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Evaluation of Bacterial Vaginosis Therapy in Pregnant Women with Vaginal Tablets Containing Lactic Acid Complexed with Eudragit® E-100 which Undergo Gelation at the Site of Application

Ocena skuteczności leczenia bakteryjnej waginozy u kobiet ciężarnych poddanych terapii globulkami dopochwowymi, zawierającymi kompleks kwasu mlekowego z Eudragit®-em E-100, ulegającymi żelowaniu w miejscu aplikacji

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Abstract

Background. Bacterial vaginosis is the most common cause of pathological vaginal discharge in women. It is of special concern in pregnant women due to its confirmed association with premature delivery and premature escape of the amniotic fluid. Bacterial vaginosis is accompanied by increased pH in the vagina. The efficacy of anti-inflammatory drugs and drugs restoring the physiological pH in the vagina depends largely on the duration of contact of the active substance with the vaginal mucosa. Due to their adhesive properties, gels based on hydrophilic polymers containing complexes of lactic acid meet the requirements and maintain normal pH.
Objectives. Evaluating the effectiveness of treating bacterial vaginosis in pregnant women with vaginal tablets containing lactic acid complexed with Eudragit® E-100, which undergo gelation at the site of application.
Material and Methods. Sixty pregnant women aged 18 to 33 years with a history of recurrent inflammatory conditions in the vagina were examined. The study group ultimately included 25 patients diagnosed with bacterial vaginosis without any accompanying vaginal conditions. The bacterial vaginosis patients were administered tablets on methylcellulose vehicle intravaginally. The vaginal tablets contained lactic acid complexed with Eudragit® E-100.
Results. The gels obtained from the tablets revealed a wide range of pH. Tablets with adequate composition and pH can be selected depending on the severity of the inflammation. The tablets do not deform at room temperature, but they do at human body temperature and this allows maintaining a normal pH in the vagina for at least 8 hours, which significantly improves the bacterial condition in the vagina.
Conclusions. Hydrophilic vaginal tablets containing lactic acid complexed with Eudragit® E-100 are useful in treating the symptoms of bacterial vaginosis in pregnant women (**Adv Clin Exp Med 2006, 15, 4, 645–651**).

Key words: bacterial vaginosis, premature delivery, lactic acid.

Streszczenie

Wprowadzenie. Bakteryjna waginoza jest najczęstszą przyczyną nieprawidłowej wydzieliny pochwowej u kobiet. Ma ona szczególne znaczenie u pacjentek ciężarnych z uwagi na udokumentowany związek z występowaniem porodu przedwczesnego i przedwczesnego odpływania płynu owodniowego. W bakteryjnej waginozie stwierdza się podwyższone pH pochwy. Skuteczność leków przeciwzapalnych i odtwarzających środowisko fizjologiczne pochwy w dużym stopniu zależy od czasu kontaktu preparatu leczniczego z błoną śluzową tego narządu. Żele na bazie

polimerów hydrofilowych, zawierające kompleksy kwasu mlekowego, dzięki swej przyczepności spełniają te wymagania, utrzymując prawidłowe pH.
Cel pracy. Ocena skuteczności leczenia bakteryjnej waginozy u kobiet ciężarnych poddanych terapii globulkami dopochwowymi zawierającymi kompleks kwasu mlekowego z Eudragit®-em E-100, ulegającymi żelowaniu w miejscu aplikacji.
Materiał i metody. Materiał badawczy stanowiło 60 pacjentek ciężarnych w wieku 18–33 lat zgłaszających w wywiadzie nawracające stany zapalne pochwy. Ostatecznie grupę badawczą stanowiło 25 pacjentek ze zdiagnozowaną bakteryjną waginozą, bez współistniejących innych schorzeń pochwowych. Pacjentkom ze stwierdzoną bakteryjną waginozą aplikowano dopochwowo globulki na bazie metylocelulozy. Globulki dopochwowe zawierały kompleks kwasu mlekowego z Eudragit®-em E-100.
Wyniki. Żele otrzymane z badanych globulek wykazują szeroki zakres pH. W zależności od zaawansowania stanu zapalnego można dobrać odpowiedni skład globulek o odpowiednim pH. Zbadane globulki nie deformują się w temperaturze pokojowej. Proces ten zachodzi natychmiast w temperaturze ciała ludzkiego i pozwala na utrzymanie prawidłowego pH pochwy przez co najmniej 8 godz. poprawia stan bakteriologiczny pochwy.
Wnioski. Zastosowanie hydrofilowych globulek dopochwowych zawierających kompleks kwasu mlekowego z Eudragit®-em E-100 jest przydatne w leczeniu objawów bakteryjnej waginozy u kobiet ciężarnych. (**Adv Clin Exp Med 2006, 15, 4, 645–651**).

