Original Article

Frequency of abnormal colposcopy cases with abnormal biopsy in people with HPV type 16 and 18

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ABSTRACT

This study investigated the frequency of colposcopy cases with abnormal biopsy in people with positive HPV type 16 and 18 infection who were referred to the specialized clinic of Imam Khomeini Hospital in Ahvaz between 2018 and 2020. According to the studied population, the sampling in this study was cross-sectional from 2018 to 2020. The number of all HPV 16 and 18 positive patients was checked during this period, which is 100, and the cases of abnormal colposcopy and biopsy samples with positive HPV 16 and 18 equal 19 people.

According to the results of the present study, the average age of people infected with HPV16, HPV18, and HPV16&18 virus was 35.72±8.49, 37.20±10.63, and 33.44±6.18, respectively. There is no significant relationship between age and type of HPV virus. The average menarche age in people infected with HPV16, HPV18, and HPV16&18 virus was 12.94±0.92, 12.80±0.77, and 13±1.03, respectively. Analysis of variance did not show a significant relationship between the age of menarche and the type of HPV virus. In addition, there is a significant relationship between HPV type and biopsy findings. According to the results of the present study, the prevalence of the HPV16 virus was higher in the studied subjects, and the most common biopsy finding observed among these subjects was CIN1. Smoking has been an influential factor in increasing the rate of HPV infection.

Keywords: Colposcopy, Abnormal biopsy, HPV 16, HPV 18, Cervical cancer

Introduction

Cervical cancer is the second leading cause of cancer-related death among women. In this cancer, more than any other type of cancer, the effects of prevention, early diagnosis, and timely treatment are evident in reducing the death rate caused by it (1). Cervical cancer is among the most common cancers in women, especially in women aged 20 to 39 (2). In several developed countries, its prevalence has decreased due to the spread of early diagnostic tests (Pap smear), but in developing countries, it is one of the most common cancers in women after breast cancer. Of the 440,000 new cases of cervical cancer reported each year, nearly 80% occur in developing and underdeveloped countries (1). According to studies, approximately 10% of all invasive cancers in women appear in the uterus, of which about 30% occur in the cervix (3). The etiology and pathogenesis of cervical cancer include many environmental and genetic factors that lead to the transformation of epithelial cells. The most important and well-known environmental cause of this cancer is the human papillomavirus (HPV), introduced in 1970 as the leading cause of cervical cancer (2). Papillomavirus has the smallest viral DNA, which can cause tumors. These viruses have different types that infect many animals and humans. So far, more than a hundred types of human papillomaviruses have been identified, and about ⅓ of them infect the epithelial cells of the genital area. More than 170 types of this virus have been reported, and more than 40 of its genotypes can be transmitted through sexual contact or contact with infection in the anogenital area (anus and penis area). Genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are carcinogenic and are introduced as high-risk genotypes (1).

Genotypes 16 and 18 are responsible for 70% of cervical cancer (2). 5-10% of infections caused by high-risk HPV genotypes can turn into precancerous lesions. The progression of a subclinical lesion to clinical infection takes years (4). It is worth noting that the critical factor related to invasive cervical cancer is the lack of

screening throughout life (in 50% of cases) and the lack of screening in the last five years (in 10% of cases) (5). The sensitivity of a traditional pap smear in cervical cancer diagnosis was estimated between 30 and 87%, and its specificity between 86 and 100%, and the sensitivity of a liquid-based pap smear in cervical cancer diagnosis was estimated between 61 and 95% and its specificity between 78 and 82% (6). The combination of the pap smear test and HPV test increases the sensitivity from 87 to 100% and decreases the specificity by 69 to 95% compared to the traditional pap smear method (7).

HPV 16 positive is the most common cause (about 50%) of cervical squamous cell carcinoma, and HPV 18 positive is the most common cause (about 55%) of cervical adenocarcinoma. HPV 16 positive, with a prevalence of 32%, and HPV 18 positive, with a prevalence of 10%, are the next causes of cervical adenocarcinoma. In 5 to 30% of cases, infection with multiple HPV genotypes is observed (8).

The risk of getting CIN3 during three years varies in different HPV genotypes and is as follows: HPV 16 has a 1 in 4 chance of getting infected, HPV 18 has a 1 in 9 chance of getting infected, and Other HR (High Risk) HPV Genotypes has a 1 in 19 chance of getting infected (9).

