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A study on the relationship of cervical cancer with vaginal microbiota and trichomoniasis infection; a single center study

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Abstract

Introduction: Cervical cancer is the most prevalent cancer associated with infection, which is provoked by the human papillomavirus (HPV). Natural vaginal microbes, known as vaginal microbiota, play an important role in regulating vaginal pH and are therefore important in the risk of cervical cancer. Trichomoniasis vaginalis is a genital infection that reports 250 million new infections worldwide each year and can increase the risk of developing cervical cancer in the general population.

Objectives: The aim of this study was to investigate the relationship between cervical cancer and vaginal microbiota or trichomoniasis infection by examining several risk factors.

Patients and Methods: This prospective case study was conducted from 2014 to 2018 from an educational pathology group in Isfahan, 200 samples are included in this study. The groups consist of women over 18 years old. The study group included patients with cervical cancer lesions. For participants, a questionnaire containing information about age, gender, abortion, age at first delivery, smoking or passive smoking and the result of Pap smear test (HPV, vaginal microbiota or *Trichomonas*) was completed.

Results: Results suggested that cancerous and precancerous lesion development is not associated with parity, age at first child's birth. However, it is statistically associated with lower vaginal microbiota, increased *Trichomonas* infection, old age, increased abortion rate, smoking, and the presence of HPV infection on Pap smear.

Conclusion: Protection from harmful factors that affect a healthy vaginal microbiome, such as *Trichomonas* infections, can reduce the risk of cervical cancer.

Introduction

Cervical cancer is the most prevalent cancer associated with infection, which is provoked by the human papillomavirus (HPV) virus. It is the second most prevalent cancer among women after breast cancer in Iran and about 2% of women before their 80's experience such a disease. Mortality rate associated with this cancer is at a range of 1.2 people every 100 000 (1).

There are different risk factors associated with cervical cancer like log period of OCP (oral contraceptive pills) use, young age at first sexual intercourse, young age at first delivery, multi partner women, high gravidity, low socioeconomic status, high parity and cigarette smoking (2).

Researches have already suggested that HPV infection is the main key in cervical cancer development, and it is also established that this type of infection is associated with high grade cervical lesions, however small

Key point

In a study on documented cervical cancer cases, we found with increasing *Trichomonas* infection, the risk of cervical cancer increases. Protection from harmful factors that affect a healthy vaginal microbiome, such as *Trichomonas* infections, can reduce the risk of cervical cancer.

quantity of HPV infections actually lead to cervical cancer (3).

Normal vaginal microbes known as vaginal microbiota play a critical role in regulating vaginal pH and thus it is crucial in the risk of cervical cancers.

In 2012, a study by Clark et al reported that the role of cervical microbiome in cervical cancer was associated with an increase in pH (4). It is possible that sexually transmitted infections, change the normal role of vaginal microbiota and consequently leads to cervical cancers (5).

Most recently, Kyrgiou et al (6) and

Kovachev, have also suggested that altered vaginal microbiota is associated with cervical cancer(7).

Trichomonas vaginalis is a protozoan parasite that causes genital infections in both men and women (8,9). Trichomoniasis is one of the most common infections in women of childbearing age and every year 250 million new trichomoniasis infections are reported worldwide (10).

The prospective epidemiological study (China), proposed that in a group of women whose HPV infection has been controlled and who have been infected with *Trichomonas vaginalis*, the risk of cervical cancer progression multiplies is two times greater than the normal population (11).

Recent studies by Silva et al and Liu & He, have shown that cervical cancer and precancerous lesions are closely related to female genital tract infection with *Trichomonas vaginalis,* which requires extensive attention (12,13).

Objectives
Due to the lack of studies in this field, especially in Iran, our aim in this study was to evaluate the role of vaginal infections and Trichomoniasis, by examining various risk factors, and its relationship with cervical cancer

Patients and Methods
Study design
In this prospective case-control study which was conducted from 2014 to 2018 in an educational pathology department in Isfahan, 200 samples were included in the study. The experimental group consisted of patients over 18 years of age who were diagnosed with cervical precancerous lesions. The control group Pap smear results were cancerous and precancerous lesions. Both experimental and control groups consisted of 100 people.

Inclusion criteria consisted of confirmed invading cancerous lesions in their pathology samples. Exclusion criteria consisted of any history of surgical procedures taken in place such hysterectomy or in case of incomplete information of the patient file.

