material was determined to be VSCP in all experimental groups. The light transmittance of resin-containing materials is related to multiple refractions and reflections at the matrix/filler interface, which is affected by the difference in refractive index between the filler particles and the matrix.[13] The VSCP we used in our study has a low filler ratio and was the material with the highest translucency. This may be explained by the view that when a material has a low filler content, light penetration will be easier.[14]

When the discoloration values were examined, coffee caused the most discoloration in all specimens. No difference was observed between coke and detox water in terms of discoloration values. Intralavan et al. evaluated the discolorations of VE and VSCP materials after exposure to distilled water, coffee and coke. In their study, coffee also caused the most discoloration in the materials.[11] Alsilani et al. evaluated the effect of coffee and coke on the color stability and surface roughness of 3 different CAD/CAM materials, including VE. In their study, coffee caused high discoloration in the VE material.[15] Sarıkaya et al. compared the color stability of 2 different hybrid ceramics, including VE, after being immersed in coffee and energy drinks, and found that coffee caused the most discoloration in the materials.[16] Abouraya et al. evaluated the effect of exposure of 3 aesthetic monolithic block materials, including VE, to coffee, coke and distilled water on the discoloration of the materials. Vita Enamic material exhibited the highest discoloration values after being kept in coffee.[17] Our results are consistent with these studies. In this study, we aimed to evaluate the mechanical and optical effects that alcohol-free beverages can have on materials. In addition, we aimed to assess the effects of detox water, which has begun to be consumed frequently due to the increasing interest of individuals in healthy nutrition, on the mechanical and optical properties of materials.

This absorption and penetration of colorants into the organic phase of the materials is likely due to the compatibility of the yellow colorants of coffee and the polymer phase.[18] In this study, the specimens immersed in detox water and coke exhibited higher roughness compared to the initial value. Chowdhury et al. studied the effect of exposure of a nanohybrid composite resin to tea, coffee and coke on the surface roughness and color stability of the material, and reported the highest surface roughness values were for specimens immersed in coke.[19] Meenakshi et al. evaluated the effect of 3 different solutions (artificial saliva, orange juice and coke) on the surface roughness and color stability of 2 composite resins. The highest surface roughness values were observed in coke groups.[20] Escamilla-Gómez et al. evaluated the relationship between the surface degradation of composite resins immersed in different acidic solutions and *Streptococcus mutans* biofilm formation. Surface degradation of composite resins was found to be related to the pH of the solution and *S. mutans* biofilm formation was associated with an increased surface roughness of composite resins.[21] Elwardani et al. evaluated the effect of exposure of 2 different composite resins to different solutions (coke, orange juice and distilled water) on the surface roughness and discoloration of the materials. The groups showing the highest surface roughness were coke groups.[22] The surface properties of the composite resin, especially microhardness and roughness, can be greatly influenced by the overall chemical composition of beverages, the type of acid present in their formulation, as well as the strength of the individual acidic components.[23] Furthermore, composite resin materials tend to wear under ascending conditions.[24] Researchers have noted that low-pH foods and beverages with acidic properties cause erosive wear of dental restoration materials. High acidity can have a greater softening effect on the resin matrix, thereby promoting the dislodging of filler particles and thus increasing the surface roughness of the composite resin.[19] In this study, the solutions with the lowest pH were coke and detox water. The reason why these 2 solutions caused the highest surface roughness in the materials may be because these solutions have lower pH values than coffee and distilled water. However, it has been reported that the larger the filler size, the greater the surface roughness.[25] Therefore, the lowest surface roughness values observed in VE groups in this study may be due to the smaller size of the fillers in this material compared to the other 2 materials.

Dayan and Çelik Güven evaluated the flexural strength and modulus of elasticity of 5 different CAD/CAM blocks, including VE. They reported that the ceramic-containing material, IPS e.max®CAD, had the highest flexural strength and modulus of elasticity values. Although the VE material contained 86% feldspathic ceramics, contrary to expectations, it exhibited lower flexural strength than resin nanoceramics. The researchers explained that this is because the ceramic part of the VE material contains porous feldspathic porcelain, unlike other hybrid materials.[26]

This study aimed to compare this disadvantage, which may affect the flexural strength of VE, with hybrid ceramic materials produced with 3D printers.

In this study, crown materials produced with 3D printing showed lower flexural strength values compared to the VE material produced using CAD/CAM. Digholkar et al. evaluated the flexural strength and microhardness of temporary restorative materials produced with 3D printing, CAD/CAM technology and traditional methods. They reported that the material produced using CAD/CAM showed the highest flexural strength values. This result is consistent with our study. The researchers reported that the reason why the material produced with CAD/CAM showed the highest flexural strength values may be due to the provision of optimum curing conditions.[27] Researchers have noted that the nature of the incremental layers in additive manufacturing technology can initiate crack propagation and cause structural failure of the material. The bond between layers is weaker than the bond within the layer itself. This is explained by the number of residual stresses and pores that accumulate during ultraviolet polymerization application and material shrinkage.[28] Park et al. evaluated the flexural strength of resin materials produced with 3D printing and found that the material produced with the stereolithography (SLA) technique exhibited higher flexural strength values. The researchers stated that the reason for the higher flexural strength of the material produced with the SLA technique is the surface morphology of the printed material. The surface of the material has a smoother structure as each layer is completed as if it is drawn with a laser beam during production with the SLA technique. In an area where the bond between layers is weak, if the surface is rough, fracture occurs faster. For this reason, the smoother surface of the material is effective in high flexural strength values.[29] The PC material produced with the 3D printer examined in this study was produced using the SLA technique. Because the flexural strength values of this material were higher than the VSCP material, one can conclude that the production of this material using SLA may be the reason.

Limitations

This study has some limitations due to the nature of in vitro research. While restorative materials are exposed to staining agents from food and drink in the oral cavity, they are constantly rinsed with saliva and brushed with oral hygiene techniques. These factors may affect the coloration of the materials or the roughness that may occur on the surface. Although this study does not include these procedures, it is among the limited number of studies evaluating the optical and mechanical properties of crown materials produced with 3D printers.

Conclusions

Since coffee causes the most color change in all materials, it can be suggested that patients with this type of restoration should be careful in terms of coffee consumption. Since VE has low roughness values, it can be recommended for dental restorations because it is advantageous in terms of fewer problems such as discoloration and plaque accumulation in the selection of restorative materials. It can be concluded that materials produced using the SLA technique among the materials produced with 3D printing can be recommended for restorations due to their higher flexural strength. Further studies are needed to evaluate the clinical success of the materials examined in this study.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.11922634. The package includes the following files:

Supplementary Fig. 1. Experimental groups as tested for color stability, translucency and surface roughness tests.

Supplementary Fig. 2. Experimental groups as tested for flexural strength test.

Supplementary File 1. Statistical analysis report.

Supplementary Table 1. Properties of the materials used in this study.

Supplementary Table 2. Properties of the solutions used in the study.

Supplementary Table 3. Means and SDs of the TP of the experimental groups.

Supplementary Table 4. ΔE means and SDs of the experimental groups.

Supplementary Table 5. Surface roughness means and SDs of the experimental groups.

Supplementary Table 6. Flexural strength means and SDs of the experimental groups.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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References

1. Erzincanlı A, Özkurt Kayahan Z, Kazazoğlu E. Hybrid ceramics for prosthetic treatment [in Turkish]. İn: Özdemir AK, ed. *Prosthetic Materials and Current Applications*. Ankara, Turkey: Türkiye Klinikleri; 2020:17–22. https://www.turkiyeklinikleri.com/article/en-protetik-tedavide-hibrit-seramikler-91041.html. Accessed December 15, 2023.
2. Pfeffer S, Jacker-Guhr S, Geurtsen W, Pfeffer TA, Lührs A. Microtensile bond strength of luting cements to a 3D printable composite: An in vitro study. *Sust Dent*. 2020;2:229–239. https://www.online-dzz.com/fileadmin/user_upload/OA_Microtensile_bond_strength.pdf. Accessed December 15, 2023.
3. Miura S, Fujisawa M. Current status and perspective of CAD/CAM-produced resin composite crowns: A review of clinical effectiveness. *Jpn Dent Sci Rev*. 2020;56(1):184–189. doi:10.1016/j.jdsr.2020.10.002
4. Zimmermann M, Ender A, Egli G, Özcan M, Mehl A. Fracture load of CAD/CAM-fabricated and 3D-printed composite crowns as a function of material thickness. *Clin Oral Invest*. 2019;23(6):2777–2784. doi:10.1007/s00784-018-2717-2
5. Murat S, Batak B. Use of three-dimensional printers in prosthetic applications [In Turkish]. İn: *From Three-Dimensional Imaging to Three-Dimensional Printing in Dentomaxillofacial Radiology*. Ankara, Turkey: Türkiye Klinikleri; 2022:37–45. https://www.turkiyeklinikleri.com/article/tr-uc-boyutlu-yazicilarin-protetik-uygulamalarda-kullanimi-99347.html. Accessed December 15, 2023.
6. Yilmaz S, Egïlmez F. Optical properties of polymer-based CAD/CAM materials [in Turkish]. *Selcuk Dent J*. 2021;8(2):580–590. doi:10.15311/selcukdentj.676441
7. Kul E, Abdulrahim R, Bayındır F, Matori KA, Gül P. Evaluation of the color stability of temporary materials produced with CAD/CAM. *Dent Med Probl*. 2021;58(2):187–191. doi:10.17219/dmp/126745
8. Paradowska-Stolarz A, Wieckiewicz M, Kozakiewicz M, Jurczyszyn K. Mechanical properties, fractal dimension, and texture analysis of selected 3D-printed resins used in dentistry that underwent the compression test. *Polymers (Basel)*. 2023;15(7):1772. doi:10.3390/polym15071772
9. Paradowska-Stolarz A, Malysa A, Mikulewicz M. Comparison of the compression and tensile modulus of two chosen resins used in dentistry for 3D printing. *Materials (Basel)*. 2022;15(24):8956. doi:10.3390/ma15248956
10. Paradowska-Stolarz AM, Wieckiewicz M, Mikulewicz M, et al. Comparison of the tensile modulus of three 3D-printable materials used in dentistry. *Dent Med Probl*. 2023;60(3):505–511. doi:10.17219/dmp/166070
11. Intralawan N, Wasanapiarnpong T, Didron PP, Rakmanee T, Klaisiri A, Krajangta N. Surface discoloration of 3d printed resin-ceramic hybrid materials against various stain beverages. *J Int Dent Med Res*. 2022;15(4):1465–1471. https://www.jidmr.com/journal/wp-content/uploads/2022/12/9-D22_1915_Nantawan_Krajangta_Thailand.pdf. Accessed December 15, 2023.
12. Guler AU, Yilmaz F, Kulunk T, Guler E, Kurt S. Effects of different drinks on stainability of resin composite provisional restorative materials. *J Prosthet Dent*. 2005;94(2):118–124. doi:10.1016/j.prosdent.2005.05.004
13. Güth JF, Zuch T, Zwinge S, Engels J, Stimmelmayr M, Edelhoff D. Optical properties of manually and CAD/CAM-fabricated polymers. *Dent Mater J*. 2013;32(6):865–871. doi:10.4012/dmj.2013-099
14. Cekic-Nagas I, Ergun Vallittu PGK, Lassila VJL. Influence of polymerization mode on degree of conversion and micropush-out bond strength of resin core systems using different adhesive systems. *Dent Mater J*. 2008;27(3):376–385. doi:10.4012/dmj.27.376
15. Alsilani RS, Sherif RM, Elkhodary NA. Evaluation of colour stability and surface roughness of three CAD/CAM materials (IPS e.max, Vita Enamic, and PEEK) after immersion in two beverage solutions: An in vitro study. *Int J Appl Dent Sci*. 2022;8(1):439–449. doi:10.22271/oral.2022.v8.i1g.1460
16. Sarıkaya I, Dilli H. The effects of coffee and energy drink on color stability of hybrid ceramics. *Eur Ann Dent Sci*. 2021;48(1):18–22. doi:10.52037/eads.2021.0006
17. Abouraya N, Sabry H. Color stability of three esthetic monolithic block materials after immersion in commonly consumed beverages. *Egypt Dent J*. 2022;68(4):3761–3770. doi:10.21608/edj.2022.161015.2244
18. Alharbi A, Ardu S, Bortolotto T, Krejci I. Stain susceptibility of composite and ceramic CAD/CAM blocks versus direct resin composites with different resinous matrices. *Odontology*. 2017;105(2):162–169. doi:10.1007/s10266-016-0258-1
19. Chowdhury D, Mazumdar P, Desai P, Datta P. Comparative evaluation of surface roughness and color stability of nanohybrid composite resin after periodic exposure to tea, coffee, and coca-cola: An in vitro profilometric and image analysis study. *J Conserv Dent*. 2020;23(4):395. doi:10.4103/JCD.JCD_401_20
20. Meenakshi C, Sirisha K. Surface quality and color stability of posterior composites in acidic beverages. *J Conserv Dent*. 2020;23(1):57. doi:10.4103/JCD.JCD_291_19
21. Escamilla-Gómez G, Sánchez-Vargas O, Escobar-García DM, et al. Surface degradation and biofilm formation on hybrid and nanohybrid composites after immersion in different liquids. *J Oral Sci*. 2022;64(4):263–270. doi:10.2334/josnusd.22-0085
22. Elwardani G, Sharaf AA, Mahmoud A. Evaluation of colour change and surface roughness of two resin-based composites when exposed to beverages commonly used by children: An in-vitro study. *Eur Arch Paediatr Dent*. 2019;20(3):267–276. doi:10.1007/s40368-018-0393-1
23. Reddy PS, Tejaswi KS, Shetty S, Annapoorna B, Pujari SC, Thippeswamy H. Effects of commonly consumed beverages on surface roughness and color stability of the nano, microhybrid and hybrid composite resins: An in vitro study. *J Contemp Dent Pract*. 2013;14(4):718–723. doi:10.5005/jp-journals-10024-1390
24. Ozdemir B, Ilday NO, Ozdemir SB, Suleyman F, Duymus ZY. The effect of erosive beverages and polishing systems on the surface properties of nanohybrid composite resin. *Niger J Clin Pract*. 2022;25(3):248. doi:10.4103/njcp.njcp_1361_21
25. Yikilgan İ, Akgul S, Hazar A, Kedıcı Alp C, Baglar S, Bala O. The effects of fresh detox juices on color stability and roughness of resin-based composites. *J Prosthodont*. 2019;28(1):e82–e88. doi:10.1111/jopr.12759
26. Dayan SÇ, Çelïk Güken M. Evaluation of flexural strength and elasticity nodules of different CAD/CAM blocks [in Turkish]. *Turkiye Klinikleri J Dental Sci*. 2020;26(1):36–42. doi:10.5336/dentalsci.2019-65318
27. Digholkar S, Madhav VNV, Palaskar J. Evaluation of the flexural strength and microhardness of provisional crown and bridge materials fabricated by different methods. *J Indian Prosthodont Soc*. 2016;16(4):328. doi:10.4103/0972-4052.191288
28. Alharbi N, Osman R, Wismeijer D. Effects of build direction on the mechanical properties of 3D-printed complete coverage interim dental restorations. *J Prosthet Dent*. 2016;115(6):760–767. doi:10.1016/j.prosdent.2015.12.002
29. Park SM, Park JM, Kim SK, Heo SJ, Koak JY. Flexural strength of 3D-printing resin materials for provisional fixed dental prostheses. *Materials (Basel)*. 2020;13(18):3970. doi:10.3390/ma13183970

Study on the role of postoperative rehabilitation based on the ERAS concept for patients undergoing pancreaticoduodenectomy: Protocol for a randomized controlled clinical trial

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Conflict of interest
None declared

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Abstract

Background. Pancreaticoduodenectomy (PD), an abdominal surgery, is known for its complexity, cost and inherent risks. Recently, there has been increasing interest in enhanced recovery after surgery (ERAS) as a therapeutic approach. However, the mechanisms underlying postoperative functional recovery remain uncertain, and there are limited data on the efficacy of ERAS in postoperative physiotherapy following complex PD.

