

## Evaluating the Pap Smear Reports of Pathology Laboratories in Ahvaz, Iran

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Original article</p>	<p><b>Background &amp; aim:</b> Pap smear is a screening procedure for cervical cancer. The regular screening in women aged 20-65 years has decreased the incidence and mortality rate of cervical cancer up to 90%. This study aimed to investigate the Pap smear reports of pathology laboratories in Ahvaz, Iran.</p> <p><b>Methods:</b> In this cross-sectional study, 1,006 Pap smear reports were collected from pathology laboratories via cluster sampling method. Data was analyzed by a researcher-made questionnaire. In addition, variables such as age, type of infection, grade of inflammation, metaplastic changes, and epithelial cell abnormalities in cervix were studied in this study. Data analysis was performed in SPSS V.16.0 using descriptive analysis, ANOVA, Fisher's exact, and Chi-square tests.</p> <p><b>Results:</b> While no infection was reported in 94.43% (n=950) of cases, the highest incidence rate of infections in the remaining samples (5.56%, n=56) was related to <i>Candida albicans</i> (4.77%, n=48). Various grades of inflammation were reported in 83.69% (n=842) of the samples. Moreover, the highest grade of inflammation in fungal infections was 2+, whereas the grades in <i>Trichomonas</i> and bacterial infections were 1+ and 3+, respectively. Cervical dysplasia and metaplasia were reported in 0.29% (n=3) and 1.19% (n=12) of the cases, respectively.</p> <p><b>Conclusion:</b> The prevalence of cervical-vaginal infections, cervical metaplasia, and dysplasia were relatively low in Pap smear reports of laboratories in Ahvaz.</p>
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### Introduction

Cervical cancer, however preventable, is still considered as the leading cause of mortality among women of developing countries (1-4). According to Global Cancer Statistics in 2012, 527,600 new cases were associated with cervical cancer and 265,700 cases were expired worldwide, with nearly 90% of cervical cancer mortalities occurring in the developing countries (2).

Despite the international reports of various screening, diagnosis, and treatment of cervical cancer in Iran (5-9), the exact rates of cervical cancer incidence and mortality are still unknown due to the lack of recorded cancer cases in the cancer registry network of Iran.

Cervical cancer typically occurs at the age of

30-55 years. However, several recent studies have reported various cases of cancer at younger ages (10).

Today, Papanicolaou screening method (Pap smear) has significantly decreased the annual incidence of invasive cervical tumors in women. A Pap smear is a screening procedure conducted to detect cervical cancer in apparently healthy women, which contributes to health promotion of the society. Pap smear can help detect the early curable stages of the disease. In addition to cervical cancer diagnosis, Pap smear was also used in a recent study for ovarian and endometrial cancer diagnosis via detecting abnormal cervical cytology (11).

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This test could be performed every 1-3 year in sexually active women. The regular conduction of this test might eventually lead to a significant decrease in the incidence and mortality rate of cervical cancer up to 90%. Therefore, Pap smear is recommended for cancer screening in sexually active women as a public health policy (12, 13).

It is noted in the latest executive guideline of Iran's Ministry of Health and Medical Education that Pap smear is required as a routine screening test for all married women aged 30-70 years (sexually active for at least three years). However, the tests must be carried out with five-year intervals. If after 15 years (three five-year periods) the results were still negative, the test would be performed every 10 years (14).

The possible association of some cervical-vaginal infections with cervical lesions has been studied since the 1950s (15). In this regard, the results of a study demonstrated that the prevalence of cervical-vaginal infections was estimated at 11% in Pap smear reports (16). Donders' study indicated an association between *Trichomonas vaginalis* infection and squamous intraepithelial lesion of the cervix (17). Another study suggested that the presence of moderate or severe cervical inflammation was mostly associated with an increased risk of major cervical cytological abnormalities (18).

