

Frequency of HR-HPV Types in Patients with High Grade Cervical Intraepithelial Neoplasia (CIN)

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ABSTRACT

Background & Objective: Cervical cancer is the second most common cancer and the fourth leading cause of death in women. Among the risk factors for cervical cancer, human papillomavirus (HPV) is the most important one.

Materials & Methods: In this cross-sectional and retrospective study conducted from 2016 to 2020, 261 women with cervical intra-epithelial neoplasia (CIN) grade two and three referred to one of the gynecological oncology clinics of Shahid Beheshti University of Medical Sciences, who were eligible to enter the study and were evaluated by the research unit of the relevant university after receiving an ethics code. During the study, patients whose cervical cancer was confirmed by colposcopic diagnostic method, HPV screening was performed by COBAS method and lesions were sampled to determine the type of HPV.

Results: Evaluation of the frequency distribution of colposcopic results compared to HPV, indicated that HPV-16 is the most common type of HPV in high grade CIN lesions. After HPV-16, other types of HPV are next in terms of frequency indicating the importance of other types of HPV. HPV-18 was also observed in people with CIN.

Conclusion: Performing a similar study with a larger number of samples at the national level is suggested. If the results of a larger study are consistent with this study, it would be for the best to highlight the role of other types of HPV in cervical cancer screening in women.

Keywords: Cervical cancer, HPV, Risk factors



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Introduction

Papilloma virus is a DNA virus (1). Human papilloma virus (HPV) causes many cancers in humans (2). HPV is the cause of warts and is found in all human populations and sometimes, the associated infection leads to cancer. These viruses infect the cutaneous and mucosal epithelial tissues of different anatomical regions (3-4). To date, more than one hundred different types of human papillomavirus have been identified, and one-third of which infect genital epithelial cells. The types of HPV that infect the genitals fall into two categories: low-risk types, such as HPV-6 and HPV-11, which cause benign warts that do not progress to cancer and usually heal, and high-risk types including HPV 16, 18, 31, 33, 45 and are associated with the development of genital cancer and are found in 99% of cervical cancers, of which HPV-16 is more prevalent

(5). Cervical cancer is the fourth leading cause of cancer deaths with 275,000 deaths in 2008 and 7.5% of cancer deaths among women worldwide in 2012 with 266,000 deaths, in some parts of the world such as Africa and South Asia, it is the leading causes of cancer-related deaths, accounting for 88% of cancer mortality in these regions. About 50 years ago, this cancer was the leading cause of cancer death in women in developed countries; at the moment it is the eighth leading cause of cancer death in developed countries of the world, and the lethality rate has increased from 30.6:100,000 deaths in 2002 to 152.2:100,000 deaths in 2008 (6). The prevalence of cervical cancer in Iran is lower than some countries in the world; according to the report of the National Cancer Registration Center of the Ministry of Health of Iran, in 2009, its

prevalence rate was 2.17:100,000 and it ranked 11th among all cancers of Iranian women, which demonstrates a slight increase compared to the 2008 report ranking it as 13th. In 2009, about 558 new cases of cervical cancer were registered in the Ministry of Health, and 286 women died in the same year due to advanced uterine cancer. It is ranked 8-7 in Tehran, however in other provinces it is not among the top 10 cancers in females (7-8). Risk factors for cervical cancer include: Initial sexual intercourse at an early age, multiple sexual partners, smoking, history of human papillomavirus infection, weakened immune system, multiparite (high number of pregnancies and deliveries), recurrent sexually transmitted diseases (infection) Herpes simplex virus type 2, exposure to diethyl acetylbestrol (DES) in the fetal period, history of intraepithelial neoplasia, infection of the partner with penile cancer, infection of the other partner with Cervical cancer, poor health and consumption of oral contraceptive pills (9-10).

