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# Types of Human Papillomavirus (HPV) in Patients with ASCUS Pap Smear

**H. Hassani (MD)[1], M. Javadian (MD)[2], M. Ranaei (MD)[3], A. Abedi (BSc)[4], Sh. Yazdani (MD)[*2]**

1.Student Research Committee, Babol University of Medical Sciences, Babol, I.R.Iran.

2.Clinical Research Development Unite of Rouhani Hospital, Babol University of Medical Sciences, Babol, I.R.Iran.

3.Cancer Research Center, Health Research Institute, Babol University of Medical Sciences, Babol I.R.Iran.

4.School of Basic Sciences, York University, Toronto, Canada.

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## ABSTRACT

**Background and Objective:** Human papillomavirus (HPV) is one of the common infectious and pathogenic agents, and some of its genotypes have the ability to cause malignant changes in the surface of mucous cells. By examining the prevalence of HPV in atypical squamous cells of undetermined significance (ASCUS) Pap smear according to the risk factors, we can make a more correct decision in choosing the evaluation method and directing the patient to report the ASCUS lesion. Therefore, the aim of this study is to investigate the types of human papillomavirus (HPV) in patients with ASCUS Pap smear.

**Methods:** This cross-sectional study was conducted on 200 patients with ASCUS pap smear results and HPV test referred to the colposcopy clinic of Babol Ayatollah Rouhani Hospital during the last two years. Demographic information, history of obstetrics, history of OCP use, history of STD disease and high-risk behaviors, cervical treatments, HPV typing, Pap smear, colposcopy and cervical biopsy results were recorded and analyzed.

**Findings:** The mean age of the patients was 33.83±8.09 years, and ASCUS pap smear showed 129 cases (64.5%) of high-risk papillomavirus, 16 cases (0.8%) of low-risk papillomavirus and 55 cases (27.5%) of both types. Cervical intraepithelial neoplasia grade 2 was in 18 cases (0.9%), cervical intraepithelial neoplasia grade 1 in 10 cases (0.5%) and cervical intraepithelial neoplasia grade 3 in 2 cases (0.1%). 17.1% of high-risk viruses caused cervical neoplasm. None of the low-risk viruses led to neoplasms and all of them were inflammatory. In both high-risk and low-risk cases, 14.5% neoplasm was seen. However, the relationship between the type of papillomavirus and the incidence of neoplasm was significant (p=0.058).

**Conclusion:** Based on the results of this study, the ASCUS pap smear needs more detailed investigation such as colposcopy and cervical biopsy under the supervision of experts because it is associated with the high-risk HPV virus in many cases.

**Keywords:** *Human Papillomavirus, Colposcopy, Pap Smear, ASCUS.*

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**[*]Corresponding Author: Sh. Yazdani (MD)**
**Address:** Infertility and Reproductive Health Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, I.R.Iran.
**Tel:** +98 (11) 33295570. **E-mail:** shahla_yazdani_1348@yahoo.com

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## Introduction

Human papillomavirus is one of the most common infectious and pathogenic agents in all parts of the world. This virus is from the family of DNA viruses and is non-enveloped, of which there are more than 200 different types. The main way of transmission of the virus is close person-to-person contact, especially sexual contact (1). The increase in the prevalence of Human Papillomavirus (HPV) as the most common sexually transmitted disease in young people has led to more attention to this disease in the field of human health (2). Studies have shown that 30-60% of sexually active adults are infected with HPV during their reproductive period (3, 4) and its rate is higher in young women than in older women (5).

Some genotypes of the HPV infection have the ability to cause malignant changes in the surface of mucous cells, which causes malignancy in both genders (1). Currently, HPV infection is the most important cause of cervical cancer, but only a few types of HPV cause cancer (6). Types 16 and 18 are one of the most important factors in causing dysplasia and cervical carcinoma (7). Cervical cancer screening was done every 3 years between the ages of 20 and 65 with the help of a Pap smear test, which has reduced the death rate from this cancer by 90% (8).