Słowa kluczowe: bakteryjna waginoza, poród przedwczesny, kwas mlekowy.

Bacterial vaginosis is the most common cause of pathological vaginal discharge in women of reproductive age. Its incidence ranges from 5–50%, depending on socio-economical status, educational level, background, etc. Bacterial vaginosis is a condition of special concern in pregnant women, as it is associated with premature delivery, late miscarriage, and premature escape of the amniotic fluid [1, 2].

The normal ecosystem in the vagina is regulated by the presence of lactic acid bacilli (*Lactobacilli*), which produce lactic acid, hydrogen peroxide, endopeptidases, immunosuppressive proteins, and protease inhibitors. This ecosystem contains numerous bacteria, both aerobic and anaerobic, which produce bacteriocins, which are macromolecular proteins. Both bacteriocins and lactocidin keep yeast-like fungi in the saprophytic or latent phase. However, lactic acid bacilli constitute about 95% of the normal flora. Lactic acid bacilli, whose hydrogen peroxide is toxic to anaerobic bacteria and viruses, plays the most significant role in the normal microflora [3]. If the levels of lactic acid are inadequate, bacteria, mostly aerobic, multiply, producing the condition referred to as bacterial vaginosis (BV). It is believed that BV in pregnant women results from bacterial invasion ascending through the cervical canal to the decidual membrane and chorion [4, 5].

In intrauterine infection, bacteria may release endotoxins, which in turn activate macrophages to release cytokines. The increase in cytokine levels in the cervical canal is responsible for prostaglandins synthesis and, consequently, for inducing premature delivery (phospholipase A released during the reaction with the deciduous membrane evokes the release of arachidonic acid, which is a prostaglandins precursor).

The prevalence of BV in pregnant women reaches 17%, but in high-risk groups it may increase to 32%. The disease may be diagnosed in patients who do not report any subjective complaints [6]. The above process may develop even when there are no bacterial colonies in the amniotic sac. How dangerous BV is in pregnancy is shown by the fact that infantile cerebral palsy is much more significantly associated with intrauterine infection than with hypoxia of the fetus. It is believed that cytokines are responsible for the foci of perinatal leukomalacia.

In vaginal inflammation, the pH of the vaginal discharge increases to 7.8, much higher than the normal pH, which ranges from 3.8 to 4.4. Gels on a hydrophilic polymer vehicle containing complexes of lactic acid were found to be useful and effective therapeutic modalities in the clinical treatment of the above-mentioned conditions. The effectiveness of anti-inflammatory drugs and agents restoring the physiological environment in the vagina depends largely on the duration of contact of the active substance with the vaginal mucous membrane. Numerous drugs fulfill the requirements only when the patient remains in the lying position. However, it is important for the drugs to act continuously, also during the daily activity of the patient. Thanks to their adhesive properties, gels on a hydrophilic polymer vehicle containing complexes of lactic acid meet the requirements and provide the normal pH.

The aim of the study was to investigate hydrophilic vaginal tablets containing lactic acid complexed with Eudragit® E-100 used in the treatment of bacterial vaginosis in pregnant patients.

Material and Methods

To investigate the tablets, a biopharmaceutical *in vitro* model was constructed to evaluate the time in which the tablets deform, the effect of glycerol

concentration on the temperature of deformation, and the effect of the hydrophilizing substance on the time of deformation of the tablet vehicle. Moreover, the effect of the concentration of the hydrophilizing substance on the pH of the vaginal tablets was evaluated by a pH-meter.

