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Modern Approach to Bacterial Vaginosis and Diagnosis of Vaginitis in Women with Vitamin D Deficiency

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Abstract

The disturbances of ecosystem are spread among all women of reproductive age and are accompanied by recidivism. Some authors consider that mon-specified vaginitis is the result of ineffective treatment of bacterial vaginosis and can lead to very serious complications in obstetrics and neonatology. Both pathologies are polyetiological diseases in which the general condition of the organism, the disturbance of immunobiological factors are indisputable. Last year's molecualar genetic methods are used in bacterial vaginosis diagnosis. By introduction of, polymerase chain reaction (PCR) in the clinics in addition to Gardnerella vag. the new agent of bacteriological vaginoses Atopobium vag. was found. Such innovations make problems, which we introduce more actual.
Keywords: bacterial vaginosis, vaginitis, lactobacilli, Gardnerella vag., Atopobium vag., key cells, PCR, vitamin D

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Современный подход к бактериальному вагинозу и диагностике вагинита у женщин с дефицитом витамина D

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Резюме

Нарушения экосистемы распространяются на всех женщин репродуктивного возраста и сопровождаются рецидивами. Некоторые авторы считают, что неспецифический вагинит является результатом неэффективного лечения бактериального вагиноза и может привести к очень тяжелым осложнениям. Обе патологии являются полиэтиологическими заболеваниями. В последние годы в диагностике бактериального вагиноза применяют молекулярно-генетические методы. При внедрении в

клинику полимеразной цепной реакции в дополнение к Gardnerella vag. обнаружен новый возбудитель бактериологического вагиноза Atopobium vag.
Ключевые слова: бактериальный вагиноз, вагинит, лактобациллы, Gardnerella vag., Atopobium vag., ключевые клетки, ПЦР, витамин D

■ INTRODUCTION

Last years in most countries of the world the disease of vaginal inflammation, especially, bacterial vaginosis is widely spread among the women of reproductive age [6, 13, 15]. Some authors consider it to be a result of non-effective treatment and both pathologies obstetrics gynecology and neonatology may be over with serious complications [8, 13].

It was determined that bacterial vaginosis and non-specific vaginitis endometritis, salpingoophoritis, after invasive procedures and operations inflame complications, colpitis and neoplastic processes of vaginal neck increase the risk of abortions, oviduct conglutination cause infertility, amnionitis, chorioamnionitis, intra-amnional infection, prejudgment opening of amniotic cavity, premature [8–10, 15].

Bacterial vaginosis and aerobe vaginitis are polyetiological diseases and during them general condition of the organism, the role of local immune features and the disturbance of immuno-biological features are irresistible [16, 18].

Nowadays in microscopic and bacteriological methods in vaginal inflammation diagnostics together with microscopic and bacteriological methods, the molecular-genetic method is also used. By entrance of PCR (Polymer Chain Reaction) besides Gardnerella vaginalis another bacterial new specific one-Atopobium vag. was detected for bacterial vaginosis [3, 5, 12]. Because of hard cultivation in well-fed conditions this microorganism before the use of PCR wasn't considered the main courser of vaginal dysbiosis. Lately in appearance of vaginal ecosystem disturbances Mobiluncus spp., Leptotrichia, Megasphera, Clostridium types participations is observed [3, 6, 14].

The latest investigations show that in gynecology vitamin D deficiency influence on polycystic ovary, premature delivery, bacterial vaginosis and other pathologies development is undeniable [4, 11, 17]. There is much information in literature confirming the association of vitamin D deficiency with bacterial vaginosis [1, 7, 11]. These pathologies, on being detected together with vitamin D deficiency speeds aggressive development of the disease and shows the important role of vitamin D deficiency in bacterial vaginoses and vaginitis pathogenesis [2, 7, 17].

■ THE PURPOSE OF INVESTIGATION

Our goal is to diagnose the bacterial vaginosis patients with vitamin D deficiency by microscopic and bacteriological way and determine suspicious diagnoses by PCR method.

