

Inna Georgiivna Ponomarova¹, Tamara Oleksandrivna Lisyana¹, Olga Ivanivna Matyashova¹, Svitlana Urijivna Krishchuk

State University „Institute of Pediatrics, Obstetrics and Gynecology named after academician OM Lukyanova, National Academy of Medical Sciences of Ukraine”, Kyiv, Ukraine

The state of the microbiota of the genital tract in women who smoke

Corresponding author:

Inna Georgiivna Ponomarova,
State University „Institute of Pediatrics,
Obstetrics and Gynecology named
after academician OM Lukyanova,
National Academy of Medical Sciences
of Ukraine”, 8 Platona Maiborody St.,
04050, Kyiv, Ukraine;
e-mail: microbiiki@gmail.com

ABSTRACT

Introduction: Tobacco use has a negative effect on the immune system and contributes to a decrease in the protective properties of a woman's body, which leads to dysbiotic changes in the microbiota of the genital tract.

Materials and methods: To assess the species and quantity composition of the microflora of the vagina in women, bacteriological studies were conducted. The research group consisted of women with inflammatory diseases of the genital tract who smoke (40 women) and 40 women who do not smoke. The control group consisted of 30 healthy women who did not smoke.

Results: The obtained results indicate that the state of the microbiota of the genital organs in women who smoke is characterized by an increase in the spectrum of isolated microflora of enterobacteria and Gram-positive cocci, anaerobic bacteria, an increase in the level of viral infection and a significant decrease in the concentration of protective microflora. The microbiota of the genital tract of women who smoke is characterized by the formation of 2–3 component associations (in 45% of women) of infectious agents in various combinations. In non-smoking women, bacterial associations were found in 17.5% of cases.

Conclusions: In women who smoke, there is a significant imbalance of protective and potentially pathogenic flora with active contamination of the genital tract with conditionally pathogenic microorganisms that form multicomponent associations of infectious agents. The obtained data indicate the need for constant monitoring of the causative agents of vaginal dysbiosis in women who smoke, as well as the need for further research to identify the impact of smoking cessation on the vaginal microbiome.

Key words: smoking, vaginal microbiota, associations of microorganisms

Med Res J 2023; 8 (2): 147–151

Medical Research Journal 2023;
Volume 8, Number 2, 147–151
10.5603/MRJ.a2023.0027
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ISSN 2451-2591
e-ISSN 2451-4101

Introduction

In the conditions of modern society with its urbanization, social and economic problems, there is an increase in smoking, especially among the female population. Active and passive smoking leads to a violation of the general and reproductive health of women [1–3].

Cigarette smoke contains many toxic chemical substances, the main component of which is nicotine. Nicotine changes the state of brain structures, worsens the production of sexual hormones and reduces body resistance against infectious viruses, bacteria and fungi [4, 5]. Tobacco use contributes to a decrease in the protective properties of the macroorganism and disruption of systemic and local immunity, which can lead to dysbiotic changes in the microbiome of the genital tract [6, 7].

Scientific studies have proven that nicotine is found not only in the blood of women who smoke but also in other body fluids, namely in cervical mucus. Nicotine has a very strong effect on the microflora of the genital tract, which leads to disruption of the regulation of the microbiome cervical canal and vagina and promotes the uncontrolled growth of pathogenic and conditionally pathogenic microorganisms [8]. Thus, in the work of scientists, it is shown that nicotine can increase the ability of biofilm formation *Staphylococcus aureus*, which is a defensive reaction from the side of *S. aureus* to adverse conditions such as exposure to nicotine [9].

The components of tobacco smoke are also able to activate the processes of proteolysis by increasing the permeability of the epithelium of the female genital tract. This is accompanied by an increase in the influx

of neutrophilic enzymes that destroy the proteins of the connective tissue matrix and leads to the emergence of an inflammatory process in this biotope [10–12].

Due to the inhibition of the growth and vital activity of the protective microflora of the genital organs in women who smoke, the destruction of the protective layer of mucus and the attachment of conditionally pathogenic bacteria to the epithelial cells of the mucous membrane is observed, which is one of the causes of the development of dysbiosis of the genital organs [13, 14].

Active proliferation of conditionally pathogenic microflora is accompanied by changes in the system of homeostasis and microcirculation, which contributes to chronic inflammation [15–17].