Nevertheless, some of the studies on cervical cancer have been performed in Iran. In this regard, in a study by Parhizgar, the prevalence of cervical-vaginal infections in Pap smear reports was estimated at 19.3% in Yasouj, Iran (19). In another study in Gorgan, Iran the prevalence of *Candida albicans* and *Trichomoniasis* infections were calculated at 14.1% and 3.6%, respectively (20). Moreover, the results of Pap smear reports in a study in Tabas, Iran revealed the prevalence of mild cervical dysplasia was 0.3% (21). However, to date, a few studies have been conducted to investigate the Pap smear reports of pathology laboratories in southern Iran. As a result, this study aimed to investigate the presence of infection, inflammation, and the rate of cervical metaplasia and epithelial cell abnormalities in the cervix based on the Pap smear reports of pathology laboratories in Ahvaz, Iran in 2013.

## Materials and Methods

In this cross-sectional study, the sample was consisted of all Pap smear results of pathology laboratories of Ahvaz, Iran in 2013. A total 1,006 Pap smear reports were collected using a multistage cluster sampling method.

At first, three pathology laboratories located at urban areas of Ahvaz were randomly selected. Afterwards, all of the Pap smear reports in 2013 were extracted from the archive of these laboratories.

We considered Pap smears for inclusion in the study which were prepared by conventional method, in which samples were smeared directly onto a microscope slide after collection. In addition, the results were reported according to the Bethesda system (2001) (22). The exclusion criterion of the study was lack of necessary information presented in the pathology reports.

Variables such as age, type of infection, grade of inflammation, presence of metaplastic changes, and epithelial cell abnormalities in the cervix were studied in this study.

According to Bethesda system, the organisms included:

- *Trichomonas vaginalis*
- Fungal organisms morphologically consistent with *Candida albicans*
- A shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with *Actinomyces* species
- Cellular changes consistent with herpes simplex virus (22)

Cervical inflammation has been defined as an increase in the average number of neutrophils per field (23). According to pathology reports, grade 1 indicated minimal inflammation and grade 2 showed mild inflammation. Meanwhile, grade 3 was considered as moderate inflammation and grade 4 was indicative of severe inflammation.

Cervical metaplasia has been identified as a process by which one fully differentiated type of epithelium appears to transform into another differentiated type (24). Epithelial cell abnormalities include atypical squamous cells of undetermined significance (ASC-US), low grade squamous intraepithelial lesion (LSIL), high grade squamous intraepithelial lesion (HSIL), squamous cell carcinoma (SCC) and atypical glandular cells (22).

The obtained data was assessed by a researcher-made questionnaire. Content validity of this questionnaire was confirmed by the judgment of experts. The reliability of data collection tool was approved by the researcher and a colleague with  $r=1$ . Every subject received a hidden code at the start of the study to protect their confidentiality.

Descriptive analysis was applied in this study to calculate the absolute frequencies of categorical variables and the mean and standard deviation of continuous variables. In addition, data was analyzed in SPSS V.16.0 using ANOVA, Fisher's exact, and Chi-square tests, and P value of less than 0.05 was considered statistically significant.

## Results

In this study, nearly 1,150 Pap smear reports were evaluated, 1,006 of which were eligible for the study. The main factor of this amount of drop-out was unsatisfactory reports due to the problems caused by bleeding or external objects.

**Table 1.** The mean and standard deviation of age (year) based on the infection type

Infection type	Mean±SD	df	F	P-value
No infection	31.5±6.5			
Candida albicans	30±6.4	3	0.8672	0.45
Trichomonas vaginalis	32.2±6			
Bacterial Vaginosis	32.6±8.6			

According to the obtained results, the mean age of the participants was 32.2±6.3 years. No

infection was reported in 94.43% (n=950) of the studied Pap smear results, while infection with *Candida albicans*, and conditions such as bacterial vaginosis or *Trichomonas* were observed in the remaining cases (5.56%, n=56). The highest incidence of infection was related to *Candida albicans*, estimated at 4.77% (n=48). In addition, *Trichomonas* and bacterial vaginosis infections were calculated at 0.49% (n=5) and 0.29% (n=3), respectively. No significant difference was observed between the mean age of the There were no reports of inflammation in 16.30% (n=164) of the samples. However, the remaining cases contained various degrees of inflammation ranged from 1-4. Grade 1 inflammation was reported in 43.33% of the cases (n=436). On the other hand, grades of 2-4 were estimated at 32.8%, 7.2%, and 0.4%, respectively.