Wang (2020) conducted a study titled "Risk of cervical lesions in high-risk women with HPV positive and normal cytology: a retrospective study in China," which included 5880 women. 59.97% had expected histological results, 19.32 had HSIL, and 1.07% had cervical cancer. The detection rate of HSIL in women infected with HPV16 (34%), HPV31 (27.5%), HPV33 (25.58%), and HPV52 (20.88%) was significantly higher than HPV18 infection (15.59%). The detection rate of +HSIL was not considerably different between HPV16 infection and multiple infections (except for HPV18) (10).

The body's immune system quickly clears most HPV infections and does not turn into cervical cancer. Transforming normal cervical cells into cancer cells is very long, and cervical cancer is usually seen in persistent infections (which generally last more than a decade or more). The longest clearing time is for HPV 16. This period depends on the strength of the immune system of individuals, especially in patients with HIV. The clearing time is longer, which makes the HPV infection more stable and thus increases the risk of invasive cervical cancer (11).

Colposcopy is one of the diagnostic methods to detect cervical cancer. A colposcopy can detect malignant and cancerous cells with an accuracy of over 99% and prevent their growth and proliferation. The percentage of correct performance of methods such as pap smear is only 60% (12).

Colposcopy is an examination of the cervix and vagina, which is graded according to the degree of the stomatitis lesion, surface contour, mosaic pattern, and punctuation marks, and the abnormality of most of these parameters is related to the severity of the lesions (13).

Colposcopy allows tissue sampling (biopsy) where abnormal areas have been seen. Indeed, sampling abnormal areas is an essential part of colposcopy because the treatment depends on the severity of the abnormality in the biopsy sample. Treatment is recommended if biopsy results show precancerous (dysplasia) or cancer. Incisional biopsy is recommended when the colposcopic appearance indicates a high-grade abnormality (14). This method has been examined in different studies. Wentzensen (2015) conducted a study titled "Multiple Biopsies and Detection of Cervical Cancer Precursors in Colposcopy" on the entire population. This study's sensitivity for detecting HSIL (high-grade squamous intraepithelial lesions) increased from 60.6% after one sampling to 85.6% after two samplings and 95.6% after three. A significant increase in the sensitivity of multiple sampling was observed in all subgroups. The greatest increase in HSIL performance was observed for women with high-grade colposcopy, HSIL cytology, and human papillomavirus (HPV) type 16. Only 2% of all HSILs were detected by sampling from a normal-appearing area (15). Paunovic (2016) conducted a study titled "Relationship of human papillomavirus infection with cytology, colposcopy, histopathology and risk factors in the development of low and high-grade cervical lesions." This study showed that patients with L-SIL and H-SIL had significantly higher HPV infections than patients with benign histological findings. The percentage of H-SIL was highest in patients infected with HPV type 16/18 who had sex with two to five sexual partners before the age of 16 (16). Burness (2020) conducted a study titled "Cervical Colposcopy: Uses and Risk Assessment" stated that colposcopy has evolved as a diagnostic method for evaluating vaginal and cervical dysplasia to incorporate patient risk factors for cervical intraepithelial neoplasia and cancer. Changes in cervical cancer screening and guidelines, human papillomavirus vaccination recommendations, and colposcopy standards from the American Society of Colposcopy and Cervical Pathology (ASCCP) have been sent to all primary care physicians. In addition to cervical cancer screening and follow-up, primary care physicians should offer HPV vaccination to all patients between 9 and 26 years of age. Clinicians should make a shared decision about Perform immediate ring electrosurgical quick removal in contrast to colposcopy For patients with the highest risk of cervical cancer and for patients who are older than 25 years and have at least two of the following (HPV-18,16 and cytology of high-grade squamous intraepithelial lesions) (17). Thomsen (2021) conducted a study titled "Probable benefits and harms of cervical cancer screening based on human papillomavirus." The HPV group needed 3.7% immediate colposcopy, and 2.8% required frequent 12-month screening. The total colposcopy visits was 6.6% more than the cytology group's 1.2%. The probability of CIN3+ among women referred to colposcopy was 21.1% lower than the cytology group 34.6% (18). Based on what was stated, this study is conducted to investigate the frequency of colposcopy cases with abnormal biopsy in people with positive HPV type 16 and 18 infection who were referred to the specialized clinic of Imam Khomeini Hospital in Ahvaz between 2018 and 2020.