■ MATERIALS AND METHODS

In the Surgical Training Clinic of AMU, maternity hospital N5 named after Sh. Aleskerova, Referans Clinical Laboratory, 132 reproductive aged patients were examined and taken examinational material. 26 of examined women were healthy, 54 bacterial vagina patients (26 women had vitamin D concentration-normal, less 28), 52 vaginitis patients (25 women had vitamin D concentration-normal, less 27). The taken pathological material

examination was done in AMU's medical microbiology and immunology departments clinical-biochemical laboratories in the Surgical Training clinic of AMU, Referans Clinical Laboratory.

Being the material of investigation the blood, sample of vaginal smear was investigated. In the investigation the complex of microbiological, biochemical, molecular-genetic methods were used.

The condition of vaginal mucosa of the patient's vaginal wall was examined with gynecological mirror. From 1/3 of vaginal and urethra was taken the examinational material for measuring vaginal pH (HANNA Germany) for pH meter, 1–2 drops 10% KOH was used for amino test. The taken smear way dyed with Gram, vaginal epithelial cells having been microscopy, were detected leukocytes, key cells, Dederlein sticks, pathogen bacteria, the pseudocelli of candida fungi. The examinational material was accessed basing on Amstel and Nugent.

For getting the pure culture of bacteria from vaginal back vault sterile cotton plug material was taken and seeded in Bio Merioux career nutrition medium to carry bacteria. For identification of facultative anaerobic microorganisms and their number, 5% donor blood was added.

Tough in clinical practice in diagnostics of vaginal inflammation the method of microscopic examination is widely used, sometimes it is difficult to identify the pathogen microbes, playing the main role in the etiology of this disease. So, during examination in the case of doubtful diagnosis of bacterial vaginosis for affirmance Real-Time CPR-Bacterial vaginosis Test panel was used.

For detecting the influence of vitamin D' on bacterial vaginosis and vaginitis pathogenesis we determined its concentration in blood (Bioactiva Diagnostic-Germany)

■ THE RESULTS OF THE INVESTIGATION

First of all, we have categorized the examined patients according to vitamin D concentration.

In examined healthy women during vaginal mucosa and microscopic examination no pathology was detected, amine test (–) negative, but vaginal pH was 3.8–4.5. Nugent score was 0–3.

The women with bacterial vaginosis didn't have any inflammation in vaginae, there was pale grey badly smelling rheum, pH more than 4.5 (5.0–7.0), in many cases amin test was positive. During microscopy there was a little amount (till 10) leukocyte, epithelium and in most cases were detected «key cells» and Gardnerella vaginalis, but lactobacteria were either very little, or none at all. The score of Nugent was estimated as 7–10. According to Amstel standards bacterial vaginosis was diagnosed.

Table 1
Examined patients' groupage according to vitamin D concentration

Diagnosis	Healthy	Bacterial vaginosis		None-specific vaginitis	
		Patients with normal vitamin D	Patients with vitamin D deficiency	Patients with normal vitamin D	Patients with vitamin D deficiency
Examination groups	I	II	III	IV	V
Number (patients)	26	26	28	25	27

The features of vaginal mucosa inflammation were detected, redness, edema and pathological extraction, amin test (–) negative, vaginal pH was more than 4.5.

But in vaginitis patients' examinational material there was epithelium, much leukocyte (30–60), little lactobacteria or even non.

During the examination in 22 from 25 was suspicious diagnosis. Having no changings in CPR classical examination, we have examined by using the Real-Times CPR Test panel-Bacterial vaginosis. 7 patients with Gardnerella vag., 1 with Bacteroides fragilis mono and 14 patients Gardnerella vag. associating with other bacteria were detected. In 3 from 7 Gardnerella vaginalis patients for absence of enough bacterial vaginosis the diagnosis was negative, in 2 pathological materials together with Megasphaera type-1, 3 Atopobium vaginae, 5 Mobiluncus spp., 4 Bacteroides fragilis was detected. It should be mentioned that together with completely used molecular – genetic method, bacteriological and microscopic method, being difficult to detect, the germs were found.

In the next stage for getting the pure culture of bacteria, we held bacteriological examination. The results are shown in the table 2.

