

MOLECULAR GENETIC DETECTION OF BACTERIAL VAGINOSIS AT KAZAKH WOMEN IN REPRODUCTIVE AGE

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Abstract

Introduction: The diagnostic of sexually transmitted infection (STI) sharply increased. Steady growth of STI at persons of young age, women of fertility age who are often accompanied by the complications leading to disability, to infertility, a pre-natal infection is observed, causing fruit and newborn diseases. The purpose of our study was to analyze the molecular genetic detection of bacterial vaginosis at women in reproductive age by noninvasive methods.

Materials and Methods: Total DNA was isolated was extracted from vaginal samples at 54 women between the ages of 15 and 45 years who were able and willing to give informed consent. The analysis of products of PCR of amplification carried out by PCR in real time.

Results: It is revealed that associations bacterial vaginosis less often met three infectious agents in comparison with one and two (18, 5±3, 5 %, 38,9±6,3 %, 42,6±6,5 %, respectively, (p<0,05).

Conclusion: Our study demonstrates that, using a panel of DNA by means of PCR in real time the identification of BV patients with minimally invasive techniques.

Keywords: Bacterial vaginosis (BV), polymerase chain reaction real time (PCR-RT), sexually transmitted infection (STI), inflammatory diseases of bodies of a small basin (IDBSB), morbidity and mortality

Introduction

Bacterial vaginosis (BV) is a common cause of vaginal discharge in women of reproductive age [1]. In fact, numerous observational studies cite BV as the most common cause of symptomatic vaginal discharge during reproductive years with a prevalence of 10–15% [2]. Depending on geographic, racial, and clinical characteristics of a given population; BV has been observed to exhibit a wide-ranging prevalence from 4 to 64%. A total of 800 000 pregnancies in the USA are affected by BV annually [3]. Similar prevalence exists in both the pregnant and non-pregnant states. Asymptomatic women, whether pregnant or not, exhibit a prevalence of 12–25% [4].

Diagnosis in the clinical setting is accomplished by two common methods: microscopic examination of vaginal discharge for clue cells and Whiff Test (sometimes referred to as the 'sniff test') in which KOH (potassium hydroxide) is applied to a vaginal swab to detect a release of amine odor that is sniffed by the clinician. The Whiff Test is deemed positive when an amine odor, sometimes described as similar to that of decaying fish, is sensed by the clinician [5]. Investigation by Hillier (nj 2000) showed microscopic finding of clue cells to be 43.1% sensitive and 99.6% specific for presence of BV. The same investigation showed the Whiff Test to be 6.58% sensitive and 73.6% specific for BV [6]. The Amsel criteria for

diagnosis of BV include the following: vaginal pH > 4.5, abnormal discharge, clue cells, and positive sniff test.⁵ Presence of three out of four criteria is considered to be diagnostic for BV in the clinical setting. The gold standard for BV screening in investigations is the Nugent score which entails generation of a scaled score 0–10 in which 0–3 is negative, 4–6 is intermediate, and ≥7 is positive for BV [7].

In recent years the importance of sexually transmitted infection (STI) sharply increased. Steady growth of STI at persons of young age, women of fertility age who are often accompanied by the complications leading to disability, to infertility, a pre-natal infection is observed, causing fruit and newborn diseases. One of the aspects testifying to the high importance of STI, their influence on the course of pregnancy, its outcomes and a state of health of the newborn is. According to the Centers for control of diseases (the CDC USA), at pregnant women most often come to light such STI as bacterial vaginosis (BV), the herpetic and chlamydial infection, is more rare trichomoniasis, gonorrhoea, syphilis and HIV infection. However frequency of the perinatal infection connected with separate STI, is defined not only their prevalence in population, but also transmission frequency. The risk of perinatal infection makes about 30 % for gonococcus, 20-50 % for mycoplasma, 20-40 % for chlamydia, 5-50 % for a herpetic infection and about 50 % for syphilis. The risk of perinatal infection of the newborn is highest at a sharp primary infection [8].