The study material comprised 60 pregnant patients aged 18 to 33 years with histories of recurrent inflammatory conditions in the vagina. The patients were between 18 and 37 weeks into pregnancy. It was the second or third pregnancy for all of them. Twenty-five patients had a history of premature delivery, while 35 had had the previous delivery at term. In this group of 60 patients, 20 reported additional symptoms of threatened premature delivery in the present pregnancy, while the remaining 40 patients were hospitalized due to other, non-infectious complications of the present pregnancy. All the patients underwent complex examination for vaginal infection. The study was performed using a pH-meter, a complex electrode, and the biopharmaceutical model. All the pregnant patients underwent: cytological examination, smear taken from the cervical canal for *Chlamydia trachomatis*, vaginal pH assessed with the use of litmus paper manufactured by Merck with a pH range of 4.0–7.0, test with KOH, Gram-stain of the smear, smear from the cervical canal taken for culture and antibiogram, smear from the cervical canal taken for mycoplasm, vaginal smear taken on McConkey's medium and agar with blood (quantitative assessment), samples investigated for the presence of fungi, clinical assessment of the condition of the vaginal mucosa, vaginal discharge evaluated according to Amsel's criteria, vaginal discharge evaluated according to Nugent's scale. Moreover, a through analysis of the clinical condition and reported complaints was performed. If massive bacterial, fungal, chlamydial, or mycoplasma infection was found, appropriate treatment was instituted.

The ultimate study group included 25 patients (41.7%) with the diagnosis of bacterial vaginosis without other associated vaginal conditions. Among the patients, 16 (64%) gave histories of premature delivery in the past, while 9 (36%) had had the previous delivery at term. Moreover, 15 patients (60%) reported vaginal discomfort or discharge at the onset of the trial, while 10 (40%) did not complain of any subjective conditions. The diagnosis of bacterial vaginosis was made on the basis of Amsel's criteria [9]. For the disease to be diagnosed, the patient should have at least 3 out of 4 criteria: 1) thin, whitish, homogenous discharge, 2) the presence of clue cells in the microvisual microscopic picture stained with 0.2% methylene blue at 400× magnification, 3) pH of the vaginal discharge above 4.5, 4) the smell of fish after the addition of alkaline solution (10% KOH) to the vaginal discharge sample.

Vaginal pH was evaluated by means of litmus paper. Moreover, the diagnostic protocol included Gram-stain of the vaginal discharge and evaluation of the material according to Nugent's scale [10]: evaluation of the proportional participation of various bacterial strains in the sample and its numerical assessment (scored 0–10). A final result above 4 indicates a normal condition, from 4 to 6 an intermediate condition, and over 6 bacterial vaginosis.

Patients with diagnosis of bacterial vaginosis were administered vaginal tablets on a methylcellulose vehicle. The tables contained lactic acid complexed with Eudragit® E-100. The first tablet was applied in the morning and vaginal pH was assessed every hour. The next tablet was inserted when the pH was above 4.5. Application of the remaining tablets was continued even when the pH was above the norm and pH evaluation was performed periodically. The therapy lasted from 7 to 18 days (usually 10 days). The outcome of the therapy was evaluated 1–2 days after termination of the treatment and involved clinical evaluation of the vaginal mucosa, repeated examinations using Amsel's criteria and Nugent's scale, as well as recording the patients' subjective evaluation of vaginal complaints prior to and after the treatment.

Results

Following application, the tablets bonded water from vaginal discharge, forming a gel with a viscosity of 114 to 887 mPas. This provided good adherence to the vaginal mucosa. *In vitro* measurements revealed that the deformation time was from 70–120 seconds. The pH of the gels formed from the tablets was an important parameter and ranged from 5.06 to 3.43, while the physiological pH according to literature ranges from 3.8 to 4.4.