In all materials, taken from healthy women Lactobacillus spp., was detected in big amount 10^7–10^9 CFU/ml. In the material, taken from patients with bacterial vaginosis lactobacillus spp., 9.3% (10^4 CFU/ml), Gardnerella vaginalis 92.6% and Bacteroides fragilis 37% cases (10^7–10^9 CFU/ml), but facultative anaerobic bacteria were in a small amount. In the material, taken from patients with bacterial vaginosis Lactobacillus spp., 9.3% (10^4 CFU/ml), Gardnerella vaginalis 92.6% and Bacteroides fragilis 37% (10^7–10^9 CFU/ml), but facultative – anaerobic bacteria were in a small amount. In patients with non – specific vaginitis Lactocillus spp., in comparison with other groups is less (7.7%) and facultative anaerobe bacteria (Staphylococcus spp., Streptococcus spp.,) was considerably high (54.8%; 21.2%).

Table 2
Vaginal microbiocenosis of healthy woman and patients with bacterial vaginosis and non-specific vaginitis

Microorganisms	Controlled groups (26)		Bacterial vaginosis (54)		Non-specific vaginitis (52)	
	Rate (number/%)	Amount (CFU/ml)	Rate (number/%)	Amount (CFU/ml)	Rate (number/%)	Amount (CFU/ml)
Lactobacillus spp.	26/100	10^7–10^9	5/9,3	till 10^4	4/7.7	till 10^4
Bifidobacterium spp.	3/11 ,5	till 10^4	15/27,8	10^3–10^4	5/9.6	10^3–10^4
G. vaginalis	4/15,4	till 10^5	50/92,6	10^7–10^9	6/11.5	10^5–10^6
Prevotella spp.	6/23,0	till 10^3	9/16,7	10^4–10^5	11/21.2	10^5–10^7
Porphyromonas spp.	5/19,2	till 10^3	–	–	3/5.8	10^4–10^5
Bacteroides frag.	3/11,5	10^3–10^4	20/37	10^3–10^4	10/19.2	10^5–10^7
Fusobacterium spp.	9/34,6	till 10^3	2/3,7	10^3–10^4	3/5.8	10^4–10^5
Veilonella spp.	4/15,4	till 10^3	3/5,6	10^4–10^5	5/9.6	10^5–10^6
Propionibacterium spp.	9/34,6	till 10^4	17/31,5	till 10^4	7/13.5	till 10^4
Staphylococcus spp.	6/23,0	till 10^4	14/25,9	till 10^4	33/63.5	10^6–10^7
Streptococcus spp.	11/42,3	till 10^4	5/9,3	till 10^5	11/21.2	10^6–10^8
Enterococcus spp.	2/7,7	till 10^3	6/11,1	till 10^4	10/19.2	10^6–10^7
Enterobacteriaceae	7/26,9	till 10^3	15/27,8	till 10^4	17/32.7	10^6–10^7
Candida spp.	4/15,4	till 10^3	8/14,8	till 10^3	19/36.5	till 10^3
The number of cultures	99	–	169	–	144	–

Table 3
The amount of vitamin D in the blood of healthy, the patients with bacterial vaginosis and non-specific vaginitis

Diagnosis	Healthy	Bacterial vaginosis		Non-pecific vaginitis	
Examining groups	I	II	III	IV	V
The number of patients	26	26	28	25	27
The concentration of vitamin D in the blood (nq/ml)	38.8±0.4	37.2±0.3	20.7±1.0	38.1±0.3	19.4±1.1

Candida fungi (C. albicans, C. tropicalis, C. krusei) in healthy, in patients with bacterial vaginosis and non-specific vaginitis was observed in less amount (<10^3 CFU/ml). Referring on this and at microscopic examination the presence of mycelium forms denied candidiasis diagnosis (Table 2). For identification of received pure culture nomenclature Berci was used.

In the next stage at the biochemical laboratory of AMU in the blood of patients the active metabolite of vitamin D was defined.

In the blood of first group patients 38.3±0.4 ng/ml, second group 37.2±0.3 ng/ml, third group 20.7±1.0 ng/ml, fourth group 38.1±0.3 ng/ml, fifth group 19.4±1.1 ng/ml.

In the result of the examination the II and V groups' patients had united association of different ethological agents, the process of the disease was concluded with relapse. In the diagnosing of these diseases together with microscopic and bacteriology method, the modern method of molecular – genetic (Real Time CPR) was more informative. So, by this method at the same time, it is possible to determine in shot time (48 hours) the quantity and quality of the main bacteria causing most of vaginosis.

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