Despite ambiguity of opinions of researchers in an pathogenic role of *Ureaplasma* spp. and *Mycoplasma hominis*, in etiological classification of World Health Organization [9] and syndromal classification of Centers for Disease Control and Prevention [10] these microorganisms are allocated as possible etiological agents nonspecific not gonococcal urethritis, inflammatory diseases of bodies of a small basin and bacterial vaginosis. Without adequate therapy at a third of the infected women develop IDBSB [11]. The question of what conditions are decisive for realization of pathogenic potential of opportunistic mycoplasmas, so far remains obscure. Numerous researches testify that it is possible to judge an etiological role of the specified activators with this or that share of probability only by results of the quantitative analysis. The standardized approaches to laboratory verification of the diagnosis of a urogenital infection are developed insufficiently.

The aim of this study is to determine the sensitivity, specificity and the predictive value of the BV and to observe the risk factors associated in the study of 54 women with BV in association with pathogenic and/or opportunistic causative agents of infections of a urogenital path (*Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis*).

Materials and Methods:

This enrollment began conducted in molecular-genetic laboratory at Scientifically Research Institute of Fundamental and Applied Medicine named by B. Atchabarov (SRIFAM) of Kazakh National Medical University named by S.D. Asfendiyarov, Almaty, (KAZNMU), Kazakhstan from September 2011 to December 2011. Funded by Ministry of Education and Science of (Republic of Kazakhstan) Grant: No. of state registration 0111PK00487 2011. Women that were eligible for project included Russian-speaking women between the ages of 15 and 45 years who were able and willing to give informed consent. Informed consent was received for all participants who were 18 years old.

As a result of the conducted complex laboratory testing at 96 (65, 8 %) women at the time of the address it was diagnosed bacterial vaginosis and at 50 (34, 2%) - a normal state of microflora of a vagina (control group). 96 patients with bacterial vaginosis were divided into 3 groups:

Group I - 30 (31, 3%) women with bacterial vaginosis;

Group II - 12 (12, 5%) women with bacterial vaginosis in association with Candidiasis vulvovaginitis;

Group III - 54 (56, 3%) women with bacterial vaginosis in association with pathogenic and/or opportunistic causative agents of infections of a urogenital path (*Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis*).

Selection criteria in group of patient's bacterial vaginosis were: reproductive age; lack of pregnancy and lactation; lack of system and local antibacterial therapy within 1 month before the real inspection; clinical-microbiological confirmation of the diagnosis bacterial vaginosis; existence of complaints.

Selection criteria of patients in control group were: reproductive age; lack of pregnancy and lactation; lack of system and local antibacterial therapy within 1 month before the real inspection; clinic microbiological confirmation of a normal state of vaginal microflora and excluded STD; absence of complaints.

At the baseline and follow-up visits, a trained nurse conducted a physical examination (including a pelvic examination) and collected endocervical specimens for testing for sexually transmitted infections. Women with bacterial or protozoan STIs were treated according to CDC guidelines [12].

Total DNA from vaginal samples was isolated using the DNA sorb-AM nucleic acid extraction kit (AmpliSens) according to the manufacturer's guidelines. DNA was allocated on an amplificatory "Rotor-Gene 6000 (Corbett Research, Australia) by set of reagents for DNA identification in a clinical material a method of PCR with gibrization-fluorescent detection of «Amplisens® *Chlamydia trachomatis*/ *Ureaplasma urealyticum* /*Mycoplasma hominis*-FL». The total volume PCR out amount of a reactionary mix – 30 mkl, including the volume of test of DNA – 10 mkl. The DNA was amplified using the following protocol «AmpliSens - 1»: an initial denaturation (95 °C for 15min), followed by 5 cycles of denaturation (95 °C for 5 s), annealing (60 °C for 20 s) and extension (72° C for 15 s), followed by 40 cycles of denaturation (95 °C for 5 s), annealing (60 °C for 20 s) with a final fluorescence detection and extension (72° C for 15 s). The diagnostic value of a set for detection of microorganisms in concentration more than 10^3 colony constitutive units in 1 ml.