Table 1 illustrates the effect of glycerol concentration on the pH of the gels formed from the vaginal tablets. As shown, the addition of 10%, 15%, 20%, and 25% glycerol to tablets containing lactic acid complexed with Eudragit® E-100 increased the pH of the gel formed from the tablets compared with the preparations not containing hydrophilizing substances. pH ranged from 4.42–4.99 for the lactic acid to Eudragit® E-100 ratio of 1 : 1 and 3.07–3.46 for the ratio of 4 : 1.

Table 2 illustrates the effect of the kind of hydrophilizing substance applied on the pH of the suppository vehicle. As shown in the analysis, 15% addition of a hydrophilizing substance to the tablets

Table 1. The influence of glycerol concentration on the pH of gels created from intravaginal globules

Tabela 1. Wpływ stężenia glicerolu na pH żeli powstałych z globulek dopochwowych

Lactic acid complex with Eudragit® E-100 stoichiometric ratio (Proporcja kwasu mlekowego do Eudragit®-u E-100)	pH				
	16% Gel. 4% MC	16% Gel. 4% MC, 10% G	16% Gel. 4% MC, 15% G	16% Gel. 4% MC, 20% G	16% Gel. 4% MC, 25% G
1 : 1	3.67	4.42	4.40	4.85	4.99
2 : 1	2.86	3.60	3.62	4.08	3.94
4 : 1	2.74	3.07	3.02	3.03	3.46

Gel. – gelatin, MC – methylcellulose, G – glycerol, PEG-200 – Polyoxyethylene glycol 200.
Gel. – żelatyna, MC – metyloceluloza, G – glicerol, PEG-200 – glikol polioksyetylenowy.

Table 2. The influence of hydrophilizing substance type on pH of the suppository base

Tabela 2. Wpływ rodzaju substancji hydrofilizującej na poziom pH z bazy preparatowej

Lactic acid complex with Eudragit® E-100 stoichiometric ratio (Proporcja kwasu mlekowego do Eudragit®-u E-100)	pH		
	16% Gel. 4% MC, 15% glycerol	16% Gel. 4% MC, 15% PEG-200	16% Gel. 4% MC, 15% Propylene-1,2--glycol
1 : 1	4.40	4.90	4.56
2 : 1	3.62	3.93	3.76
4 : 1	3.02	3.51	3.30

Gel. – gelatin, MC – methylcellulose, PEG-200 – Polyoxyethylene glycol 200.
Gel. – żelatyna, MC – metyloceluloza, PEG-200 – glikol polioksyetylenowy.

Table 3. Amsel's criteria of bacterial vaginosis diagnosis in pregnant patients

Tabela 3. Kryteria rozpoznania bakteryjnej waginozy według Amsela u pacjentek ciężarnych

No of patients (Liczba pacjentek) 25	Thin, whitish, homogenous discharge (Rzadka, biaława, jednorodna wydzielina)	Presence of clue cells in preparation (Komórki *clue cells* w preparacie)	Vaginal pH above 4.5 (pH pochwy powyżej 4,5)	Positive trial with KOH (Dodatni wynik próby z KOH)
Prior to treatment (Przed leczeniem)	14 (56%)	25 (100%)	25 (100%)	11 (44%)
After 10-day treatment (Po 10-dniowym leczeniu)	5 (20%)	2 (8%)	4 (16%)	0 (0%)

affected the pH of the produced gels in comparison with the preparations without any addition. With increasing lactic acid to Eudragit ratio from 1 : 1 to 4 : 1, the pH for propylene glycol-1,2 ranged from 4.56 to 3.30, for PEG-200 from 4.90 to 3.51, and for glycerol from 4.40 to 3.02.

Moreover, the effect of glycerol concentration on the temperature of suppository deformation was evaluated. The analysis revealed that the deformation time at 30°C increased from 3 min 30 sec. to 11 min with an increase in glycerol concentration from 10% to 15%.

