Trichomonas, Candida, and *Gardnerella* in Cervical Smears of Iranian Women for Cancer Screening

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Abstract

Background: *Trichomonas vaginalis, Gardrenella vaginalis,* and *Candida* sp are responsible for 90% of vaginitis which has been reported as important risk factors for cervical cancer. **Aim:** This study aimed to find the prevalence of *T. vaginalis, Candida* sp, and *G. vaginalis* in women attended the cancer clinic charity, Babol, Iran and to identify the associated risk factors. **Materials and Methods:** This retrospective study carried out from 1996 to July 2012 on women who attended to a cancer screening program at the cancer clinics charity, Babol, Iran. Papanicolaou test and clinical examinations were performed for each woman. In addition to Papanicolaou test results, demographic data were collected. The data were analyzed with X² test using SPSS software, version 18. **Results:** In total, 2511 out of 33600 (7.5%) cases had vaginal infections. A total of 71 (0.2%), 2248 (6.7%), and 192 (0.6%) of subjects were infected by *T. vaginalis, Candida* sp, and *Gardnerella*, respectively. The highest rate of infection was seen in 20-30 and 30-40 years age group. The frequency of vaginal ulcers was higher in trichomoniasis (14.1%). **Conclusion:** This study demonstrated that the prevalence of *T. vaginalis, Candida* sp, and *Gardnerella* was low among the studied population. Moreover, malignant cytological alternations were not seen in any infected women.

Keywords: Candida sp, Gardnerella vaginalis, pap smear, T. vaginalis

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Introduction

The Papanicolaou smear has been widely accepted as the model screening test for cervical cytology. However, recent reports have suggested some limitations for the test, such as low sensitivity and high false negative rates, which have forced many to revisit the utility of cytology as a primary screening.^[1] The Papanicolaou smear, which is a cost-effective test, fast and acceptable to most patients, can also be use in the diagnosis of genital tract infections.

In addition to *Chlamydia trachomatis*, herpes simplex virus and human immunodeficiency virus, *Trichomonas*

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vaginalis, is the most common sexually transmitted diseases over the world. These agents have been proposed to act as cofactors to influence the progression of cervical human papillomavirus infection to high-grade lesions and cervical cancer.^[2,3] Additionally, bacterial vaginosis (BV) and *Candida* sp are the most frequent vaginal disorders which may play a role in cervical carcinogenesis.^[4,5]

Although, *T. vaginalis*, BV, and *Candida* sp are responsible for 90% of infectious vaginitis; but the prevalence found for these infections are widely varied among different populations.^[6] For example, *T. vaginalis* and *Candida* sp were diagnosed in 10% and 0.5% of the cytology tests in 1968 and in 3.4% and 22.5% in 1998 in Brazil.^[7] Another study demonstrated that *T. vaginalis*, *Candida sp* and *Gardnerella/Mobiluncus* were respectively found in 12%, 5.8%, and 21.8% of pap smears of women in a prison in Brazil.^[8] However, many studies have investigated the prevalence of infectious agents among the genital tract using Papanicolaou smear, but there are few studies on large populations of women who have been referred for cervical cancer screening. In Iran, based on our best knowledge, most of the studies on genital tract infections by Papanicolaou smear were performed among symptomatic cases. The objective of this work was to study prevalence of *T. vaginalis, Candida* sp, and *Gardnerella* in women subjected to Papanicolaou smear at a clinic supported by Cancer Patients Aid Association, Babol, Iran and to identify the risk factors associated with each infective agent.

