Etiologic agents of cervicovaginitis in Turkish women

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ABSTRACT

Objective: To investigate the distribution of microbiologic agents causing cervicovaginitis.

Methods: We conducted the study between October 2002 and December 2004 in Abant Izzet Baysal University, Duzce School of Medicine Hospital, Turkey. The samples were obtained from the posterior vaginal fornix and cervix by swabs in 828 patients. Direct microscopic examination, culture and enzyme immune assay (EIA) methods were performed in all patients for diagnosis of microbiologic agents.

Results: *Gardnerella vaginalis (G. vaginalis)* were diagnosed in 254 (30.7%) patients, *Candida albicans (C. albicans)* in 152 (18.4%), *Candida glabrata (C. glabrata)* in 36 (4.3%), *Candida species* in 52 (6.3%), *Staphylococcus aureus (S. aureus)* in 62 (7.5%), *Streptococcus* group B in 28 (3.4%), *Escherichia coli (E. coli)* in 42 (5.1%), *Klebsiella species* in 24 (2.9%), and *Streptococcus* group D in 8 (1%) patients in culture. Less frequent enterobacteria in 30 (3.6%) were: *Pseudomonas species, Proteus species Enterobacter species*, *Hafnia alvei and Nonfermenter species*. *Neisseria gonorrheae (N. gonorrheae)* was detected in one patient (0.1%) in culture. The *Chlamydia trachomatis (C. trachomatis)* antigen was detected by EIA methods in 130 (15.7%) patients and *Trichomonas vaginalis (T. vaginalis)* was observed in 8 (1%) patients by direct microscopic examination.

Conclusion: Performing the etiologic diagnosis of cervicovaginitis is necessary in order to take appropriate therapeutic and preventive measures. Therefore, we recommend *G. vaginalis, C. albicans* and *C. trachomatis* should be investigated in patients having a diagnosis of cervicovaginitis in our population, since these were detected in a considerable number of cases. Additionally, *C. glabrata* and *T. vaginalis* should be kept in mind as possible pathogens.

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Cervicovaginal infection constitutes one of the most common problems in clinical medicine and it is one of the main motives that lead women to seek out a gynecologist.[1] Cervicitis is a syndromic diagnosis derived from the observation of a mucopurulent discharge from the cervical os, as well as other signs of inflammation such as edema and easily induced endocervical bleeding.[2] *Gardnerella vaginalis (G. vaginalis), Trichomonas vaginalis (T. vaginalis), Candida species*, *Chlamydia trachomatis*, and *Neisseria gonorrhea* are the most prevalent agents of vaginitis and mucopurulent cervicitis.[3] The syndrome of bacterial vaginosis, consisting of copious and malodorous vaginal discharge, pelvic inflammatory disease (PID), postabortal PID, premature rupture of the membranes, chorioamnionitis, and postcesarean endometritis has recently been implicated in the etiology of premature labor and low birth weight.[4,5]

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Chlamydia trachomatis (C. trachomatis) is a parasite of columnar epithelial cells and a common cause of mucopurulent cervicitis. While many women who have *Chlamydia* isolated from the cervix have no signs or symptoms of infection, at least one–third experience an increase in vaginal discharge.[6] An association between *C. trachomatis* and pelvic inflammatory disease, subsequent infertility, and preterm births is also known.[3,6]

Enterobacteriaceae and *Staphylococcus aureus (S. aureus)* for vulvovaginitis, *Enterococcus species, Streptococcus agalactiae (S. agalactiae)* for salpingitis, *Escherichia coli (E. coli)*, *Proteus mirabilis*, *S. aureus* and *Streptococci* for bartholinitis, *S. agalactiae* for cervicitis and *Enterococcus species*, *E. coli*, *Klebsiella species,* and *S. agalactiae* for endometritis were not frequently accepted among female genital infectious disease syndromes associated microbial pathogens.[7]

Most gynecologists begin treatment as empiric anti-microbial chemotherapy for cervicovaginal infections. However, the identification of the microbial agents of cervicovaginitis will lead us to success in treatment and avoid unnecessary drug usage. Therefore, we aimed to investigate the usage of microscopic examination and cultures together in the diagnosis of etiologic factors of cervicovaginitis.