In many cases of Pap smears, Atypical Squamous Cells of Undetermined Significance (ASCUS) lesions are reported (9), which actually have more obvious changes than reactive changes in these cases, but do not have the specific characteristics of intracellular neoplasia (10). There are three ways to evaluate patients with ASCUS lesions: immediate patient referral for colposcopy, repetition of cervical cytology one year later, and HPV typing assessment (9). In a study, about 10.9% of pap smears were ASCUS, and in the study of the high-risk HPV type, it was different in different races and it was 5.6% to 6.5% (11). In a study, postmenopausal women were examined based on the result of ASCUS pap smear test, and it was recommended to perform HPV test in these people and to perform colposcopy after estrogen therapy (12).

By examining the prevalence of HPV in ASCUS patients, we can make a more accurate decision regarding the selection of the evaluation method and guiding the patient to report the ASCUS lesion according to the climatic and epidemiological conditions and various risk factors in the region.

## Methods

This cross-sectional study was conducted after approval by the ethics committee of Babol University of Medical Sciences with the code IR.MUBABOL.REC.1399.120 on a total of 200 patients with ASCUS pap smear results and registered HPV typing.

Demographic information, history of obstetrics, history of OCP use, history of STD disease, cervical treatments such as cryosurgery, HPV typing, results of pap smear, colposcopy and cervical biopsy were entered into the checklist. The results of ASCUS pap smear was done for all patients with Based-Liquid method and HPV typing with hybrid soft kit and reverse dot blot technique in a single laboratory (Pasteur laboratory in Babol) and colposcopy of all patients was performed by a gynecologic oncologist at Babol University of Medical Sciences and the results of colposcopy were recorded and cervical biopsy samples were taken and recorded in the pathology center of Ayatollah Rouhani Hospital and the final pathology report was recorded. Then, the data were analyzed using SPSS 22 software. Chi-square and T-test were also used and $p<0.05$ was considered significant.

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## Results

In this study, 200 women with pap smear results (ASCUS) with a mean age of 33.83±8.09 years were included in the study (Table 1). 29 people (14.5%) mentioned the history of OCP consumption. 77 people (38.5%) had a history of genital warts in the patient or spouse (Table 2). 123 cases (61.5%) had high-risk papillomavirus, 23 cases (11.5%) low-risk and 54 cases (27.0%) had both types. Out of 123 cases of high-risk papillomavirus, type 16 in 19 cases (15.4%), type 51 in 15 cases (12.2%) and types 58 and 59 in 14 cases (11.4%) were the most prevalent. In the next ranks were virus type 52 in 13 cases (10.6%) and type 18 in 11 cases (8.9%), respectively (Table 3).

**Table 1. Descriptive indices of basic variables in studied women with ASCUS pap smear results**

| Variables | Mean±SD | Median | Min. | Max. |
|---|---|---|---|---|
| Age (years) | 33.83±8.09 | 34 | 17 | 59 |
| Menarche age (years) | 12.43±1.58 | 12 | 9 | 17 |
| Age of marriage (years) | 20.12±6.03 | 18 | 13 | 45 |
| Age of first pregnancy (years) (145 people) | 21.07±4.65 | 20 | 14 | 34 |
| Number of previous pregnancies (145 people) | 2.07±1.21 | 2 | 1 | 5 |
| Number of living children (145 people) | 1.50±0.89 | 1 | 0 | 4 |
| Number of abortion cases (145 people) | 0.52±0.95 | 0 | 0 | 4 |

**Table 2. Frequency of studied variables in women with ASCUS pap smear**

| Variables | Number(%) |
|---|---|
| **History of OCP use** | |
| No | 171(85.5) |
| Yes | 29(14.5) |
| **History of genital warts in patient and spouse** | |
| No | 123(61.5) |
| Yes | 77(38.5) |
| **History of smoking in patient and spouse** | |
| No | 156(78.0) |
| Yes | 44(22.0) |