Materials and Methods

The present study is a descriptive cross-sectional and retrospective study that was performed to determine the prevalence of high-risk HPV types in women with high cervical intra-epithelial neoplasia (CIN) grade. The study population consisted of women with high grade CIN (grade 2 and 3), who referred to the gynecology and oncology clinic of Imam Hossein Hospital in 2016-2020. After the approval of the ethics committee, the files were reviewed and patients' informed consent to review the file information. They were obtained and used in the study. Inclusion criteria was as follows: colposcopy during the previous 3 years of referral, CIN pathology of high grade (grades 2 and 3), HPV examination during one month prior to referral, HPV test by COBAS method. Exclusion criteria including virginity and history of total hysterectomy. The patients who underwent colposcopy in the last three years with a high CIN grade and examined by COBAS method in the last one month were included in the study. In this study, all colposcopies were examined by the same specialist, all HPV infections were confirmed by one laboratory, and all pathology reports were performed by one pathologist. By reviewing the files, firstly, individual-social information and risk factors for HPV infection were recorded. Information recorded from the files includes: age, city of birth and place of residence in the last 10 years, year of referral, marital status, patient education, employment status, number of sexual partners, age of onset of sexual intercourse, number of pregnancies and delivery method, type of contraception Menstrual status, smoking and hookah, genital warts, immune deficiency, Pap smear results, colposcopy and HPV type. In this study, using the data in the file, interviewing and examining the patient, in addition to gathering information about HPV diagnostic tests, research variables were recorded in a

checklist prepared for each patient. The study population consisted of women with CIN 3 and 2 (as precancerous lesions of the cervix). After collecting and classifying the data, statistical analysis of the data was performed using SPSS 20 (SPSS Inc., Chicago, IL., USA) and MedCal 15.3 software. Statistical analysis of data was performed on quantitative and qualitative variables using t-test and chi-square test, respectively.

Demographic Characteristics of Patients

The study was conducted among 261 patients with a mean age of 34.18, among whom 31 (12%) of women with high CIN grade had a history of hookah use. Out of a total of 261 patients with high CIN grade, 31 (12%) had a history of smoking and the rest had no such history. Among 29 patients with high CIN grade and a history of smoking, the highest number of cigarettes smoked was 5 cigarettes by 10 patients. A total number of 148 patients had only one sexual partner, the rest had more than one sexual partner. Frequent evaluation of the duration of contraceptive use showed that only 17 patients had such a history, of whom four patients had consumed it for one year, four patients for two years, three patients for four years, and two patients for 10 years. Also, in cases of use at five, six, eight and nine years, only one patient reported such a history. Out of 261 patients evaluated with high grade CIN, in terms of gravid history (pregnancy), 102 patients had no pregnancy history, 76 patients had a history of one pregnancy, 39 patients had a history of 2 pregnancies, 32 patients had a history of 3 pregnancies, and 12 patients had a history of more than 3 pregnancies. Regarding the history of Para (delivery), 128 patients were negative, 68 patients had one case, 45 patients had two cases, 15 patients had three cases and five other patients had a history of more than three cases of Para. Regarding the history of abortion, 203 patients did not experience it. A number of 41 patients mentioned one case, 13 patients mentioned two cases, one patient mentioned six cases and one other patient mentioned 10 cases. External uterine pregnancy (EP) was also reported for three patients with a frequency of one. In addition, 198 patients had no history of NVD (normal delivery), 33 patients had one case of NVD, 19 patients had two cases of NVD, six patients had three cases of NVD and five patients had a history of more than three cases of NVD. The history of cesarean section was 1, 2 and 3 for 56, 22 and 5 patients, respectively. A total of 255 patients had never had a history of curettage, four patients had a history of one curettage and two patients had a history of three curettages. The rest had a history of two and one curettages. The prevalence of sexually transmitted diseases (STD) among patients indicated 44 cases (64.7%) of genital warts and 10 cases of herpes infection (14.7%). Other causes accounted for 14 cases (20.6%). In the present study, other causes besides risk factors for high-grade precancerous lesions were also investigated, including STD in the sexual partner, smoking in the partner, sex, menopausal age,

menstrual status and method of contraception. The majority of cases registered as STDs in a sexual partner include genital warts in 29 people (78%), herpes infection in four people (11%) and four other sexually transmitted diseases. High-grade CIN and smoking in sexual partners were reported in 12 patients, and for 249 others, there was no history of smoking in sexual partners. According to the results recorded for menopausal age, six people were menopausal. Regarding menstrual status, 223 people were normal and 19 had oligomenorrhea and other cases of menstrual status were not very common. In most cases, high grade CIN is the natural method or WD. Also, 54 patients used condoms.