A modern feature of inflammatory diseases of the pelvic organs is the polymicrobial nature of the lesion with the formation of multicomponent associations of conditionally pathogenic microorganisms and other infectious agents on the mucous membrane of the genital tract, which are more resistant to such protective factors as phagocytosis and lysozyme [18–20].

Lactic acid bacteria (*Lactobacillus spp.*). They act as antagonists to many opportunistic bacteria, stimulate the synthesis of secretory IgA and lysozyme, suppress the toxicity of some microorganisms, and produce substances of an antibiotic nature [21, 22].

But under the influence of various negative endogenous and exogenous factors, the amount of *Lactobacillus decreases spp.* and the number of conditionally pathogenic microorganisms increases [23, 24].

A change in the protective properties of the endogenous microflora of the vagina in women who smoke poses a threat of ascending infection, which can be realized by the development of chronic endometritis, inflammatory diseases of the pelvic organs, and subsequently by infertility, pregnancy losses, and adverse perinatal consequences [25, 26].

Therefore, this work aimed to study the state of the vaginal microbiota in women with pelvic inflammatory disease who smoke.

Material and methods

Bacteriological studies were carried out in order to evaluate the species and quantitative composition of the microflora of the vagina in 80 women of childbearing age with chronic inflammatory diseases of the lower genital organs (chronic vaginitis, chronic endocervicitis) depending on the use of tobacco. Diagnosis of chronic vaginitis and chronic endocervicitis verified according to the data of clinical and laboratory research methods. When establishing a gynaecological diagnosis, women used the International Classification of Diseases: 10, created by the recommendations of

WHO experts and adopted in Ukraine, according to which there are the following forms of inflammatory diseases of the genital organs of the lower part: chronic vulvitis, chronic vaginitis, chronic endocervicitis, chronic cervicitis.

The age of the study participants ranged from 24 to 27 years, the BMI was 18.5–24.9, and at the time of the examination, the women were not pregnant and did not take medication.

According to the gynaecological history, 45% of women were previously treated for colpitis of various aetiologies, 12.5% of women had a history of cervical erosion, and 22.5% of patients suffered from menstrual cycle disorders.

There was a somatic history weighted in 37.5% of women: 12.5% had disease gastrointestinal tract, 15% were diagnosed with endocrine pathology and 10% suffered from chronic tonsillitis.

The examined patients were divided into groups: Group I — women who smoke and have inflammatory diseases of the genital organs (40 women), and Group II — women who do not smoke and have inflammatory diseases of the genital organs (40 women).

The data obtained during the examination of 30 healthy women who do not smoke were used as a control.

Conducting microbiological analyses and recording the results was carried out by Order No. 234 of the Ministry of Health of Ukraine dated May 10, 2007, and other regulatory documents.

The following differential diagnostic media were used for vaginal mucus culture: blood agar, yolk-salt agar, Endo media, Saburo media and MRS media for *Lactobacillus spp.*

Sowing was carried out by the method of sectoral sowing on dense nutrient media, which allows for determining the degree of microbial insemination and revealing the maximum possible spectrum of aerobic and facultatively anaerobic microflora.

The identification of the selected microorganisms was carried out according to their cultural, morphological, tincture and biochemical properties on the automatic microbiological analyser Vitek 2 Compact (France).

Gardnerella vaginalis was diagnosed by the bacterioscopy method by staining smears according to Romanovsky with subsequent counting of „key” cells, performing an amine test and determining p N.

Diagnosis of *Chlamydia trachomatis* *Ureaplasma urealyticum* and *HSV 1/2* were performed by the luminenscent method using the „Chlamy-Scan”, „Urea-Scan” and „Herpes-Scan” kits.

Statistical processing of the obtained research results was carried out with the help of the standard computer package „Data Analysis” of Microsoft Excel for Windows 2007. The value of the arithmetic mean

Table 1. Indicators of microbiocenosis of the vagina of women who smoke and have inflammatory diseases of the genital organs (% , lg CFU/mL)