Participants were divided into two groups. For both groups, a questionnaire containing information about age, gender, abortion, age at first delivery, smoking or passive smoking and the result of Pap smear test (HPV, vaginal microbiota or *Trichomonas*) was completed.

Data analysis
Data were analyzed using SPSS version 21 and *P* value less than 0.05 is considered statistically significant. Independent *t* test, chi-square and logistic regression methods were used to analyze the data.

Results
Two-hundred women at childbearing age were included in the study.

Sixty-six percent of those with precancerous lesions or cancer as a result of vaginal biopsies were over 45 years old (Table 1). Eight percent of the case group had abortions more than two, while this number in the control group was 2% (Table 2). Moreover, 53% of the case group had more than two children, which the number did not differ statistically in the control group (56%) (Table 3). In addition, the percentage of women at the age of first childbirth was almost equal (Table 4).

Smoking or passive smoking was seen in 87% of the patients with cancerous and precancerous lesions and it was statistically associated with lesion development (*P* = 0.0023; Table 5).

In the results, the presence of vaginal microbiota was recorded in 27 women who had cancer or precancerous lesions, while the number was 60 in the other group (the control group). HPV infection was observed in 88% of the patients with cancerous and precancerous lesions, and this type of infection was statistically associated with lesion development (*P* = 0.001; Table 6).

Table 7 shows the distribution of the number of vaginal microbiota cases among the different types of cervical cancer.

Of the patients with cervical cancer or lesions, 29 had a history of *Trichomonas vaginitis* as a result of Pap smear, while only four cases of Trichomoniasis infection were reported in the control group (Table 8). Therefore, statistically, it is clear that, with increasing *Trichomonas*

Table 1. Age groups

Age (years)		Groups		Total
		Case	Control	
18-30	No.	12	10	22
	%	12.00	10.00	11.00
30-45	No.	22	40	62
	%	22.00	40.00	31.00
45-60	No.	28	26	54
	%	28.00	26.00	27.00
>60	No.	38	24	62
	%	38.00	24.00	31.00
Total	No.	100	100	200
	%	100	100	100

P value = 0.003.

Table 2. Abortion rates

			Groups		Total
			Case	Control	
Number of abortion	0	No.	80	75	155
		%	80.00	75.00	77.50
	2-0	No.	12	23	35
		%	12.00	23.00	17.50
	>2	No.	8	2	10
		%	8.00	2.00	5.00
Total		No.	100	100	200
		%	100	100	100

P value = 0.027.

Table 3. Statistics of number of parity

| | | | Groups | | Total |
			Case	Control	
Parity	0	No.	10	7	17
		%	10.00	7.00	8.50
	2-0	No.	37	41	74
		%	37.00	41.00	37.00
	>2	No.	53	52	109
		%	53.00	52.00	54.50
Total		No.	100	100	200
		%	100.00	100.00	100.00

P value = 0.736.

Table 4. Age at birth of first child distribution

| | | | Groups | | Total |
			Case	Control	
Age at birth of first child (years)	15-35	No.	88	90	178
		%	88	90.00	89.00
	>35	No.	12	10	22
		%	12	10.00	11.00
Total		No.	100	100	200
		%	100	100	100.00

P value = 0.651.

Table 5. Statistics of smoking or passive smoking in this study

| | | | Groups | | Total |
			Case	Control	
Smoking or passive smoking	Yes	No.	87	15	102
		%	87.00	90.00	89.00
	No	No.	13	85	89
		%	13.00	85.00	49.00
Total		No.	100	100	200
		%	100	100.00	100.00

P value = 0.002.

infection, the risk of cervical cancer increases. Results also suggested that cancerous and precancerous lesion development is not associated with parity, age at first child's birth. Moreover, as the number of abortions increases, cancerous and precancerous lesions are likely to be found (*P* = 0.027). However, it is statistically associated with age, smoking, HPV infection and vaginal microbiota. The less vaginal microbiota exists, the more precancerous and cancerous lesions develop (*P* < 0.001).