Objectives. This study aims to examine the feasibility and effectiveness of conducting a large powered randomized controlled trial (RCT) to evaluate a 2-week postoperative rehabilitation program based on the ERAS concept for patients undergoing pancreaticoduodenectomy.

Materials and methods. This study is a RCT with a single treatment group. From June 2022 to June 2024, 28 PD patients will participate in the trial. Patients will be randomly assigned to either a control group receiving standard clinical care or an intervention group undergoing a 2-week postoperative rehabilitation program. Cardiopulmonary function will be assessed using the 6-minute walk test (6MWT), and gastrointestinal (GI) recovery will be evaluated using the Intake, Feeling nausea, Emesis, physical Exam, and Duration of symptoms (I-FEED) scoring system.

Results. Secondary outcomes, including changes in recovery quality post-surgery, will be evaluated using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC-QLQ-C30) and the Quality of Recovery Questionnaire (QOR-40). Additional recorded items will include time to first flatus and feces, daily volume of stomach fluid, time to gastric tube removal, length of hospital stay (LOS), and postoperative complications.

Conclusions. The study will utilize the I-FEED score, a novel tool for assessing GI function, to monitor the impact of a 2-week postoperative rehabilitation exercise program on patients. The primary outcome will focus on improvements in cardiopulmonary capacity following postoperative rehabilitation activities.

Key words: quality of life, pancreaticoduodenectomy, postoperative rehabilitation, gastrointestinal function

Introduction

Pancreaticoduodenectomy (PD) is necessary for treating diseases of the pancreatic head, jugular abdomen, duodenum, and distal bile duct. It is a challenging abdominal surgical procedure with high operational costs and risks.[1] The PD involves significant surgical trauma and multiple gastrointestinal (GI) anastomoses, which can cause considerable surgical stress to patients, thereby increasing the risk of postoperative complications in 30–60% of cases. These complications include pancreatic leak, bile leak, postoperative bleeding, and delayed gastric emptying.[2] Delayed gastric emptying has an incidence rate as high as 44%; although it is not life-threatening, it can impact the patient's postoperative nutritional status and lead to postoperative malnutrition.[3] This effect prolongs postoperative bed rest and contributes to issues such as pulmonary infection, reduced physical fitness and decreased quality of life (QoL). These factors result in longer hospital stays and increased medical costs.[4,5] Therefore, multidisciplinary management during the perioperative period is crucial.[6]

Enhanced recovery after surgery (ERAS) has gained increasing attention in clinical settings in recent years. This concept refers to a series of perioperative management measures within a multidisciplinary collaborative treatment model aimed at promoting early patient recovery, reducing traumatic stress responses, shortening hospital stays, and reducing medical costs.[7] Although ERAS protocols are mainly surgeon- or technique-based, many optimal clinical management measures adopted during the perioperative period align with the principles of prevention and early rehabilitation advocated by rehabilitation medicine. Surgical interventions impose significant stress on patients, particularly those undergoing PD, who are often elderly and have multiple comorbidities. These factors can significantly reduce their functional ability. Additionally, following the operation, patients may encounter various emergent complications that lead to multisystem dysfunctions such as GI, motor and cardiopulmonary dysfunction.[8] Therefore, it is essential to achieve the minimum functional level necessary for discharge and subsequent independent living in the community or at home, which encompasses all biological and cognitive functions. Rehabilitation interventions play a decisive role in attaining this goal.[9] Therefore, postoperative rehabilitation for these patients is crucial.

Early postoperative activity is an integral part of ERAS. Scholars advocate that patients should mobilize within 24 h after surgery to accelerate postoperative recovery, in conjunction with other optimal clinical measures. Early postoperative bed activities can help patients maintain normal muscle tone, alleviate postoperative pain, promote GI tract recovery, enhance overall metabolism and blood circulation, and expedite the resumption of daily activities.[10,11] Research by Na et al.[12] showed that early moderate exercise positively affects natural killer cell function in vitro following radical surgery in gastric cancer patients. Additionally, a short-term prospective randomized controlled trial (RCT) by Allgayer et al.[13] indicated that a short-term moderate exercise program reduces oxidative DNA damage and boosts the immune system. These findings suggest that early rehabilitation programs following abdominal surgery are safe and feasible interventions that can mitigate potential infectious complications. In an RCT involving patients with stage I–III colon cancer, Ahn et al. compared postoperative exercise with standard medical care, revealing that low- to moderate-intensity exercise can shorten hospital stay and improve bowel motility following colectomy.[14] This finding shows the importance of postoperative rehabilitation exercise programs in improving functional recovery and reducing hospitalization duration. However, there is limited supporting evidence regarding the application of these interventions in the context of complex PD. Therefore, further investigation based on the ERAS concept is required into the effects of postoperative rehabilitation on GI function, cardiopulmonary function and quality of recovery in PD patients. The results of such studies are expected to inform clinical practice, potentially accelerating postoperative rehabilitation, shortening hospital stay, alleviating the burden of high medical costs, and facilitating an early return to family and community life.

Objectives

This study aims to examine the feasibility and effectiveness of conducting a large, powered RCT to determine the role of a 2-week postoperative rehabilitation program based on the ERAS concept for patients undergoing PD.

Materials and methods

Design

This study is a RCT with a single treatment group, which will enroll a total of 28 PD patients between June 2022 and June 2024. Participants will be randomly assigned to either a control group receiving standard clinical care or a trial group participating in a 2-week postoperative rehabilitation program. Cardiopulmonary function will be assessed using the 6-minute walk test (6MWT) at preoperative day 0 (T0), postoperative day 7 (T1), postoperative day 14 (T2), and postoperative month 1 (T3) in both groups. The primary outcome measure for both groups will be GI recovery at 2 weeks postoperative (T0–T2), measured using the Intake, Feeling nausea, Emesis, physical Exam, and Duration of symptoms (I-FEED) symptom score. Quality of recovery from T0 to T3 will be compared between the 2 groups using secondary outcomes measured with QOR-40 and EORTC-QLQ-C30. Secondary

outcomes, including time to first flatus and defecation, daily volume of stomach fluid, time to gastric tube removal, length of hospital stay (LOS), and postoperative complications, will be measured 1 and 14 days post-surgery (Tm).

The conceptual flowchart of the study is shown in Fig. 1. The study design conforms to the The SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) checklist and SPIRIT diagram (Fig. 2).

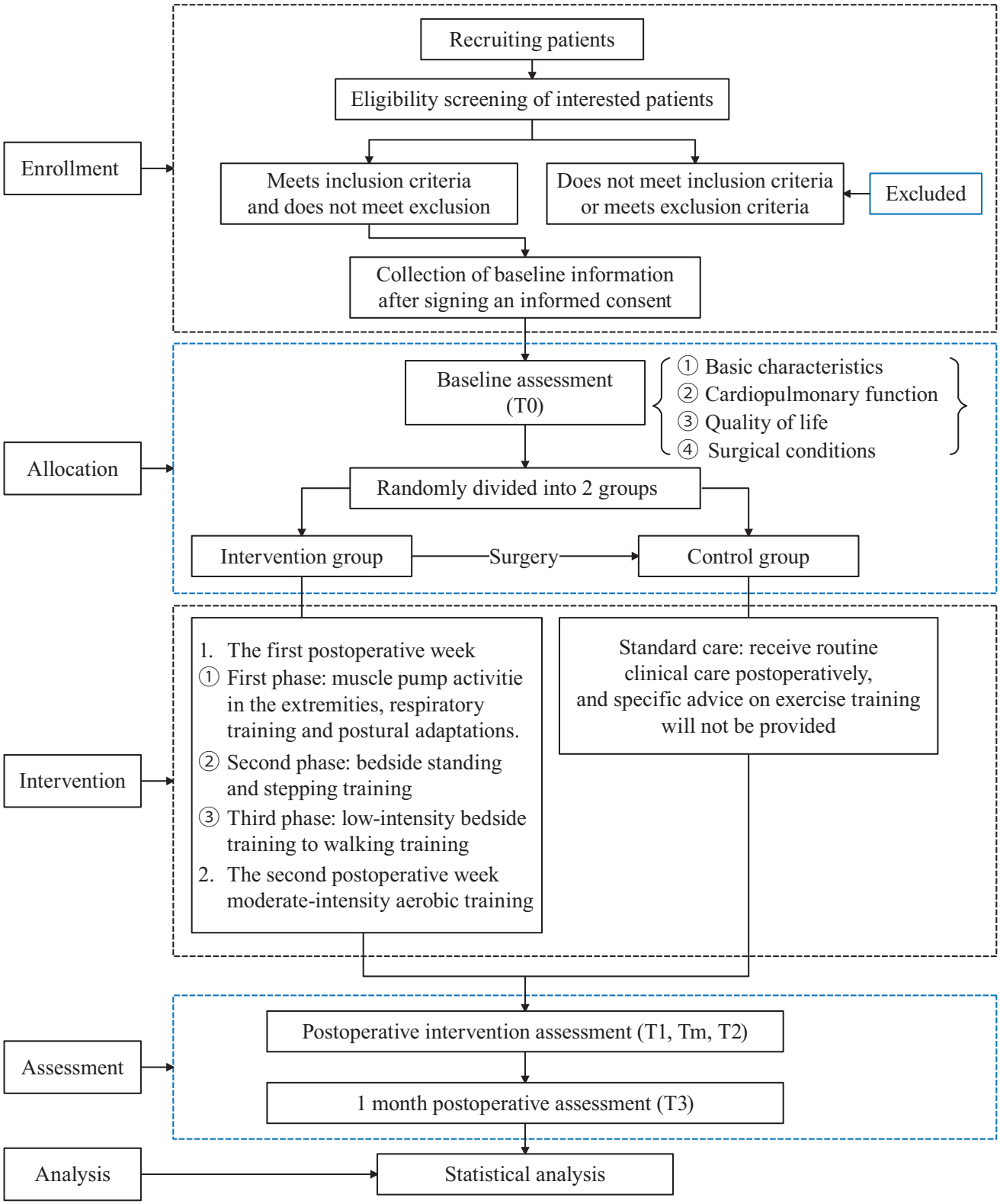

Fig. 1. Flow diagram of the study design

T0 – baseline (1 day preoperatively); T1 – 1 week postoperatively; Tm – sometime between 1 and 14 days postoperatively; T2 – 2 weeks postoperatively; T3 – 1 month postoperatively.

	Study period					
	Enrollment and allocation		Post-operative period			
Time point	**Pre-T_0**	**T_0**	**T_1**	**T_m**	**T_2**	**T_3**
Enrollment						
Eligibility screening	●					
Informed consent	●					
Allocation		●				
Interventions						
Intervention group (postoperative rehabilitation			●	—	●	
Control group (usual clinical care group)			●	—	●	
Assessments						
Basic characteristics*		●				
Primary outcomes						
I-FEED score			●		●	
6WMT		●	●		●	●
Secondary outcomes						
Quality of postoperative recovery (QoR-40)		●	●		●	●
Quality of life (EORTC QLQ-C30)		●	●		●	●
First exhaust time				○		
Time of the first defecation				○		
Daily gastric fluid volume			●	—	●	
Time of gastric tube removal				○		

Fig. 2. SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) checklist shows the time points for enrollment, interventions and assessment

* – basic characteristics include demographic information collection and baseline perioperative details such as duration of surgery, surgical approach and intraoperative bleeding; ● – fixed assessment time point; ○ – potential assessment time point; I-FEED – Intake, Feeling nauseated, Emesis, physical Exam, and Duration of symptoms scoring; QOR-40 – Quality of Recovery Score-40; EORTC QLQ-C30 – European Organization for Research and Treatment of Cancer Quality of Life Questionnaire Core 30; T0 – baseline (1 day preoperatively); T1 – 1 week postoperatively; Tm – sometime between 1 and 14 days postoperatively; T2 – 2 weeks postoperatively; T3 – 1 month postoperatively.

Study population and recruitment

Commencing in June 2022, subjects will be recruited from the Department of Hepatobiliary and Pancreatic Surgery at the Second Affiliated Hospital of Hainan Medical College (Haikou, China). Initially, the project manager will brief the team on the study's goals and eligibility requirements. Then, patients scheduled for PD due to hepatobiliary and pancreatic tumors will receive information about the study's goals, methods, potential benefits, and risks. Individuals interested in participating in the current research will undergo an interview with recruiting investigators to verify their eligibility. Finally, eligible participants or their guardians will sign an informed consent form to ensure confidentiality.

No patients or members of the public have been involved in the design, conduct, reporting, or dissemination plans of this research. Ethical approval has been obtained from the Ethics Committee of the Second Affiliated Hospital of Hainan Medical College (approval No. LW2022040) and is registered on ClinicalTrials.gov (No. ChiCTR2200060468). Written informed consent will be obtained from each participant before performing any procedures. The results of the study will be published in a peer-reviewed journal. This trial is currently in the recruitment phase.

Randomization, allocation and blinding

Eligible subjects will be randomly assigned to either the postoperative quantitative rehabilitation protocol or the control group at a 1:1 ratio based on their inclusion numbers using the PROC PLAN function of SAS v. 9.1 statistical software (SAS Institute, Cary, USA). The principal investigator will maintain confidentiality by placing random numbers inside opaque envelopes to determine the sequence of allocations. The allocation will remain undisclosed to the data analyst and appraiser; if they do become aware, they will be replaced. Participants will be instructed to keep their assigned interventions confidential from the evaluator.

Sample size

According to literature and previous studies such as "Changes in motor function and quality of life after

surgery in patients with pancreatic cancer", the mean 6MWT distance after treatment is 498 m for the rehabilitation program group and 403 m for the control group, with a standard deviation (SD) of 80 m.[15] A two-sided test with equal sample sizes (κ = 1), a type I error probability (α) of 0.05 and a type II error probability (β) of 0.2 will be used for both groups. The sample size was calculated using the following equation (Equation 1):

$$n_B = \left(1 + \frac{1}{\kappa}\right)\left(\sigma \frac{Z_{1-\alpha/2} + Z_{1-\beta}}{\mu_A - \mu_B}\right)^2 \quad (1)$$

This calculation showed that 12 participants were required for the rehabilitation program group and 14 participants for the control group, totaling 28 participants and accounting for a 10% missed visit rate.

Participants

Inclusion criteria

Participants must meet the following criteria: 1) age 18–75 years; 2) absence of distant organ metastases; 3) no history of abdominal surgery; 4) absence of pancreatic, bile duct or jugular occupancy; and 5) signed informed consent for PD diagnosed through preoperative imaging.

Exclusion criteria

Patients will be excluded if they: 1) do not meet the indications for surgery; 2) have other serious complications incompatible with general anesthesia; 3) require immediate surgery; 4) have severe heart, lung, liver, kidney, or other organ insufficiencies; 5) have had recent cerebrovascular accidents resulting in limb or cognitive dysfunction; or 6) have a diagnosis of PD combined with serious infections, endocrine dysfunction or other factors limiting participation in physical activities.