The investigations also involved the effect of the kind of hydrophilizing substance applied on the time of deformation of the suppository vehicle. The longest deformation time (11 minutes) was observed in tablets containing 15% glycerol. Tablets containing 15% polypropylene glycol-1,2

Table 4. Evaluation of the vaginal suppository, stained with Gram method, Nugent scale

Tabela 4. Ocena preparatu z pochwy, barwionego metodą Grama w skali Nugenta

No of patients (Liczba pacjentek) 25	Score 0–3 – neutral (0–3 pkt – neutralne)	Score 4–6 – intermediate (4–6 pkt – pośrednie)	Score 7–10 – BV (7–10 pkt – BV)
Prior to treatment (Przed leczeniem)	0 (0%)	20 (80%)	5 (20%)
After treatment (Po leczeniu)	17 (68%)	6 (24%)	2 (8%)

underwent deformation in 4 minutes 30 seconds. The shortest deformation time, 4 minutes 15 seconds, was observed with 15% PEG-200 content in the tablets. When the tablets were prepared (according to a technology protected by patent), they were subjected to clinical analysis in a selected group of patients with bacterial vaginosis.

In the analyzed material, all the patients reported a history of persistent vaginal discharge, discomfort during intercourse, and recurrent inflammatory conditions in the vagina prior to pregnancy, which necessitated frequent gynecological consultations.

In the group of 60 women, the ultimate diagnosis of bacterial vaginosis was made in 25 patients (41.7%). Of this group, 15 (60%) reported vaginal discomfort or discharge at the onset of the trial, while 10 (40%) patients did not report any subjective complaints. All the patients had vaginal pH above 4.5. Vaginal pH evaluated on preliminary examination was from 4.7 to 7.0. Eleven patients of the 25 (44%) had a positive KOH trial. Fourteen (56%) had homogenous, profuse vaginal discharge. All 25 patients revealed the presence of a clue cell in the samples (Table 3). In the group of 25 patients, Gram-stains of the vaginal smear evaluated according to Nugent's scale (Table 4) prior to the treatment revealed a score of 4–6 in 20 cases (80%) and 7–10 in 5 cases (20%).

The tablets were inserted intravaginally at a time when normal pH was maintained in the vagina. The application was repeated every time pH was higher than 4.4. In 15 cases (60%) the vaginal pH remained normal for 2–3 hours in the first 2 days; later it remained normal for 8 hours. In 5 cases (20%), vaginal pH remained normal for 8 hours, while in the remaining 5 cases (20%) it was normal for 12 hours.

Four patients, despite normal vaginal pH at a level below 4.5 when the tablets were applied, did not reveal any improvement in vaginal pH or smear parameters after 10 days of therapy, when the treatment was stopped. Two patients did not complete the therapy and in a further two the therapy was prolonged and the desired effect was achieved after 18 days of treatment. A positive KOH result was not found on check-up in any of the analyzed cases. Of 11 patients who reported unpleasant smell, the symptoms were relieved after termination of treatment in all of them. Homogenous, whitish discharge was present in 5 of the 25 patients (20%) (Table 3). On evaluation of the samples according to Nugent's scale after termination of therapy (Table 4), scores of 0–3 were found in 17 (68%) patients, 4–6 in 6 (24%) patients, and 7–10 in 2 (8%) patients.

The treatment resulted in a significant improvement in the condition of the vaginal mucosa in all the patients and those women who had suffered from persistent vaginal complaints prior to the therapy reported a significant improvement in vaginal comfort. No side effects associated with the use of the vaginal tablets were reported, apart from the necessity of using panty liners during therapy. Between successive applications of the tablets, the vaginal walls were lined with the formed gel which, in the patients' opinion, produced a sensation of effective moistening.

Discussion

Bacterial vaginosis is believed to be a common problem in women consulting a gynecologist for recurrent inflammatory conditions in the vagina [11–13]. In the analyzed material, among 60 patients with histories of vaginal complaints, bacterial vaginosis was diagnosed in 25 women, which constitutes 41.7%. The etiology of BV remains unexplained, but it is known to occur and subside spontaneously [14]. Some patients with bacterial vaginosis do not suffer from any complaints. In presented material, 15 (60%) of the 25 patients reported complaints, the remaining 10 (40%) were free of any complaints.

