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Vitamin D_3 and Bone Remodeling in Children with Chronic Kidney Disease Treated Conservatively

Witamina D_3 a przebudowa kostna u dzieci z przewlekłą chorobą nerek leczonych zachowawczo

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Abstract

Background. One of the basic pathologies observed during the progression of chronic kidney disease (CKD) is changes in vitamin D_3 metabolism.
Objectives. The aim of this study was to compare the serum concentrations of calcidiol and calcitriol with selected exponents of parathyroid gland function and to assess bone density in children with CKD treated conservatively.
Material and Methods. The study was performed on 36 children and adolescents with CKD aged 4–18 years (average age: 12.4 years) and treated conservatively. The patients were divided into four groups according to the stage of disease (KI/DOQI 2003 division). Group I comprised 12 patients with CKD stage II, group III 12 patients with stage III, group IV 12 patients with stage IV, and a control group (group I) of 19 healthy children. Total calcium (Ca^{2+}), inorganic phosphorous (P_i), intact parathormone (iPTH), calcidiol ($25(OH)D_3$), and calcitriol ($1.25(OH)_2D_3$) were determined in the serum of all the subjects. Densitometry (total body, TB) was performed with a Lunar DPX-L system and the skeleton was investigated.
Results. The average serum concentrations of iPTH and P_i in the CKD patients treated conservatively were significantly higher than in the control group. Ca concentration in the CKD group was lower than in the controls, but the difference was not statistically significant. Average $1.25(OH)_2D_3$ concentrations in the group of children with CKD (3rd and 4th stage) were lower than in the controls. Average $25(OH)D_3$ concentrations were also decreased in the children with CKD compared with controls.
Conclusions. Decreased serum concentrations of vitamin D_3 are observed in children with CKD with moderate stages of renal failure. These disturbances increase with continuing impairment of glomerular renal filtration. With the impairment of renal function, active vitamin D_3 deficiency induces disturbances in calcium-phosphate metabolism, leading to changes in bone structure (**Adv Clin Exp Med 2008, 17, 2, 141–146**).

Key words: vitamin D_3, children, chronic kidney disease, bone mineral density.

Streszczenie

Wprowadzenie. Jedną z podstawowych patologii obserwowanych w przebiegu pogłębiania się przewlekłej choroby nerek (p.ch.n.) są zmiany z zakresu metabolizmu witaminy D_3.
Cel pracy. Ocena surowiczych stężeń kalcydiolu i kalcytriolu u dzieci z przewlekłą chorobą nerek leczonych zachowawczo w powiązaniu z wybranymi wykładnikami funkcji przytarczyc i oceną gęstości mineralnej kośćca.
Materiał i metody. Badaniami objęto 36 dzieci i młodzieży z p.ch.n. leczonych zachowawczo w wieku 4–18 lat (średnia wieku 12,4 lata). W zależności od stopnia zaawansowania choroby pacjenci zostali przydzieleni (kryteria podziału wg KI/DOQI 2003) do danej grupy. Grupa II – 12 chorych z p.ch.n. w II stadium choroby; grupa III – 12 chorych z p.ch.n. w III stadium choroby; grupa IV – 12 chorych z p.ch.n. w IV stadium choroby. Grupa kontrolna (grupa I) – 19 dzieci zdrowych. U wszystkich dzieci oznaczano w surowicy stężenie: wapnia całkowitego, fosforu nieorganicznego, *intact* parathormonu, kalcydiolu, kalcytriolu. Wykonano ponadto badanie densytome-

tryczne całego szkieletu (TB – *total body*) w projekcji AP z użycem aparatu LunarDPX-L firmy Lunar. W grupie pacjentów z p.ch.n. leczonych zachowawczo średnie wartości iPTH i P_i w surowicy krwi były znamiennie większe w porównaniu z grupą kontrolną. Stężenie Ca^{2+} w grupie dzieci z p.ch.n. było zmniejszone w stosunku do grupy I, ale bez istotności statystycznej. Średnie wartości stężenia $1.25(OH)_2D_3$ w grupie dzieci z p.ch.n. (grupa III, IV) były zmniejszone w porównaniu z grupą I. Zmniejszone również były średnie stężenia $25(OH)D_3$ u dzieci z p.ch.n. w porównaniu z grupą I.