Intervention

Patients in both groups will receive preoperative education, including explaining surgical precautions and coordination points and assisting patients in completing surgery-related examinations or preparations. The advantage of the intervention group lies in its ability to promptly engage in the postoperative rehabilitation program, with the aim of facilitating swift recovery of postoperative function.

Control group

Subjects in the control group will receive standard postoperative care, which involves advising patients to rest in bed after surgery and gradually mobilize as their condition stabilizes. No personalized exercise programs will be recommended.

Interventional study of postoperative rehabilitation

According to the following guidelines, a trained physical therapist will assess all patients starting on the day of surgery. Patients must meet the following criteria: 1) they must be awake and responsive to calls; 2) their blood pressure, heart rate and oxygen saturation were stable during surgery; 3) they did not experience any postoperative bleeding, with abdominal drainage of 600 mL or less over 24 h; 4) their muscle strength was assessed at level 3 or above; and 5) their wound pain score (on a numerical scale) was less than 4. During the first week after surgery, patients can participate in a 30–40-min early activity rehabilitation program with a physical therapist. The rehabilitation regimen during the 1st week after surgery (postoperative days 1–7 (POD1–7)) consists of 3 stages. First, patients will engage in a series of "muscle pump" activities designed to stimulate blood flow to the extremities. Second, they will practice standing and stepping on the bed. Third, they will transition gradually from low-intensity bedside training to walking training within the hospital setting. Table 1 shows the postoperative exercise schedule for the 1st week (T1).

The results of the 6MWT at T1 will inform the development of moderate-intensity aerobic training to be implemented during the 2nd postoperative week (T2). Table 2 displays specific recommendations for aerobic activities. Vital signs and patient complaints will be monitored during one-on-one exercises; however, if patients are unable to remain upright or report feeling fatigued, the activity will be terminated immediately. Based on the results of the 6MWT at T1, moderate-intensity aerobic training will be continued into T2. The time interval from illness to intermittent participation will be noted on the case report form (CRF) and illustrated during data interpretation, allowing participants to resume exercise intermittently if they have to stop for 1 or 2 days due to illness.

Outcome measurements

Primary outcomes

To assess the functionality of a patient's digestive system after surgery, a novel objective evaluation tool called the I-FEED scale will be implemented.[16] This scale focuses on 5 areas: postoperative intake, nausea, vomiting, physical examination findings, and symptom duration. Postoperative GI intolerance was defined as a score of 3, and GI dysfunction as a score of 6 on this 5-factor scale.[17]

Condition of the heart and lungs

The 6MWT is a walking speed and distance test conducted over 6 min. It is an easy-to-administer and reliable test that can be repeated with consistent results. This test predicts the likelihood of postoperative complications with high accuracy and correlates strongly with peak oxygen

Table 1. Exercise program for postoperative patients from day 1 to day 7

Time	Activities and specific content
POD1–7 rehabilitation program schedule	
1st phase (POD1)	Pre-treatment transcutaneous electrical nerve stimulation for analgesia **1. Visceral exercise training:** **Ankle pump:** lower limb flattened, dorsal foot hyperflexion and hyperextension, toe down and up for 5 s each time, both feet simultaneously. 20/set, 2 sets/session/2–3 h. **Fist pump**: simultaneous clenching and unclenching of both hands. 10/set, 2 sets/reps/2–3 h. **Bridge exercise:** 8–10/set, 3 sets/reps/2–3 h. **Upper and lower limb alternate flexion and extension training** (upper limb alternate flexion and extension training: double upper limb elevation, double elbow flexion-extension exercise, 10/set, 2 sets/session/2–3 h; lower limb alternate hip flexion and knee slide training: 3–4 sets/session, 4–5 sessions/day). **2. Turning training:** axial turning (turning the trunk in a straight line to avoid the shear force generated by the twisting of the trunk affecting the wound), 1 session/2 h. **3. Respiratory function exercise:** **Lip retraction breathing:** Inhale deeply through the nostrils, discard the air for 2–3 s, retract the lips, and exhale slowly, just like whistling, inhalation to exhalation ratio 1:2, 5/set, 4 sets/session, 5–10 min/session/2 h. **Effective coughing and coughing up sputum:** After a group of deep breaths, inhale slowly and deeply while leaning your upper body forward. Inhale once, cough 2–3 times continuously, and then stop coughing. When coughing, press the wound with both hands and a pillow to reduce the tension of the wound. **Postural adaptation training:** Starting from 30°, gradually increase the angle of the bed's head until the patient is in a sitting position in bed, 2 h/time. At each increasing angle, allow the patient to sit for 5 min and observe whether there is dizziness, nausea and other discomforts. When there is no discomfort observed, bedside sitting can be performed.
2nd phase (POD2–3)	1. Execute the first phase of the training with a pre-activity transcutaneous electrical nerve stimulation treatment. **2. Bedside standing**: The patient sits up at the bedside for 5–10 min, and if there is no dizziness or other discomfort noticed, the therapist and family members assist the patient to stand up; during the process, allow the patient to support the wound with both hands to reduce pain. **3. Bedside stepping training:** Stand at the bedside for 5–10 min and observe any discomfort such as dizziness and nausea. If there is no discomfort noticed, perform bedside stepping training in place, 10–15 sets/group, 2–3 sets/session, 2–3 times/day. After each set of stepping training, rest for 1 min at the bedside in a seated position, then repeat the training one more time.
3rd phase (POD3–7)	1. Implement the first phase and second phase of training. **2. Bedside walking, indoor walking and outdoor walking**: based on the patient's condition, gradually change the walking location, intensity and distance, and slowly increase the amount of activity.

POD – postoperative day.

Table 2. Aerobic exercise prescription in the 2nd postoperative week

Type	Walk
Intensity	Moderate intensity (40–60% of heart rate reserve), determined according to maximal/resting heart rate, and 6MWT results. Patients were given walking distance, and self-perceived exertion (Borg 11–13 points) was applied to monitor walking intensity during the exercise
Time	Continuous or intermittent walking for 40 min (includes 5 min warm-up, 30 min exercise and 5 min relaxation)
Frequency	1 per day

6MWT – 6-minute walk test.

intake.[18] The risk of significant postoperative complications due to hepatopancreatobiliary malignancy can be determined using the 6WMT.[19]

Secondary consequences

To compare the postoperative recovery quality between the 2 groups, we will administer the 40-item Quality of Recovery Questionnaire (QoR-40). The QoR-40 is considered the best QoL evaluation questionnaire for Chinese patients undergoing surgery due to its excellent reliability, validity and responsiveness.[20] It covers 5 domains: emotional state (9 items), physical comfort (12 items), psychological support (7 items), self-care ability (5 items), and pain (7 items). Higher total scores indicate successful rehabilitation, with a possible range of 40–200 points.

Quality of life

The Quality-of-Life Questionnaire (QLQ-C30), developed by the European Organization for Research and Treatment of Cancer (EORTC), will be used to evaluate postoperative improvements in patients' QoL.[21] It includes the Global Health Status and Quality of Life scales, as well as the eight symptom scales (fatigue, pain, nausea/vomiting, dyspnea, sleep problems, loss of appetite, constipation, and diarrhea) among its 30 items. Responses for items 29 and 30 will be rated on a scale from 1 to 7, whereas the rest will be categorized into 4 levels (none, a little, more, and a lot) and rated from 1 to 4. Higher scores in symptom categories indicate worse health and functioning, whereas higher scores in functioning and overall health suggest better QoL.[22]

Clinical evaluation indices

Additional clinical evaluation indices will include time to first flatus and feces, daily volume of stomach fluid and time of gastric tube removal. Anaerobic digestion and elimination are signs that intestinal peristalsis is beginning to function again post-surgery. While they are closely associated with the recovery of intestinal function, they cannot independently determine it. Therefore, time to exhaustion and defecation remain crucial indicators for assessing digestive tract health post-surgery.[23] Nurses or researchers will record patients' exhaustion and defecation times, daily volume of gastric juices, and gastric tube removal times from postoperative day 1 to day 14 to calculate the time to first flatus and defecation.

Length of hospital stay

The LOS for patients undergoing PD will be evaluated during routine clinical checks. It was defined as the time from surgical resection completion until the patient's discharge. Data will be retrieved from patients' medical records post-discharge.

Postoperative complications

Routine clinical assessments of PD patients will be used to assess the frequency and severity of postoperative complications. Surgical complications were defined and will be assessed for severity using the Clavien–Dindo classification.

Safety

Methods for tracking and reporting medical mishaps are essential. An adverse event was defined as any unexpected medical issue or damage that occurs during the trial. On the observation form, the patient's condition, noting symptom severity, onset, duration, and interventions taken are recorded. Rapid notification of adverse events during rehabilitative exercises will allow the investigator to pause the intervention, adjust the training cycle and provide focused treatment, while also informing the research unit and ethics committee as needed. Event occurrence will be analyzed.

Data collection and management

Data in the CRF should be promptly, accurately and truthfully recorded by the researcher based on participants' original records. The monitoring person will verify that all CRFs are correctly filled out and are consistent with the original data; any discrepancies will prompt immediate correction by the investigator. The investigator's signature and correction date are required to maintain the integrity of the original records.

Statistical analyses

Participants were blindly randomized, and data will be analyzed using the full sample. A complete case analysis will be performed if the data missing rate is less than 10%. For data missing more than 10%, extensive interpolation will be used to fill gaps. Data processing will utilize IBM SPSS v. 25.0 statistical software (IBM Corp., Armonk, USA). Mean and SD will be used to represent normally distributed data; Student's t-tests will be used to compare data between groups; analysis of variance (ANOVA) hybrid design, using general linear model (GLM) tests, will be used to compare data across multiple time points; non-normally distributed data will be represented by median and interquartile range (M(IQR)) and compared using the χ^2 test. The threshold for statistical significance was set at $p < 0.05$.

Discussion

The 5-year survival rate for patients treated with PD, the principal treatment for pancreatic and Vater ampulla carcinoma (VPC), ranges only from 8% to 17%.[24] The PD is a highly invasive surgical procedure. Despite continuous advancements in surgical techniques that have increased the overall survival rate,[25] patients still face challenges in recovering after surgery.[26] A major obstacle to full recovery post-surgery is the increased likelihood of postoperative complications, including reduced physical function, lung infections, GI dysfunction, and other systemic dysfunctions arising from prolonged bed rest due to factors such as postoperative discomfort and the presence of tubes. Surgeons' recognition of the impact of their patients' functional status on postoperative QoL and their pursuit of improved clinical outcomes have led to a surge in interest and demand for post-surgery rehabilitation.

The ERAS concept heavily emphasizes early postoperative mobilization, aligning with functional recovery post-surgery. Exercise regimens designed for postoperative rehabilitation following abdominal surgeries such as colonoscopy, thoracotomy and hernia repair have been shown to be successful.[27] A single-blind, parallel-arm, RCT comparing early postoperative mobility programs with standard care among patients undergoing major abdominal tumor surgery demonstrated the safety and feasibility of early mobilization programs, incorporating exercises such as core stability, gait training, upright training, and aerobic and resistance training, twice daily, which also improves patient functions.[28] Furthermore, in a meta-analysis and retrospective analysis of the ERAS program for PD, it has been universally concluded that postoperative ERAS programs are feasible and safe and can improve postoperative outcomes.[29,30] These studies highlight the importance of early mobilization for patients undergoing this procedure.[31] Patients participating in postoperative

rehabilitation programs experience lower risks of major complications, shorter hospital stays and reduced out-of-pocket medical expenses. Early, intensive physical therapy after surgery has been shown to significantly enhance QoL by restoring or improving muscle strength, reducing fatigue and enhancing physical functioning among cancer patients.[32] Physical therapists can more effectively monitor patient progress and ensure adherence to daily exercise regimens during hospital-based postoperative rehabilitation.

Conclusions

In this study, the I-FEED score, a novel tool for assessing GI function, will be used to monitor the impact of a 2-week postoperative rehabilitation exercise program on patients' GI function. The primary outcome measure will focus on improvements in cardiopulmonary capacity following postoperative rehabilitation activities. Secondary outcomes include examining the effects of postoperative rehabilitation on recovery quality, complications and LOS. This experimental evidence will contribute to larger hypotheses guiding future multicenter RCTs.