When using sets of reagents for identification of DNA of each microorganism analyze curve accumulation of a fluorescent signal on two channels:
- on the channel for a fluorofor of FAM the signal testifying to accumulation of a product of amplification of a fragment of DNA of the revealed microorganism is registered,
- on the channel for a fluorofor of JOE the signal testifying to accumulation of a product of amplification of DNA of eternal control sample is registered.

Principle of interpretation of results the following

- DNA of a microorganism is found if for this test in the table of results of the channel for a fluorofor of FAM value of the threshold cycle Ct is defined. Thus the curve of fluorescence of this test has to cross the threshold line on a site of characteristic exponential lifting of fluorescence.
- DNA of a microorganism isn't found if for this test in the table of results of the channel for a fluorofor of FAM it isn't defined there (is) no value of the threshold cycle Ct (the curve of fluorescence doesn't cross the threshold line), and in the table of results of the channel for a fluorofor of JOE the value of the threshold cycle Ct which isn't exceeding specified (boundary) value is defined.

Statistical analysis

The data were analyzed using criteria Student tests. The results are expressed with calculated standard deviations (SD). We considered p values of $\leq 0,05$ to indicate statistical significance.

Results of researches:

In studied group of women were observed moderated (66, 7±7, 5%), ochroleucous color (68, 5±8, 3%), homogeneous (70, 4±11,2 in %), viscous (70,4±11,2 in %) allocation from sexual ways more often. At survey of mucous membranes of genitals the vagina hyperemia (48, 1±6, 7 %) and uterus necks (59, 3±7, 1 %) more often came to light, and also contact bleeding of a neck of a uterus (50, 0±6,4 by %) was noted. At bimanual survey more often in this group of women morbidity and increase in appendages of a uterus, existence of adhesive process in a small basin (51, 9±6, 6 by % and 70, 4±11, 2 in %, respectively), ($p < 0,05$).

Table 1 - Results of a combination bacterial vaginosis with pathogenic and/or opportunistic microorganisms

Infectious agents	Number of patients (N=54)	
	Absolute number	%
Chlamydia trachomatis	32	59,3±7,1
Ureaplasma urealyticum	39	72,2±11,4*
Mycoplasma hominis	26	48,1±6,7
Association bacterial vaginosis with:		
One infectious agent	21	38,9±6,3
Two infectious agents	23	42,6±6,5
Three infectious agents	10	18,5±3,5*
Remarks:* - distinctions are authentic, $p < 0,05$		

Discussion of results:

Apparently from the presented data in table 1, in studied group of women the PCR method and/or a cultural method with a quantitative assessment found Chlamydia trachomatis at 32 (59, 3±7, 1 %) patients, U. urealyticum at 39 (72,2 ±11,4 %) and M. hominis in high titers – at 26 (48,1± 6,7 %). It is revealed that is more often bacterial vaginosis associated with Ureaplasma urealyticum in comparison with Mycoplasma hominis ($p < 0,05$).

Only Ureaplasma urealyticum in high titers is found at 8 (14, 8 %) the women, only by Mycoplasma hominis in high titers – at 5 (9,3 %), only Chlamydia trachomatis – at 8 (14,8 %).

Thus, the association bacterial vaginosis with one infectious agent in the studied group of women was observed in 21 (38, 9 ± 6, 3 by %) a case. Association with two infectious agents - at 23 (42, 6 ± 6, 5 the %) patients, from them at 9 (39, 1 %) bacterial vaginosis was combined with Ureaplasma urealyticum and Mycoplasma hominis in high titers, at 12 (52,2 %) with C. trachomatis and U. urealyticum, at 2 (8,7 %) Chlamydia trachomatis and Mycoplasma hominis.