Bacterial vaginosis is known to be responsible for premature delivery, premature escape of the amniotic fluid, and intrauterine infections. The diagnosis of bacterial vaginosis. especially in women with a history of miscarriage or premature delivery, is an indication for obstetricians for special care of the pregnant woman and the institution of adequate therapy [6, 4, 15]. In the analyzed material as many as 41.6% of the patients were women with a history of premature delivery.

The methodology of BV treatment in pregnancy is a great obstetric problem. The administration of antibiotics to pregnant women is controversial.

Many authors reported oral administration of metronidazole which was found to be 80% effective four weeks after termination of the therapy. A similar effectiveness was reported for topical application of metronidazole or clindamycin cream. Some of the reports indicate a reduced incidence of premature deliveries in cases of antibiotic therapy for BV. Other authors do not see any difference between groups of women treated and untreated for BV. Some of them even state that among multiparous women from high-risk groups suffering from BV and treated with metronidazole, the duration of pregnancy was shorter than in untreated women. This may result from the elimination of natural bacterial flora and subsequent colonization of the vagina with opportunistic bacteria, the effect of which may be more pathogenic than BV [16, 17].

Safe new alternative methods of BV treatment are still being sought. In the present trial a preparation in the form of vaginal tablets containing lactic acid complexed with Eudragit® E-100 on methylcellulose and gelatin was investigated. This preparation is characterized by unique properties. To protect normal vaginal biocenosis it is essential to maintain normal pH in the vagina [19]. Normal pH prevents the multiplication and invasion of pathogenic bacteria. At higher pH, *Lactobacillus* species are displaced from the receptor sites in vaginal cells while the adhesion of *Gardnerella vaginalis* is increased. Application of the investigated preparation allowed maintaining pH within the normal limits for about 8–12 hours until its repeated application in the majority of cases. Control Gram-stains assessed according to Nugent's scale were normal in 17 of the 25 cases. The control examination was performed several days after termination of treatment.

As recurrences are common in BV, the problem that remains to be solved is how often the therapy should be repeated in order to achieve a prophylactic effect. The evaluation requires long and tedious investigations on the vaginal biocenosis in the course of pregnancy.

The use of a preparation in the form of vaginal tablets containing lactic acid complexed with chitosan provides a safer opportunity of its administration. No side effects were found during the therapy. The tablets transformed in the vaginal environment into gel which revealed physicochemical properties approaching those of natural mucus, which is especially significant in view of its tolerance by delicate vaginal mucosa. For this reason, application of the tablets may be safely repeated and maintained as long as necessary. After the therapy, the vaginal mucosa did not show any evidence of irritation or allergic reactions. Throughout the whole therapy, the vagina was moistened with gel closely adhering to its walls.

Further investigations shall concentrate on evaluating the time between termination of therapy and recurrence of BV infection. This is difficult, as the causes and frequency of BV recurrences have not yet been explained. The lack of side effects of the applied preparation as well as its physicochemical properties resembling the natural vaginal mucus permit its frequent use, probably in repeated therapeutic cycles.

The gels obtained from the investigated vaginal tablets show a wide pH range. Depending on the severity of the inflammatory condition, tablets with an adequate composition may be selected to obtain the desired pH. The investigated tablets do not undergo deformation at room temperature. However, this process occurs at body temperature, which allows maintaining a normal vaginal pH for at least 8 hours and improves the bacteriological condition in the vagina.

Hydrophilic vaginal tablets containing lactic acid complexed with Eudragit® E-100 are useful in the treatment of symptoms of bacterial vaginosis in pregnant women.

References

[1] McDonald HM, O'Loughlin JA, Vigneswaran R, Parker R, Patterson E, Draper D: Impact of metronidazole therapy on preterm birth in women with bacterial vaginosis flora (*Gardnerella vaginalis*): A randomised, placebo controlled trial. Br J Obstet Gynecol 1997, 104, 1391–1397.

[2] McGregor JA, French JI: Bacterial vaginosis in pregnancy. Obstet Gynecol Survey 2000, 55, 1–19.