Discussion

Vaginal microbiota refers to several species of lactobacillus, which dominate normal vaginal. They play a crucial role in normalizing pH level of the vagina, thus creating a defense system using antibacterial substances and cytokines (14). Infectious vaginitis seems to be an immunological disorder altering normal vaginal microbiota and several studies have suggested that alterations related to this biological defensive mechanism may trigger cancerous lesions to develop. We have conducted this study in order to determine the role of vaginal microbiota in reducing risk of cancerous lesion development. In previous studies, Kyrgiou et al (6) and Kovachev (7) suggested that altered vaginal microbiota is associated with cervical cancer. In 2011, Gillet et al conducted a study showing this fact is reachable through HPV infection, meaning that altered vaginal microbiota predisposes patients to HPV infection as bacterial vaginosis is a link between HPV infection and altered microbiota of the vagina (15).

According to our results, the vaginal microbiota is less variable in the healthy controls group. Moreover, in Pap smears containing cancerous and precancerous lesions, we detected changes in the vaginal microbiota. Thus, our study has the same results as previous studies. As for *Trichomonas* infections, previous studies showed that, through the negative effect of infections on the vaginal microbe, it could be the cornerstone for the beginning of the growth of cancerous lesions. These studies were also suggested by Silva et al and Liu & He (12,13).

Our statistical results showed the presence of this type of infection in vaginal Pap smear results in patients with cancerous and precancerous cervical lesions.

As for the rest of the other factors that were studied in this study, the statistics in the above tables showed a

Table 6. Pap smear results

| | | | Groups | | Total | *P* value |
			Case	Control		
Vaginal microbiota	Yes	No.	27	60	87	0.0021
		%	27.00	60.00	43.50	
	No	No.	73	40	113	
		%	73.00	40.00	56.50	
Total		No.	100	100	200	
		%	100.00	100.00	100	
HPV infection	Yes	No.	88	15	51.2	0.001
		%	88.00	15.00	13.50	
	No	No.	12	85	173	
		%	12.00	85.00	48.50	
Total		No.	100	100	200	
		%	100.00	100.00	100%	

Table 7. Distribution of the number of vaginal microbiota cases among the different types of cervical cancer

| | | | Presence of vaginal microbiota | | Total |
			Yes	No	
Cancer types	CIN2	No.	7	14	21
		%	25.90	19.20	21.00
	CIN 3	No.	11	24	35
		%	40.70	32.90	35.00
	HSIL	No.	6	15	21
		%	22.20	20.50	21.00
	AIS	No.	1	7	8
		%	3.70	9.60	8.00
	SCC	No.	2	13	15
		%	7.40	17.80	15.00
Total		No.	27	73	100
		%	100.00	100.00	100.00

CIN; cervical intraepithelial neoplasia, HSIL; high-grade squamous intra-epithelial lesions, AIS; adenocarcinoma in situ, SCC; squamous cell carcinoma

Table 8. *Trichomonas vaginitis* in Pap smear results in two groups

| | | | Groups | | Total |
			Case	Control	
Trichomonas vaginitis	Yes	No.	29	4	33
		%	29.00	0.00	16.50
	No	No.	71	96	167
		%	71.00	96.00	83.50
Total		No.	100	100	200
		%	100.0	100.00	100.00

close correlation between the presence of HPV infections, increase in age, smoking and increase in the number of abortions.

However, there are still many questions about the exact effect of vaginal infections like Trichomoniasis infections on the cells of the vagina, and the real reason for the increased risk of developing precancerous lesions. Therefore to answer this question and others, more studies and specialized experiments are needed.

Conclusion
According to statistical results, trichomoniasis infection was associated with an increased risk of cervical cancer. In addition, our study suggests that vaginal microbiota is a defensive mechanism against different conditions like bacterial vaginitis and progression of precancerous and also cancerous lesions. Hence, it is important to lower the risk of cervical cancer by protecting against factors interfering with normal healthy microbiome of vagina. Risk factors such as previous HPV infections, age and smoking also play an important role in causing cancerous lesions.

Limitations of the study
Patients who underwent hysterectomy or in case of incomplete information of the patient file, were not included in this study.

Authors' contribution
MD, PH and RB were the principal investigators of the study. MD, PH and RB were included in preparing the concept and design. PH and RB revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the work.

Conflicts of interest
The authors declare that they have no competing interests.

Ethical issues
The research followed the tenets of the Declaration of Helsinki. The Ethics Committee of Isfahan University of Medical Sciences approved this study (IR.MUI.MED.REC.1399.242). Accordingly, written informed was consent taken from all participants before any intervention. This study was extracted from M.D thesis of Reda Bazzal at this university (Thesis# 399137). Moreover, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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