**Table 2.** The mean and standard deviation of age (year) based on the inflammation grade

Inflammation grade	Mean±SD	Df	F	P-value
No inflammation	32.1±9.2			
1+	33.7±8.6	4	1.1053	0.35
2+	33.2±8			
3+	33.4±6			
4+	33±6.4			

Samples and various types of cervical infections (P=0.45) (Table 1).

There was no significant difference between the age of the participants and various degrees of inflammation on Pap smear reports (P=0.35). In this regard, the highest frequency of infection and inflammation were observed in the age group of 30-35 years (Table 3).

**Table 3.** The frequency of infection types and inflammation grades in different age groups\*

Variable	Age groups (year)				P-value
	25>	25-30	31-35	35<	
<b>Infection type</b>					
Candida albicans	10 (20.8)	17 (35.4)	17 (35.4)	4 (8.3)	0.48
Trichomonas vaginalis	0 (0)	1 (20)	3 (60)	1 (20)	
Bacterial Vaginosis	1 (33.3)	0 (0)	1 (33.3)	1 (33.3)	
<b>Inflammation grade</b>					
1+	1 (0.2)	89 (20.4)	280 (64.2)	66 (15.1)	0.61
2+	1 (0.3)	92 (27.8)	185 (56.1)	52 (15.7)	
3+	0 (0)	18 (25)	44 (61.1)	10 (13.9)	
4+	0 (0)	1 (25)	2 (50)	1 (25)	

\* Data is shown as n (%). Variables were analyzed using exact Chi-square test

The highest grade of inflammation in fungal infections was 2, while it was 3 and 1 in Trichomonas and bacterial vaginosis infections, respectively (Table 4).

Moreover, cervical dysplasia and metaplasia were reported in 0.29% (n=3) and 1.19%

(n=12) of the cases, respectively. There was no report of ASC-US, HSIL, SCC or glandular cell abnormalities in this study. The majority of the metaplasia cases were reported in the mean age of  $36 \pm 7.4$  years.

**Table 4.** The frequency of infection type according to the inflammation grade of the study population\*

Variable	Infection type			P-value
	Candida Albicans	Trichomonas Vaginalis	Bacterial Vaginosis	
<b>Inflammation grade</b>				
1+	3 (6.2)	1(20)	2 (66.6)	0.003
2+	32 (66.7)	0 (0)	0 (0)	
3+	10 (20.8)	3 (60)	1 (33.4)	
4+	3(6.2)	1(20)	0 (0)	

\* Data is shown as n (%). Variables were analyzed using exact Chi-square test

## Discussion

According to the results of the current study, some of the cervical-vaginal infections could be easily diagnosed from cervical cytology, by identification of either the organism or cytological characteristic of the sample. The Pap smear results of a study by Cheraghi (2014) marked that 8.8% of women in southern Iran were infected with one of the microorganisms such as Chlamydia, Candida albicans, Trichomona, and bacterial agents (25), which were in line with the results of the present study.

In this study, the highest incidence of infections was related to fungal infection of Candida albicans. Vulvovaginal candidiasis is a disease caused by abnormal growth of yeast-like fungi in the mucosa of the female genital tract (26). In addition, it has been reported affected by candidiasis at least once and twice (or more) per year, respectively (27, 28). The results obtained by Makvandi and Zargar Shoushtari (2012) revealed that the highest incidence rate of infection in Pap smear results of women in Ahvaz, Iran was associated with Candida Albicans, which is in congruence with the results of the current study (16).