Materials and Methods

This study was descriptive-analytical and retrospective, conducted after obtaining the necessary permissions from the Research Council and the Research Ethics Committee. The research statistical population included all women with positive HPV type 16 and 18 infections referred to the specialized clinic of Imam Khomeini Hospital in Ahvaz between 2018 and 2020. In this study, all people with positive HPV type 16 and 18 infection were selected and included in the study using a cross-sectional sampling method. The number of all 100 positive HPV 16 and 18 patients was checked, and the cases of abnormal colposcopy samples with positive HPV 16 and 18 are equal to 19 people.

The inclusion criteria are the completeness of the patient's medical record so that 50% of the information in the list can be completed. In addition, patients who were only infected with HPV type 16 and 18 were positive. Exclusion criteria include the lack of completeness of the patient's medical record.

The researcher referred to the specialized clinic of Imam Khomeini Hospital in Ahvaz and identified all the people who were positive for HPV type 16 and 18 infection and examined their medical records based on the prepared checklist. The checklist included patient demographic variables and disease-related variables.

After collecting, the data were used using SPSS version 22 statistical software and descriptive statistics methods (for qualitative data of frequency and percentage, for quantitative data of mean and standard deviation). The chi-square test will be used to compare nominal variables; the Kolmogorov-Smirnov test will be used to determine the normality of quantitative data distribution and an independent t-test will be used to compare quantitative variables.

Results and Discussion

Table 1. Correlation between HPV type and age

HPV type	Mean ± standard deviation	confidence interval		P-value
		lower limit	upper limit	
16	8.49±35.72	33.66	37.78	
16,18	6.18±33.44	30.14	36.73	0.46
18	10.63±37.20	31.31	43.09	

In **Table 1**, the relationship between HPV type and age has been measured using a one-month variance analysis test. As observed, there is no significant relationship between age and the type of HPV virus.

Table 2. Correlation between HPV type and menarche age

HPV type	Mean ± standard deviation	confidence interval		P-value
		lower limit	upper limit	
16	0.92±12.94	12.72	13.16	
16,18	13±1.03	12.45	13.55	0.81
18	12.80±0.77	12.37	13.23	

In **Table 2**, the relationship between HPV type and age of menarche has been measured using a one-month analysis of variance test. As observed, there is no significant relationship between the age of menarche and the type of HPV virus.

Table 3. Correlation between HPV type and smoking

Smoking	type **HPV**			P-value
	16	16,18	18	
yes	44(63.8)	16(100)	10(66.7)	0.016
no	25(36.2)	0(0)	5(33.3)	
Experience of STDs (sexually transmitted diseases)				
yes	13(18.8)	4(25)	3(20)	0.85
no	56(81.2)	12(75)	12(75)	
Family experience of malignancy				
yes	5(7.2)	0(0)	2(13.3)	0.34
no	64(92.8)	16(100)	13(86.7)	

Table 3 shows the relationship between HPV type and smoking, STD experience, and family experience of malignancy using the chi-score test. As observed, there is a significant relationship between HPV type and smoking. However, there is no significant relationship between HPV type and experience of STD (sexually transmitted diseases) and family experience of malignancy.

Table 4. Correlation between HPV type and biopsy findings

Biopsy findings	HPV type			P-value
	16	16,18	18	
Atypical glandular cell	0(0)	0(0)	2(13.3)	
cin i	7(10.2)	0(0)	0(0)	
cin iii	2(2.9)	1(6.3)	0(0)	
Focal koylosytic atypia	0(0)	2(12.5)	0(0)	0.03
High-grade dysplasia	2(2.9)	0(0)	0(0)	
Low-grade srtomal neoplasm	1(1.4)	0(0)	0(0)	
Normal	55(79.7)	13(81.2)	13(86.7)	
Severe dysplasia	2(2.9)	0(0)	0(0)	

Table 4 shows the relationship between HPV type and biopsy findings using the chi-score test. As observed, a significant relationship exists between HPV type and biopsy findings.

