JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Comparison of cervico-vaginal colonization among sexually active women by intrauterine device use

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Abstract

Introduction: In this study we aimed to evaluate the effect of intrauterine devices (IUDs) on cervico-vaginal colonization.

Methodology: Cervico-vaginal samples of 96 patients with vaginal discharge were included in the study. Microscopic evaluation, bacteriological and fungal culture, and antigen testing for *Chlamydia trachomatis* using an immunochromatographic test method were performed.

Results: *Trichomonas vaginalis* was not detected by wet mount examination. Gram smear revealed that seven patients (7.3%) had *Candida* spp. and five (5.2%) had clue cell. Of the 96 swabs tested for conventional culture, pathogenic microorganisms were isolated from 24 patients. While *Neisseria gonorrhoeae* was not found in any of the sample, five (5.2%) were positive for *Gardnerella vaginalis*. Five (5.2%) were positive for *C. trachomatis* antigen, while three positivity only for *C. trachomatis* antigen, one had *G. vaginalis* additionally, and the other had a mixed infection. Chlamydial antigen positivity was higher among women over 30 years of age ($p = 0.157$). Increase in polymorphonuclear leukocytes (PNL) was detected 40% and 35.2% of samples, positive and negative, for chlamydial antigen, respectively ($p = 1.000$). Among IUD+ cases, increase in PNL, fungal elements, *E. coli* and Gram-positive bacteria and decrease in *Lactobacillus* spp. were observed, compared to IUD-cases. No statistically significant relationship was detected between IUD and chlamydial antigen with the reported rates of 4.8% and 5.6% for IUD+ or IUD-, respectively ($p > 0.05$).

Conclusion: Statistically significant relationship was not detected between IUD and cervico-vaginal colonization. More comprehensive studies using specific test methods should be conducted to better understand the relationship.

Key words: *Gardnerella vaginalis*; *Chlamydia trachomatis*; *Neisseria gonorrhoeae*, vaginal discharge; intrauterine device.

J Infect Dev Ctries 2015; 9(9):930-935. doi:10.3855/jidc.6006

(Received 03 October 2014 – Accepted 13 March 2015)

Introduction

Many aerobic and facultative-obligate anaerobic species can be colonized in the normal vaginal flora of asymptomatic women of reproductive age. Among these species, anaerobic bacteria show dominance, found ten times more often than aerobic bacteria. The reason for bacterial colonization is not clear. Bacteria could be in a symbiotic relationship with the host and can show variation due to the microenvironment [1].

Several factors are responsible for variation in vaginal flora among sexually active women. These factors cause several problems on a wide scale, ranging from asymptomatic vaginal discharge to severe pelvic infection. Chlamydial infection is one of the most common bacterial sexually transmitted infections, affecting approximately 215 million people globally as of 2010 [2-4]. The primary sites of infection are the endocervix and urethra [5]. Infection is more common in persons 25 years of age and younger, especially in sexually active adolescent females due the incompletd cervical anatomic development and sensitivity to infection by certain sexually transmitted pathogens, and to some other features that characterize sexual and healthcare behavior of young people [3,4,6]. Risk factors include a history of chlamydial or other sexually transmitted infection, new or multiple sexual partners, and inconsistent condom use [7,8]. Between 70% and 80% of cases are asymptomatic. Additionally, *Chlamydia trachomatis* infections have been associated with increased rates of transmission of HIV [9]. Occasionally, the infection spreads to the upper genital tract in women, causing pelvic inflammatory disease, ectopic pregnancy, infertility, or to the epididymis in men, causing epididymitis [5,7,9-16]. Bacterial vaginosis is believed to be the most common vaginal disorder affecting women and is characterized by the overgrowth of several anaerobic and microaerophilic

bacteria and the decreased prevalence of *Lactobacillus* species due to alteration of the vaginal bacterial morphotypes [5].

Although the intrauterine device (IUD) is a highly reversible method, it has a limited usage due to the several complications related to its use. In this study, we aimed to investigate whether IUDs have an effect on cervico-vaginal colonization among sexually active women.

