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ORIGINAL ARTICLE

DETECTION OF PREDEFINED BACTERIAL SPECIES IN THE VAGINAL MICROBIOTA IN SARS-COV-2-POSITIVE PATIENTS

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Abstract. *Whether severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) can be found in the vagina of infected women remains unclear; moreover, the impact of the virus on the normal vaginal microbiota is not known.* ***The aim*** *of our study was to identify the vaginal presence of SARS-CoV-2 and detection of predefined bacterial species changes in the vaginal flora of women that tested positive for SARS-CoV-2 infection.* ***Materials and Methods.*** *This prospective study included 40 women, aged 24-47 years, tested for SARS-CoV-2 via nasopharyngeal and vaginal culture (TaqPath ™COVID-19 CE-IVD RT-PCR), and vaginally tested for changes in the vaginal microbiota using the Femoflor® 16 REAL-TIME PCR Detection Kit.* ***Results.*** *No one of women in this study was tested positive for vaginal presence of SARS-CoV-2. Three (7.5%) women with sexually transmitted disease were excluded. Irregularities were observed in the vaginal microbiota of 8 (21.6%) out of 37 patients included in the study: 3 (8.1%) from the SARS-CoV-2-positive group and 5 (13.5%) from the SARS-CoV-2-negative group. The remaining 29 (78.4%) women had normal vaginal flora; lactobacilli were found to be dominant. Although results revealed a difference in the vaginal microbiota between the two groups, the differences were not statistically significant ($p \geq 0.05$).* ***Conclusions.*** *Even though it remains unclear whether SARS-CoV-2 invades the vagina of infected women, there is no significant evidence to suggest that it causes a more frequent disturbance in the vaginal microbiota of infected women compared to that in healthy women.*

Key words: *SARS-CoV-2, vaginal presence, vaginal flora, microbiota, microbial changes*

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INTRODUCTION

Vaginal flora in healthy women forms a dynamic ecosystem dominated by lactic acid bacteria (lactobacilli), and this ecosystem undergoes continuous changes in its structure and composition under the influence of many exogenous and endogenous factors [1-3]. Reduction or disappearance of vaginal lactobacilli unlocks a pathological microbial spiral, leading to a disruption in the existing equilibrium with the possibility of developing a local infection [1-3]. Besides local factors, such as exogenous

import of pathogenic microbes and viruses, allergic reactions, operative interventions, various other diseases, and external factors can cause a disturbance in the vaginal flora [4-11]. In 2019–2020, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was established as the causative agent for some life-threatening serious diseases [12]. In March 2020, the disease caused by SARS-CoV-2, termed coronavirus disease (COVID-19), was officially declared a pandemic by the World Health Organization [12]. COVID-19 exhibits different trends in terms of morbidity and symptoms in humans, based on their locations [13]. The symptoms also vary depending on individual genetics, ethnicity, age, and co-morbidities [14]. Recent studies reported that SARS-CoV-2 can be found in the vagina of infected women [15]. According to other studies, it is not found in the vagina of infected patients [16]. However, to the best of our knowledge, this is the first study that aimed to investigate the impact of the virus (direct or indirect) on vaginal microbial flora.

The purpose of our study was to identify the vaginal presence of SARS-CoV-2 and detection of predefined bacterial species changes in the vaginal flora of women that tested positive for SARS-CoV-2 infection.

MATERIALS AND METHODS

This prospective study was conducted in the Gynecology and COVID-19 Departments of Military Medical Academy, Sofia in October–December 2020. The study included a total of 40 women aged 24-47 who visited an emergency COVID-19 office of the Military Medical Academy with symptoms of mild and severe SARS-CoV-2 infection [17]. The medical history of each study participant was recorded; a general medical examination, gynecological examination, and microbiological tests were performed thereafter. A swab for the Multiplex real-time RT-PCR test intended for qualitative detection of nucleic acids from SARS-CoV-2 (TaqPath™ COVID-19 CE-IVD RT-PCR Kit, Applied Biosystems, Thermo Fisher Scientific, Life Technologies Corporation, 6055 Sunol Blvd, Pleasanton, CA 94566) was taken from the nasopharynx of every patient [18]. The women were divided into two groups depending on their nasopharyngeal PCR SARS-CoV-2 test results. The first group included 19 women who tested positive for SARS-CoV-2. The second group included 21 women who tested negative for SARS-CoV-2. During the gynecological check, a vaginal examination was performed to evaluate vaginal secretion for persistent infection according to clinical symptoms and following specifications: quantity, consistency, color, and odor. At the same time, a sample for the Femoflor® 16 REAL-TIME PCR test and a swab for the Multiplex real-time RT-PCR test intended for qualitative detection of nucleic acids from SARS-CoV-2 (TaqPath™) was taken from vagina of every study participant prior to any antibiotic treatment.

