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ORIGINAL ARTICLE

Age and sex trends of *Gardnerella vaginalis* infection in patients with sexually transmitted infections in Korea

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ABSTRACT

Background and Objectives: *Gardnerella vaginalis* and *Candida albicans* are the most common causative agents of bacterial vaginosis, and infections with these pathogens lead to inflammation, endometritis, and pruritus. The aim of this retrospective study was to determine the trends of *G. vaginalis* infections based on real-time PCR data according to age and sex in patients with sexually transmitted diseases.

Materials and Methods: A total of 59,381 specimens isolated at a clinical laboratory from September 2018 to December 2020 were subjected to real-time PCR for the detection of *G. vaginalis* DNA. Sample types included catheter, pus, tissue, swab, and urine samples.

Results: Among 59,381 samples, 20,718 (34.8%) were positive for *G. vaginalis*. Of the positive samples, 13,186 (63.7%) were from male patients and 7,532 (36.3%) were from female patients. Average patient age was 39.1 years (the average age of male and female patients was 38.34 and 40.43 years, respectively). Female patients younger than 19 years exhibited the highest incidence of *G. vaginalis*, at 71.57%, followed by 68.46% incidence in those aged 20-29 years; the lowest incidence was in women aged 40-49 years. Further, among specimen types, the highest number of *G. vaginalis*-positive specimens was obtained by the swab sampling method.

Conclusion: From 2018 to 2020 in Korea, the number of tests conducted for bacterial vaginosis has increased, while the incidence of *G. vaginalis* infections appears to have decreased. the finding that female adolescents have a high tendency to carry the pathogen is important. and for effective surveillance of BV, sampling by cotton swabs and detection by multiplex PCR might be a good approach.

Keywords: Bacterial vaginosis; Dermatology; *Gardnerella vaginalis*; Multiplex real-time polymerase chain reaction; Sexually transmitted disease

INTRODUCTION

Bacterial vaginosis (BV) is caused by anaerobic pathogens, such as *Gardnerella vaginalis (G. vaginalis), Mycoplasma hominis,* and *Prevotella* spp. and it occurs due to bacterial proliferation in the vagina (1, 2). BV causes abnormal odor and discharge in the vagina, affecting women's sexual relations and quality of life (3). Moreover, BV is associated with a higher susceptibility to pelvic inflammatory diseases

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and sexually transmitted infections (STIs) (4, 5). *G. vaginalis* and *Candida albicans* are the most common causative agents of vaginitis in women and can lead to pelvic infections, urinary tract infections, and endometritis in women. In addition, vaginitis causes vulvar pruritus, which makes daily life uncomfortable for patients (6). *G. vaginalis*, an anaerobic bacterium found in specimens isolated from patients with BV, is a well-studied pathogen (2, 7, 8). It produces several virulence factors, including sialidase A (9, 10) and vaginolysin (9), and it generates biofilms by attachment to vaginal epithelial cells (11, 12). In a study of patients with BV and those with healthy vaginal microflora, *G. vaginalis* was found in 87.5% of the women with BV (13), and 26.4% of women with a healthy vaginal microbiome were positive for *G. vaginalis* (13).

It has been hypothesized that vaginal microflora may be associated with the progression of cervical carcinogenesis (CC). One study showed that the presence of *Gardnerella* and *Streptococcus* spp., as detected by amplicon sequencing, may be associated with the advancement of CC in Korean women (14). Patients with psoriatic skin or those treated with UV-B light therapy show decreased levels of *Firmicutes, Staphylococcus, Finegoldia, Anaerococcus, Peptoniphilus, Gardnerella, Prevotella*, and *Clostridium* spp. (15). Only a few epidemiological studies have employed PCR for the diagnosis of BV. Accurate diagnosis and information on epidemiological trends are important to effectively curb this disease. The aim of the present study was to determine the trends of *G. vaginalis* infections in a Korean study population according to age and sex based on real-time PCR data. We believe that the findings will contribute to the improvement of public health and the development of new therapeutic approaches and preventive strategies for BV.