Wnioski. U dzieci z p.ch.n. już w stadium umiarkowanej niewydolności nerek dochodzi do zmniejszenia stężenia metabolitów witaminy D_3 w surowicy. Zaburzenia te pogłębiają się przy dalszym upośledzeniu filtracji kłębuszkowej. Wraz z upośledzeniem czynności nerek niedobór witaminy D_3 skutkuje zaburzeniami gospodarki wapniowo-fosforanowej, doprowadzając do zmian w strukturze kostnej (**Adv Clin Exp Med 2008, 17, 2, 141–146**).

Słowa kluczowe: metabolity, witamina D_3, dzieci, przewlekła choroba nerek, densytometria.

In each period of human growth about 10–20% of all the bone mass undergoes rebuilding processes. The rate of rebuilding and the activity of bone cells is determined by several endo- and exogenous factors which become of great importance during chronic kidney disease. The factors which play a role in the regulation of bone remodeling are resorption-stimulating hormones, such as parathormone, calcitriol, glycocorticosteroids, and thyroid hormones, and the bone tissue reconstruction-stimulating hormones calcitonin, insulin, and estrogens [1]. The full cycle of rebuilding takes a couple of months and results in new "packets" in spongy bone and new Harvers systems in compact bone. Disturbances in bone rebuilding in any part of the bone rebuilding process in patients with chronic renal disease lead to loss of bone mass and progression of uremic osteopathy, which in these patients is primarily a result of the parathormone-vitamin D-magnesium and phosphorus ion axes [2]. Vitamin D_3 has an undisputed role in bone growth and mineralization processes through its activity on receptors located in different organs, i.e. the intestine, kidneys, bones, skin, and parathyroid glands [1, 3]. Under physiological conditions there are three main vitamin D_3 metabolites in the blood: 25-hydroxycholecalciferol [$25(OH)D_3$], 1,25-dihydroxycholecalciferol [$1,25(OH)_2D_3$], and 24,25- dihydroxycholecalciferol [$24,25(OH)_2D_3$], which, after conversion in the target tissue, are eliminated from the organism. $25(OH)D_3$ (calcidiol) undergoes hydroxilation in the 1 α position in the kidney, which leads to the creation of the most active form of vitamin D_3, $1,25(OH)_2D_3$. Parathormone, PTH-similar peptide, hypocalcemia, and hyperphosphatemia are among the most effective stimulators of calcitriol synthesis [1, 2], which is why the network of interrelationships between vitamin D_3, parathormone, calcium ions, and inorganic phosphorus ions in patients with chronic renal failure effects, among other things, bone remodeling disturbances.

The problem with changes in the skeletal system in patients during the progress of chronic renal failure already occurs with a creatinine clearance depression just under 60 ml/min/1.73 m^2. In children with chronic kidney disease, calcium-phosphate metabolism and, therefore, osteal balance influence the growing organism in a most unfavorable way [4]. To assess bone changes in this group of patients during the process of CKD, the most useful in everyday practice are biochemical parameters of calcium-phosphate metabolism and bone densitometric exercision.

The aim of this study was to determine the serum concentrations of calcidiol and calcitriol in children with CKD treated conservatively in relation to selected exponents of parathyroid gland function and to assess mineral bone density.

Material and Methods

The study was performed on 36 children and adolescents aged 4–18 years (mean: 12.4 years) with CKD treated conservatively. The patients were divided into four groups according to the stage of CKD (KI/DOQI 2003 division criteria [5]). Group II comprised 12 patients with CKD in stage II, group III 12 patients with stage III, group IV 12 patients in stage IV, and the control group (group I) was made up of 19 healthy children (mean age: 13.1 years). Total calcium (Ca^{2+}), inorganic phosphorous (P_i), intact parathormone (iPTH), calcidiol ($25(OH)D_3$, and calcitriol ($1.25(OH)_2D_3$) were determined in the serum of all subjects. Serum Ca^{2+} and P_i were tested by standard colorimetric methods (Boehringer Mannheim Systems). iPTH was tested with an immunoradiometric assay (IRMA) kit and $25(OH)D_3$ and $1.25(OH)_2D_3$ with a radioimmunoassay (RIA-CT) kit, all from BioSource Europe S.A.