Inclusion criteria: Clinical symptoms suspected of SARS-CoV-2 infection, preserved ovarian steroidogenesis.

Exclusion criteria: Pregnant women; women taking corticosteroids, antibiotics, imidazoles, probiotics or vaginal medications in the last month; immunocompromised patients; and those with autoimmune diseases, endocrine diseases, or diabetes, contraception HIV infection and the researched and established sexual transmitted diseases (STD).

The patients were provided with information regarding the purpose of the study and the inclusion/exclusion criteria; all patients provided informed consent for participation in the study. Ethical approval (№ 27/12 Nov. 2020) was sought and granted by the Ethical Review Board of Military Medical Academy, Sofia.

A sample of vaginal secretion was collected with a dry sterile swab for microbiological testing with a Femoflor 16® REAL-TIME PCR Detection Kit (DNA-Technology Research & Production, LLC, Moscow, Russia) to detect vaginal microbiota changes, during vaginal examination from the back vaginal vault [19]. The sample was then transferred to plastic tubes containing 300 µl of physiological saline solution or in tubes containing the “DNA-Technology” PREP-RAPID DNA Extraction Kit (P-001/1EU) solution, according to the manufacturer’s instructions [19]. Overall time from the sample intake until analysis did not exceed 24 hours at storage temperatures between 2 °C and 8 °C. The Femoflor® Real-time PCR Kit is a qualitative in vitro nucleic acid test and uses one biological sample for quantitative assessment of the total bacterial mass, urogenital normoflora-lactobacilli, combinations of aerobic and anaerobic microorganisms typically found in the urogenital tract of women, mycoplasma, and fungi in the Candida genus, involved in the development of dysbiotic processes in urogenital microbiocenosis [19]. The test results reveal information about the total vaginal bacterial mass by measuring *Lactobacillus spp.; Enterobacterium spp.; Streptococcus spp.; Staphylococcus spp.; Gardnerella vaginalis/Prevotella bivia/Porphyromonas spp.; Eubacterium spp.; Sneathia spp./Leptotrichia spp./Fusobacterium spp.; Megasphaera spp./Veillonella spp./Dialister spp.; Lachnobacterium spp./Clostridium spp.; Mobiluncus spp./Corynebacterium spp.; Peptostreptococcus spp.; Atopobium vaginae;*

Mycoplasma hominis; Mycoplasma genitalium; Ureaplasma (urealyticum + parvum); Candida spp., and T. vaginalis; N. gonorrhea vaginal colonization [19].

Diagnostic sensitivity of Femoflor: 97%. Diagnostic specificity of Femoflor: 97% [19].

Statistical methods

The Chi-square test was used to evaluate independent variables. The result was considered statistically significant at $p < 0.05$.

RESULTS

None of the women in this study were tested positive for vaginal presence of SARS-CoV-2. Of the 40 women enrolled in our study, 3 (7.5%) were found to have a sexually transmitted disease (exclusion criteria) with T. vaginalis (1/2.5% from the SARS-CoV-2-positive group and 2/5% from the SARS-CoV-2-negative group); therefore, they were excluded from the study.

Consequently, we presented and discussed the results for 37 patients: 18 (48.6%) in the first SARS-CoV-2-positive group and 19 (51.4%) in the second SARS-CoV-2-negative group. None of the women tested positive for other sexual transmitted diseases (*Mycoplasma genitalium; N. gonorrhea*) after the Femoflor®-16 vaginal test.

The vaginal microbiota was found to be disturbed in 8 (21.6%) patients: 3 (8.1%) from the SARS-CoV-2-positive group and 5 (13.5%) from the SARS-CoV-2-negative group; the remaining 29 (78.4%) women showed normal vaginal flora dominated by Lactobacillus spp. Although a difference in vaginal microbiota disturbances was observed between the two groups, the results were not statistically significant ($p \geq 0.05$).

Table 1. Patient distribution based on nasopharyngeal and vaginal SARS-CoV-2 RT-PCR, and STD test results

All patients included n-40/100%	Vaginal SARS-CoV-2 positive n (%)	Vaginal SARS-CoV-2 negative n (%)	STD positive n (%)	STD negative n (%)
nasopharyngeal SARS-CoV-2 positive	0 (0)	19 (47.5)	1 (2.5)	18 (45)
nasopharyngeal SARS-CoV-2 negative	0 (0)	21 (52.5)	2 (5)	19 (47.5)
Total	0 (0)	40 (100)	3 (7.5)	37 (92.5)

Table 2. Patient distribution based on SARS-CoV-2 RT-PCR test results and vaginal microbiota status.