**Table 3. Frequency distribution of the types of high-risk human papillomavirus**

| High risk type of human papillomavirus | Number(%) | Low risk type of human papillomavirus | Number(%) |
|---|---|---|---|
| 16 | 19(15.4) | 6 | 22(40.7) |
| 18 | 11(8.9) | 11 | 11(20.4) |
| 31 | 1(0.8) | 40 | 2(3.7) |
| 33 | 5(4.1) | 42 | 2(3.7) |
| 35 | 3(2.4) | 43 | 6(11.1) |
| 39 | 4(3.3) | 54 | 4(7.4) |
| 45 | 6(4.9) | 61 | 3(5.6) |
| 51 | 15(12.2) | 72 | 2(3.7) |
| 52 | 13(10.6) | 81 | 2(5.6) |
| 56 | 5(4.1) | | |
| 58 | 14(11.4) | | |
| 59 | 14(11.4) | | |
| 66 | 1(0.8) | | |
| 68 | 9(7.3) | | |
| 82 | 3(2.4) | | |

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In the analysis of the colposcopic pathology report, the results showed that acute and chronic cervical inflammation were seen in 89 cases (44.5%), chronic cervical inflammation in 81 cases (41.5%), cervical intraepithelial neoplasia II in 18 cases (0.9%), cervical intraepithelial neoplasia I in 10 cases (0.5%) and cervical intraepithelial neoplasia III was seen in 2 cases (0.1%).

17.9% of HR viruses caused cervical neoplasm. However, none of the LR viruses led to neoplasms and all of them had inflammatory pathology. In cases with both HR and LR, 14.8% neoplasm was seen (Table 4). However, the relationship between the type of papillomavirus and the occurrence of neoplasm was borderline significant (p=0.058).

In the evaluation of the type of high-risk human papillomavirus based on the pathology report, according to 22 cervical neoplasm pathology results, virus number 18 was the most neoplasmic agent in 4 cases (18.2%). In addition, virus types 16, 58 and 59 were involved in the occurrence of neoplasm with a frequency of 3 cases each (13.6%).

Of the 29 people who took OCP, 6 people (20.7%) had CIN and 23 people (79.3%) did not have CIN. However, among people who did not take OCP, 24 people (14%) had CIN and 147 people (86%) did not have CIN, and the difference was not statistically significant.

**Table 4. Comparison of papillomavirus type based on pathology results**

| Pathology report<br>Type of virus | Cervical inflammation<br>Number(%) | Cervical neoplasm<br>Number(%) | p-value* |
|---|---|---|---|
| HR | 101(82.1) | 22(17.9) | 0.058 |
| LR | 23(100) | – | |
| HR+LR | 46(85.2) | 8(14.8) | |

*Using the Chi-square test

## Discussion

In the present study, the most common high-risk papillomavirus was type 18 and low-risk papillomavirus was type 11. The low-risk genotypes of the human papillomavirus are types 6 and 11, which often lead to the creation of benign lesions such as genital warts and precancerous lesions with a low degree of differentiation, and the high-risk types, including types 16 and 18, are the most common causes of cervical and other anogenital cancers (13, 14).

Hajibagheri et al. stated in their study that the frequency of genotype 18 was higher in high-risk types compared to other genotypes, which is similar to the present study, and in the low-risk type, genotype 6 was the highest (15), but in our study, genotype 11 was more frequent than type 6. The reason for this difference can be associated with the difference in sample size. In the review of similar studies by Khodakarami et al., Moradi et al., Safaei et al., and Ghaffari et al., conducted in different cities of Iran, the prevalence of HPV types 6 and 11 among women was 3-6%, type 16 was about 2.1-24% and type 18 was 0.2-10% (16-19).

The relationship between tissues infected with human papillomavirus and histopathological appearance and other prognostic factors of cervical neoplasia has been the focus of researchers, and considering the importance of quick and correct diagnosis of the cause of the disease before its complications, the molecular method can be used for this purpose and is useful in cases with low levels of pathogenic agent. The most important finding of the current research was that high-risk human papillomavirus was related to the outcome of cervical neoplasm in patients. The results of the study of Mostafavizadeh et al. strengthen the relationship between HPV and cervical cancer. According to the protocol used in DNA extraction and PCR,

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this test can usually be performed in the pathology laboratory with simple and cheap facilities (20). In the study by Cheng et al., out of 182 patients with high-risk HPV, about 129 (70.8%) had CIN or cervical cancer in the biopsy sample (21). Several reports show the role of viral factors in the occurrence and evolution of uterine cancer. Human papillomavirus, especially its carcinogenic types, is seen with epithelial malignancies of the genital system and outside of it (22). In this study, ASCUS pap smear was detected in 17.9% of investigations by colposcopy, and HR viruses caused cervical neoplasm. However, none of the LR viruses gave neoplasms and all of them had inflammation-type pathology. In cases with both HR and LR, 14.8% neoplasm was seen.