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References

1. Karim SAM, Abdulla KS, Abdulkarim QH, Rahim FH. The outcomes and complications of pancreaticoduodenectomy (Whipple procedure): Cross sectional study. *Int J Surg*. 2018;52:383–387. doi:10.1016/j.ijsu.2018.01.041
2. Lubrano J, Bachelier P, Paye F, et al. Severe postoperative complications decrease overall and disease free survival in pancreatic ductal adenocarcinoma after pancreaticoduodenectomy. *Eur J Surg Oncol*. 2018;44(7):1078–1082. doi:10.1016/j.ejso.2018.03.024
3. El Nakeeb A, Askr W, Mahdy Y, et al. Delayed gastric emptying after pancreaticoduodenectomy: Risk factors, predictors of severity and outcome. A single center experience of 588 cases. *J Gastrointest Surg*. 2015;19(6):1093–1100. doi:10.1007/s11605-015-2795-2
4. Wang J, Ma R, Churilov L, et al. The cost of perioperative complications following pancreaticoduodenectomy: A systematic review. *Pancreatology*. 2018;18(2):208–220. doi:10.1016/j.pan.2017.12.008
5. Dai J, Jiang Y, Fu D. Reducing postoperative complications and improving clinical outcome: Enhanced recovery after surgery in pancreaticoduodenectomy. A retrospective cohort study. *Int J Surg*. 2017;39:176–181. doi:10.1016/j.ijsu.2017.01.089
6. Melloul E, Lassen K, Roulin D, et al. Guidelines for Perioperative Care for Pancreatoduodenectomy: Enhanced Recovery After Surgery (ERAS) Recommendations 2019. *World J Surg*. 2020;44(7):2056–2084. doi:10.1007/s00268-020-05462-w
7. Tian Y, Li Q, Pan Y. Prospective study of the effect of ERAS on postoperative recovery and complications in patients with gastric cancer. *Cancer Biol Med*. 2022;19(8):1274–1281. doi:10.20892/j.issn.2095-3941.2021.0108
8. Yuan F, Essaji Y, Belley-Cote EP, et al. Postoperative complications in elderly patients following pancreaticoduodenectomy lead to increased postoperative mortality and costs: A retrospective cohort study. *Int J Surg*. 2018;60:204–209. doi:10.1016/j.ijsu.2018.11.016
9. Zhao LY, Liu XT, Zhao ZL, et al. Effectiveness of enhanced recovery after surgery in the perioperative management of patients with bone surgery in China. *World J Clin Cases*. 2021;9(33):10151–10160. doi:10.12998/wjcc.v9.i33.10151
10. Fang A, Ding W, Zeng W, et al. Impact of early postoperative activities on postoperative recovery in patients undergoing abdominal surgery: A protocol for systematic review and meta-analysis. *Medicine (Baltimore)*. 2021;100(44):e27556. doi:10.1097/MD.0000000000027556
11. Ni CY, Wang ZH, Huang ZP, et al. Early enforced mobilization after liver resection: A prospective randomized controlled trial. *Int J Surg*. 2018;54(Pt A):254–258. doi:10.1016/j.ijsu.2018.04.060
12. Na YM, Kim MY, Kim YK, Ha YR, Yoon DS. Exercise therapy effect on natural killer cell cytotoxic activity in stomach cancer patients after curative surgery. *Arch Phys Med Rehabil*. 2000;81(6):777–779. doi:10.1016/S0003-9993(00)90110-2
13. Allgayer H, Owen RW, Nair J, et al. Short-term moderate exercise programs reduce oxidative DNA damage as determined by high-performance liquid chromatography-electrospray ionization-mass spectrometry in patients with colorectal carcinoma following primary treatment. *Scand J Gastroenterol*. 2008;43(8):971–978. doi:10.1080/00365520701766111
14. Ahn KY, Hur H, Kim DH, et al. The effects of inpatient exercise therapy on the length of hospital stay in stages I–III colon cancer patients: Randomized controlled trial. *Int J Colorectal Dis*. 2013;28(5):643–651. doi:10.1007/s00384-013-1665-1
15. Nakashima Y, Kawae T, Iwaki D, et al. Changes in motor function and quality of life after surgery in patients with pancreatic cancer. *Eur J Cancer Care*. 2021;30(2):e13368. doi:10.1111/ecc.13368
16. Alsharqawi N, Alhashemi M, Kaneva P, et al. Validity of the I-FEED score for postoperative gastrointestinal function in patients undergoing colorectal surgery. *Surg Endosc*. 2020;34(5):2219–2226. doi:10.1007/s00464-019-07011-6
17. Hedrick TL, McEvoy MD, Mythen MG, et al. American Society for Enhanced Recovery and Perioperative Quality Initiative Joint Consensus Statement on Postoperative Gastrointestinal Dysfunction Within an Enhanced Recovery Pathway for Elective Colorectal Surgery. *Anesth Analg*. 2018;126(6):1896–1907. doi:10.1213/ANE.0000000000002742
18. ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS Statement: Guidelines for the Six-Minute Walk Test. *Am J Respir Crit Care Med*. 2002;166(1):111–117. doi:10.1164/ajrccm.166.1.at1102
19. Hayashi K, Yokoyama Y, Nakajima H, et al. Preoperative 6-minute walk distance accurately predicts postoperative complications after operations for hepato-pancreato-biliary cancer. *Surgery*. 2017;161(2):525–532. doi:10.1016/j.surg.2016.08.002
20. Chen Y, Wang J, Liu S, et al. Development and validation of the Chinese version of the Quality of Recovery-40 Questionnaire. *Ther Clin Risk Manag*. 2020;16:1165–1173. doi:10.2147/TCRM.S281572
21. Fitzsimmons D, Kahl S, Butturini G, et al. Symptoms and quality of life in chronic pancreatitis assessed by structured interview and the EORTC QLQ-C30 and QLQ-PAN26. *Am J Gastroenterol*. 2005;100(4):918–926. doi:10.1111/j.1572-0241.2005.40859.x
22. Fayers P, Bottomley A. Quality of life research within the EORTC: The EORTC QLQ-C30. *Eur J Cancer*. 2002;38:125–133. doi:10.1016/S0959-8049(01)00448-8
23. Bai Y, Li DF, Wang SL, et al. Chinese expert consensus on perioperative medications for endoscopic retrograde cholangiopancreatography (ERCP). *J Digest Dis*. 2019;20(3):103–113. doi:10.1111/1751-2980.12699
24. Bengtsson A, Andersson R, Ansari D. The actual 5-year survivors of pancreatic ductal adenocarcinoma based on real-world data. *Sci Rep*. 2020;10(1):16425. doi:10.1038/s41598-020-73525-y
25. Nakeeb AE, Askar W, Atef E, et al. Trends and outcomes of pancreaticoduodenectomy for periampullary tumors: A 25-year single-center study of 1000 consecutive cases. *World J Gastroenterol*. 2017;23(38):7025–7036. doi:10.3748/wjg.v23.i38.7025
26. Kurokawa H, Akezaki Y, Tominaga R, et al. Changes in physical function and effects on QOL in patients after pancreatic cancer surgery. *Healthcare*. 2021;9(7):882. doi:10.3390/healthcare9070882
27. Castelino T, Fiore JF, Niculiseanu P, Landry T, Augustin B, Feldman LS. The effect of early mobilization protocols on postoperative outcomes following abdominal and thoracic surgery: A systematic review. *Surgery*. 2016;159(4):991–1003. doi:10.1016/j.surg.2015.11.029

28. De Almeida EPM, De Almeida JP, Landoni G, et al. Early mobilization programme improves functional capacity after major abdominal cancer surgery: A randomized controlled trial. *Br J Anaesth*. 2017;119(5): 900–907. doi:10.1093/bja/aex250
29. Abu Hilal M, Di Fabio F, Badran A, et al. Implementation of enhanced recovery programme after pancreatoduodenectomy: A single-centre UK pilot study. *Pancreatology*. 2013;13(1):58–62. doi:10.1016/j.pan.2012.11.312
30. Kuemmerli C, Tschuor C, Kasai M, et al. Impact of enhanced recovery protocols after pancreatoduodenectomy: Meta-analysis. *Br J Surg*. 2022;109(3):256–266. doi:10.1093/bjs/znab436
31. Fukushima T, Adachi T, Hanada M, et al. Role of early mobilization on the clinical course of patients who underwent pancreaticoduodenectomy: A retrospective cohort study. *Tohoku J Exp Med*. 2021; 254(4):287–294. doi:10.1620/tjem.254.287
32. Weyhe D, Obonyo D, Uslar V, Tabriz N. Effects of intensive physiotherapy on Quality of Life (QoL) after pancreatic cancer resection: A randomized controlled trial. *BMC Cancer*. 2022;22(1):520. doi:10.1186/s12885-022-09586-1

An umbrella analysis assessing the risk of acute kidney injury in COVID-19 patients

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Abstract

A number of research studies have indicated a potential association between COVID-19 and acute kidney injury (AKI). However, the methodologies employed and the risk estimates derived from these studies vary. Therefore, an umbrella review of systematic reviews and meta-analyses was conducted to determine the incidence of AKI in COVID-19 patients and AKI-associated mortality. A complete literature search was undertaken in PubMed, Embase, Scopus, and the Cochrane Library. The methodological rigor of the included papers was evaluated using the Assessment of Multiple Systematic Reviews (AMSTAR-2) instrument. The pooled risk ratio (RR) and odds ratio (OR) of the included studies were calculated to establish the strength of the association between AKI cases and COVID-19 infections. This umbrella review included 20 studies. Two of the 20 studies assessed adult COVID-19 patient risk factors for AKI, 1 examined survival rates and 7 examined the incidence of AKI. The remaining 10 investigations revealed that patients with coronavirus were susceptible to AKI. The umbrella analysis comprised reviews that contained a range of 6 to 54 papers. The AMSTAR-2 ratings yielded a total of 14 studies deemed to be of high quality, with 6 studies classified as intermediate quality. Statistical analysis of included reviews revealed a 1.50 RR for AKI incidence in COVID-19 patients (95% confidence interval (95% CI): 1.40–1.60, I^2 69%, $p < 0.0001$) and a 2.02 RR (95% CI: 1.79–2.29, I^2 56%, $p < 0.0001$) for AKI-associated death. This umbrella review revealed that individuals infected with the novel coronavirus often develop AKI. SARS-CoV-2 infections were associated with AKI due to advanced age, male gender, coronary artery disease, diabetes, and hypertension. However, AKI and a renal replacement therapy (RRT) requirement independently predicted unfavorable COVID-19 results.

Key words: COVID-19, renal replacement therapy, chronic kidney disease, acute kidney injury, incidence of AKI

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Introduction

The COVID-19 epidemic has developed into a disaster that affected healthcare systems all across the world. The SARS-CoV-2 virus, which causes COVID-19, was initially identified in Wuhan, China, in December 2019. Since then, it has rapidly spread across the world.[1,2] The World Health Organization (WHO) decided to officially classify COVID-19 as a pandemic in March 2020.[3] The clinical trajectory of SARS-CoV-2 infections is exceptionally erratic and diverse, encompassing subclinical infection, asymptomatic infection, productive cough, dyspnea, fatigue, and imaging indications of pneumonia, as well as multi-organ systemic failure and mortality.[4,5] Several epidemiological studies have demonstrated that individuals with comorbidities such as diabetes, arterial hypertension, metabolic syndrome, and cardiovascular disease are more likely to manifest symptoms related to SARS-CoV-2 infections.[6–9] This is especially true for older patients. In addition to this, the probability of these patients acquiring a severe condition is significantly higher than in most patients.[10]

COVID-19 is known to cause deterioration in kidney function, in addition to the negative effects it has on the respiratory system. There is a significant relationship between acute kidney injury (AKI) and coronavirus infections,[11] as seen in this correlation study. According to the findings of the research, AKI affects 5–15% of patients infected with SARS-CoV2 and is linked to a mortality rate of 70–90%.[12] Systemic inflammatory responses are triggered when viruses such as influenza, SARS CoV-2, etc., infect and replicate in cells that are the focus of their infection, which ultimately leads to malfunction in a number of organs.[13,14]

Hypoxemia, dehydration, underlying conditions, and the deleterious effects of drugs supplied to these patients[15] are the leading causes of AKI. Several studies have documented the occurrence of AKI and other serious renal complications in hospitalized individuals with COVID-19.[16–20] However, the epidemiological burden of AKI in people who have been infected with COVID-19 and the involvement of the kidney are still unknown and require additional investigation.

Despite the fact that a significant number of research studies have been conducted to investigate the connection between COVID-19 and AKI, the designs of these studies are extremely diverse and the risk estimations produced by these studies are quite diverse. Therefore, it is problematic to focus on the highest quality of evidence, the multitude of published meta-analyses and systematic reviews, the duplication of patient data, and the variety of evidence sources, as this could impede the identification and implementation of evidence-based strategies in medical practice. Consequently, it is a significant challenge to concentrate on the highest quality evidence. Therefore, we conducted umbrella review of systematic reviews and meta-analyses to synthesize findings from selected systematic reviews and meta-analyses[21–40] concerning the effects of COVID-2019 on kidney health.

Objectives

The purpose of this umbrella analysis of systematic reviews and meta-analyses was to ascertain the frequency of AKI among COVID-19 patients and its correlation with disease severity and mortality.

Materials and methods

In accordance with the guidelines that were recently released,[41] an umbrella review was carried out. We adhered to the Preferred Reporting Items for Overviews of Reviews (PRIOR) protocol[42] when it came to the reporting process. Guidelines from the Assessing the Methodological Quality of Systematic Reviews (AMSTAR)[43] were utilized to conduct a quality evaluation of the studies.

Eligibility criteria

For studies to be considered, they had to meet all of the following criteria. The criteria used to assess the impact of COVID-19 on kidney health and the risk of AKI and AKI-related mortality in COVID-19 patients were: (1) systematic reviews that incorporated individual participant meta-analyses or meta-analyses of meta-analyses; (2) included individuals who had been diagnosed with COVID-19; (3) reported incidence of AKI in COVID-19 patients; (4) examined any of the outcomes listed below in COVID-19 patients: i) the incidence of AKI; ii) odds ratio (OR) of AKI; iii) AKI-associated mortality; and iv) risk ratio (RR) of AKI; and (5) risk factors associated with AKI in COVID-19 patients. Bibliographic references that were obsolete, anecdotal or solely relied on expert assessments were excluded from the selection process. Furthermore, studies that were dependent on animal experiments or trials were excluded, along with those in which the authors lacked access to primary data and critical information. Furthermore, non-research publications, qualitative studies, studies involving patients with HIV and other systemic diseases, and papers published in languages other than English were likewise omitted.

Information sources

A search of the literature was carried out in a number of different databases, such as PubMed, Embase, Scopus, Web of Science, and the Cochrane Library. In accordance with the PICOS methodology, the key words were identified and checked to ensure that they were consistent throughout both the MEDLINE and Embase databases.

Search strategy

The search covered the years 2020–2023 and utilized specific key words such as "acute kidney injury" OR "AKI" OR "COVID-19" OR "kidney injury" OR "incidence of AKI" OR "prevalence of AKI" OR "RRT" OR "SARS-CoV2" OR "kidney replacement therapy" OR "kidney disease" OR "kidney injury" OR "chronic kidney disease" OR "CKD" OR "mortality" OR "fatality" OR "disease severity" OR "recovery" OR "risk of AKI" OR "kidney failure" OR "meta-analysis" OR "systematic review and meta-analysis" OR "systematic review". The key words were identified and verified for consistency in both the MEDLINE and Embase databases, in accordance with the PICOS framework (Table 1). The aforementioned key words were entered into the Title (ti)-Abstract (abs)-keyword (key) field in the Scopus search. Cochrane search terms included "AKI" and "COVID-19 patients". The PICO structure was applied to establish specific criteria for selection. "P" in this context represented patients infected with COVID-19, "I" denoted the incidence of AKI, "C" represented a control, and "O" comprised the clinical outcomes, specifically the incidence of AKI in COVID-19 patients and mortality. The research design incorporated in this study was limited to the implementation of systematic reviews and meta-analyses. The identification of relevant studies was conducted through an unbiased and thorough examination of the related literature by 2 researchers (Q.G. and Y.Z.). Additional relevant papers were identified by carefully screening the references listed in the final research for analysis.

Selection process

A first screening of the titles and abstracts of the publications that were acquired was followed by an examination of the complete texts of references that had the potential to be eligible. The screening procedure was carried out by 2 researchers, and in case of any inconsistencies, the decision regarding whether or not to include the material under consideration was made through discussion.

Data items

The data extraction process was carried out by 1 author, and 2 other authors subsequently verified the extracted information. Disputes were resolved through deliberation. Information pertinent to our study was selectively gathered regarding the incidence of AKI and mortality associated with AKI in COVID-19 patients from publications that met the inclusion criteria and contained data on multiple disorders. Initially, we gathered pertinent details from the eligible reviews, such as author information, publication year, journal of publication, study type, number of included studies, study aim, age of participants, search engines used, quality assessment tools used, primary outcomes, and study conclusions. Furthermore, to encompass the full geographic range of evidence, we collected data regarding the specific locations of the individual studies that were included in the relevant reviews. This involved gathering information about the countries where the research was carried out.

Table 1. Database search strategy

Database	Search strategy
Scopus	#1 "acute kidney injury" OR "AKI", OR "COVID-19" OR "kidney injury" OR "incidence of AKI" OR "prevalence of AKI" OR "renal replacement therapy" OR "SARS-CoV2" OR "kidney replacement therapy" OR "kidney disease" OR "kidney injury" OR "chronic kidney disease". #2 "mortality" OR "fatality" OR "disease severity" OR "recovery" OR "risk of AKI" OR "kidney failure" OR "meta-analysis" OR "systematic review and meta-analysis", OR "systematic review". #3 #1 AND #2
PubMed	#1 "acute kidney injury" OR "AKI" OR "COVID-19" [MeSH Terms]* OR "kidney injury" OR "incidence of AKI" [All Fields] OR "prevalence of AKI" OR "renal replacement therapy" OR "SARS-CoV2" OR "kidney replacement therapy" [All Fields] OR "kidney disease" OR "kidney injury" [All Fields] OR "chronic kidney disease" [All Fields] #2 "mortality" OR "fatality" [MeSH Terms] OR "disease severity" OR "recovery" OR "risk of AKI" [All Fields] OR "kidney failure", OR "meta-analysis", OR "systematic review and meta-analysis" [All Fields], OR "systematic review" [All Fields]. #3 #1 AND #2
Embase	#1 "acute kidney injury"/exp[$] OR "AKI"/exp OR "COVID-19"/exp OR "kidney injury"/exp OR "incidence of AKI"/exp OR "prevalence of AKI"/exp OR "renal replacement therapy"/exp OR "SARS-CoV2"/OR "kidney replacement therapy"/exp OR "kidney disease"/exp OR "kidney injury"/exp OR "chronic kidney disease"/exp #2 "mortality"/exp OR "fatality"/exp OR "disease severity"/exp OR "recovery"/exp OR "risk of AKI"/exp OR "kidney failure"/exp OR "meta-analysis"/exp OR "systematic review and meta-analysis"/exp OR "systematic review"/exp #3 #1 AND #2
Cochrane Library	#1 (acute kidney injury): ti, ab, kw[@] OR (AKI): ti, ab, kw OR(COVID-19): ti, ab, kw OR (kidney injury): ti, ab, kw OR (incidence of AKI): ti, ab, kw OR (prevalence of AKI): ti, ab, kw OR (renal replacement therapy): ti, ab, kw OR (SARS-CoV2) OR (kidney replacement therapy): ti, ab, kw OR (kidney disease):OR (kidney injury):ti, ab, kw OR (chronic kidney disease): ti, ab, kw (word variations have been searched) #2 (mortality): ti, ab, kw OR (fatality): ti, ab, kw OR (disease severity): ti, ab, kw OR (recovery): ti, ab, kw OR (risk of AKI): ti, ab, kw OR (kidney failure): ti, ab, kw OR (meta-analysis): ti, ab, kw OR (systematic review and meta-analysis): ti, ab, kw OR (systematic review) (word variations have been searched) #3 #1 AND #2

* MeSH terms – Medical Subject Headings; [$] exp – explosion in Emtree – searching of selected subject terms and related subjects; [@] ti, ab, kw – either title or abstract or keyword fields.