All patients included n-37/100%	Normal vaginal microbiota n (%)	Disturbed vaginal microbiota n (%)	Total n (%)
SARS-CoV-2 positive	15 (40.6)	3 (8.1)	18 (48.7)
SARS-CoV-2 negative	14 (37.8)	5 (13.5)	19 (51.3)
Total	29 (78.4)	8 (21.6)	37 (100)

*X2 (1, n = 37) = 0.5078, p = 0. 47608. *This statistic is used for all vaginal microbiota changes and all group I/group II patients

Table 3. Microbial findings of patients from the first and second groups with disturbed vaginal microbiota.

Patients with disturbed vaginal microbiota	Vaginal infections – patients n (%)	Detected microbial species – patients n (%)
n-3 (8.1%) SARS-CoV-2 positive	2 (5.4) Obligate anaerobes	1 (2.7) *Gardnerella vaginalis/ Prevotella bivia/Porphyromonas spp.*
		1 (2.7) *Mixed anaerobe infection: Gardnerella vaginalis/Prevotellabivia/Porphyromonas spp.; Peptostreptococcus spp.; Mycoplasma hominis; Lachnobacterium spp./ Clostridium spp.*
	1 (2.7) Candida spp.	1 (2.7) Candida albicans
n-5 (13.5%) SARS-CoV-2 negative	2 (5.4) Obligate anaerobes	1 (2.7) *Mixed anaerobe infection: Gardnerella vaginalis/Prevotellabivia/Porphyromonas spp.; Peptostreptococcus spp.; Mycoplasma hominis; Lachnobacterium spp./ Clostridium spp.*
		1 (2.7) *Atopobium vaginae*
	1 (2.7) Streptococcus species	1 (2.7) *Streptococcus species*
	2 (5.4) Mixed vaginal infections	2 (5.4) *C. albicans; Gardnerella spp.; Peptostreptococcus spp.; Ureaplasma spp.*
Total	8 (21.6)	8 (21.6)

Microbial species in patients with impaired vaginal microbiota presented at Table 3, were not significantly different between the two groups. There were the prevalence of obligate anaerobes, Candida spp, and mixed infections.

DISCUSSION

The world's most common pathogens that infect humans are viruses. The mechanism underlying viral infections includes promotion and suppression and other less-understood pathophysiological steps [20]. Many studies suggest mutual interactions between viruses and the human microbiota [7, 20-25]. Normal Lactobacillus spp. dominated the microbiota and could prevent and suppress some of the sexually transmitted and other viral infections through distinct mechanisms, such as competitive adhesion, interactions with the local immunity and plasminogen-plasmin system, and production of lactic acid, hydrogen peroxide, and antibacterial substances [2, 3, 20-23]. There is evidence that disturbance of the vaginal microbiota potentiated sexually transmitted virus infections such as HIV, HSV and HPV infections; however, it is still unclear whether these microbial disturbances are a result of local virus infections or they arise as a consequence of a different stimulus [23-25]. In this study we did not find vaginal presence of SARS-CoV-2 in none of the women even at those 3 (8.1%) with disturbed vaginal flora, nasopharyngeal SARS-CoV-2 positive patients. From this fact, we can make an assumption that normal Lactobacillus spp. dominated vaginal microbiota, have no impact on the vaginal invasion of SARS-CoV-2 in COVID-19 positive patients.

Microbial species detected in patients with impaired vaginal microbiota in both groups were almost the same. These were the anaerobes and Candida spp isolated most often in women with impaired vaginal flora. From these results, it can not be concluded that patients positive for SARS-CoV-2 have more often than negative, infection of a certain microbial species.

Some studies did, while others did not, report a vaginal presentation of SARS-CoV-2 in infected women [15, 16, 26]. Therefore, it is still unclear whether SARS-CoV-2 is found in the vagina of infected women; moreover, clarification is required regarding the type of impact of the virus on the normal vaginal microbiota, the circumstances and factors that facilitate the vaginal entry of this virus (direct or indirect), and whether COVID-19 can be transmitted sexually [27].

A limitation of our study is the small number of patients included. Similar randomized studies are needed to establish the vaginal presentation of SARS-CoV-2 in infected women and effect of SARS-CoV-2 infection on the vaginal microbiota.

In conclusion, even though it is still unclear whether SARS-CoV-2 invades the vagina of infected women, there is no significant evidence suggesting that it causes a more frequent disturbance in the vaginal microbiota of infected women when compared to that in healthy women.

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