Hassani et al. stated in their study that most of the samples (52.2%) were infected with high-risk types of viruses, most of which were type 16 viruses. According to the results of this study, HPV can be considered as the main factor responsible for the symptoms of cervical cell changes that can turn into cancer. Also, in most cases, the main cause of contamination are high-risk types of viruses. The most common type of virus infecting the cervical epithelium is HPV 16 (23). Mehr Afza et al. reported in their study that pathological intraepithelial lesions were observed in 14 cases (66.6%) of 21 patients with ASCUS pap smear results; among them one person had HSIL and 13 had LSIL (24).

In a study conducted by Tsedenbal et al., 12% of women had ASCUS pap smear results, 8% LSIL (low grade squamous intraepithelial lesion), 7% HSIL (high grade squamous intraepithelial lesion) and 14% SCC (25). In this study, patients who had ASCUS pap smear results were included in the study. In this study, 30% of the obtained pathology results were neoplasia and 70% were cervical inflammation. In their research, Chen et al. found that the prevalence of neoplasia in patients with ASCUS pap smear results was equal to 76% (21). The findings of the study of Keyhani et al. reinforce previous reports on the relationship between HPV and cervical cancer (1).

The prevalence of abnormal pap smear (cervical neoplasia) in the study of Khodakarami et al. was 4.1%. They suggested that by carrying out epidemiological studies at the country level, its actual prevalence should be extracted and planned for the prevention and control of this dangerous disease (16). In the study of Monsefi et al., the prevalence of dysplastic or cancerous lesions was 0.4%. According to the findings of the present study and the peculiar family culture of Iran, HPV infection among women with cervical cancer is less common than in other countries. The predominant genotype was HPV 16, which is one of the carcinogenic genotypes (26). In a study conducted by Sahin et al., it was concluded that 12.9% of abnormal Pap smears were found to be involved in cervical cancer, and the most prevalent pathologies were ASCUS, Atypical glandular cells, ASC-H, LSIL, and HSIL, respectively (27). Since the prevalence of neoplasia and the type of HPV in women with abnormal pap smear results are different based on geographic region, race, and socio-economic conditions, some differences may be justified.

One of the strengths of the present study is the examination of the type of human papillomavirus. Based on the results, high-risk virus type 16 in 19 cases (15.4%), type 51 in 15 cases (12.2%) and types 58 and 59 in 14 cases (11.4%) were the most prevalent. Low risk virus type 11 in 6 cases (26.1%) followed by virus type 81 in 5 cases (21.7%) were the most prevalent.

In their study, Krashias et al. stated that women with abnormal pap smear results had High risk HPV, Low risk HPV and known HPV types, respectively (28). Studies have shown that the human papillomavirus in the environment outside the body causes the immortalization of epithelial cells with the occurrence of E6 and E7 oncogenes (29).

HPV 16 was the most common type among women with normal cervical cancer cytology in the study of Tsedenbal et al. However, in women with abnormal cervix, which included low grade squamous intraepithelial lesion and high grade squamous intraepithelial lesion, HPV 52 was predominant (25).

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The broad perspective of this study will be an analysis of the diagnosis, epidemiology and identification of human papillomavirus infections in women. Based on the results of the study, it is suggested that patients with risk factors and ASCUS pap smear should be further examined with colposcopy to detect cervical lesions early.

According to the results of the present study, the most common virus associated with ASCUS pap smear was the high-risk type of genotype 16 and the low-risk type of genotype 11, and in the examination with colposcopy and biopsy, 17.9% CIN was reported. In this study, the role of high-risk human papillomavirus infection in the occurrence of cervical premalignant lesions was confirmed.

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