Methods. A total of 828 patients who attended the gynecology clinic at the Abant Izzet Baysal University, Duzce School of Medicine Hospital, Turkey. between October 2002 and December 2004 with complaints of cervicovaginitis were included in the study. The ethical committee of the university hospital approved the study and informed consent was obtained from the patients.

All women enrolled in the study presented with complaints (vulvovaginal irritation, itching, odorous or abnormal discharge) suggestive of cervicovaginal infection. Exclusion criteria were postmenopausal women, the presence of vulvar dystrophy, dermatological disorder in genital area and the use of any local vaginal medication or any oral antimicrobial drug within 2 weeks before the visit. Pelvic examination was performed and subjects were screened for cervicovaginal infection. Lower abdominal tenderness with or without rebound, cervical motion tenderness, and adnexal tenderness were used to establish the diagnosis of PID. Suspected cases were confirmed by laparoscopic or histological criteria. Infertility was defined as one year of unprotected intercourse without pregnancy. A standardized questionnaire for sociodemographic characteristics, medical and sexual history, and subjective symptoms were administered to all patients.

Gardnerella vaginalis infection was diagnosed with gram stain, amine or fishy odor elicited by addition of a drop of 10% potassium hydroxide to the discharge and vaginal pH was examined by pH paper. Moreover, all samples were cultured on human blood bilayer plate and isolated colonies morphology. Direct microscopic examination for *T. vaginalis*, enzyme immune assay (EIA) methods for *C. trachomatis* and culture for *G. vaginalis*, *Neisseria gonorrhea* (*N. gonorrhea)* and *Candida* species were performed in all patients for diagnosis. Samples were collected from posterior vaginal fornix and endocervical canal. The swabs for microscopic evaluation were placed into a tube containing 0.5 ml of sterile physiologic saline and hand delivered to the laboratory immediately. Microscopic evaluation was carried out by either wet-mount preparation or Gram and Giemsa stains. *Trichomonas vaginalis* and clue cells were investigated by direct microscopic examination. The examination of Gram stained smears were evaluated according to Nugent's criteria, for bacterial vaginosis and *G. vaginalis*.[8] The absence of a predominance of lactobacilli and the presence of *Gardnerella*-like bacteria on Gram stain are sign of bacterial vaginosis. Swabs collected for isolation of bacterial agents were transported to the laboratory in modified Stuart's medium. The swabs were inoculated with 5% Blood agar (Oxoid-England) and Chocolate agar (Oxoid-England) - New York City Medium plates (Oxoid-England) for the culture of *G. vaginalis* and *N. gonorrhea*. The plates were incubated at 35°C in an atmosphere of 10% CO_2 and 5% O_2, between 48 and 72 hours. The colonies where *N. gonorrhea* was suspected were tested by the API NH System (Biomerieux-France). The isolated *G. vaginalis* strains were identified by API 20 CORYNE (Biomerieux-France). In addition, the swabs were inoculated on Sabouraud dextrose agar for culturing of the *Candida species*. The plates were incubated and examined at 30°C for 48-72 hours. The isolated *Candida* strains were identified by API 20 C Aux (Biomerieux-France). The swabs were inoculated with 5% Blood agar (Oxoid-England) and Eosine methylene Blue agar (Oxoid-England) for the culture of *S. aureus*, *Streptococcus* group B and D, *E. coli*, *Klebsiella species* and *Enterobacteriaceae*. The plates were incubated at 37°C for 24 hours. Suspected colonies were separated by Gram staining, catalase and plasma coagulase tests and identified by API 20 STAPH, rapid ID 32 STREP and ID32 E (Biomerieux-France) identification strips. The *C. trachomatis* antigen enzyme linked immunosorbent acid (DRG Diagnostic-Germany) test was used for the detection of the *C. trachomatis* antigen. After

cleaning the exocervical area, an appropriated swab was inserted into the endocervical canal and rotated vigorously for 5-10 seconds at the columnar epithelial junction, and the tip of the swab was then immersed into DRG *Chlamydia* transport medium. Specimens were stored at –70°C until the assay was performed.

Numerical data were analyzed with Student's t-test. Chi-square, and Fisher's exact probability test was applied for the categorical data. A p value <0.05 was considered significant. Analyses were performed using commercially available software (Statistical Package for Social Sciences version 12 Demo, SPSS Inc. Chicago, Illinois).