Materials and Methods

Population study

After obtaining approval letter with number 8929916 from the Ethical Committee of the Research Council of our organization, this retrospective study was carried out on women who attended to the cancer screening program at a clinic supported by Cancer Patients Aid Association, Babol, Iran. This center is a charity organization and established in 1996 in order to help people to increase their knowledge on cancer, diagnosis, and treatment. Breast and genital cancer were screened in the diagnostic clinic by clinical examinations and Papanicolaou test which were completely free of charge. Pap smear test was performed for any woman who attends this clinic, in addition to other clinical examinations, annually. Vaginal examination and taken smear was carried out by physician. The smears were fixed with methanol and send to clinical pathology laboratory. The smears were evaluated by a pathologist. This clinic also collects demographic data and other information based on clinical examinations and the Pap smear reports using a Bethesda system. This information includes the sample adequacy, the type of epithelium (e.g. squamous, glandular, and metaplastic), benign cellular alterations (e.g. inflammation), and the type of genital infections (e.g. T. vaginalis and Candida sp). Between 1996 and July 2012, the information from 33600 cases was archived. The files for all of the cases of T. vaginalis, Candida sp, and Gardnerella infections were retrieved. Also, files of 200 women without any infection (control group) were randomly recovered in order to calculate the association of risk factors with vaginal infections.

Data analysis

The data were analyzed by SPSS software, version 18.0 using descriptive analysis and Chi-squared test with a 5% statistical significance level and 95% confidence interval.

Results

Overall, 2511 out of 33600 (7.5%) cases had vaginal infection. A total of 71 (0.2%), 2248 (6.7%), and 192 (0.6%) of the women were infected by T. vaginalis, Candida, and Gardnerella, respectively. A total of 142 out of 2511 (5.7%) cases had mixed infection. The minimum and maximum of age among women with trichomoniasis was 18 and 61 years and the mean of age was 34.7 years. The mean age of women infected with Candida and Gardnerella were 32.7 ± 8 and 32.8 ± 8.5 , respectively which ranged from 14 to 67 and 17-60 years in that order. The mean age of women with coinfection and healthy women (control group) was 32.4 ± 9.2 and 34.6 ± 10.5 years, respectively which ranged from 17 to 67 and 18 to 73 years in that order. The age of one case infected with T. vaginalis and 12 cases infected with Candida were obtained as missing data. The highest infection rate of T. vaginalis and Candida were observed in the 30-40 age groups and for Gardnerella was seen in the 20-30 years age group. The highest rate of coinfection was also seen in the 20-30 years age group. The highest frequency rate of women in the control group was demonstrated in the 20-30 years age group followed by the 30-40 age groups [Table 1].

The lowest rate of trichomoniasis (1.4%) was demonstrated in women who used condom as their preferred choice of contraception, while the highest rate (2.7%) was seen in women who used other forms of contraception such as tubectomy or oral contraceptives. Furthermore, 13.8% of women who used intrauterine device (IUD) were infected with

Table 1: Prevalence of Trichomonas vaginalis, Candida sp, Gardnerella and coinfection in women	attending to the
cancer clinics charity, Babol, Iran, by age	

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Infection	Mean± σ		N %						
Age group (years)		<20	20-30	30-40	40-50	>50	Total		
T. vaginalis	34.7±10.2	57.1	2028.6	2738.6	1420	45.7	70100	0.09	
Candida	32.7±8	873.9	86938.9	92341.2	31914.3	381.7	2236100	0.001	
Gardnerella	32.8±8.5	105.2	7941.1	6835.4	3116.2	42.1	192100	0.95	
Coinfection	32.4±9.2	107	6143	5135.9	1611.3	42.8	142100	-	
Control	34.6±10.5	157.4	6633	6030	4321.6	168	200100	-	

N. B: The mean age, standard deviation, and frequency of healthy women (control group) among different age group also mentioned. The numbers of trichomoniasis and candidiasis cases were 71 and 2248, but there was missing data on age of 1 tricomoniasis and 12 candidiasis cases. *T. vaginalis: Trichomonas vaginalis*

Gardnerella and the lowest infection rate was observed in those used condom and other methods (0.05%). The infection rate of candidiasis was approximately similar in women who used different contraception methods and ranged from 82.7% to 87.3% [Table 2]. A total of 1.2% and 12.3% of healthy women used condom and IUD, respectively and the rest used other methods. Vaginal ulcers were demonstrated in 14.1% (10/71), 7.4% (167/2246), and 8.9% (17/192) of women infected with *T. vaginalis, Candida* sp, and *Gardnerella*, respectively [Table 2 and Figure 1]. Prevalence of genital infections in association with other factors including number of gravid, abortion, inflammation, residency, and presence of squamous cells are also shown in Table 2.