MATERIALS AND METHODS

Materials. From September 2018 to December 2020, 59,381 specimens were collected from outpatients across primary and secondary hospitals in Korea who had requested U2Bio (Seoul, Korea) to conduct molecular biology venereal disease testing. The specimens were analyzed after classification as catheter, pus, tissue, swab, or urine samples. Specimen collection was performed with the prior consent of the patients.

Ethics approval. This study was approved by the Institutional Review Board of Dankook University, Republic of Korea (DKU 2021-03-056), and was conducted in accordance with the principles of the Declaration of Helsinki.

Nucleic acid extraction. The clinical specimens were stored at -70°C until DNA isolation for real-time multiplex PCR (mPCR). DNA for the mPCR assay was extracted using an ExiPrep Dx Bacteria Genomic DNA Kit (Bioneer, Daejeon, Korea) according to the manufacturer's instructions. The concentrations of the extracted DNA samples were measured using an AccuPower STI4C-Plex Real-Time PCR Kit (Bioneer).

Real-time PCR analysis. Real-time PCR analysis was performed using the AccuPower STI4C-Plex Real-Time PCR Kit with an Exicycler 96 Real-Time Quantitative Thermal Block (Bioneer) according to the manufacturer's protocol. The amplification protocol consisted of one cycle at 95°C for 5 min, followed by 45 cycles of 95°C for 5 s and 55°C for 5 s. The threshold cycle was determined according to the manufacturer's instructions. Four pathogens, namely, *C. albicans, G. vaginalis, Ureaplasma parvum*, and *Treponema pallidum*, were evaluated. This was a retrospective study based on results obtained from an institution that received the patient data. Since a follow-up study is currently in progress, only the data of a single item, i.e., *G. vaginalis*, which had the highest positivity among STI samples, was analyzed.

RESULTS

Effect of sample collection methods. Among the different collection methods, the swab method yielded the highest number of *G. vaginalis*-positive specimens for both male and female patients. *Gardnerella vaginalis*-positive swab samples were obtained from 66.1% and 33.2% of the female and male patients, respectively. The second-highest number of *G. vaginalis*-positive specimens was obtained in urine samples, i.e., 45.8% and 29.2% of female and male patients, respectively, in the study population. A comparison of the positivity rates for *G. vaginalis* in male and female

patients based on different sample collection methods is shown in Fig. 1.

Positivity rate for *G. vaginalis* based on sex and age. Of the 59,381 specimens, 45,833 were from men and 13,548 were from female patients. Overall, 20,718 (34.8%) specimens were positive for *G. vaginalis*, of which 13,186 (63.7%) and 7,532 (36.3%) were from male and female patients, respectively. Although the total number of tests conducted for male patients was higher than for female patients, the latter accounted for more than half of the total positive cases. The average age of patients who tested positive for *G. vaginalis* was 39.1 years, with 38.3 years being the average for male patients and 40.4 years for female patients. The individuals of the *G. vaginalis*-positive group were classified by sex and age (Table 1). Overall, the percentage of *G. vaginalis*-positive patients was the highest in the 40-49 age group (37.8%), followed closely by the 0-19 and 50-59 age group. The lowest incidence was found in the >70 years group.

The incidence rate of *G. vaginalis* among female patients under the age of 19 was the highest (71.5%), followed by 68.4% for 20-29 years, 61.4% for 40-49 years, 59.4% for 30-39 years, and 52.2% for 50-59 years. In patients aged 60-70 years and above, the incidence rate sharply decreased to 31.5% and 26.7%, respectively. The number of samples and the average positivity rate of the patients were classified by age and sex (Figs. 2 and 3).

Trends of the mean incidence of *G. vaginalis*. The average number of *G. vaginalis*-positive tests per year was 574.5 in 2018, which increased to 778.6 in 2019 and 1250.8 in 2020. It is worth noting that the results for this time period indicate a gradually decreasing trend in the positivity rate, from 38.9% in 2018 to 34.4% in 2020 (Figs. 4 and 5).