Results. The study consisted of 828 patients who were clinically diagnosed with cervicovaginitis. The mean age of the patients was 33 years (range 17-46 years). The sociodemographic characteristics and description of the study population are shown in **Table 1**. Most of the patients were educated up to 11 years (88.4%). Thirteen percent of the patients were pregnant women. Most of the study group were married and had only one partner. Infectious agents were detected in 774 (93.5%) out of 828 patients. *Gardnerella vaginalis* was the most frequent isolate whereas *N. gonorrhea* was detected in one patient (0.1%). The distribution of infectious agents detected in the study group and the agents in 774 patients diagnosed with infection is shown in **Table 2**. We detected the *C. trachomatis* antigen from endocervical material in 18 (42.9 %) of 42 samples with PID and in 10 (22.7%) of 44 infertility patients. The difference between the *C. trachomatis* infection rate in PID patients and in non-PID patients was statistically significant (p<0.001). The difference between the *C. trachomatis* infection rate in infertile patients and non-infertile patients was also found to be statistically significant (p<0.05).

Discussion. Cervical inflammation is characterized by mucopurulent discharge from the cervical os during physical examination and is a condition frequently affecting young and sexually active women.[9] Some prospective studies demonstrate that vaginitis or lack of lactobacilli is associated with an increased risk of Human Immunodeficiency virus infection.[10] Evidence supporting the biologic plausibility of these findings is provided by in vitro studies indicating that H_2O_2-producing lactobacilli inhibit HIV and bacterial vaginosis organisms are associated with the activation of HIV.[11,12] Due to the importance of lactobacilli in the defense mechanisms of the body, cervicovaginal infections should be treated. Isolation of the causative agents is important for the success of the treatment. Previous studies performed for establishing the

Table 1 - The sociodemographic characteristics and description of the study population (n=828).

Characteristics	n	(%)
Education (years)		
0-5	444	(53.6)
6-11	288	(34.8)
>11	96	(11.6)
History of cervicovaginitis		
First episode	512	(62.8)
Previous episodes	316	(38.2)
Previous birth		
0	165	(19.9)
1	240	(29.0)
>1	424	(51.2)
Pregnancy	110	(13.3)
Infertility	44	(5.3)
Pelvic inflammatory disease	42	(5.1)
Sexual partners		
1	812	(98.1)
>1	16	(1.9)
Age (mean [range])	33.80 ± 8.82	[17- 46]

Table 2 - The distribution of infectious agents detected in the study group (n=828).

Infectious diseases	n	(%)
Gardnerella vaginalis (G. vaginalis)	222	(26.8)
Candida albicans	152	(18.4)
Chlamydia trachomatis (C. trachomatis)	110	(13.3)
Candida glabrata	36	(4.3)
Trichomonas vaginalis	8	(1)
G. vaginalis + Candida spp.	32	(3.9)
C. trachomatis+ Candida spp	20	(2.4)
Staphylococcus aureus	62	(7.5)
Group B *Streptococcus*	28	(3.4)
Group D *Streptococcus*	8	(1)
Escherichia coli	42	(5.1)
Klebsiella spp.	24	(2.9)
Other Enterobacteria	30	(3.6)
Neisseria gonorrheae	1	(0.1)
No agents detected	53	(6.5)