This study demonstrated that 64.8% and 5.6% of women infected with *T. vaginalis* were also infected with *Candida* sp and *Gardnerella*, respectively. Coinfection was also demonstrated in women with candidiasis and *Gardnerella* infection [Figure 1]. The findings obtained in this study showed that about 4%-8.3% of

infected women with *T. vaginalis* remained infected in subsequent refers. Also, 59.3% of infected with *Candida* sp and 51.2% of infected women with *Candida* sp and *Gardnerella* remained infected in the next refer. Malignant cytological alternations were not seen in any infected women.

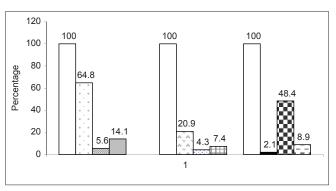


Figure 1: Prevalence of coinfection and ulcer in women attending to a clinics supported by Cancer Patients Aid Association, Babol, Iran. T, I, C, G, and U are Trichomonas, Infection, Candida, cancer Gardnerella, and ulcer, in that order

Table 2: Prevalence of *Trichomonas vaginalis, Candida sp*, and *Gardnerella* related to some risk factors and sign, in women attending to a clinics supported by cancer patients aid association, Babol, Iran

Risk Infection		N %	Control	P value	
	T. vaginalisa ^a	Candida ^b	<i>Gardnerella</i> ^c		
Inflammation					
Severe	79.9	1516.8	18 9.4	-	0
Moderate	37 52.1	88539.7	89 46.6		
Mild	2129.6	77334.6	189		
no	68.4	42218.9	6634.6		
Ulcer					
Yes	1014.1	1677.4	178.9	-	a, 0.3; b, 0.002; c, 0.9
No	6185.9	207993	17591.1		
Squamous cells					
Yes	0	4100	1100	-	
No					
Abortion					
Yes	1725.8	52924.3	4423.9	56 28	a, 0.9; b, 0.000; c, 0.8
No	4974.2	164776	14076.1	14472	
Gravid no					
<1	1322.8	47423	3318.7	3518.8	a, 0.35; b, 0.16; c, 0.000
1-3	245.6	112555	9453.1	9048.4	
>3	1831.6	46322.4	5028.2	6132.8	
Contraception					
IUD	2/942.1	81/9882.7	13/9413.8	212.9	a, 0.01; b, 0.02; c, 0.05
Condom	4/2861.4	255/29287.3	15/2850.05	61.2	
Natural and other	55/20062.7	1699/203383.6	138/19876.9	15586.1	
Residency					
Urban	6088.2	193388	16988	179 89.5	a, 0.7; b, 0.09; c, 0.9
Rural	811.8	27712.5	2312	2110.5	

N.B. *P* value arranged in order of *Trichomonas vaginalis, Candida sp,* and *Gardnerella* infections. Note in regarding the association between candidiasis and number of gravidity and abortion and contraception, the *P* values were 0.000 and 0.02 which may resulted from number of cases in non infected women (200 cases). Also notice that missing data of Gravid number and contraception in noninfected population. *a, b,* and c represent *P* value of Trichomonas vaginalis, Candida sp and Gardnerella and the factors, respectively. IUD: Intrauterine device, *T. vaginalis: Trichomonas vaginalis*