Methodology

Specimens of 96 sexually active women that were sent to the microbiology laboratory of Ahi Evran University Research and Training Hospital, Kirsehir, Turkey, for cervico-vaginal culture between May and August 2013 were evaluated. During pelvic examination, two specimens, one endocervical and one vaginal, were collected from each patient using a Dacron swab. While the endocervical specimen was used for a chlamydial rapid antigen test (Chlamy-check, VEDALAB, Alencon, France), the vaginal sample was used for wet mount examination, Gram staining, and culture. Classification of bacterial vaginosis was assessed according to the Nugent score, a Gram-staining scoring system of vaginal smears, and bacterial culture results. A score of 7 to 10 is consistent with bacterial vaginosis. The specimen was inoculated onto 5% sheep blood agar, eosine-methylene blue agar, sabaroud-dextrose agar, and chocolate agar, and incubated for up to three days in an aerobic and microaerophilic environment. Strain identification and antimicrobial susceptibility testing was performed by conventional tests and VITEK-2 Compact automated system (bioMerieux, Marcy-l'Etoile, France). Patients were classified into two groups based on IUD use. Vaginal culture, chlamydial antigen testing, and Gram-smear results were compared for both groups.

Statistical analyses

Statistical comparisons were performed with the Statistical Package for Social Sciences (SPSS) version 15.0. Associations and comparisons were analyzed using the χ^2 test or Fisher's exact test. All hypotheses were two-tailed and were considered significant at the $p < 0.05$ level.

Results

A total of 96 women, of whom 42 (43.8%) were using an IUD, were included in the study. The average age of the patients was 35.77 ± 10.03 years, ranging between 18 and 57 years. Of the patients, 34 (35.4%) were under 30 years and 62 (64.6%) were over 30 years of age. More than 10 leukocytes on each microscope area were found in 34 (35.4%) patients. While 67 samples had normal numbers of *Lactobacillus* spp., 29 (30.2%) had decreased numbers. *Trichomonas vaginalis* was not detected in any of the samples by wet mount microscopy examination. Gram smear revealed that 7 (7.3%) had *Candida* spp., and clue cell was detected in 5 (5.2%) smears. Of the 96 swab tested for conventional culture,

Table 1. Microbiological testing results of the samples (n = 96) and distribution of the percentages by intrauterine device (IUD) usage

Microbiological diagnostic test	Total		IUD+ (n = 42)		IUD- (n = 54)	
	N	%	N	%	N	%
Bacterial culture						
Escherichia coli	6	6.3	4	9.5	2	3.7
Streptococcus agalactia	4	4.2	1	2.4	3	5.6
Staphylococcus aureus	2	2.1	2	4.8	-	-
Enterococcus faecalis	2	2.1	1	2.4	1	1.9
Gardnerella vaginalis	5	5.1	2	4.8	3	5.6
Staphylococcus hominis	1	1	1	2.4	-	-
Staphylococcus lugdunensis	1	1	1	2.4	-	-
Yokenella spp.	1	1	1	2.4	-	-
Sphingomonas paucimobilis	1	1	-	-	1	1.9
No growth	26	27.1	10	23.8	16	29.6
Normal vaginal flora	46	47.9	18	42.9	28	51.9
Fungal culture						
C. albicans	3	3.1	3	7.1	-	-
C. sphaerica	1	1.0	-	-	1	1.8
Chlamydial antigen test	5	5.2	2	4.8	3	5.6

26 (27.1%) showed no growth and 46 (47.9%) had normal genital flora, while pathogenic microorganisms were isolated from 24 patients. A total of 5 (5.2%) *G. vaginalis* strains were isolated from patients; 3 of them had only *G. vaginalis*, and the rest had mixed pathogenic organisms. *Neisseria gonorrhoeae* was not found in any patient. Distribution of the microorganisms is shown in Table 1.