G. vaginalis - Gardnerella vaginalis,
C. trachomatis - Chlamydia trachomatis

frequency of the common infectious agents for vaginitis have shown varying results. The rates found for *G. vaginalis* have varied between 8% and 75%, *C. albicans* has presented rates between 2.2% and 30%, and *T. vaginalis* between 0% and 34%.[13-15] These results are similar to those of the present study. Spinillo et al[16] found that *T. vaginalis* or *C. albicans* were detected in 53.3% of 1564 childbearing age women. They reported the prevalence of bacterial vaginosis in 210 of the women, and emphasized that the high rate of agents of vaginitis in their sample was due to the low socioeconomic level of the study population. We detected at least one infectious agent in 93.5% of our study group. Candidiasis was detected in 29% (240/828), of these 152 were *C. albicans* 36 were *C. glabrata* and 52 where other *Candida* species. These 36 cases were recurrent candidiasis. *Candida glabrata* may be the reason behind recurrent vaginal candidiasis. Oyarzun et al[15] detected *Candida species* in 16.8%, *Gardnerella* in 11.1% and *Trichomonas* in 1.6% of patients encountering at least 3 of the following 4 criteria for diagnosis: homogeneous vaginal discharge, vaginal pH >4.5, an odor of fish when the vaginal secretion was alkalinized, and the presence of 'clue cells'. They reported that the finding of 'clue cells' had 100% sensitivity and 96% specificity for the diagnosis of bacterial vaginosis. Our study also supports microscopic examination for 'clue cells' and Nugent's criteria, of bacterial vaginosis. *Gardnerella vaginalis* detected in patients has been compared with other infection agents. It was found in 222 out of 828 (26.8%) patients and significantly high from other reports (p<00.5) **Table 2**. Levett et al.[17] investigated the presence of genital tract infections, including *C. trachomatis* in 35 of 98 women presenting with symptoms of genital tract infection. *Neisseria gonorrhea* was detected in one patient and *T. vaginalis* in 6. *Candida albicans* and *C. trachomatis* were each detected in 18 patients. *Chlamydia trachomatis* was the most common sexually transmitted pathogen detected in this population. They also emphasized the need for an aggressive approach to the diagnosis and treatment of *Chlamydia* infection in females. *Chlamydia trachomatis* has received significant attention as a primary etiological factor, responsible for approximately 40-50% of PID and salpingitis, 25% of ectopic pregnancies and 50% of tubal infertility.[18-20] Kouri et al[21] and Paavonen et al[22] reported that *C. trachomatis* is one of the principal causes of PID and cervicitis. Forty to fifty percent of tubal infertility is a result of PID.[20] Thongkrajai et al[23] reported the prevalence of *C. trachomatis* infection in rural Thai women as 6.7% (12/179). In our study, we detected the *C. trachomatis* antigen from endocervical material in 18 (42.8%) of 42 patients with PID and in 14 (31.8%) of 44 infertility patients. The difference between the *C. trachomatis* infection rate in PID patients and in non-PID patients was found to be statistically significant (p<0.001). The difference between the *C. trachomatis* infection rate in infertile patients and in non-infertile patients was found as not statistically significant (p>0.05). These results are similar to those of other studies.[21,23]

Neisseria gonorrheae is a sexually transmitted infection and one of the most common causative agents of cervicitis. Mayaud et al[24] reported the prevalence of *N. gonorrhea* as 2.8%. In our study, *N. gonorrhea* was detected in one patient (0.06%). We consider that this difference of results is probably associated with differences between traditional rules, and most of our patients having a single partner. Flores-Paz et al[25] were not able to isolate *N. gonorrhea* from any patients. The group B *Streptococcus* can be an etiological agent of newborn's infections and vaginitis.[26] In similar studies group B *Streptococcus* incidence was found between 0.8-12.3% that these findings correlate with our result (3.4%).[24,26-28] In our study, other agents that can cause cervical discharge were *S. aureus*, *E. coli, Klebsiella species, Streptococcus* group D and less frequent enterobacteria [*Pseudomonas species* (7.5%), *Proteus species* (5.1%), *Enterobacter species* (2.9%), *Hafnia alvei* (1%) and *Nonfermenter species* (3.6%)]. These results correlated with similar study performed by Flores-Paz et al[25] who reported data in cervical discharge specimens from 6811 patients. In another study, Brook et al[29] reported 913 isolates from patients with obstetric and gynecologic infection with *E. coli* in 85, *S. aureus* in 59.

In conclusion, lactobacilli found in normal vaginal flora have a protective role in the prevention of sexually transmitted diseases such as *T. vaginalis* and *C. trachomatis*. Similarly, bacterial vaginosis appears when the normal flora is damaged and transmissions of these conditional infections become easier. In the present study, lactobacilli flora was decreased or lacking in all patients with infectious agents detected. These results were scored according to Nugent's criteria,.[8] Since cervicovaginal infections appeared simultaneously in patients with vaginal discharge, the possible etiologic agents resulting in both kinds of infections should be searched. A variety of microbial agents causes cervicovaginitis and the identification of these microbial agents is important for successful treatment and also, to avoid unnecessary drug usage. We suggest usage of microscopic examination and cultures together in the diagnosis of etiologic factors of cervicovaginitis.

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