

Nagornaya V. F., Moskalenko T. Ya., Gritsenko A. A. Descriptiveness of vaginal secretions pH in the vaginal microbiota assessment in pregnant women. *Journal of Education, Health and Sport*. 2016;6(7):67-74. eISSN 2391-8306. DOI <http://dx.doi.org/10.5281/zenodo.57113> <http://ojs.ukw.edu.pl/index.php/johs/article/view/3659>

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part B item 755 (23.12.2015).
755 Journal of Education, Health and Sport eISSN 2391-8306 7

© The Author (s) 2016;

This article is published with open access at Licensee Open Journal Systems of Kazimierz Wielki University in Bydgoszcz, Poland

Open Access. This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited. This is an open access article licensed under the terms of the Creative Commons Attribution Non Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

This is an open access article licensed under the terms of the Creative Commons Attribution Non Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted, non commercial use, distribution and reproduction in any medium, provided the work is properly cited.

The authors declare that there is no conflict of interests regarding the publication of this paper.

Received: 25.05.2016. Revised 25.06.2016. Accepted: 28.06.2016.

UDC 618.3-06: 618.15-022.7] -07-085

DESCRIPTIVENESS OF VAGINAL SECRETIONS pH IN THE VAGINAL MICROBIOTA ASSESSMENT IN PREGNANT WOMEN

V. F. Nagornaya¹, T. Ya. Moskalenko^{1,2}, A. A. Gritsenko²

¹Odessa National Medical University, Department of Obstetrics and Gynecology №1;

²KU maternity hospital №7, Odessa

Summary

143 pregnant and 20 non-pregnant women have been examined. The objective: to determine the information content of the vaginal fluid pH. Methods used: Cytological, standard microbiological, real time PCR, the measurement of vaginal fluid pH. Results: five types of vaginal microbiota have been revealed, as well as a clear link of pH and the quantitative characteristic of microflora, high inverse correlation with estradiol content was shown, and correspondence to the number of gestation complications and the number of inflammatory diseases of the somatic plan was proved. The authors recommend to use pH of the vaginal fluid as an indicator of the state of vaginal biotope in the course of pregnancy.

Keywords: pH of the vaginal secretion, vaginal biotope pregnancy.

Vaginal secretions (*fluor vaginalis*) is a transudate with admixed thereto vaginal epithelial cells and microflora of different types - obligate protective, transient opportunistic, aerobic and anaerobic, the color of discharges is transparent, milky white, odorless or with a faint odor of moisture. The last two parameters are determined by the vaginal microflora: its character, the degree of contamination and the percentage in the biotope. Representations of the microbiota has undergone significant changes in recent years: from total denial of the possibility of the presence of pathogenic, opportunistic, anaerobic flora to moderately loyal when this flora presence in a certain concentration (10^3) is permissible [1,2].

At the conditions of the glands' increased secretion to differentiate norm from disease by the presence and quantity of leucorrhoea is difficult for both the pregnant and for the doctor and further examinations are needed [3]. All existing methods of diagnosis are of little descriptiveness, some are professionally dependent, others are related to pre-laboratory errors (violation of the rules of the material preparation, correctness of its gathering), and other financially burdensome. The doctor, interpreting the results of studies, will face certain difficulties [4].

MATERIALS AND METHODS.

Currently the following methods are used:

Cytological methods. A smear of vaginal secretions is often the first and the only diagnostic method that provides qualitative to a very small extent but not quantitative characterization of vaginal biotope and empirical approach to the treatment.

Does a smear, in which white blood cells are counted in the field of view, belonging to cocci and rods is determined, to Gram's stain, identified "key cells" for the doctor focuses mainly on the number of white blood cells to make a diagnosis?

To a certain extent it does, but at the same time it is a source of significant errors that lead to neglecting of patient's complaints, late diagnosis of bacterial vaginosis or medical aggression, particularly harmful during pregnancy and which is determined by a proverbial phrase "a smear cures" [4].

Bacteriological examination. If done at specialized laboratory the method under discussion allows to determine the content of certain types of opportunistic aerobic flora,

expressed it in the degree of contamination (degrees I-V or 10^{2-8}), show the physician the percentage of lactobacilli, while sometimes this is expressed by the words "a lot", "a little." Usually sensitivity to antibiotics, some antiseptics is determined. This method allows to determine the type of biocenosis and move from the empirical to the de-escalation targeted therapy. Bacteriological method is standard for evidence-based medicine. At the same time it needs a lot of time, is rather expensive and in each case the result depends on the laboratory capabilities.