Study risk of bias assessment

Regarding methodological rigor, we collected data on whether the authors assessed this aspect of the studies included in each systematic review using a pre-validated instrument or a supplemental set of extracted questions. We documented the specific tool that was used, and the primary findings of the evaluation were categorized into 3 broad groups: studies that exhibited weak methodological rigor, studies that demonstrated a high level of methodological rigor, or studies that displayed intermediate or mixed patterns from the 2 above groups. If the answer was affirmative, we documented the specific tool that was used. The methodological rigor of the included systematic reviews was evaluated by 2 reviewers using the AMSTAR-2 program. Any differences were resolved with the assistance of a 3rd reviewer. AMSTAR-2 utilizes a checklist consisting of 16 items or domains, of which 7 are deemed crucial for ensuring the overall validity of a review. The essential domains to be accounted for are as follows: (1) ensuring protocol registration prior to commencing the review; (2) conducting a thorough and comprehensive literature search; (3) providing a rationale for excluding specific studies; (4) assessing the risk of bias in the included studies; (5) employing suitable statistical techniques for conducting a meta-analysis; (6) considering the influence of bias when interpreting the findings; and (7) evaluating the existence and consequences of publication bias. Finally, utilizing abstracts and a full-text analysis, we retrieved pertinent information regarding the primary conclusions drawn from each of the reviews included. Moreover, if the review involved multiple disease areas, we only selected the primary outcomes from the individual studies concerning the incidence of AKI in COVID-19 patients.

Statistical analyses

A descriptive analysis was conducted due to the substantial variability observed in the study designs, research inquiries, findings, and metrics. The methodological assessment, summary estimates, 95% confidence intervals (95% CIs), and heterogeneity estimates, as well as the characteristic information, were compiled for each of the included studies. The elevated risk of AKI in COVID-19 patients was documented in every single study that was included. However, because the strategies used were so different, the overall OR and RR of the studies were also calculated to evaluate how strongly the incidence of AKI was linked to coronavirus infections. The subjects' health conditions (presence or absence of AKI) served as the basis for grouping the studies. An OR value higher than 1 was considered statistically significant and showed that COVID-19 patients were at high risk of AKI and AKI-associated mortality.

Results

Study selection

The process of selecting studies, in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines,[44] has been presented in Fig. 1. An electronic scanning technique was employed to perform an exhaustive search across multiple databases; this led to the identification of 315 articles that satisfied the inclusion criteria specified in the PICOS framework.[45] Prior to screening, 44 duplicate documents were eliminated, bringing the total number of papers screened to 271. One hundred and thirty-one papers were subsequently excluded for having invalid titles and abstracts, and 140 records were requested for retrieval. Twenty-five records were not retrieved in their entirety; the eligibility of the remaining 115 reports was evaluated. Upon implementation of the inclusion-exclusion criteria, 95 articles were determined to be ineligible and were consequently excluded. The principal determinants leading to the exclusion of research studies were their failure to provide essential outcome measures, no access to the complete texts, unsuitable study designs, and being published in a language other than English. Finally, 20 systematic reviews and meta-analyses that satisfied the predetermined inclusion criteria and spanned the time period from 2020 to 2023 were incorporated into this umbrella review.

Characteristics of included reviews

The included studies were published from 2020 to 2023. The articles included in the study were retrieved from Scopus (n = 68), PubMed (n = 135), Embase (n = 45), and the Cochrane Library (n = 67). Among the 20 reviews that were included, 2 studies, Cai et al.[22] and Hidayat et al.,[26] investigated the risk factors associated with acute renal injury in adult patients with COVID-19, and 1 study, Ali et al.,[21] discussed the survival rate in patients with AKI and COVID-19. The incidence of AKI in COVID-19 infections was reported in 7 additional studies.[23,32–34,36,39,40] Individuals with coronavirus infections were found to have a higher risk of experiencing AKI, according to the remaining 10 investigations.[24,25,27–31,35,37,38] All the reviews included in this analysis included articles without any geographical limitations. The evaluations included a range of study counts, varying from 6 to 54. Each of the 20 reviews involved data pooling, meta-analyses and qualitative analysis. Table 2 presents a comprehensive summary of the characteristics of the studies that were included in this umbrella review.

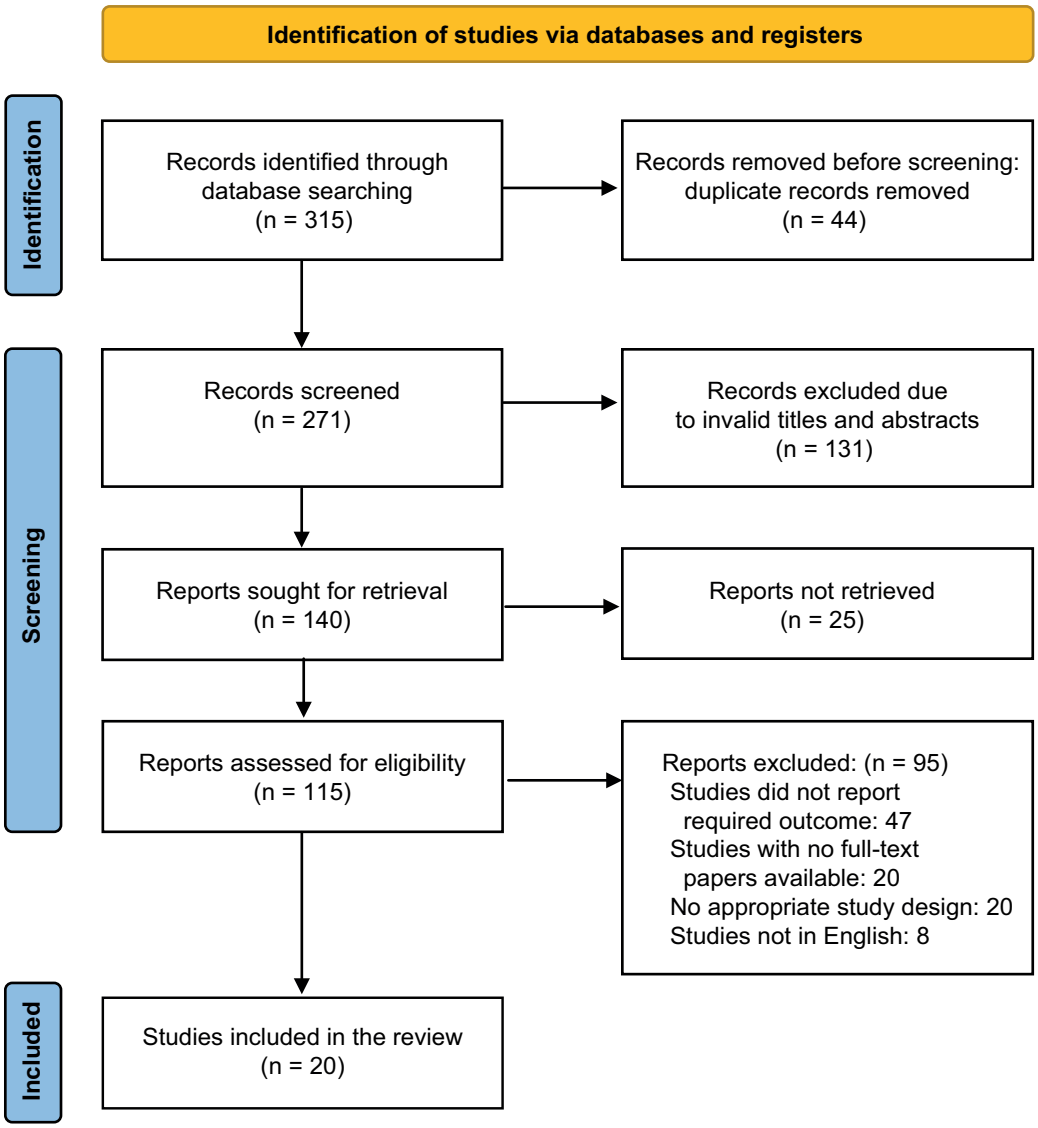

Fig. 1. Flowchart of the study selection

Methodological quality

Figure 2 provides a summary of the frequency of each AMSTAR2 rating for each domain across all of the evaluations. Table 3 shows the domain-specific methodological quality evaluations for the individual study. These evaluations are provided for each review. Twenty of the included studies[21–40] addressed the review questions using the PICO elements, provided an explanation of their study design selection, compiled a list of excluded studies, evaluated their conclusions, employed appropriate statistical methods, assessed the possible impact of bias risk in the individual studies, and conducted a quantitative synthesis. Sixteen reviews[21,24–29,31–37,39–40] stated that the review methods were established prior to use and provided justification for substantial deviations from the protocol, while articles by Cai et al.[22] and Chen et al.[23] partially met this criterion, and papers by Lim et al.[30] and Wang et al.[38] failed to do so. Each of the studies included in the review provided a comprehensive account of the rationale behind the selection of study designs for inclusion. With the exception of Ali et al.,[21] which could benefit from a more exhaustive search strategy, every article employed a comprehensive literature search approach. Six studies[21,24,29,30,33,37,38] did not disclose their funding sources. All studies used appropriate methods for the statistical combination of the results. With the exception of Chen et al.,[23] Hidayat et al.[26] and Saghafi et al.,[34] all other studies employed duplicate study selection; nevertheless, the methodology of Lim et al.[30] and Wang et al.[38] was also ambiguous, thus they received a partial yes. Likewise, with the exception of studies Ali et al.,[21] Menon et al.[32] and Zhou et al.,[40] all remaining studies extracted data in duplicate; however, Saghafi et al.[34] was also assessed as a partial affirmation. The research included in this study provides adequate summaries of the studies included and utilizes satisfactory techniques to evaluate the potential for bias. All studies, except Menon et al.,[32] accounted for the possibility of bias in the individual studies when interpreting or analyzing the results, and provided a satisfactory explanation for and discussion

Table 2. Characteristics of the included studies (all of them were systematic reviews and meta-analyses)

Study ID	Year of publication	Journal of publication	Country of study	Number of included studies	Aim of study	Age of participants	Search engines used	Quality assessment tool	Primary outcome	Conclusions
Ali et al.[21]	2020	*Renal Failure*	UK	6	Survival rate in acute kidney injury superimposed COVID-19 patients	>18 years	PubMed, MEDLINE, Embase, and Cochrane databases	NOS	mortality	Severe AKI in patients with COVID-19 is an ominous clinical predictor and is associated with high mortality.
Cai et al.[22]	2021	*Frontiers in Medicine*	China	38	Risk factors for acute kidney injury in adult patients with COVID-19	>16 years	PubMed, Embase, Web of Science, the Cochrane Library, CNKI, VIP, and Wan Fang Data	NOS	prevalence of AKI	AKI is a common and serious complication of COVID-19.
Chen et al.[23]	2020	*Critical Care*	Taiwan	20	Incidence of acute kidney injury in COVID-19 infection	45–70 years	PubMed and Embase	–	incidence of AKI	COVID patients are at high risk of AKI.
Cheruiyot et al.[24]	2020	*Acta Biomedica*	Kenya	15	Acute kidney injury is associated with worse prognosis in COVID-19 patients	40–78 years	PubMed and CNKI	Methodological index for non-randomized studies (MINORS) tool	mortality, prevalence of AKI	AKI is a relatively frequent manifestation in COVID-19 patients.
Fabrizi et al.[25]	2020	*Pathogens*	Italy	39	Acute kidney injury in COVID-19 patients	35–70 years	MEDLINE and manual searches	–	incidence of AKI (%), all-cause mortality	AKI is a common complication in hospitalized COVID-19-positive patients.
Hidayat et al.[26]	2023	*Pathophysiology*	Indonesia	30	Risk factors and clinical characteristics of acute kidney injury in patients with COVID-19	35–90 years	PubMed and DOAJ databases	NIH quality assessment tools	AKI prevalence, mortality rate	AKI carries high morbidity and mortality in COVID-19.
Hsiao et al.[27]	2023	*Frontiers in Medicine*	USA	12	Acute kidney injury in patients with COVID-19	>18 years	PubMed, Embase and Cochrane databases	NOS	mortality, incidence of AKI	COVID-19 patients had higher risk of developing AKI.
Juarez et al.[28]	2020	*Kidney International Reports*	USA	20	Outcomes for patients with COVID-19 and acute kidney injury	45–71 years	MEDLINE, Embase, Scopus, and MedRxiv databases	Study Quality Assessment Tool for Case Series Studies	mortality, incidence of AKI	Kidney dysfunction is common among patients with COVID-19.
Lee et al.[29]	2021	*Nature Scientific Reports*	South Korea	16	Increased risk of acute kidney injury in coronavirus disease patients	35–90 years	PubMed, Embase, Scopus, and Cochrane databases	NOS	mortality, incidence of AKI	Coronavirus disease patients have high risk of AKI.
Lim et al.[30]	2020	*Canadian Journal of Kidney Health and Disease*	Indonesia	15	Acute kidney injury and severity of COVID-19	40–60 years	PubMed, Scopus, Europe PMC, and the Cochrane Central Database	NOS	mortality, incidence of AKI	High risk of AKI was associated with COVID-19 patients.
Liu et al.[31]	2021	*PLoS One*	China	36	The chronic kidney disease and acute kidney injury involvement in COVID-19 pandemic	30–90 years	PubMed, CNKI and WanFang databases	NOS	mortality, incidence of AKI	CKD and AKI are susceptible to occur in patients with severe COVID-19.