Bone mass densitometry (BMD) of the total skeleton (TB, total body) in the anterior-posterior (AP) projection was carried out in all the children of groups III and IV using DEXA performed on Lunar DPX-L scanners (Lunar Company). The

BMD standard deviation score (Z-score) was calculated using age- and sex-matched control data and software supplied with the machine. It expresses the result in the form of concentrated bone mineral on a unit of surface (g/cm^2). A Z-score for BMD below 2 *SD* from the average (Z-score < –2.0) is indentified as osteoporosis (OP) and from < –1.0 to –2.0 as osteopenia (OPE) [6].

All of the patients with CKD in whom disturbances in Ca-P metabolism were observed were put on a phosphate-poor diet (phosphate intake did not exceed 500–800 mg/day) and were treated with compounds which bind phosphate in the intestine (calcium carbonate at a dose of 35–200 mg/kg/day). The doses of these therapeutic agents were individualized so that the serum concentration of phosphate was 4.5–5.2 mg/dl and of calcium 8.8–10.8 mg/dl. Dietary calcium intake was defined as 500–600 mg of elementary calcium per day. Additionally, patients with enhanced levels of iPTH were treated with vitamin D_3 analogues (Alphacalcidol, Polfa, Poland) in doses ranging from 0.25–1.2 µg/day. The subjects gave their informed consent and the study was approved by the local ethics committee.

Statistics

The results are expressed as mean values and standard deviation ($x \pm SD$). A value of $p < 0.05$ was considered statistically significant. Analysis of variance was used to analyze differences in the parameters. Post hoc comparison was made using the LSD Tukey test. Dependencies among the parameters were investigated using Pearson's correlation coefficient. Significance was tested with the *t* test.

Results

In the group of patients with CKD treated conservatively, the average serum iPTH and P_i levels were significantly higher than in the control group. The Ca^{2+} concentration in the CKD group was lower than in the healthy children. Mean $1.25(OH)_2D_3$ concentrations were lower in groups III and IV than in the healthy children, especially in group IV. There were no statistically significant differences between mean $25(OH)D_3$ concentrations in the CKD children compared with the healthy children, especially compared with group III and IV. The average $25(OH)D_3$ concentration in groups III and IV were statistically significantly different.

The correlation study showed statistically significant positive correlation between $1.25(OH)_2D_3$ and $25(OH)D_3$ ($r = 0.86$, $p < 0.01$), $1.25(OH)_2D_3$ and BMD ($r = 0.32$, $p < 0.12$) (Fig. 2), and $25(OH)D_3$ and BMD, but without statistical significance ($r = 0.21$, $p < 0.31$) (Fig. 1). Statistically significant negative correlation was observed between $1.25(OH)_2D_3$ and $25(OH)D_3$ and between iPTH, P_i, and creatinine.

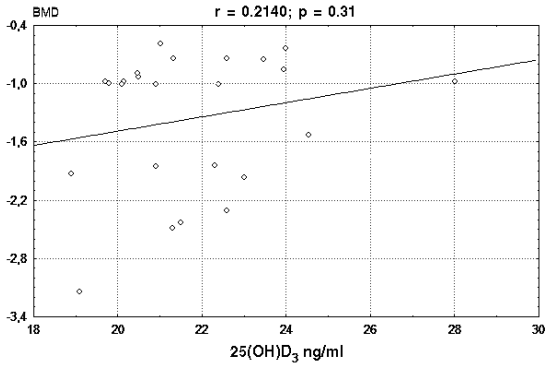

Fig. 1. Relationship between $25(OH)D_3$ and BMD in III and IV groups

Ryc. 1. Zależność między $25(OH)D_3$ a masą kostną w III i IV grupie

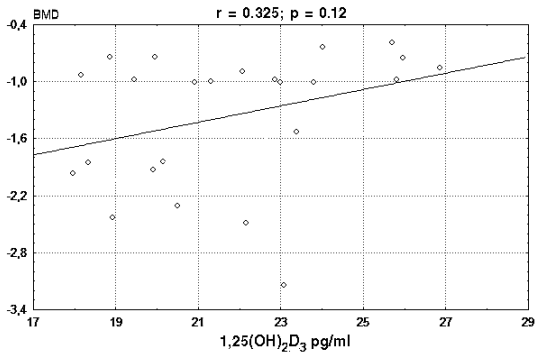

Fig. 2. Relationship between $1.25(OH)_2D_3$ and BMD in III and IV groups

Ryc. 2. Zależność między $1.25(OH)_2D_3$ a masą kostną w III i IV grupie

The BMD values of the group III patients indicated osteopenia in two patients and osteoporosis in one, while the BMDs of the remaining eight patients were within the range of the age norms. However, in group IV the BMD values of four children were characteristic of osteopenia and of three of osteoporosis, while the BMDs of the remaining patients were within the norm.