The method of qualitative polymerase chain reaction (PCR), abused by the doctors, is a marker of infection, gives the qualitative characteristics, but does not allow it to clarify its stage, which gives the relative value to the method. The use of PCR is appropriate if quantitative characteristics of pathogens that should not be present in the vagina (STI) [2] is necessary.

ELISA method allows to determine the stage of the disease caused by the virus.

Thus, the most commonly used in clinical practice, diagnostic techniques do not allow doctors to make a diagnosis correctly and on time and assign the correct treatment.

In the work presented we used the following methods:

- general clinical (complaints, the development of the disease, medical and life history, general and gynaecological diseases history), colposcopy;

- microbiological: cytology (smears from the vagina and cervix), bacteriological (biopsy specimens from the posterior vaginal vault);

- PCR in real time (multiplex PCR) [1] provides to get a qualitative and quantitative characterization of vaginal biotope as a whole and its individual components: lactic bacteria from the group of opportunistic aerobic, anaerobic flora, STIs. These results allow us to solve treat or not to treat and how to treat, for example, bacterial vaginosis effectiveness of treatment depends on the pathogen, the chosen drug. However, the investigations should be carried out in a specialized laboratory by trained biologists, the method is expensive. Therefore, for all its virtues, multiplex PCR method has certain limitations.

- Method for determining the acidity of vaginal secretions pH- indicator with graded test strips. The method's essence is studied from the beginning of the 20th century to the present day (Italian Projects Study on pH and Hygiene - Sophy) [3]. The pH level is due to the number of lactobacilli and glycogen in vaginal epithelium. Accordingly, the method to a certain extent can assess vaginal biotope [5] and hormonal background [6]. To determine pH test strips were used, index value was determined on a graduated colour scale.

RESULTS AND DISCUSSION.

Under supervision there were 143 patients aged 21-39 years. 123 pregnant women were in different stages of gestation, they have made I clinical group. Research in the group I was of randomized nature at informing consent to participate in research. Criteria for inclusion in the research: age not older than 39 years, the lack of severe somatic pathology, the absence of indications of pharmacological correction of inflammatory diseases of the genitalia in the last 6 months. The file of primary observation was filled on the day of appealing to the doctor. Requirements for the collection of material: lack of special preparation and sexual contacts during the last 3 days. Criteria for exclusion from the study: STI in acute phase.

The results of the study of vaginal microbiota of the patients under observation has been analyzed. The data obtained allowed to determine five types of microbiota in the clinical group I. pH of the vaginal secretion was a basis for formation of subgroups. The content of lactic bacteria, pathogenic and conditionally pathogenic aerobic and anaerobic flora; concentration of *Candida*; white blood cell count were taken into account.

The following types of vaginal biocenosis: have been determined:

type I - pH <4.0; lactobacilli content up to 100%. Total bacterial mass (TBM) and the number of lactobacilli 10^8 . Pathogenic and opportunistic aerobic and anaerobic flora are absent. In smears - white blood cell count to 5 per HPF, a lot of lysed squamous epithelium cells. Patients complain of leucorrhoea, discomfort, a burning sensation in the vagina. Type I corresponds to cytolytic vaginosis, which is mentioned in rare sources of literature [3].

Type II - a pH of 4.0 -4.4; lactobacilli content > 78%. TBM 10^8 and the number of lactobacilli 10^{6-8} . Flora conditionally pathogenic, aerobic up to 30% of the biotope total mass, 10^3 - 10^4 , concentration does not exceed 10^3 , log <2, anaerobes to 10^2 , log 0,2 - 2,0 (*Gardnerella vag* to log 2,0; *Atopobium vag.* log 0,2 -1,3). Here were no complaints, vaginal examination revealed no abnormal discharge and visual signs of inflammation. Type II corresponds to the normocenosis.