Of all the samples tested, *C. trachomatis* was detected in five (5.2%) patients. While three patients had *C. trachomatis* only, one also had *G. vaginalis*, and the other had a mixture of the pathogenic organisms *Yokenella regensburger* and *Staphylococcus lugdunensis*. *Candida* spp. was not detected from the samples found to be antigen-positive for *C. trachomatis*. Chlamydial antigen positivity was higher among women over 30 years (n = 5; 8.1%) of age compared to women under 30 years (p = 0.157) of age. Major symptoms of the patients positive by the chlamydial antigen test were dysmenorrhea and itching. Decreases in the numbers of *Lactobacillus* spp. were observed in two (40%) and three (60%) patients positive and negative for the chlamydial antigen, respectively (p = 0.636). Increase in polymorphonuclear leukocytes (PNL) were detected two (40%) and 32 (35.2%) of samples positive and negative for the chlamydial antigen, respectively (p = 1.000). Mixed bacterial infection was detected only in the sample of one patient with an IUD. Distribution of wet mount and Gram-staining results of the samples by IUD usage are shown in Table 2.

Increase in PNL, presence of fungal elements, and decrease in *Lactobacillus* spp. were observed more frequently among samples from samples patients with an IUD compared to samples of patients without an IUD, although no statistically significant relationship was detected. Higher rates of bacterial recovery, especially for *Escherichia coli*, were detected among cases with an IUD (9.5%) compared to cases without an IUD (3.7%). Additionally, Gram-positive bacterial colonization with *Staphylococcus* spp. was frequent among cases with an IUD (Table 2). Chlamydial antigen test positivity was higher among cases without an IUD, compared to cases with an IUD, with reported rates of 5.6% and 4.8%, respectively (p > 0.05) (Table 1).

Discussion

Enzyme-linked immunosorbent assay (ELISA) tests, culture, direct immunofluorescence, and polymerase chain reaction (PCR) are available for the detection of *C. Trachomatis* from endocervical and urethral swab specimens [17,18]. Although culture of the microorganism is the gold standard for detection, culture was replaced by nucleic acid amplification test (NAAT) because of improved test accuracy and ease of specimen management and screening. To detect chlamydial infections, healthcare providers frequently rely on screening tests. Annual screening of all sexually active women 25 years of age and older is recommended, as is screening of older women with risk factors such as having a new sex partner or multiple sex partners [8,12].

From an epidemiological point of view, the microorganisms more frequently isolated in this study were *E. coli* (6.3%), *G. vaginalis* (5.1%), and *Streptococcus agalactiae* (4.2%). While *N. gonorrhoeae* was not detected from any of the samples, chlamydial antigen prevalence was 5.2%, with frequencies of 4.8% and 5.6% among IUD users and nonusers, respectively; no statistically significant relationship was detected between the groups. In previous studies, *N. gonorrhoae* frequency was 0.1%–2.2% in symptomatic patients [19-21], and *C.*

Table 2. Wet mount and Gram staining evaluation of the samples (n = 96) and distribution of percentages by intrauterine device (IUD) usage

Microbiological analysis	Result	IUD+ (n = 42)		IUD- (n = 54)		P
		N	%	N	%	
Wet mount						
PNL	*Increase*	16	38.1	18	33.3	0.628
	Rare/absent	26	61.9	36	66.7	
Yeast	+	4	9.5	3	5.6	0.695
	-	38	90.5	51	94.4	
Gram smear						
***Lactobacillus* spp.**	*Decrease*	9	21.4	20	37	0.098
	Normal	33	78.6	34	63	
Clue cell	+	2	4.8	3	5.6	1.000
	-	40	95.2	51	94.4	

trachomatis and *N. gonorrhoeae* were more prevalent in patients over 25 years of age [22]. In this study, chlamydial antigen positivity was higher among women over 30 years of age compared to women under 30 years of age. In previous studies, *C. trachomatis* prevalence was found to be 2.5%–30.3% in vaginal discharge by various diagnostic techniques [3,16,19,21]. In Turkey, frequency of chlamydial infections in women is low, between 5.1% and 24% [20,22,23] for symptomatic patients and 1.11% [22] for asymptomatic patients. In this study, lower rates were detected for chlamydial antigen positivity, similar to the reports published previously.