Microorganisms	Women with inflammatory diseases of the genital organs				Healthy women who do not smoke (CONTROL) n = 30	
	who smoke (I group) n = 40		who do not smoke (II group) n = 40		%	lg CFU/mL
	%	lg CFU/mL	%	lg CFU/mL		
<i>S. epidermidis</i>	20	4.5 ± 0.04■	22.5	3.8 ± 0.02♦	20.0	2.2 ± 0.02
<i>S. epidermidis</i> (hem +)	22.5	4.8 ± 0.03■	17.5	4.0 ± 0.4♦	6.6	2.5 ± 0.02
<i>S. aureus</i>	12.5	4.5 ± 0.02*■	7.5	3.6 ± 0.06♦	3.3	2.0 ± 0.03
<i>S. agalactiae</i>	10	4.4 ± 0.04*■	5	3.2 ± 0.03	6.7	2.5 ± 0.03
<i>S. pyogenes</i>	15	4.3 ± 0.02	2.5	3.8 ± 0.02	–	–
<i>Enterococcus spp.</i>	15	4.2 ± 0.03■	10	4.0 ± 0.03	10	3.3 ± 0.02
<i>E. coli</i>	25	4.3 ± 0.04	12.5	3.8 ± 0.07	13.3	3.5 ± 0.02
<i>E. coli</i> (hem+)	20	4.8 ± 0.05	10	3.6 ± 0.01	–	–
<i>Klebsiella spp.</i>	15	4.3 ± 0.03■	7.5	3.8 ± 0.06	6.6	3.0 ± 0.02
<i>Proteus spp.</i>	5	5.9 ± 0.02*	2.5	3.6 ± 0.01	–	–
<i>Candida fungi</i>	25	4, 5 ± 0.02■	7.5	4.1 ± 0.04♦	10	3, 1 ± 0.03
<i>Lactobacillus spp.</i>	65	3.6 ± 0.04*■	82.5	4.6 ± 0.03♦	100	6.8 ± 0.04
<i>Gardnerella vaginalis</i>	30*■		10		10	
<i>Chlamydia trachomatis</i>	15*■		7.5		6,7	
<i>Ureaplasma urealyticum</i>	20■		15		10	
HSV 1/2	15■		10		6.7	

*The difference is statistically significant between the indicators of women of the I and II groups ($p < 0.05$)

■The difference is statistically significant between the indicators of women of group I and healthy women ($p < 0.05$)

♦The difference is statistically significant between the indicators of women of the II group and healthy women ($p < 0.05$)

was calculated — the value (M), the average error of the mean value (m) and the probability value (p). The reliability of the obtained data was assessed by the generally accepted method using the Student's criterion. Reliability was considered established if its probability was at least 95% (0.05).

Results

Bacteriological studies conducted on women who smoke with inflammatory diseases (group I) testify to the substantial insemination of the vagina by opportunistic pathogens.

When examined women of the I group *Staphylococcus spp.* with haemolytic properties were recorded with a moderate frequency: *Staphylococcus epidermidis* (hem) — 22.5%, *Staphylococcus aureus* — 12.5%, various types of *Streptococcus spp.* were sown in 5–15% of examined women. Among *Enterobacteriaceae* were found: *Escherichia coli* (25%), *E. coli* with haemolytic properties (20%) and *Klebsiella spp.* (10%), *Proteus*

spp. (5%). *Candida fungi* contaminated the vagina of women of this group in 25% of cases. Representatives of normal microflora are *Lactobacillus spp.* found in 65% of the examined. Decreased *Lactobacillus* seeding rate *spp.* registered in 45% of women, and their absence in 35% of patients.

Lactobacillus concentration *spp.* was lg 3.6 CFU/mL and was lower than in women, who do not smoke but have inflammatory diseases of the genital organs (Tab. 1), from which it can be concluded that smoking has a significant effect on the number of *Lactobacillus spp.* as part of the microbiota urogenital tract. Smoking has negative effects on immune reactivity and may indirectly reduce the competitive ability of *Lactobacillus spp.* in the vaginal biotope.

The analysis of quantitative indicators of the microflora of the vagina in patients of the 1st group shows that the concentrations of Gram-positive *Staphylococcus spp.* and *Streptococcus spp.* were registered in the range of lg4.0–lg4.5 CFU/mL. Intestinal microflora was cultured in concentrations of lg4.3–lg5.9 CFU/mL. The level of sowing *Candida fungi* exceeded the diagnostic level –lg4.5 CFU/mL.

By the method of bacterioscopy, taking into account "key cells" and auxiliary tests of *Gardnerella vaginalis* was diagnosed in 30% of the examined. *Chlamydia* registration frequency *trachomatis* was 15%, *Ureaplasma urealyticum* 20%. HSV ½ was found in 15% of patients.

In 45% of sick women who smoke and have inflammatory diseases of the genital organs, the presence of opportunistic microflora in 2–3 species associations was recorded. The associations included *Staphylococcus* spp., *Enterobacteriaceae* and *Gardnerella vaginalis* or *Chlamydia trachomatis*.