Discussion

During the progress of chronic kidney disease, uremic toxins begin to accumulate, among others PTH. In this study, the serum concentrations of PTH in children with stage II chronic kidney dis-

Table 1. Mean values (x ± SD) of biochemical parameters in the study groups

Tabela 1. Wartości średnie (x ± SD) wskaźników biochemicznych w badanych grupach

Groups (Grupy) / Parameters (Wskaźniki)	I – control (Grupa kontrolna) n = 19	II group (Grupa II) n = 12	III group (Grupa III) n = 12	IV group (Grupa IV) n = 12
25(OH)D_3 ng/ml	37.37 ± 2.99	36.61 ± 1.82	23.21 ± 1.62[a]	21.61 ± 1.98[b]
1.25$(OH)_2D_3$ pg/ml	31.75 ± 2.06	30.97 ± 1.64	25.48 ± 1.96[a]	21.46 ± 2.91[a]
Ca^{2+} mg/dl	9.45 ± 0.51	9.58 ± 0.58	8.79 ± 0.25[a]	8.38 ± 0.45[a]
P^{3+} mg/dl	4.79 ± 0.24	4.86 ± 0.87	5.48 ± 0.39	5.71 ± 0.46
cr mg/dl	0.76 ± 0.18	1.29 ± 0.17	1.83 ± 0.18	2.87 ± 0.26
iPTH pg/ml	27.56 ± 5.65	55.52 ± 13.45[a]	92.66 ± 42.65[b]	115.80 ± 22.25[b]
GRF ml/min	110.86 ± 9.43	76.28 ± 7.11 [b]	52.11 ± 4.27 [b]	24.72 ± 3.37 [b]

$p < 0.05^a$; $p < 0.01^b$.

$p < 0,05^a$; $p < 0,01^b$.

ease were within the normal range except for one patient. However, in the children with stages III and IV, a gradual accumulation of this hormone in the blood was noted. This excessive secretion of PTH in patients with chronic kidney disease, which has been described by other authors, is related, among others, to proliferation of the parathyroid glands, a reduction in the amount of active kidney parenchyma as the clearance organ for this hormone, hypocalcemia, hyperphosphatemia, and decreased 1.25$(OH)_2D_3$ secretion [1, 7, 8]. The study by Ishumura E. et al. in patients with chronic kidney disease treated conservatively also found elevated PTH blood concentrations in both diabetics and non-diabetics [9]. Moreover, there was no difference between these groups of patients. The biological results of excessive PTH secretion include an intensification of osteolytic processes over osteogenesis.

The observations of the present study concerning blood calcium levels clearly showed a reduction in children with stage IV compared with healthy children and the other patients with CKD. In the other patient groups the concentrations of this ion changed, but were within the limits of laboratory norms. Therefore, in children with stage II CKD a physiological mechanism probably occurred in which, after the binding of Ca^{2+} to the calcium receptor (Ca-R) through the activation of phospholipase C, there was an increase in Ca^{2+} in parathyroid gland cells and suppression the PTH secretion. According to Herbert and de Luca, the greatest expression of Ca-R is found in the large cortex section of the ascending limb of the loop of Henle [10, 11]. In children with advanced CKD (groups III and IV), disturbances in the PTH-Ca-P axis probably cannot be corrected by the afflicted organism.

The current study showed elevated blood levels of P_i in the children of groups III and IV which were statistically significant compared with group II patients and the control group. The elevated P_i concentrations were accompanied by high levels of PTH, which has also been confirmed by several researchers [1, 8]. The children of the present study were recommended low-phosphorous diet with phosphate binders (Renagel, Calcium carbonicum) the moment blood changes were noted in beyond the range of laboratory norms for calcium and phosphate concentrations in serum. In patients with stages III and IV CKD, a statistically significant negative correlation between P_i and 1.25$(OH)_2D_3$ was found.