According to the results of the present study, Trichomonas was the second most common observed organism in women, which had a rather broadly diverse prevalence rate among the different populations. Kalantari (2014) stated in a study that the Pap smears results of 0.2% of Iranian females revealed Trichomonas vaginalis infection (29). Another study (2012), conducted on female prisoners of Brazil,

demonstrated that Trichomonas vaginalis was found in 12% of Pap smears of the samples (30). Trichomonas vaginalis could be diagnosed using cytological characteristics in the Pap smear results. However, this approach is less sensitive (36–60%) and less specific (90%) compared to cell culture (31).

The results of the present study marked that the mean age of Candidiasis infected samples was 30 years, while it was 32.2 years in Trichomonas infections. The prevalence of this infection was mostly reported at mean age of 30 to 32.6 years. However, this finding might not be indicative of a higher infection rate in this age range, since the majority of the samples in the present study were of a similar age.

In this study, 83.7% of the samples suffered from different degrees of inflammation. However, normal cervical tissue mostly contains a certain amount of inflammatory cells, which was not considered in the test interpretation. Inflammation and infection are two distinct biological phenol-mena. Inflammation can be caused by infectious, chemical, allergic, or mechanical agents, whereas infection is generated by infectious agents, such as bacteria, fungi, viruses, or parasites. Inflammation on Pap smear has a relatively low predictive value for the presence of vaginal pathogens in asymptomatic women (32). In a study by Moasheri et al. conducted on 2,918 of Pap smears during two consecutive years, 96.6% and 0.14% of women had inflammatory grade of 1+ and 3+ with the highest and lowest frequencies, respectively (33).

The obtained results of the present study revealed that the frequency of cervical metaplasia and epithelial cell abnormalities was relatively low. Squamous cell metaplasia should not be considered as dysplasia, since no increase is observed in mitotic activity in metaplasia. In addition, the metaplasia would not progress to invasive cancer (34). The prevalence of cervical dysplasia detected by Pap smear tests varies in different regions of the world, which is estimated at 18.3% in Nigeria (35) and 3-6% in the U.S (36). In this regard, in a study in India, the prevalence of LSIL and HSIL in Pap smears were reported to be 1.3% and 0.9%, respectively (37).

The exclusion criterion of the present study was the existence of unsatisfactory reports in which blood or foreign materials were barriers to pathological tests. It is suggested that causes of bleeding (e.g., violent surface of spatula, having fragile cervical mucosa, and suffering from vaginal spotting) be explained in such cases (38).

According to the current study, no paramount information, such as occupational status of the samples and their partners, literacy level, type and duration of contraception, and type of previous deliveries, was provided in the pathology sheets. Therefore, it is recommended that the relevant sheets be prepared and completed for Pap smear reports to evaluate the data more accurately.

This study was carried out on a relatively large population, which was the main strength of the present study. Some factors, such as sample collection and preparation, type of used fixative, and the method of its application, might affect the adequacy of the conventional Pap smears. These factors might affect the efficiency of the samples and limit the ability of pathologists and cytologists to fully interpret the results (39), which was considered as one of the limitations of our study. Another limitation of the present study was collecting Pap smear results from different laboratories in the country, which significantly affected the accuracy and precision of the results.

## Conclusion

According to the results of the present study, the prevalence of cervico-vaginal infections,

metaplastic changes, and epithelial cell abnormalities were relatively low in Pap smear reports in Ahvaz. While further investigation is required to epidemiologically evaluate Pap smear reports and determine the factors related to abnormal results, periodical Pap smear screening could be effectively applied in early detection of various cervical lesions. In addition, this method can promote the reduction of cervical cancer incidence in women.

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## Conflicts of Interest

The authors declare no conflicts of interest.

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