When comparing the results of the examination of women who smoke and have inflammatory diseases of the genital organs (group I) with the data of women who do not smoke but have inflammatory diseases of the genital organs (group II), a lower frequency of detection of Gram-positive cocci was established in patients of group II. Thus, *S. epidermidis* (heme) was detected in 17.5% of the examined, at a concentration of lg 4.0 CFU/mL, *S. aureus* — 7.5% in an amount of lg 3.6 CFU/mL, *Streptococcus agalactiae* was cultured in 5% (lg 3.2 CFU/mL), and *Streptococcus pyogenes* in 2.5% of examined women in the amount of lg 3.8 CFU/mL. *Enterococcus* spp. was detected in 10% of women in this group and its level was lg 4.0 CFU/mL.

The frequency of cultivation of *Enterobacteriaceae* in women of the II group was 2.5–12.5%, and their quantitative indicators were –lg 3.6 to –lg 3.8 CFU/mL, which did not exceed the diagnostic level. Mushrooms *r. Candida* was cultured in women of the II group in 7.5% of cases. The concentration of *Candida* fungi was lg 4.1 CFU/ml. In patients of the II group, a decrease in the level of lactoflora seeding was also established. *Lactobacillus* spp. was detected in 82.5% of women, and their absence was registered in 17.5%.

Chlamydia diagnosis frequency *trachomatis* in non-smoking women was 7.5%, *Ureaplasma urealyticum* 15%. *Gardnerella vaginalis* and HSV ½ were registered in 10% of patients.

In 17.5% of women of the II group, the isolated microflora was found in associations that included *Enterobacteriaceae*, *Candida* fungi, *Gardnerella vaginalis* and HSV ½ in various combinations.

Discussion

The obtained results showed that the state of the microecology of the genital organs in women who smoke and have inflammatory diseases of the genital organs is characterized by an increase in the level of viral infection, an increase in the specific weight in the general spectrum of isolated microflora of anaerobic bacteria, enterobacteria and a significant decrease in the concentration of protective microflora [27, 28].

Inflammatory diseases of the genital organs are a very urgent problem in modern gynaecology, as they occupy the first place in its structure (up to 70%), and also lead to serious long-term complications, such as infertility, chronic pelvic pain syndrome and ectopic pregnancy.

The conducted research revealed pathological changes in the vaginal microbiota of various degrees in women who smoke. These negative factors cause shifts in the composition of the microbiota at the level of bacterial communities and can increase the pathogenic potential of microbial populations [29, 30]. The obtained results indicate the formation of complex associations of microorganisms, which lead to dysbiotic disturbances of the microflora of the urogenital tract of women.

This indicates the need for constant monitoring of causative agents of vaginal dysbiosis in women in order to select optimal therapy schemes taking into account the biological properties of individual strains that cause imbalances.

It was found that smoking is associated with a vaginal microbiota that contains low proportions of *Lactobacillus* spp., however, future research is needed to establish whether smoking cessation can positively affect the vaginal microbiome.

The current research has several limitations. The research was designed as a pilot; therefore the size of samples and funds were limited. Wide testing for infections that are transmitted sexually could not be held. In addition, the size sample size was reduced due to the exclusion of women who used hormonal contraception, because the use of hormonal contraception was associated with a reduced risk of bacterial vaginosis in most epidemiological studies [31].

A larger smoking cessation study is needed to establish a causal relationship that smoking directly affects the vaginal microbiome.

Conclusions

The negative influence of smoking on the state of micro biocenosis of the genital tract was established. Women who smoke have a significant imbalance of protective and potentially pathogenic bacterial flora and active contamination of the genital tract with conditionally pathogenic microorganisms.

In women who smoke with inflammatory diseases of the genital organs, there is a significant violation of the microbiota of the vagina, which is characterized by the formation of bacterial associations. With a greater frequency of association of infectious agents in various combinations, it was found in examined smokers (45%). In women who do not smoke, but have inflammatory diseases of the genital organs, bacterial associations were found in only 17.5% of cases.

Studies have shown the need for constant monitoring of causative agents of vaginal dysbiosis in women, as well as their associations for choosing optimal therapy schemes.

Further research is needed to determine whether smoking cessation can have a positive effect on the vaginal microbiome.

Conflict of interest: *None.*

Funding: *None.*

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