Type III - pH to 4.5; content of lactic acid bacilli is 53 - 77% in biotope. TBM 10^{6-5} . White blood cells in the smears 4-6-7-10 in field of vision. A small number of the surface epithelial cells (up to 5 per HRF). Flora is conditionally pathogenic, anaerobic up to 30%, to 10^3 , (log 1,2 -2,0 -2,5). Anaerobes to 10^3 , (log 0,3 - 2,5 to 3.0). In this group 96% of pregnant women did not complain, there were no visual signs of inflammation of the external genitalia and vaginal mucosa, exocervix. 4% of them had periodically light discharges, soiling underwear with a sour smell. This type of biocenosis is related to the intermediate type.

Type IV - pH > 4.7 to 5.0; TBM 10^8 . Lactic bacilli content in the biotope is dramatically reduced - 36% to the total absence. White blood cells 0 - 5 - 7 in the field of view. Anaerobic flora predominates: pH 4.7 *Gardnerella vag.* at concentration $> 10^{3-6}$, log 3.1 - log 5.7; *Atopobium vag.* $< 10^3$, log 0.2 -1.3. At pH 5.0 concentration of anaerobic flora is much higher: *Gardnerella vag.* at concentration $> 10^5$, log 5.4 - log 8.5; concentration of *Atopobium vag.* $< 10^5$, log 3.2 -5.1; *Candidae* to 10^3 . Anaerobic flora is pathogenic, opportunistic, to 20% of the total biotope, concentration is up to 10^3 (log 2.5 -3.0), conditionally pathogenic flora (*E. coli*) prevails (log to 3.0). At pH 4.7 only 8.3% of women complained of abnormal vaginal discharge with unclear characteristics (smell, color, quantity).

At pH 5.0 74% of women complained of leucorrhoea gray-white in colour, heavy soiling underwear with an unpleasant odour. This type of biocenosis matches for microbiological and clinical characteristics of dysbiotic condition and is called bacterial vaginosis.

Type V - pH > 5.0; TBM content 10^8 , content of lactobacilli is drastically reduced or they are absent. The biotope is represented by aerobic diverse flora: cocci, rods at concentration of more than 10^3 (log 3.5); aerobes in varying concentrations above 10^3 . Patients complain of pronounced discomfort, discharges with the smell of rot, soiling underwear, burning, itching, pain. In vaginal smears there is many desquamated epithelium of surface and deeper layers. The number of leukocytes per HPF is usually sharply increased from 15 to 100, sometimes by layers, but in few patients white blood cells count was between 2 - 10. This type of biocenosis corresponds to nonspecific or mixed type of vaginitis.

In terms of clinical and paraclinical manifestations the Vth clinical subgroup was divided into 2 parts:

Va - pH > 5.0 -5.5. TBM 10^8 , leukocytes 10-30 per HPF. The number of lactobacilli in the biotope is reduced to 53-20% log 3.0-2.0; leukocytes more than 10 per HPF. Microbial diverse association 10^{4-5} (log 3.0 - 5.5), 50 to 70% in the biotope, anaerobes to 10^5 , fungi, mycelium $> 10^2$. Many desquamated epithelial cells.

Vb - pH > 5.5. TBM 10^8 . Lactobacilli are absent. White blood cells are more than 30 - 100 per HPF, situated in layers; some patients had no leukocytes. Microbial associations in the biotope, pathogenic, conditionally pathogenic flora, aerobic one is different (Gramm's staining), concentration 10^{6-8-9} log 5.0. Anaerobic flora is present in high concentration, fungi $> 10^3$. There is a lot of surface and deeper layers (parabasal, basal) epithelium.

Va type is designated as non-specific vaginitis of moderate severity and Vb - as severe vaginitis of mixed aetiology.

All patients with STI pathogens were treated to group V of biocenosis's type and have been excluded from the major clinical group.

A high reverse correlative link of vaginal secretions pH and estradiol content in blood serum in non-pregnant women's group [6] has been revealed.

Later the general somatic status at inflammation was analyzed and compared with features of pregnancy course. 6 clinical subgroups were formed.

In subgroup I (cytolytic type of biocenosis and pH <4.0; n = 2 (1.6%)), there were no inflammatory diseases, complications of gestation, these women did not receive drug therapy.

In subgroup II (n = 9 (7.3%)) with vaginal normocenosis pH 4.0 - 4.4 in 2 (1.6%) of 123 and 22.2% with respect to the number of subgroups kidney disease, caries in 2.4% and 33.3%, vaginal infection in 22.3% were marked. In this group, 8 women had physiological pregnancy, one had missed miscarriage and besides she had disturbances of luteal phase (LF) before pregnancy. This group pregnant women were sanitized in related professionals.