Ishumura E. et al. obtained similar result in a population of patients with CKD treated conservatively [9]. According to Andress D. et al., accumulation of P1 in the blood already begins with GFR below 45 ml/min, which has a suppressive influence on the activity of alpha-hydroxylase, a kidney enzyme, and reduces the synthesis of 1.25$(OH)_2D_3$ from 25(OH)D_3 [12]. Andress et al. emphasized in their observations that even in second-stage CKD one can find increased serum PTH; however, significant increases in P_i concentration in the blood take place in the third and fourth stages of CKD. Furthermore, hyperphosphatemia, regardless of Ca concentration, causes an increase in PTH gene expression and cell proliferation of the parathyroid glands [2, 13].

During the process of increasing renal insufficiency, the degradation observed in the active renal parenchyma impairs calcidiol hydroxylation

in the alpha position in the proximal renal tubules. Suppression of this enzyme causes a drop in $1.25(OH)_2D_3$ synthesis, which was confirmed in the present study by the patients' $25(OH)D_3$ and $1.25(OH)_2D_3$ serum levels, especially in stage IV CKD. What is more, similarly to other studies, it was found that with the drop in GFR were decreased serum concentrations of $25(OH)D_3$ and $1.25(OH)_2D_3$. Ishimura E. et al. showed statistically significant positive correlation between $1.25(OH)_2D_3$ and GFR [9]. The most active stimulators of $1.25(OH)_2D_3$, besides PTH and PTHrP (PTH-similar peptide), are lowered Ca^{2+} and P_i serum levels. In the present study. lowered concentrations of $1.25(OH)_2D_3$ were coincident with hypocalcemy, hyperphosphatemy, and hyperparathyroidism. In patients with stage IV CKD, significant negative correlation between $1.25(OH)_2D_3$ and PTH, P_i was found. These observations are in accord with those made by other researchers [9, 14]. What is more, Ishimura E. et al. found in studies of patients in the pre-dialysis period no significant correlation between Ca and vitamin D_3 metabolites [9]. In the present study, only children with stage IV disease showed reduced blood concentrations of Ca, which was statistically significant compared with the other patients.

The natural pathomechanism of the progression of renal osteodystrophy is complex and multifactorial, but an absolute or relative $1.25(OH)_2D_3$ deficiency is regarded as the most important cause [14]. With regard to the histopathological structure of the bone, osteodystrophy is not a homogenous disease entity, and in children one mostly observes forms of renal osteodystrophy which are dependent on hyperparathyroidism. The assessment of osteoporotic changes is based on radiological imaging of the bone and also on densitometric examination. Quantitative estimation of the bone mineral density (BMD) allows the determination of Ca^{2+} shortage in the skeleton earlier than on x-ray images. In the patients with stage III disease, the BMD values indicated osteopenia in two patients and osteoporosis in one, while the BMDs of the other eight patients were within the normal range for their age. However, in group IV the BMDs of four children were characteristic of osteopenia and in three of osteoporosis, while the remainder had normal BMDs. It is supposed that these lowered BMDs were caused not only by the osteolytic activity of PTH, but also by vitamin D. According to Ljunghall et al., $1.25(OH)_2D_3$ activity is about 100 times more destructive on the skeletal system than $25(OH)D_3$ [15]. The lowered blood levels of $1.25(OH)_2D_3$ found in the present study, which were accompanied by PTH hypersecretion, doubtlessly caused changes in bone rebuilding, and bone tissue defects were verified. Densitometric examination allows monitoring bone mineral density, which is significant for pharmacotherapy. The pathogenesis of CKD contains multilevel changes for which pharmacological therapy can have a beneficial effect for a patient. An example of this is vitamin D_3 supplementation; where its anabolic influence on bone tissue counteracts the progression of osteoporosis, "the silent bone thief".

The authors conclude that decreased serum concentrations of vitamin D_3 were observed in children with CKD with moderate stages of renal failure. These disturbances increased with continued impairment of glomerular renal filtration. With the impairment of renal function, active vitamin D_3 deficiency induces disturbances in calcium-phosphate metabolism, leading to changes in bone structure.

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