Table 2. Characteristics of the included studies – cont

Study ID	Year of publication	Journal of publication	Country of study	Number of included studies	Aim of study	Age of participants	Search engines used	Quality assessment tool	Primary outcome	Conclusions
Menon et al.[32]	2021	*The Cureus Journal of Medical Science*	USA	20	The association of acute kidney injury with disease severity and mortality in COVID-19	43–72 years	PubMed, Embase, Google Scholar, and clinicaltrial.gov	NOS	mortality, incidence of AKI	AKI develops in a considerable number of COVID-19 patients, and the condition is significantly associated with adverse outcomes in patients with COVID-19.
Passoni et al.[33]	2022	*Nefrologia*	Brazil	28	Occurrence of acute kidney injury in adult patients hospitalized with COVID-19	>18 years	PubMed, Embase, Web of Science, Scopus, and Lilacs	Joanna Briggs Institute's critical appraisal tool	mortality, incidence of AKI	The occurrence of AKI is frequent among adult patients hospitalized with COVID-19.
Saghafi et al.[34]	2021	*Journal of Renal Injury Prevention*	Iran	22	Acute kidney injury in hospitalized COVID-19 patients	10–94 years	Web of Science, PubMed, Embase, Scopus, and Google Scholar databases	–	mortality, incidence of AKI	AKI is prevalent in hospitalized COVID-19 patients.
Shao et al.[35]	2020	*Pharmacological Research*	China	40	Acute kidney injury in patients with COVID-19	23–80 years	PubMed, Web of Science, ScienceDirect, MedRxiv and COVID-19, academic research communication platforms	–	fatality, incidence of AKI	AKI is a crucial complication in patients with COVID 19.
Silver et al.[36]	2020	*Kidney Medicine*	China	54	The prevalence of acute kidney injury in patients hospitalized with COVID-19 infection	45–70 years	MEDLINE, Embase, Cochrane Library, and a registry of preprinted studies	NIH Quality Assessment Tool for Case Series Studies	prevalence, mortality of AKI	Risk of AKI was higher in critically ill COVID-19 patients.
Singh et al.[37]	2021	*Clinical and Experimental Medicine*	Switzerland	29	Kidney disease and COVID-19 disease severity	40–80 years	PubMed, Web of Science, Scopus, and MedRxiv	NOS	prevalence, mortality of AKI	COVID-19 patients are at high risk of AKI.
Wang et al.[38]	2021	*Kidney and Blood Pressure Research*	China	42	The involvement of acute kidney injury in disease severity and mortality in patients with COVID-19	40–70 years	PubMed, Embase, Cochrane Library, medRxiv, Social Science Research Network, and Research Square databases	NOS	mortality and prevalence of AKI	AKI was associated with higher risks of severity and mortality in COVID-19 patients.
Yang et al.[39]	2021	*International Immunopharmacology*	China	58	Acute kidney injury and renal replacement therapy in COVID-19 patients	40–75 years	PubMed, Embase, Web of Science, medRxiv, and bioRxiv databases	NOS	mortality and prevalence of AKI	AKI and RRT use among COVID-19 patients represent a major public health concern.
Zhou et al.[40]	2021	*Frontiers in Medicine*	China	37	Chronic kidney diseases and acute kidney injury in patients with COVID-19	>18 years	PubMed, Embase, bioRxiv, and medRxiv	NOS	prevalence, mortality of AKI	AKI is associated with COVID-19 prognosis.

DOAJ – Directory of Open Access Journals; CNKI – China National Knowledge Infrastructure; NOS – Newcastle–Ottawa Scale; AKI – acute kidney injury; NIH – National Institutes of Health; CKD – chronic kidney injury; RRT – renal replacement therapy.

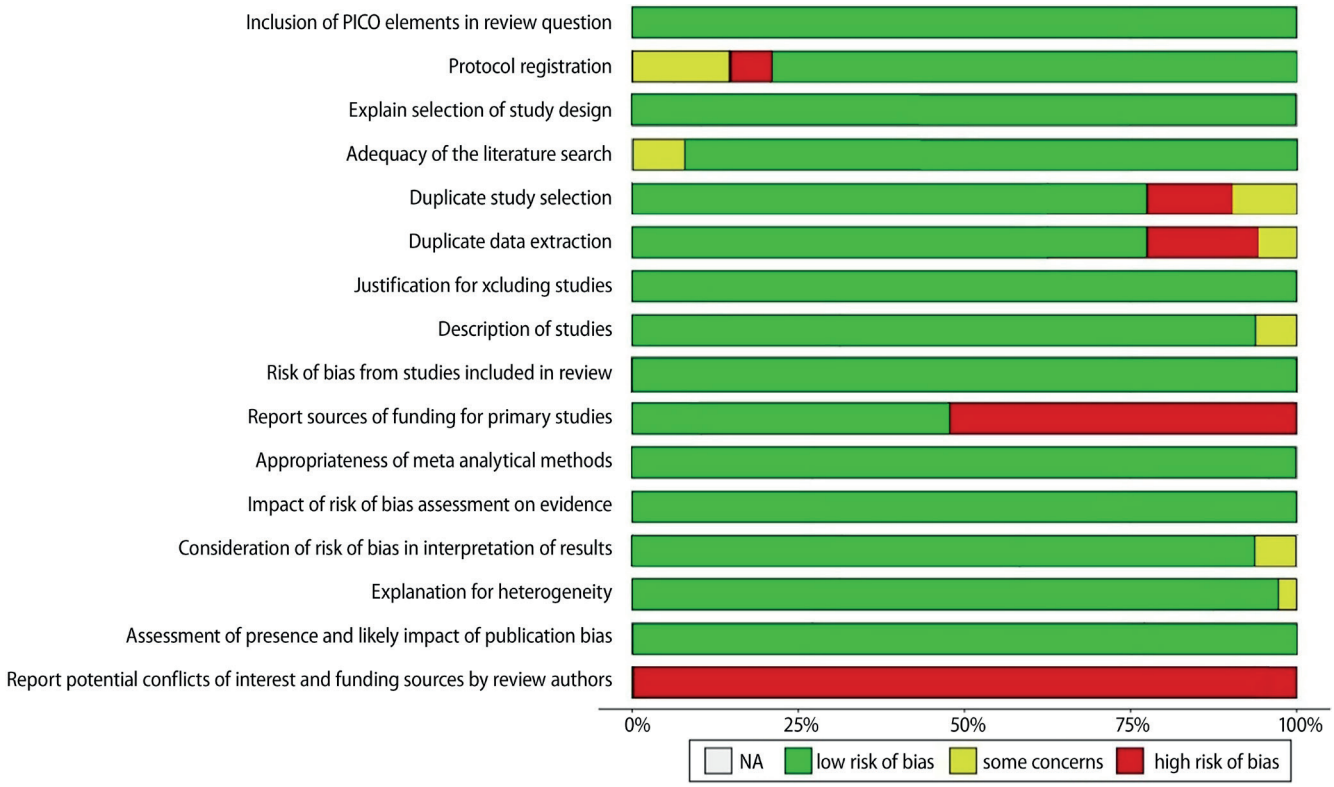

Fig. 2. Frequency of the risk of bias for Assessment of Multiple Systematic Reviews (AMSTAR-2) parameters

of any heterogeneity seen in the results of the review, with the exception of Saghafi et al.,[34] which partially satisfied this requirement. Based on the aforementioned evaluations, 14 of 20 studies[22,24,25,27–29,31–33,35–39] received a high overall assessment, whereas the remaining 6[21,23,26,30,34,40] were deemed to be of moderate quality.

Characteristics of the patients included in the umbrella review

The present umbrella analysis analyzed a total of 20 selected systematic reviews and meta-analyses, encompassing an overall cohort of 3 and 17,538 COVID-19 patients, to assess the risk of AKI in these individuals. The COVID-19-infected participants analyzed in these studies had a high frequency of AKI (about 60%) and a high mortality rate (around 72%). The populations that were taken from the included studies came from a variety of nations, with the bulk of the research being conducted in countries with high or moderate incomes, including the UK, China, Taiwan, Kenya, Italy, Indonesia, the USA, South Korea, Brazil, Iran, and Switzerland. For the reviews that were included, the summary estimates of the occurrences of AKI in COVID-19 patients are presented in Table 4.

Statistical analysis of extracted data

Using the results extracted on the incidence of AKI in COVID-19 patients and associated mortality, the overall OR of the included studies was calculated to assess how strongly the incidence of AKI was linked with COVID-19. Figure 3 displays the forest plot for the RR of the incidence of AKI in COVID-19 patients. The pooled RR was 1.50 (95% CI: 1.40–1.60, I^2 = 69%, p < 0.0001) with a heterogeneity of Tau^2 0.07, χ^2 60.60, degrees of freedom (df) = 19 and Z = 4.35. Figure 4 shows the pooled OR for AKI-associated mortality of COVID-19 patients with an OR of 2.02 (95% CI: 1.79–2.29, I^2 = 56%, p < 0.0001) with a heterogeneity of Tau^2 0.11, χ^2 43.44, DF: 19, and Z = 4.89. The pooled RR and OR values were greater than 1, indicating a strong correlation between the incidence of AKI and COVID-19. This suggests that patients infected with COVID are at high risk of AKI and associated mortality.

Discussion

COVID-19, a coronavirus disease that predominantly impacts the respiratory system, has evolved into a global pandemic and is now widespread.[46] Acute kidney injury, which carries an increased risk of mortality, has been documented as a severe complication of COVID-19. Kidney impairment can manifest in a wide range of ways, including a gradual deterioration of renal function or complete cessation of kidney activity. The term 'AKI' denotes the sudden cessation of renal function.[47,48] The RIFLE classification is the optimal framework for defining AKI. The 3 progressive stages of AKI, as outlined in this system, are risk (class R), injury (class I) and

Table 3. Methodological assessment of the studies was conducted using the Assessment of Multiple Systematic Reviews (AMSTAR-2) evaluation

Study ID	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Overall assessment
Ali et al. [2020][21]	Y	Y	Y	PY	Y	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	moderate
Cai et al. [2021][22]	Y	PY	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Chen et al. [2020][23]	Y	PY	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Cheruiyot et al. [2020][24]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	high
Fabrizi et al. [2020][25]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	high
Hidayat et al. [2023][26]	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Hsiao et al. [2023][27]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Juarez et al. [2020][28]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Lee et al. [2021][29]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	high
Lim et al. [2020][30]	Y	N	Y	Y	PY	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	moderate
Liu et al. [2021][31]	Y	Y	Y	Y	Y	Y	Y	PY	Y	Y	Y	Y	Y	Y	Y	N	high
Menon et al. [2021][32]	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	PY	Y	Y	N	moderate
Passoni et al. [2022][33]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	high
Saghafi et al. [2021][34]	Y	Y	Y	Y	N	PY	Y	Y	Y	Y	Y	Y	Y	PY	Y	N	moderate
Shao et al. [2020][35]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Silver et al. [2020][36]	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	high
Singh et al. [2021][37]	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	high
Wang et al. [2021][38]	Y	N	Y	Y	PY	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	moderate
Yang et al. [2021][39]	Y	Y	Y	Y	Y	Y	Y	PY	Y	Y	Y	Y	Y	Y	Y	N	high
Zhou et al. [2020][40]	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	PY	Y	Y	N	moderate

AMSTAR-2 overall assessment rating
1. High – the review accurately and comprehensively summarizes the studies on the topic.
2. Moderate – the review has multiple problems but no major ones. It may accurately summarize study outcomes.
3. Low – the review has a critical flaw and may not provide an accurate and comprehensive summary of the studies that address the question of interest.
4. Critically low – the review has multiple critical flaws and should not be relied on.

Questions:
N – no; PY – partial yes, Y – yes.
Q1: Did the research questions and inclusion criteria for the review include the components of PICO?
Q2: Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?
Q3: Did the review authors explain their selection of the study designs for inclusion in the review?
Q4: Did the review authors use a comprehensive literature search strategy?
Q5: Did the review authors perform study selection in duplicate?
Q6: Did the review authors perform data extraction in duplicate?
Q7: Did the review authors provide a list of excluded studies and justify the exclusions?
Q8: Did the review authors describe the included studies in adequate detail?
Q9: Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?
Q10: Did the review authors report on the sources of funding for the studies included in the review?
Q11: If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?
Q12: If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?
Q13: Did the review authors account for RoB in individual studies when interpreting/discussing the results of the review?
Q14: Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?
Q15: If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?
Q16: Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?

failure (class F).[49] An increasing body of data indicates that AKI, even when moderate and transitory, is linked to the onset of chronic renal illness and a higher likelihood of long-term death in COVID-19 patients.[50–52] Moreover, acute renal damage imposes substantial economic burdens in addition to its detrimental health consequences, prolonged hospital stays, admission to an intensive care unit (ICU), and the necessity for renal replacement therapy (RRT).[53–55]

The present study identified a strong correlation between AKI and mortality in patients diagnosed with COVID-19. Additionally, the study identified key risk factors for AKI development in these patients.

The survival rate of patients with AKI and COVID-19 was determined by Ali et al.,[21] who performed a comprehensive review and meta-analysis of 6 trials. No evidence of publication bias was found (Egger's test: $p < 0.05$).

Table 4. Summary estimates of the included studies

Study ID	Outcome	Number of studies	OR (95% CI)	I^2 [%]	p-heterogeneity
Ali et al.[21]	RR	6	3.08 (1.54–6.19)	90	<0.001
Cai et al.[22]	OR	38	1.37 (1.25–1.49)	40	<0.001
Chen et al.[23]	I (%)	20	8.9 (4.6–14.5)	97.8	<0.001
Cheruiyot et al.[24]	OR	15	23.9 (18.84–30.31)	0	0.47
Fabrizi et al.[25]	OR	39	15.4 (11.4–20.99)	97.26	<0.001
Hidayat et al.[26]	OR	30	3.24 (2.20–4.79)	74	<0.001
Hsiao et al.[27]	OR	12	1.67 (1.56–1.80)	92.42	<0.01
Juarez et al.[28]	OR	20	15.27 (4.82–48.36)	97.9	<0.001
Lee et al.[29]	OR	16	1.68 (1.19–2.36)	79.65	<0.01
Lim et al.[30]	RR	15	13.38 (8.15, 21.95)	24	<0.001
Liu et al.[31]	OR	36	13.29 (4.69–41.26)	0	<0.001
Menon et al.[32]	I (%)	20	11 (0.07–0.15)	98	<0.01
Passoni et al.[33]	I (%)	28	50.4 (17.0–83.9)	98.9	<0.005
Saghafi et al.[34]	I (%)	22	24 (17–31%)	97	<0.01
Shao et al.[35]	OR	40	14.63 (9.94–21.51)	55	<0.00001
Silver et al.[36]	I (%)	54	28 (22–34)	99	<0.001
Singh et al.[37]	OR	29	8.28 (4.42–15.52)	73	<0.00001
Wang et al.[38]	OR	42	30.46 (18.33–50.59)	98.3	<0.001
Yang et al.[39]	I (%)	58	12.3 (9.5–15.6)	97	<0.001
Zhou et al.[40]	I (%)	37	11.43 (6.93–16.94)	95	<0.001

RR – relative risk of AKI; OR – odds ratio of AKI; I2 [%] – percentage of incidence of AKI; AKI – acute kidney injury; 95% CI – 95% confidence interval.