Subgroup III (intermediate type of biocenosis, pH to 4.5, n = 24 (19.5%)) 72% of the patients under observation suffered bowel disease, 4.2% had kidney pathology, 4.2% - ENT diseases, at 8.4% had caries, periodontal disease, 16.5% of them had different infections. At 20.8% of the pregnant women pregnancy was complicated by the threat of interruption, the formation of retroplacental hematoma - 4.2% and placental dysfunction was in 12.5%. Recommendations in this group were concerned hygiene of pregnancy and drug therapy aimed at the treatment of gestational complications.

In 62 (48,8%) patients of the IVth subgroup (pH > 4.7 - 5.0) and in 28 (22.8%) women of the Vth subgroup (pH > 5.0) a sharp increase of inflammatory diseases and gestation complications were marked. The majority of them had co-morbid conditions, among them colitis were diagnosed in 96.7% of the patients with bacterial vaginosis and 100% vaginitis patients, caries, periodontal disease (20% at bacterial vaginosis and 39.3% at vaginitis), vaginal infections in the past history (70% at bacterial vaginosis and 100% at vaginitis), ENT pathology had 50% of vaginitis patients. In subgroups Va and Vb incidence of inflammatory diseases dependent on the severity of vaginitis.

In subgroups IV and V (violated biocenosis and pH > 4.7 and especially pH > 5.0) the number of women with disorders of gestation is clearly seen. The number of patients with placenta dysfunction had dramatically increased and constituted 89.3% in the groups with vaginitis (normocenosis 0%; intermediate type - 12.5%); with the threat of interruption (75% at

normocenosis - 0%, the intermediate type - 20.8%), hydramnion, arrest of intrauterine development - up to 17.9%; premature rupture of fetal membranes - 42.9%. A large number of pre-eclampsias in the group with severe vaginitis (57.3%) cannot be ignored. This may be explained from the point of view of pre-eclampsia pathogenesis (violation of gestational changes in the spiral arteries, vascular endothelial damage). The last position gives reason to include vaginal infection as risk factor for preeclampsia.

Patients of group IV sanitation with drugs of imidazole group and vitamin C locally was held. At *Gardnerella vag.* was used dequalinium chloride locally, at *Atopobium vag.* probiotics topically and orally were obligatory used.

In group V sanitation was carried out by vitamin C, antiseptics of broad-spectrum action permitted for the use during pregnancy, the treatment was obligatory followed by probiotics for topical and oral administration.

CONCLUSIONS:

1. Index pH of vaginal secretion coincides with quantitative estimation of vaginal microbiota and may be used as an indicator of vaginal biotope.

2. Index pH of 4.0 - 4.7 can be considered as an indicator of norm. pH above 4.7 indicates violation of biocenosis and clearly corresponds to the increase of gestational complications and higher number of inflammatory diseases.

3. pH of the vaginal secretions can be used for screening, monitoring of the vaginal biotope during pregnancy, before delivery, caesarean section without additional bacteriological support.

References

1. Urogenital infections based by conditionally pathogen biota in fertility aged women: Clinical and laboratory diagnosis / Ye. V. Lipova et al. - Moscow, 2009.-44 p. (Rus.).
2. Kira Ye. F. Bacterial vaginosis. – Moscow: MIA, 2012.- 471p. (Rus.).
3. Cervix, vagina, vulva. Physiology, pathology, colposcopy, esthetic correction / Ed. S. I. Rogovskaya, Ye. V. Lipova. – Moscow: Status praesens, 2014. – 832 p. (Rus.).
4. Radsynsky V. Ye. Obstetrical aggression. – Moscow: Status praesens, 2011.-688 p. (Rus.)
5. Guaschino S., Benvenuti C., SOPHY project: an observational study of vaginal pH and lifestyle in women of different ages and in different physiopathological conditions // Minerva Ginecol. - 2008. - Apr. 60(2).-P.105-114.

6. V. F. Nagornaya, T. Ya. Moskalenko, A. A. Gritsenko. pH level-integral index of health of women`s sexual system // Medical aspects of family`s health. – 2015.- N 6 (92).- P. 48 – 52 (Ukr.)