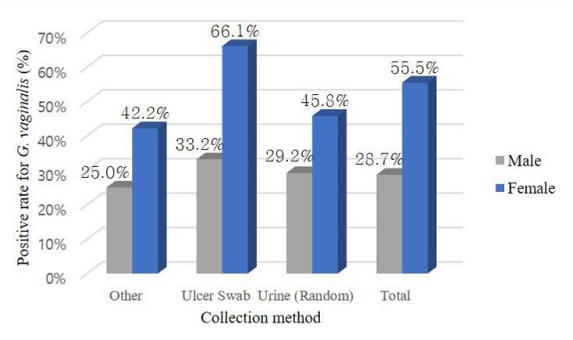

Fig. 1. Comparison of *Gardnerella vaginalis* positivity rates for specimens derived from male and female patients based on different sample collection methods. Gray bars represent male patients, and blue bars represent female patients.

Table 1. Total *Gardnerella vaginalis*-positive specimens

		Positive specimens (%)
Sex	Male	13,186 (28.7)
	Female	7,532 (55.5)
Age	0-19	376 (36.6)
	20-29	5,742 (35.7)
	30-39	5,635 (36.0)
	40-49	4,210 (37.8)
	50-59	3,094 (36.5)
	60-69	1,227 (26.2)
	≥70	434 (18.3)

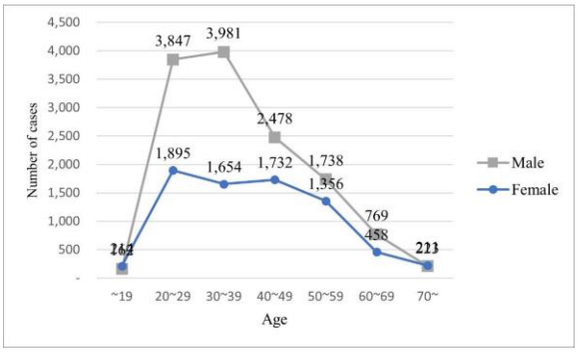

Fig. 2. Comparison of the number of *G. vaginalis*-positive reactions classified on the basis of age. The gray line indicates the number of positive cases in male patients, and the blue line indicates the number of positive cases in female patients.

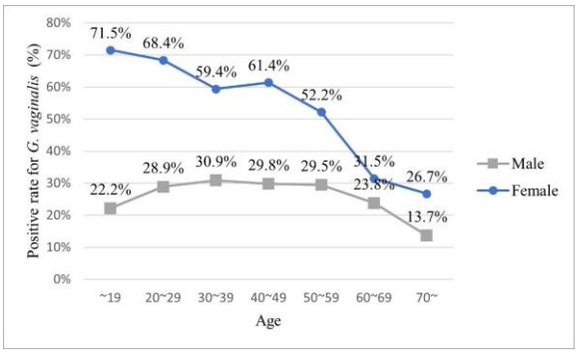

Fig. 3. Comparison of the mean positivity rate for *G. vaginalis* on the basis of age. The gray line represents the *G. vaginalis* positivity rate in male patients, and the blue line represents the *G. vaginalis* positivity rate in female patients.

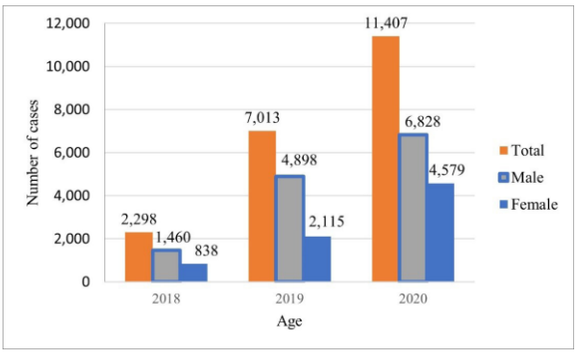

Fig. 4. Annual average testing trend of *G. vaginalis*. The orange bar represents the total number of conducted tests. The gray bar represents the number of tests conducted on male patients, and the blue bar represents the number of tests conducted on female patients.