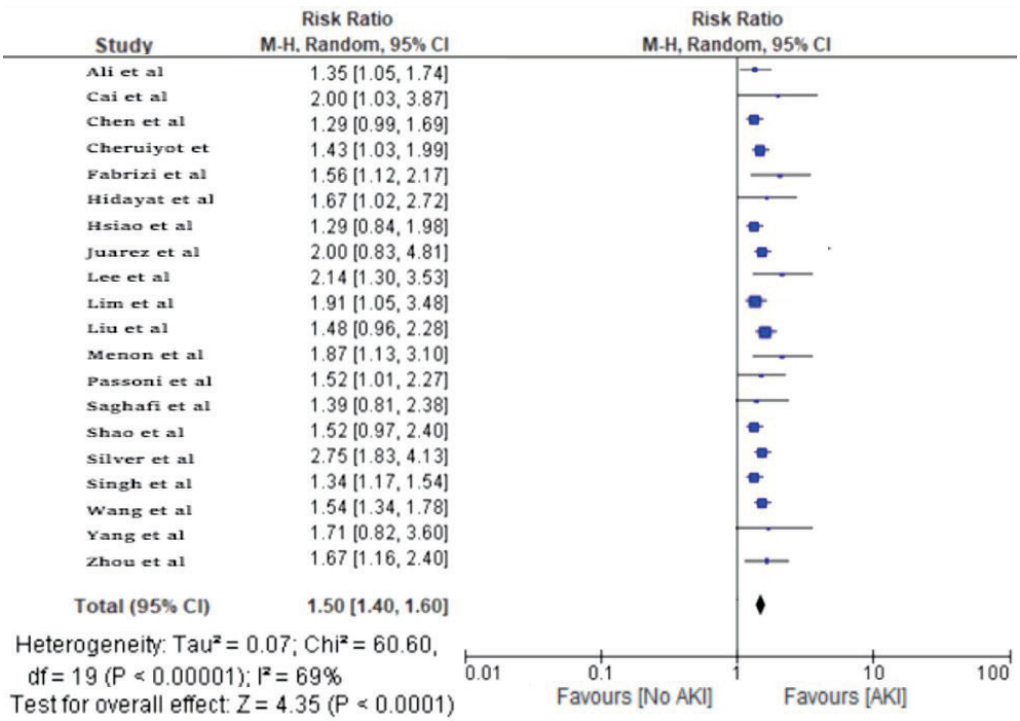

Fig. 3. Forest plot for relative risk (RR) of acute kidney injury (AKI) in COVID-19 patients

Severe AKI was associated with a higher risk of mortality (95% CI: 3.08; 1.54–6.19). Cai et al.[22] conducted a comprehensive review and meta-analysis of 38 studies involving 42,779 participants to assess the parameters linked to AKI in adult COVID-19 patients. The scientists discovered several important risk factors for AKI. These risk factors included advanced age (mean difference = 5.63), male gender (OR = 1.37), smoking (OR = 1.23), obesity (OR = 1.12), hypertension (OR = 1.85), diabetes (OR = 1.71), pneumopathy (OR = 1.36), cardiovascular disease (OR = 1.98), cancer

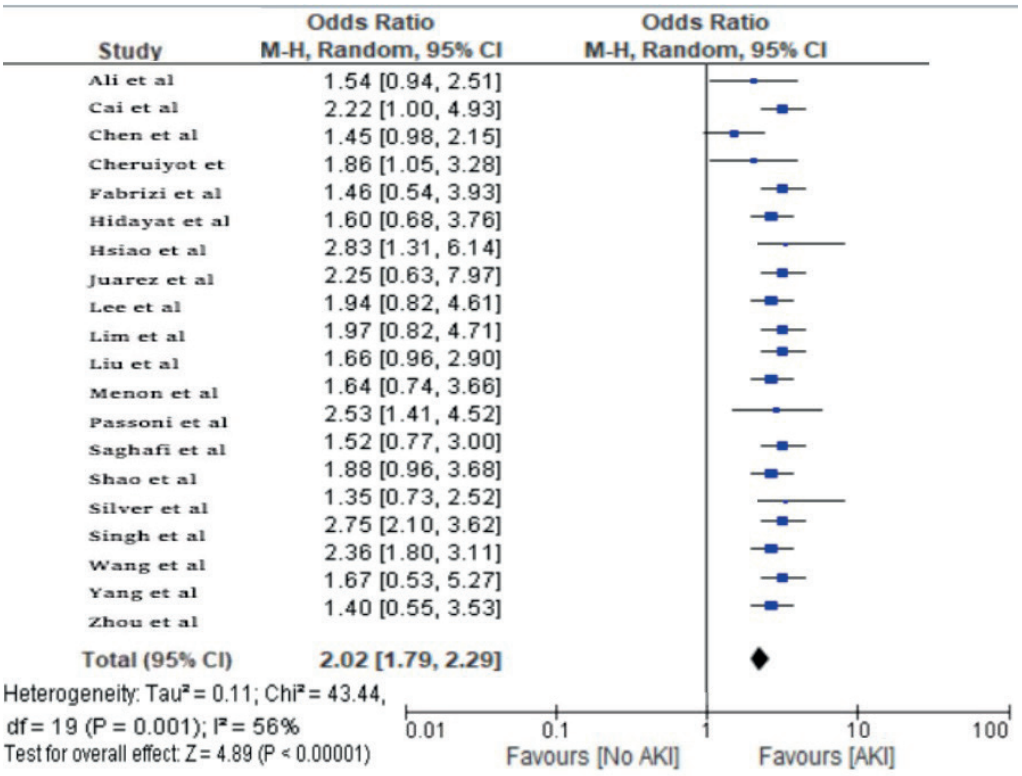

Fig. 4. Forest plot for odds ratio (OR) of mortality due to acute kidney injury (AKI) in COVID-19 patients

(OR = 1.26), chronic kidney disease (CKD) (OR = 4.56), mechanical ventilation (OR = 8.61), and the use of vasopressors (OR = 8.33).

In a comprehensive review and meta-analysis of 20 articles involving 6,945 patients, Chen et al.[23] examined the occurrence of AKI in individuals with COVID-19 infections. The occurrence of AKI in patients with COVID-19 was 8.9%, with a 95% CI of 4.6–14.5. The statistical heterogeneity of the studies was demonstrated by the fact that the I^2 value was 97.8% and the p-value was less than 0.001. Acute kidney injury was claimed to have occurred in approx. 9/100 COVID-19 individuals, according to the analysis of Chen et al.[23]

In a comprehensive review and meta-analysis of 15 studies involving 5,832 patients, Cheruiyot et al.[24] did a thorough review and meta-analysis of 15 studies with 5,832 patients to explore the association between AKI and adverse outcomes in COVID-19 patients. Acute kidney injury was found to be substantially linked with increased odds of COVID-19 severity (OR = 18.5; 95% CI: 8.99–38.08) and death (OR = 23.9; 95% CI: 18.84–30.31). With Cochran's Q = 4.56, p = 0.47 and I^2 = 0%, and Cochran's Q = 6.21, p = 0.52 and I^2 = 0%, respectively, for the 2 outcomes, it was concluded that AKI was associated with a poorer outcome in COVID-19 individuals.

Fabrizi et al.[25] carried out a comprehensive review and meta-analysis of 39 clinical investigations, which included a total of 25,566 patients. There was a significant amount of variability (p = 0.0001) in the aggregated incidence of AKI across all of the trials, which was 0.154 (95% CI: 0.107; 0.201; p < 0.0001). This study did not find any instances of publication bias, as determined with Egger's test (p = 0.11). The prevalence of COVID-19-positive individuals undergoing RRT was 0.043 (95% CI: 0.031–0.055; p < 0.0001). Acute kidney injury occurrence was significantly associated with age (p < 0.007) and arterial hypertension (p < 0.001) in hospitalized patients diagnosed with COVID-19.

Hidayat et al.[26] conducted a meta-analysis of 30 studies involving 22,385 confirmed COVID-19 patients to examine the risk factors and clinical characteristics of AKI in COVID-19 patients. Patients diagnosed with AKI exhibited proteinuria (OR = 3.31; 2.59; 4.33) and hematuria (OR = 3.25; 2.59; 4.08), as well as the need for invasive mechanical ventilation (OR = 13.88; 8.23; 23.40). Diabetes, hypertension, ischemic cardiac disease, heart failure, CKD, chronic obstructive pulmonary disease (COPD), peripheral vascular disease, and a history of using nonsteroidal anti-inflammatory drugs (NSAIDs) are all factors that enhance the risk of AKI in male COVID-19 patients.

Hsiao et al.[27] conducted a comprehensive study of 12 retrospective cohort studies, including 17,618 hospitalized patients with influenza and COVID-19. This study aimed to investigate the parallels and distinctions in acute renal impairment across patients with influenza and COVID-19. Patients with COVID-19 had a greater incidence of AKI compared to influenza patients with AKI (29.37% vs 20.98%, OR = 1.67, 95% CI: 1.56–1.80, p < 0.01, I^2 = 92.42%), as well as a higher in-hospital mortality rate (30.95% vs 5.51%, OR: 8.16, 95% CI: 6.17–10.80, p < 0.01, I^2 = 84.92%). Juarez et al.[28] investigated 20 cohorts that included a total of 13,137 individuals. The majority

of these patients were hospitalized and diagnosed with COVID-19. It was established that 17% of patients with AKI had developed severe COVID-19 infections, and 52% of those patients died. The prevalence of AKI was found to be 17%. Despite the fact that there was a significant amount of variation between studies and locales, AKI was found to be related to an elevated risk of mortality among COVID-19 patients (pooled OR = 15.27; 95% CI: 4.82–48.36). Their findings indicated that RRT was necessary for approx. 5% of all patients.

In a comprehensive study and meta-analysis, Lee et al.[29] examined the increased risk of AKI in patients with coronavirus infection who were using renin–angiotensin–aldosterone pathway (RAAS) blockers. According to their analysis of 14 studies that included 17,876 patients, the utilization of RAAS blockers was substantially linked with an increased risk of AKI in hospitalized COVID-19 patients (OR = 1.68; 95% CI: 1.19–2.36). Regarding multiorgan failure, more notably AKI and the severity of COVID-19, Lim et al.[30] carried out a comprehensive study that included 3,615 patients who were the participants of 15 different investigations. It was documented by the researchers that AKI was associated with a higher composite outcome (RR [7.68, 14.50], $p < 0.001$; I^2: 0%), an increased mortality rate (RR: 13.38 [8.15, 21.95], $p < 0.001$; I^2: 24%), severe COVID-19 (RR: 8.12 [4.43, 14.86], $p < 0.001$; I^2: 0%), and the need for ICU treatment (RR: 5.90 [1.32, 26.35], $p = 0.02$; I^2: 0%).

An investigation of the role that acute renal damage and CKD in the COVID-19 pandemic was the focus of a comprehensive review and meta-analysis that was carried out by Liu et al.[31] The research project included a total of 36 trials and included 6,395 patients who were diagnosed with COVID-19. The severity group had significantly higher odds of having preexisting CKD (OR = 3.28), complications of AKI (OR = 11.02), abnormal serum creatinine (OR = 4.86), blood urea nitrogen (BUN; standard mead difference (SMD) = 1.95), and continuous RRT (OR = 23.63) compared to the non-severe group, according to the findings of their overall analysis.

Menon et al.[32] conducted a study in which they investigated the relationship between AKI, the severity of the disease and mortality from COVID-19. A total of 14,415 patients participated in the trial, and they were divided into 20 different groups. There were 3,820 patients from the total who experienced AKI. This is equivalent to a pooled prevalence of 11% (95% CI: 0.07–0.15; $p = 0.01$; $I^2 = 98\%$). A pooled OR of 8.45 (95% CI: 5.56–12.56; $p < 0.00001$; $I^2 = 0\%$) suggested that there was a significant link between AKI and severe COVID-19 disease. Such a correlation was detected. Patients diagnosed with COVID-19 and experiencing AKI had a significantly elevated risk of mortality, as evidenced by an OR of 13.52 (95% CI: 5.43–33.67; $p < 0.00001$; $I^2 = 88\%$).

The incidence of AKI in adult patients who were hospitalized with COVID-19 was the focus of a comprehensive review and meta-analysis that was conducted by Passoni et al.[33] Researchers examined information obtained from 28 studies that included a total of 18,043 adult patients diagnosed with COVID-19. According to their report, the overall incidence estimates for AKI were 9.2% (4.6–13.9), while the critical care unit incidence estimates were 32.6% (8.5–56.6). The estimated death rate from AKI was 50.4% (17–83.9), whereas the estimated incidence of patients requiring RRT was 3.2% (range: 1.1–5.4). Saghafi et al.[34] focused on AKI in COVID-19 patients hospitalized in Iran. They performed a systematic review and meta-analysis of 4,069 verified cases, with ages ranging from 10 to 94, extracted from 22 distinct investigations. Acute kidney injury was shown to occur in 24% (95% CI: 17–31%) of hospitalized patients in Iran who were infected with COVID-19. Shao et al.[35] conducted a study that established a correlation between severe infections, mortality and AKI in patients infected with COVID-19. The conclusions drawn were derived from the results of 40 studies comprising a total of 24,527 patients. They discovered that the frequency of AKI in individuals infected with COVID-19 was 10% (95% CI: 8–13%). Patients who were infected with COVID-19 had considerably greater rates of severe illness and mortality compared to those who were not infected with the virus (55.6% vs 17.7% and 63.1% vs 12.9%, respectively, all $p < 0.01$). In patients diagnosed with COVID-19, AKI was found to be a significant predictor of both death (OR = 14.63, 95% CI: 9.94–21.51, p-value < 0.00001) and severe infections (OR = 8.11, 95% CI: 5.01–13.13, p-value < 0.00001).

Silver et al.[34] conducted a study comprised of 30,639 participants sourced from 54 distinct studies. The purpose of their investigation was to ascertain the incidence of AKI across hospitalized patients infected with COVID-19. The pooled prevalence of RRT was 9% (95% CI: 7–11%; $I^2 = 97\%$), and AKI occurred at a rate of 28% (95% CI: 22–34%; $I^2 = 99\%$).

Singh et al.[37] conducted a study of the pertinent literature concerning the association between AKI and the severity of COVID-19 disease. These analyses encompassed a cohort of 39 investigations, which comprised 15,017 positive COVID-19 patients. The total prevalence of AKI was 11.6% (430/3693), concomitant CKD was 9.7% (1,342/13,728), and the utilization of RRT was 2.58% (102/3,946). Drawing from the aforementioned results, it was determined that the severity of COVID-19 disease was significantly related to concurrent CKD, AKI and RRT use.

A meta-analysis was undertaken by Wang et al.[38] regarding the correlation between AKI and CKD and the sternness of illness and fatality among COVID-19 patients. The meta-analysis encompassed 42 studies comprising a total of 8,932 participants. In patients infected with COVID, AKI was linked to a significantly elevated probability of progression to severe disease (OR = 11.88, 95% CI: 9.29–15.19) or death (OR = 30.46, 95% CI: 18.33–50.59).

To examine the association between RRT and AKI in patients inflicted with COVID-19, Yang et al.[39] conducted a meta-analysis involving 21,531 patients. Acute kidney injury was observed in 12.3% (95% CI: 9.5–15.6%) of the total study population. Among the 1,745 patients who had passed away, the incidence was 42.0% (95% CI: 30.3–54.9%), while it was 38.9% (95% CI: 27.3–51.9%) among 290 kidney transplant patients and 39.0% (95% CI: 23.2–57.6%) among 565 ICU patients.

Chronic kidney disease and AKI in COVID-19 patients were the subject of the analysis conducted by Zhou et al.[40] including 52 studies and a total of 31,164 individuals. It was established that the composite values for the prevalence of AKI were 11.46% (95% CI: 6.93–16.94%). Both the risk of mortality (45.79, 36.88–56.85; I^2 = 17%) and the incidence of severe cases were substantially greater among patients with AKI (OR = 6.97; 95% CI: 3.53–13.75; interaction coefficient = 0%).

The statistical analysis of the current umbrella review demonstrates that patients infected with COVID-19 have an increased likelihood of developing AKI. The pooled RR of AKI incidence in COVID-19 patients is greater than 1, with an observed RR of 1.50 (95% CI: 1.40–1.60). Likewise, the probability of mortality associated with AKI is significantly increased in patients inflicted with COVID-19, as indicated by an OR value of 2.02 (95% CI: 1.79–2.29). Furthermore, the study revealed a correlation between AKI and an increased prevalence of CKD and mortality. Nevertheless, the majority of information was obtained from heterogeneous research of moderate quality. Therefore, it is imperative to conduct well-executed research endeavors covering a wider geographical area and conduct thorough systematic reviews accompanied by rigorous meta-analyses to obtain vital insights in this field.