At 10 (18, 5±3, 5 the %) patients observed association bacterial vaginosis with three infectious agents: Chlamydia trachomatis, Ureaplasma urealyticum and Mycoplasma hominis.

It is revealed that associations bacterial vaginosis less often met three infectious agents in comparison with one and two (18, 5±3, 5 %, 38, 9±6,3 %, 42,6±6,5 %, respectively, ($p < 0,05$).

To important features of urogenital chlamidiosis, besides its not enough symptoms and high frequency of complications, often meeting association with other STI activators, and also a many loci treats with involvement in pathological process not only urogenital bodies, but also quite often rectum, a throat, eyes, joints, heart, skin.

All 32 patients with the revealed urogenital clamidiosis were surveyed for an exception of a many loci of infectious process. Thus, PCR method Chlamydia trachomatis were found in rectum – at 15 (46, 9±4, 3 by %), in a oropharynx – at 21 (65, 6±7, 5 in %) women.

Thus, Chlamydia trachomatis came to light in the urogenital center (an urethra and the cervical channel) in comparison with chlamydial defeat rectum and oropharynx (98, 4±9, 3 %, 46, 9±4, 3 %, 65,6±7,5 %, respectively), ($p < 0,05$) more often.

Conclusion

Recently often applied method of diagnostics of causative agents of urogenital infections is PCR, allowing identifying them in liquids and organism fabrics. The method is based on the analysis of nucleotide sequence and it is considered the most sensitive (94-100 %) and specific (97-100 %). The main problem in use of PCR is connected with their exclusively high sensitivity of a method that demands observance of rigid rules of work. Besides, at interpretation of results, it is necessary to consider that PCR reveals only a small part of a genome of a microorganism and, therefore, isn't criterion of its viability [13, 14].

We are demonstrated an association between increased severity of BV and the future incidence of contracting an STI. Although the use of the scoring systems used in this article may not be practical in the hospital, the studies' conclusions are most helpful in counseling the patient, as having all more severe cases of BV puts that patient at risk for future STIs.

Thus, bacterial vaginosis now it is necessary to consider not only as frequent independent nozological unit, but also as a background for additional development of STI. In this regard the importance has careful laboratory inspection of each patient bacterial vaginosis on STI, including carrying out screening on existence of the extra genital centers of a chlamydia infection. Attracts attention, what even in the absence of STI, at patient's bacterial vaginosis, besides vagina defeat, signs cervicitis and/or urethritis take place. It can be caused by realization of pathogenic properties of opportunistic microorganisms.

Our study provides support for the interpretation that sexual behavior has a causal role in the development of BV. Whereas this study was not designed to track the transmission of specific microorganisms that might be necessary for the development of BV, the longitudinal design and the data analysis allowed to separately assess three possible causal links: 1) that sexual behavior commonly associated with the acquisition of STIs is also associated with the development of BV; 2) that the acquisition of STIs is a risk factor for the subsequent development of BV; and factor for incident three infectious agents) differed in their associations with the two study outcomes, supporting the hypothesis of a similar etiologic role of sexual behavior in the acquisition of STIs and BV.

Although this study is somewhat limited by both the inherent recall bias of the participants and the recognized fact that young women tend to underreport sexual behaviors, it does add to a growing body of evidence that supports BV as a sexually transmitted illness. Findings are consistent with a causal role of sexual behavior in the acquisition of BV and confirm that BV facilitates acquisition of three infectious agents and vice versa independently from other risk factors. An important note is that Gram staining results are compatible with PCR results, since this method is fast, easy and inexpensive, so that it could be used in developing countries, where and when molecular techniques are not available. The high frequency in Kazakh young women found in this study is alarming, since BV increases woman's susceptibility to HIV, HPV and other important sexually transmitted diseases. Therefore BV has to be correctly and timely diagnosed in order to be adequately treated. Further investigations regarding other pathogens involved in BV such as *A. vaginae* and *Mobiluncus* spp. are warranted.

Conflict of interest

All authors declare to have no conflict of interest.

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