Table 5. Correlation between HPV type and colposcopy

Colposcopy	HPV Type			P-value
	16	16.18	18	
satisfactory	46(66.7)	13(81.3)	12(80)	0.36
Un satisfactory	23(33.3)	3(18.7)	3(20)	

In **Table 5**, the relationship between HPV type and colposcopy has been analyzed using the chi-score test. As observed, there is no significant relationship between HPV type and colposcopy.

Today, cancer is the second leading cause of death in developed countries after cardiovascular diseases and the third leading cause of death in developing countries (1). In general, cervical cancer can have various causes, and one of the risk factors for this cancer is HPV. HPV is a large family of viruses and the most common sexually transmitted infection. In most cases, HPV infection is asymptomatic and self-limiting. This infection is associated with benign or malignant proliferation of squamous mucosa. Therefore, this study aimed to investigate the frequency of colposcopy cases with abnormal biopsy in people with positive HPV type 16 and 18 infections who were referred to the specialized clinic of Imam Khomeini Hospital in Ahvaz between 2018 and 2020.

According to the results, the average age of the people infected with HPV16, HPV18, and HPV16&18 virus was 35.72±8.49, 37.20±10.63 and 33.44±6.18, respectively. HPV virus has no significant relationship. Ahmad *et al*. (2017) conducted a study based on the relationship between age and HR-HPV type 16, in which the highest percentage was observed in the age range of 35-45 years (36.5%) (19). Many studies show that HPV16 positivity is significantly associated with younger age, mainly when early detection is used, such as CIN diagnosis. This increasing infection decreases with age. With increasing age, the prevalence of HPV type 16 decreased ($P < 0.008$) (19). This means HPV type 16 is more common in young women with high-grade cervical lesions (19, 20). HR-HPV 18 shows a relatively similar relationship with age, but in general, HPV16 is more common at older ages, but this difference was not significant. Some other studies also agreed with our findings that HPV18 is more common in relatively older ages (20).

In this study, the average menarche age in people infected with HPV16, HPV18, and HPV16&18 virus was 12.94±0.92, 12.80±0.77 and 13±1.03, respectively. Analysis of variance showed no significant relationship between the age of menarche and the type of HPV virus, and no similar study was found in this regard.

In the present study, the relationship between HPV type and smoking was analyzed using the chi-score test. It was shown a significant relationship between HPV type and smoking, so 44% of people who were infected with the HPV16 virus smoked. Schabath *et al*. (2012) stated that current smoking was associated with an increased risk of any HPV infection (21). Utami *et al*.. (2021) showed a statistical association between smoking and HPV 16 infection in the normal cervix (22). Smoking can also potentially increase viral load by weakening the cellular immune response because previous studies have shown that smoking has harmful effects on systemic and local immunity (23-25). Smoking leads to the recruitment of inflammatory cells and the subsequent release of proinflammatory cytokines, chemotactic factors, oxygen radicals, and proteases that alter immune cell function (26). Nicotine is the main compound responsible for the addictive properties of smoking, which has also been shown to be immunosuppressive in both animals (27) and humans (28).

In addition, the present study has been used to measure the relationship between HPV type and family experience of malignancy using the chi-score test, which showed no significant relationship between HPV type and family experience of malignancy. No similar study was found in this field. According to the findings of the present study, the most biopsy findings were observed in patients infected with the HPV16 virus, cin i, and in those infected with the HPV18 virus, atypical glandular cells, and between the patients infected with both viruses focal koylosytic atypia. The Chi-score test was used to measure the relationship between HPV type and biopsy findings, which showed a significant relationship between HPV type and biopsy findings. Masoumi *et al*. (2016) showed that malignant lesions in the squamous tissue (CIN1, CIN2, CIN3, HPV) are more than the malignant lesions found in the glandular tissue, which is consistent with the results of the present study. CIN1 lesions are related to a mild cervical squamous lesion, CIN2 is related to a medium cervical squamous malignant lesion, and CIN3 is associated with a high-grade cervical squamous malignant lesion (29). Finally, the results showed no significant relationship between HPV type and colposcopy. No similar study was found in this field.

Conclusion

According to the results of the present study, the prevalence of the HPV16 virus was higher in the studied subjects, and the most common biopsy finding observed among these subjects was CIN1. Smoking has been an influential factor in increasing the rate of HPV infection. For further investigation, it is suggested that this study be conducted comparatively in other provinces.

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