[3] Eschenbach DA, Davis PR, Williams BL, Klebanoff SJ, Young-Smith k, Crithlpw CM, Holmes KK: Prevalence of hydrogen peroxide-producing Lactobacillus species in normal women and women with bacterial vaginosis. J Clin Microbiol 1989, 27, 251–256.

[4] Chaim W, Mazor M, Leiberman JR: The relationship between bacterial vaginosis and preterm birth. A review. Arch. Gynecol. Obstet., 1997, 259, 51–58.

[5] Klebanoff MA, Hillier SL, Nugent RP, MacPherson CA, Hauth JC, Carey JC, Harper M, Wapner RJ, Trout W, Moawad A, Leveno KJ, Miodovnik M, Sibai BM, Vandorsten JP, Dombrowski MP, O'Sullivan MJ, Varner M, Langer O: Is bacterial vaginosis a stronger risk factor for preterm birth when it is diagnosed earlier in gestation? Am J Obstet Gynecol 2005, 2, 470–477.

[6] Leitich H, Bodner-Adler B, Brunbauer M, kaider A, Egarter C, Husslein P: Bacterial vaginosis as a risk factor for preterm delivery: A meta-analysis. Am J Obstet Gynecol 2003, 189, 139–147.

[7] Doderlein A: Das Scheiden Sekret und fur seine Beteutung fur das Puerperal fieber. Durr. Leipzig 1892.
[8] Thomason JL, Gelbart SM, Broekhuizen FF: Advances in the understanding of bacterial vaginosis. J Reprod Med 1989, 34, 581–587.
[9] Amsel R, Totten PA, Spiegel CA, Chen KC, Eschenbach D, Holmes KK: Nonspecific vaginitis. Diagnostic criteria and microbial and epidemiologic associations. Am J Med 1983, 74, 14.
[10] Nugent RP, Krohn MA, Hillier SL: Reliability of diagnosis bacterial vaginosis in improved by a standardized method of Gram stain interpretation. J Clin Microbiol 1991, 29, 297–301.
[11] Schmidt H, Hansen JG: A wet smear criterion for bacterail vaginosis. Scand J Prim Health Care 1994, 12, 223–233.
[12] Hay PE, Taylor-Robinson D, Lament RF: Diagnosis of bacterial vaginosis in a gynaecology clinic. Br J Obstet Gynecol 1992, 99, 63–66.
[13] Gutman RE, Peipert JF, Weitzen S, Blume J: Evaluation of clinical methods for diagnosing bacterial vaginosis. Obstet Gynecol 2005, 3,105(3), 551–556.
[14] Cook RL, Redondo-Lopez V, Schmitt C, Meriwether C, Sobel JD: Clinical, microbiological, and biochemical factors in recurrent bacterial vaginosis. J Clin Microbiol 1992, 30, 870–877.
[**15] Balaka B, Agbere A, Dagnra A, Baeta S, Kessie K, Assimadi K:** Genital bacterial carriage during the last trimester of pregnancy and early-onset neonatal sepsis. Arch Pediatr 2005, 5, 12(5), 514–519.
[**16] Hanson JM, McGregor JA, Hillier SL, Eschenbach DA, Kreutner AK, Galask RP, Martens M:** Metronidazole for bacterial vaginosis. A comparison of vaginal gel vs oral therapy. J Reprod Med 2000, 45, 889–896.
[17] Ferris DG, Litaker MS, Woodwar L, Mathis D, Hendrich J, Parker R, Patterson E, Draper D: Treatment of bacterial vaginosis: A comparison of oral metronidazole, metronidazole vaginal gel and clindamycin vaginal cream. J Fam Pract 1995, 41, 443–449.
[18] McGregor JA, French JI, Jones W: Association of cervico/vaginal infections with increased vaginal fluid phospholipase A2 activity. Am J Obstet Gynecol 1992, 167, 1588–1594.
[19] Peterek J: Wpływ zaburzeń ekosystemu pochwy na przebieg ciąży, porodu i połogu. Klin Perinatol Ginekol 2002, supl. XXV, 88–92.

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