Persons who undergo testing and are diagnosed with chlamydia should be tested for other sexually transmitted diseases (STDs). Treating infected patients prevents sexual transmission of diseases. Co-infection with *C. trachomatis* frequently occurs among patients who have gonococcal infection; therefore, presumptive treatment of such patients for chlamydia is appropriate [16]. False-negative results might occur in the presence of persistent infections involving limited numbers of organisms [18]. In this study, among chlamydial antigen-positive patients, co-infection with *N. gonorrhoeae* was not detected, but *G. vaginalis* and mixed infection with Gram-negative bacilli and Gram-positive cocci was detected. Previous studies on evaluation of risk factors of chlamydial infection showed that coital frequency and cervical ectopy were associated with chlamydial infection [3]. It has been shown that chlamydial infection significantly reduces the purity of vaginal discharge, which is more pronounced in nulliparae women, and that the Pap smear is not specific enough to demonstrate chlamydial infection [19]. *C. trachomatis* antigen positivity was detected in 17% of the patients with > 10 PNL and mucopurulent discharge [16]. It has been reported that mucopurulent discharge and friability were not reliable indicators for *C. trachomatis*, and that PNL was a significant indicator associated with *C. trachomatis* in black patients but not in white patients [16]. In this study, similar results were achieved, and of 34 (35.4%) patients with increased levels of PNL, two were positive for *C. trachomatis* antigen ($p > 0.05$).

Vaginal cultures for *G. vaginalis* are sensitive but not specific, as 50%–60% of healthy asymptomatic women will be culture positive. As Amsel criteria are dependent on the clinician, the Nugent scoring system has been favored. Evaluation of smears, however, is also subjective and requires experience. In previous studies, bacterial vaginosis was associated with an increased risk of pelvic inflamatory disease [21], and prevalence rates of *G. vaginalis* were found to be higher among IUD users (19.7%–32.5%) [21,24]. *G. vaginalis* frequency from culture of symptomatic patients was 10.2%–30.7% [25]. In this study, of all the samples tested, 5.1% showed growth of *G. vaginalis*, and no statistically significant relationship was detected between the presence of *G. vaginalis* and the presence (4.8%) or absence (5.6%) of an IUD ($p > 0.05$).

Candida spp.-related vaginitis is more common compared to other pathogens in women with diabetes mellitus and women receiving antimicrobial treatment [5]. In this study, yeast cells were detected in 7.3% of all the samples tested; 3.1% were positive for fungal culture, and 7.3% showed positive smear and wet mount for yeast. Although a statistically significant relationship was not detected, a higher percentage of yeast presence either by culture or wet mount and Gram smear was observed for samples collected from patients with an IUD (9.5%) compared to patients without an IUD (5.6%) ($p < 0.05$).

T. vaginalis is one of the common causes for genital discharge. Wet mount evaluation and modified Diamond's base are routinely used for diagnosis, and higher sensitivity was detected for the latter test method [26]. A wide range of presence was reported (1%–23.3%) [20,21]. In this study, we used only wet mount examination for *T. vaginalis* detection and any microorganism was not detected.

In some cases, bacterial culture of the vaginal discharge demonstrated Gram-negative bacilli or Gram-positive cocci dominance and a simultaneous absence of *Lactobacillus* spp. flora on microscopic examination [5]. In view of the relatively low carriage rate in normal flora, these findings suggest that *Enterobacteriacea*, *Enterococcus* spp., or *S. agalactiae* have roles in the infection and should not be considered as part of the normal vaginal flora in symptomatic women with microscopic evidence of inflammation [5]. The causitive role of *S. aureus* in vulvovaginitis has not been well researched, since the bacteria is known to colonize the genital tract in 4%–18% of healthy women [27]. Some studies showed that it is related to vaginal symptoms and signs [28]. In this study, *E. coli* and *S. agalactiae* were detected in 6.3% and 4.2% of the samples, respectively. *E. coli* identification was higher among IUD users (9.5%) compared to IUD non-users (5.6%). In this study, no statistically significant relationship was detected between vaginal colonization and IUD usage.

Conclusions

More specific testing methods and more comprehensive studies conducted in large patient groups should be performed to better understand the relationship between IUD and infection. It is crucial to screen patients with genital discharge so as to limit transmission of sexually transmitted diseases. If more sensitive tests are used widely, more infected persons will be identified and treated. More comprehensive studies should be conducted on *C. trachomatis* screening to estimate the real situation.

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Conflict of interests: No conflict of interests is declared.