Limitations

The present investigation is not without inherent limitations. It is crucial to acknowledge that while we adhered to recent guidelines concerning the optimal databases for umbrella reviews encompassing AKI, CKD, prognosis, aggravating variables, incidence/prevalence of AKI, and kidney transplant recipients infected with COVID-19, we might have overlooked other noteworthy considerations. These include the potential for CKD progression and non-recovery of AKI during post-acute COVID-19. Moreover, this umbrella review is primarily comprised of systematic reviews that include research articles exhibiting a moderate-to-high degree of bias. In addition, retrospective observational, cross-sectional and case-series research designs, which are all susceptible to residual confounding and influence temporal associations, provided the majority of the conclusions in these reviews. Furthermore, evaluations of the risk of bias indicated substantial variation in the quality of the studies, with specific interventions exhibiting particularly pronounced disparities in quality. Additionally, probability estimates could be affected by selection biases. Moreover, it is critical to acknowledge the potential existence of selection bias in this study, given that a substantial proportion of papers were excluded. Therapeutic possibilities for COVID-19 patients who suffered from kidney impairments were subsequently not examined, as this topic was outside the purview of the current umbrella review. To gain an improved comprehension of the overall incidence and possibility of AKI in COVID-19-infected patients, further research is required.

Conclusions

It is evident from the available evidence that AKI is a prevalent complication among individuals infected with COVID-19. Substantial risk factors associated with AKI in SARS-CoV-2-infected individuals included progressive age, male gender, coronary artery disease, hypertension, and diabetes. Acute kidney injury and the requirement for RRT were both autonomous predictors of adverse COVID-19 outcomes. It is critical to conduct additional research to ascertain the specific effects of different SARS-CoV-2 variants on the kidneys and the long-term prognosis of these individuals. As a result, future high-quality research studies must incorporate meticulous meta-analyses and systematic reviews to acquire insightful information in this area.

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References

1. Velavan TP, Meyer CG. The COVID-19 epidemic. *Trop Med Int Health*. 2020;25(3):278–280. doi:10.1111/tmi.13383
2. Baloch S, Baloch MA, Zheng T, Pei X. The coronavirus disease 2019 (COVID-19) pandemic. *Tohoku J Exp Med*. 2020;250(4):271–278. doi:10.1620/tjem.250.271
3. Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomed*. 2020;91(1):157–160. doi:10.23750/abm.v91i1.9397
4. Da Rosa Mesquita R, Francelino Silva Junior LC, Santos Santana FM, et al. Clinical manifestations of COVID-19 in the general population: Systematic review. *Wien Klin Wochenschr*. 2021;133(7–8):377–382. doi:10.1007/s00508-020-01760-4
5. Healey Q, Sheikh A, Daines L, Vasileiou E. Symptoms and signs of long COVID: A rapid review and meta-analysis. *J Glob Health*. 2022;12:05014. doi:10.7189/jogh.12.05014
6. Sanyaolu A, Okorie C, Marinkovic A, et al. Comorbidity and its impact on patients with COVID-19. *SN Compr Clin Med*. 2020;2(8):1069–1076. doi:10.1007/s42399-020-00363-4
7. Gęca T, Wojtowicz K, Guzik P, Góra T. Increased risk of COVID-19 in patients with diabetes mellitus: Current challenges in pathophysiology, treatment and prevention. *Int J Environ Res Public Health*. 2022;19(11):6555. doi:10.3390/ijerph19116555
8. Bigdelou B, Sepand MR, Najafikhoshnoo S, et al. COVID-19 and preexisting comorbidities: Risks, synergies, and clinical outcomes. *Front Immunol*. 2022;13:890517. doi:10.3389/fimmu.2022.890517
9. Fitero A, Bungau SG, Tit DM, et al. Comorbidities, associated diseases, and risk assessment in COVID-19: A systematic review. *Int J Clin Pract*. 2022;2022:1571826. doi:10.1155/2022/1571826

10. Gupta A, Marzook H, Ahmad F. Comorbidities and clinical complications associated with SARS-CoV-2 infection: An overview. *Clin Exp Med*. 2022;23(2):313–331. doi:10.1007/s10238-022-00821-4
11. Bansode J, Sayed S, Ahmad S, Sinha S, Swami R, Mehta K. Acute kidney injury in COVID-19: Clinical profile and outcome. *Indian J Nephrol*. 2022;32(4):291. doi:10.4103/ijn.IJN_21_21
12. Aroca-Martínez G, Musso CG, Avendaño-Echavez L, et al. Differences between COVID-19-induced acute kidney injury and chronic kidney disease patients. *Braz J Nephrol*. 2022;44(2):155–163. doi:10.1590/2175-8239-jbn-2021-0161
13. Faist A, Janowski J, Kumar S, et al. Virus infection and systemic inflammation: Lessons learnt from COVID-19 and beyond. *Cells*. 2022; 11(14):2198. doi:10.3390/cells11142198
14. Hall MW, Joshi I, Leal L, Ooi EE. Immune immunomodulation in coronavirus disease 2019 (COVID-19): Strategic considerations for personalized therapeutic intervention. *Clin Infect Dis*. 2022;74(1):144–148. doi:10.1093/cid/ciaa904
15. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. *Compr Physiol*. 2012;2(2):1303–1353. doi:10.1002/cphy.c110041
16. Hirsch JS, Ng JH, Ross DW, et al. Acute kidney injury in patients hospitalized with COVID-19. *Kidney Int*. 2020;98(1):209–218. doi:10.1016/j.kint.2020.05.006
17. Al Abri SY, Burad J, Al Wahaibi MM. The incidence of acute kidney injury (AKI) in critically ill COVID-19 patients: A single-center retrospective cohort study at a tertiary level hospital in Oman. *Cureus*. 2023;15(6):e40340. doi:10.7759/cureus.40340
18. Kolhe NV, Fluck RJ, Selby NM, Taal MW. Acute kidney injury associated with COVID-19: A retrospective cohort study. *PLoS Med*. 2020; 17(10):e1003406. doi:10.1371/journal.pmed.1003406
19. Procaccini FL, Alcázar Arroyo R, Albalate Ramón M, et al. Acute kidney injury in 3182 patients admitted with COVID-19: A single-center, retrospective, case-control study. *Clin Kidney J*. 2021;14(6):1557–1569. doi:10.1093/ckj/sfab021
20. Wan YI, Bien Z, Apea VJ, et al. Acute kidney injury in COVID-19: Multicentre prospective analysis of registry data. *Clin Kidney J*. 2021;14(11): 2356–2364. doi:10.1093/ckj/sfab071
21. Ali H, Daoud A, Mohamed MM, et al. Survival rate in acute kidney injury superimposed COVID-19 patients: A systematic review and meta-analysis. *Ren Fail*. 2020;42(1):393–397. doi:10.1080/0886022X.2020.1756323
22. Cai X, Wu G, Zhang J, Yang L. Risk factors for acute kidney injury in adult patients with COVID-19: A systematic review and meta-analysis. *Front Med (Lausanne)*. 2021;8:719472. doi:10.3389/fmed.2021.719472
23. Chen YT, Shao SC, Hsu CK, Wu IW, Hung MJ, Chen YC. Incidence of acute kidney injury in COVID-19 infection: A systematic review and meta-analysis. *Crit Care*. 2020;24(1):346. doi:10.1186/s13054-020-03009-y
24. Cheruiyot I, Henry B, Lippi G, et al. Acute kidney injury is associated with worse prognosis in COVID-19 patients: A systematic review and meta-analysis. *Acta Biomed*. 2020;91(3):e2020029. doi:10.23750/abm.v91i3.10222
25. Fabrizi F, Alfieri CM, Cerutti R, Lunghi G, Messa P. COVID-19 and acute kidney injury: A systematic review and meta-analysis. *Pathogens*. 2020;9(12):1052. doi:10.3390/pathogens9121052
26. Hidayat AA, Gunawan VA, Iragama FR, et al. Risk factors and clinical characteristics of acute kidney injury in patients with COVID-19: A systematic review and meta-analysis. *Pathophysiology*. 2023;30(2): 233–247. doi:10.3390/pathophysiology30020020
27. Hsiao CY, Pan HC, Wu VC, et al. Acute kidney injury in patients with COVID-19 compared to those with influenza: A systematic review and meta-analysis. *Front Med (Lausanne)*. 2023;10:1252990. doi:10.3389/fmed.2023.1252990
28. Robbins-Juarez SY, Qian L, King KL, et al. Outcomes for patients with COVID-19 and acute kidney injury: A systematic review and meta-analysis. *Kidney Int Rep*. 2020;5(8):1149–1160. doi:10.1016/j.ekir.2020.06.013
29. Lee SA, Park R, Yang JH, et al. Increased risk of acute kidney injury in coronavirus disease patients with renin–angiotensin–aldosterone-system blockade use: A systematic review and meta-analysis. *Sci Rep*. 2021;11(1):13588. doi:10.1038/s41598-021-92323-8
30. Lim MA, Pranata R, Huang I, Yonas E, Soeroto AY, Supriyadi R. Multiorgan failure with emphasis on acute kidney injury and severity of COVID-19: Systematic review and meta-analysis. *Can J Kidney Health Dis*. 2020;7:205435812093857. doi:10.1177/2054358120938573
31. Liu YF, Zhang Z, Pan XL, et al. The chronic kidney disease and acute kidney injury involvement in COVID-19 pandemic: A systematic review and meta-analysis. *PLoS One*. 2021;16(1):e0244779. doi:10.1371/journal.pone.0244779
32. Menon T, Sharma R, Kataria S, et al. The association of acute kidney injury with disease severity and mortality in COVID-19: A systematic review and meta-analysis. *Cureus*. 2021;13(3):e13894. doi:10.7759/cureus.13894
33. Passoni R, Lordani TVA, Peres LAB, Carvalho ARDS. Occurrence of acute kidney injury in adult patients hospitalized with COVID-19: A systematic review and meta-analysis. *Nefrologia (Engl Ed)*. 2022; 42(4):404–414. doi:10.1016/j.nefroe.2022.11.005
34. Saghafi A, Aghaali M, Saghafi H. Acute kidney injury in hospitalized COVID-19 patients in Iran: A systematic review and meta-analysis. *J Renal Inj Prev*. 2021;10(2):e09. doi:10.34172/jrip.2021.09
35. Shao M, Li X, Liu F, Tian T, Luo J, Yang Y. Acute kidney injury is associated with severe infection and fatality in patients with COVID-19: A systematic review and meta-analysis of 40 studies and 24,527 patients. *Pharmacol Res*. 2020;161:105107. doi:10.1016/j.phrs.2020.105107
36. Singh J, Malik P, Patel N, et al. Kidney disease and COVID-19 disease severity: Systematic review and meta-analysis. *Clin Exp Med*. 2022; 22(1):125–135. doi:10.1007/s10238-021-00715-x
37. Silver SA, Beaubien-Souligny W, Shah PS, et al. The prevalence of acute kidney injury in patients hospitalized with COVID-19 infection: A systematic review and meta-analysis. *Kidney Med*. 2021;3(1):83–98.e1. doi:10.1016/j.xkme.2020.11.008
38. Wang B, Luo Q, Zhang W, et al. The involvement of chronic kidney disease and acute kidney injury in disease severity and mortality in patients with COVID-19: A meta-analysis. *Kidney Blood Press Res*. 2021;46(1):17–30. doi:10.1159/000512211
39. Yang X, Tian S, Guo H. Acute kidney injury and renal replacement therapy in COVID-19 patients: A systematic review and meta-analysis. *Int Immunopharmacol*. 2021;90:107159. doi:10.1016/j.intimp.2020.107159
40. Zhou Y, Ren Q, Chen G, et al. Chronic kidney diseases and acute kidney injury in patients with COVID-19: Evidence from a meta-analysis. *Front Med (Lausanne)*. 2020;7:588301. doi:10.3389/fmed.2020.588301
41. Belbasis L, Bellou V, Ioannidis JPA. Conducting umbrella reviews. *BMJ Med*. 2022;1(1):e000071. doi:10.1136/bmjmed-2021-000071
42. Pollock M, Fernandes RM, Pieper D, et al. Preferred Reporting Items for Overviews of Reviews (PRIOR): A protocol for development of a reporting guideline for overviews of reviews of healthcare interventions. *Syst Rev*. 2019;8(1):335. doi:10.1186/s13643-019-1252-9
43. Shea BJ, Reeves BC, Wells G, et al. AMSTAR 2: A critical appraisal tool for systematic reviews that include randomised or non-randomised studies of healthcare interventions, or both. *BMJ*. 2017;358:j4008. doi:10.1136/bmj.j4008
44. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *BMJ*. 2009;339:b2700. doi:10.1136/bmj.b2700
45. Schardt C, Adams MB, Owens T, Keitz S, Fontelo P. Utilization of the PICO framework to improve searching PubMed for clinical questions. *BMC Med Inform Decis Mak*. 2007;7(1):16. doi:10.1186/1472-6947-7-16
46. Van Damme W, Dahake R, Delamou A, et al. The COVID-19 pandemic: Diverse contexts, different epidemics. How and why? *BMJ Glob Health*. 2020;5(7):e003098. doi:10.1136/bmjgh-2020-003098
47. Turgut F, Awad A, Abdel-Rahman E. Acute kidney injury: Medical causes and pathogenesis. *J Clin Med*. 2023;12(1):375. doi:10.3390/jcm12010375
48. Mercado MG, Smith DK, Guard EL. Acute kidney injury: Diagnosis and management. *Am Fam Physician*. 2019;100(11):687–694. PMID:31790176.
49. Venkataraman R, Kellum JA. Defining acute renal failure: The RIFLE criteria. *J Intensive Care Med*. 2007;22(4):187–193. doi:10.1177/0885066607299510

50. Schiffl H, Lang SM. Long-term interplay between COVID-19 and chronic kidney disease. *Int Urol Nephrol*. 2023;55(8):1977–1984. doi:10.1007/s11255-023-03528-x
51. Chung EYM, Palmer SC, Natale P, et al. Incidence and outcomes of COVID-19 in people with CKD: A systematic review and meta-analysis. *Am J Kidney Dis*. 2021;78(6):804–815. doi:10.1053/j.ajkd.2021.07.003
52. Khoshdel-Rad N, Zahmatkesh E, Shpichka A, Timashev P, Vosough M. Outbreak of chronic renal failure: Will this be a delayed heritage of COVID-19? *J Nephrol*. 2021;34(1):3–5. doi:10.1007/s40620-020-00851-9
53. Ostermann M, Cerdá J. The burden of acute kidney injury and related financial issues. In: Ding X, Rosner MH, Ronco C, eds. *Acute Kidney Injury: Basic Research and Clinical Practice*. Vol. 193. Contributions to Nephrology. Berlin, Germany: Karger; 2018:100–112. doi:10.1159/000484967
54. Dasta JF, Kane-Gill S. Review of the literature on the costs associated with acute kidney injury. *J Pharm Pract*. 2019;32(3):292–302. doi:10.1177/0897190019852556
55. Altawalbeh SM, Wali LM, Alshogran OY, Hammad EA, Tahaineh L. Incidence, predictors, clinical outcomes, and economic burden of recurrent acute kidney injury: A retrospective cohort study. *Curr Med Res Opin*. 2023;39(3):399–407. doi:10.1080/03007995.2023.2175997