Discussion

This study found that infection rate among the women attending to cancer screening program was low (7.5%). This result was obtained by Papanicolaou test which has some limitations such as less sensitivity.^[1-9] It also demonstrated that Candida sp was the main cause of vaginal infection (6.7%) and T. vaginalis (0.2%) and Gardnerella (0.6%) had low prevalence rates among these women. Although, our study is confirmed by publications which indicate that candidiasis is the most common vaginal infection in most countries, but the prevalence rate of infective agents obtained from the current study was lower in comparison with other studies in the word and Iran.[10-13] For example, one study reported that the amount of candidiasis, trichomoniasis, and Gardnerella infection were 9.8%, 1.9%, and 0.7% in pap smear of women who participated in screening.^[12] Adad et al.,^[7] studied 20,356 cases using Papanicolaou test in four decades in Brazil and found that low and high indices of infections belong to trichomoniasis (3.4%) and candidiasis (22.5%) from 1988 to 1998. Mehmetoglu et al.,^[14] reported that vaginal infection identified in 17.7% (59 out of 332) of the cases by Pap smear and the prevalence of trichomoniasis was 0.6%. Also, Lessa et al.,^[8] studied 672 patient records and demonstrated that the main cervical-vaginal colonization was due to Gardnerella/Mobiluncus (21.8%), followed by T. vaginalis (12%), and Candida sp (5.8%). Furthermore, the prevalence of BV, candidiasis, and trichomoniasis in asymptomatic subjects were found 0.4%, 1.1%, and 0.7%, in that order.^[15] Although, different diagnostic methods were used in this study, its findings support our results. With consideration of the prevalence rate of *T. vaginalis*, Candida, and Gardnerella infections among asymptomatic cases in several reports,^[5,13,15,16] the differences of the studied populations are the main possible explanation for dissimilarity between our findings and other publications.

The current study showed that the highest prevalence rate of *T. vaginalis* (38.6%) and *Candida* (41.3%) infections and *Gardnerella* infection (41.1%) was seen in 20-30 years and 30-40 and age group, respectively. This finding is in agreement with results obtained from several studies which indicated that highest rate of trichomoniasis demonstrated in women at sexually active ages.^[17-19] or BV was observed at reproductive ages.^[20] This study found that, although moderate inflammation was a general manifestation in infected women,^[8] trichomoniasis causes more inflammation (mild to sever) and ulcers in comparison to candidiasis and *Gardnerella* [Table 2]. Indeed, *Trichomonas, Candida*, and *Gardnerella* are considered as the simultaneous inflammatory events in genital tract.^[12] In regard to T. vaginalis and Gardnerella infections, the analysis of the behavioral marker showed that the prevalence of the infection was significantly lower in women whose husbands use condom as a contraception method. These results are in agreement with other publications which indicate that the rate of trichomoniasis and BV was less in those whose husbands used condoms than who did not.^[18-22] Moreover, the current study found that the Gardnerella infection may be associated with IUD. This finding is supported by other studies^[23-25] which indicate that IUD is a risk factor for vaginal infections. Furthermore, this study did not find any association between different methods of contraception and candidiasis. These results are comparable with other published data which indicated that using an IUD could increase the risk of both acute and recurrent vulvovaginal candidiasis.[26,27]

However, coinfection, that is, two infections occurring simultaneously in the same case, was common. Hence, 64.8% and 5.6% of women infected with *T. vaginalis* were also infected with *Candida* sp and *Gardnerella* in that order or 20.9% and 4.3% of women with candidiasis also had *Trichomonas* and *Gardnerella* infections, respectively [Figure 1]. These finding are supported by Jones *et al.*,^[28] which indicates that the prevalence of BV is significantly associated with trichomoniasis or Mendoza-Gonzalez's studies which found that fourteen subjects had combined *Candida* and *Gardnerella* infection^[29] but this was in contrast with another study.^[11]

The current study also demonstrated that *T. vaginalis*, *Candida* sp, and *Gardnerella* infections were persisting in some women after treatment. The persistence of the infections in the case may be related to improper treatment, unsanitary behaviors, and emerging of drug resistance strain of the infection agents.^[30-32]

Conclusion

Although our study was based only on the Papanicolaou smear test, the prevalence of *T. vaginalis* (0.2%), *Candida* sp (6.7%), and *Gardnerella* (0.6%) was nearly similar to asymptomatic subjects reported by others. Therefore, infections can be diagnosed in cervical smears through identification either of the organism or of characteristic cytological cellular changes. Moreover, vaginal infections produced by drug resistance of infectious agents should be noticed in treatment.

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