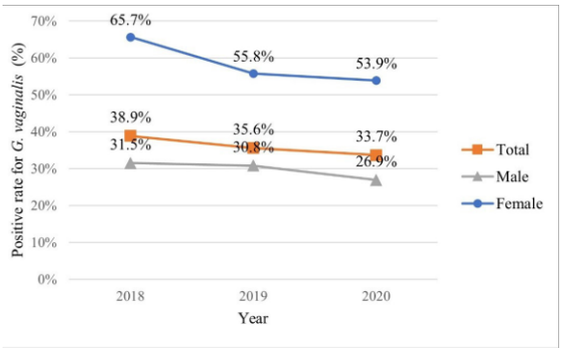

Fig. 5. Annual average positivity rate of *G. vaginalis* in 2018, 2019, and 2020. The orange line represents the total positivity rate for *G. vaginalis*. The gray line represents the positivity rate for *G. vaginalis* in male patients, and the blue line represents the positivity rate for *G. vaginalis* in female patients.

DISCUSSION

This retrospective study was conducted to determine the trends of *G. vaginalis* infections according to age and sex based on real-time PCR data obtained from Korean patients with STIs from 2018 to 2020. In a previous study involving 49 patients, researchers identified the microbial flora of the bladder using the urea method (16). These microbes were not similar to those of the urethra and urine. In a study wherein 50 paired swabs and urine samples of pregnant women were analyzed for the presence of *G. vaginalis*, droplet digital PCR was employed for the absolute quantification of the pathogen. The average number of copies of *Gardnerella* was quantified as 241,598 copies/μL in urine and 441,655 copies/μL in cotton swabs (17). The results were similar to those of the present study in that the maximum *G. vaginalis*-positive rates were obtained by the swab method. The swab collection might be the most suitable collection method for this bacterium.

The same study further revealed that *Lactobacillus* and *G. vaginalis* were detected more frequently in young women than in the elderly. Young women in their teens to thirties were found to be more likely to be positive for *G. vaginalis* than women in their 60s and 70s (17). This is consistent with the results of the present study. In a retrospective study on 487 girls aged 14-18 years with adolescent vulvovaginitis (18), the incidence of BV in the 17-year-old group was higher than that of other age groups. The incidence of vulvovaginal candidiasis and *Candida* infections in the 17- and 18-year-old groups was higher than that in the 14-year-old group. In our study, no subdivision was made in the teenage group; however, this is worth referencing because it was the age group with the highest positivity rate. In a Japanese study, Prevalence of *G. vaginalis* in males was 14% (19). *G. vaginalis* was found in approximately one out of 7 men with urethritis (19). In previous studies of the 108 vaginal samples collected from BV positive women, we observed that *G. vaginalis* was present in 96.2% (104/108) females and amongst 208 BV negative women, it was present in 52.6% (109/208) (20). This is consistent with our results that showed the highest positivity rates of 71.57% and 68.46% for teenaged girls and 20-29-year-old women, respectively.

A rare case of osteomyelitis due to *G. vaginalis* and *Streptococcus parasanguinis* has also been observed in a postmenopausal woman aged 61 years (21). This case involves a peculiar co-infection of two pathogens, usually present in the genitourinary tract and oral cavity, and highlights the risks that arise when these organisms penetrate the body's normal barriers. A sizeable number of men were found to harbor *G. vaginalis* in our study. Therefore, medical professionals should acknowledge *G. vaginalis* as a causative agent of infection, even in male patients with or without urogenital disease and offer adequate treatment, particularly when a Gram-variable organism is detected in the blood or urine of men (22).

A limitation of our study might be that the data were from the last three years and were not collected over a longer period of at a single inspection center. Owing to the absence of patient information such as

other underlying diseases, the data cannot represent the overall positive results of *G. vaginalis*; however, the trends of infection can be examined through age- and sex-specific analysis and the trend of *G. vaginalis* over the last three years.

CONCLUSION

Gardnerella vaginalis is the most common causative agent of BV. STIs potentially contribute to the occurrence of several diseases, and their occurrence may vary depending on sex and age. Our study findings could aid in devising strategies to protect public health and in reducing the incidence and transmission of STIs (e.g., by using improved sampling and detection techniques). We highlight the need to focus on accumulating long-term analysis data on STIs based on geographic region, age, and sex, together with continuous monitoring of infection trends.

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