Results

To assess the prevalence of HPV-16 among individuals, all patients who were diagnosed with 16-HPV infection, regardless of whether or not they had other types of HPV, were considered HPV-16 positive and the others were considered HPV-16 negative. Then, the frequency of positive and negative cases among people with precancerous lesions was evaluated. The results showed that HPV-16 was significantly more common among people with CIN (55.9%). Also, the evaluation of odds ratio indicated that HPV-16 increases the chances of people getting precancerous lesions by about 2.67 times. To assess the prevalence of HPV-18 among individuals, all patients who were diagnosed with HPV-18 in their samples, regardless of whether or not they had other types of HPV, were considered HPV-18 positive and the others were considered HPV-18 negative. Then, the frequency of positive and negative cases among patients with precancerous lesions was assessed. Assessing the odds ratio showed that in the statistical sample of the present study, HPV-18 did not increase the chance of developing precancerous lesions. To assess the prevalence of HPV among individuals, all patients who were diagnosed with other types of HPV, regardless of whether or not they had other types of HPV, were classified as positive for other types of HPV and the others were considered negative for other types of HPV. Then, the frequency of positive and negative cases among people with precancerous lesions was assessed. The odds ratio assessment indicated that in the statistical sample of the present study, other types of HPV could be a risk factor for precancerous lesions. Most of the people examined with Pap smear method had normal results. Then, ASCUS, LSIL and Inflammation modes have the most Pap smears. In order to have an overview of the status of Pap smear report, Pap smear results were generally classified into abnormal and non-abnormal. Normal and inflammation cases were called non-abnormal and the rest were abnormal. Then, we evaluated the frequency of these two situations. The results of comparing the frequency of abnormal and non-abnormal Pap smears showed that 56.3% of patients with high grade CIN

(case group) had abnormal Pap smears and 43.7% had non-abnormal Pap smears. ASCUS smear was reported at 20.7% and HSIL was reported at 12.3%. As mentioned earlier, in order to evaluate colposcopic results compared to Pap smear, Pap smear results were initially classified as non-abnormal (normal and inflammation) and the rest were abnormal. In women with CIN, the abnormal category was also divided into three subgroups under the headings of subgroup 1, 2, and 3. Subgroup 1 included ASC-H and HSIL, subgroup 2 included ASCUS and LSIL, and subgroup 3 included other abnormal cases. The results showed that the distribution of CIN type between different types of Pap smears is statistically significant; High grade CIN is significantly higher in some types of Pap smears. CIN2 colposcopic results were reported in non-abnormal Pap smear. In abnormal Pap smears, the majority of people were ASCUS with CIN2 colposcopy results. Evaluation of the frequency distribution of colposcopic results in relation to high-risk HPV type indicated that HPV-16 is the most common type of HPV in people with CIN2 colposcopy; 46 patients with HPV-16 had a CIN2 colposcopy result; 33.3% of CIN2 people had a diagnosis of "only HPV-16". In people with CIN3 colposcopy, 49.1% of patients had a diagnosis of "only HPV-16", which indicates that this type of virus is associated with greater pathogenesis of the disease and the development of precancerous lesions. In fact, HPV-16 is the most abundant and most pathogenic agent identified in precancerous lesions in the present study. After HPV-16, other types of HPV were the most prevalent in CIN2 group (26.8%) and in the CIN3 group (19.3%). This demonstrates the importance of the type of HPV Others.

Discussion

Today, cancer is the second leading cause of death in developed countries after cardiovascular disease and the third leading cause of death in developing countries (11). Cervical cancer is one of the most common cancers in women, especially in women aged from 20 to 39 years (12). In a number of developed countries, its prevalence has decreased due to the prevalence of early diagnostic tests (Pap smears) (13), but in developing countries, after breast cancer, it is one of the most common cancers in women. Of the 440,000 new cases of cervical cancer reported each year, nearly 80% occur in developing and underdeveloped countries (14). According to studies, approximately 10% of all invasive cancers in women occur in the uterus, of which about 30% occur in the cervix (15-16). In general, cervical cancer can have different causes, and one of the risk factors for this cancer is the HPV. HPV is a large family of viruses and the most common sexually transmitted infection, and in most cases, HPV infection is asymptomatic and self-limiting. This infection is associated with benign or malignant proliferation of squamous mucosa (17). Genital HPV types are divided into low-risk and high-

risk categories based on their association with cervical cancer. There are 15-20 types of high-risk HPV, the most common of which are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59. HPV types 68 and 73 are also high-risk types, but there is little evidence that they are carcinogenic (18-19). Most HPVs are low-risk, and types 11 and 6 are responsible for 90% of genital warts. About 5% of cancers in men and women are associated with HPV infection. Low-risk types such as 6 and 11 cause low-potential cervical lesions, genital warts, and pharyngeal lesions (20). Recurrent infections occur in 5-10% of these women which may progress to precancerous lesions. The interval between infection and progression to malignancy is 10 years or more (21). HPV DNA is found in 80-90% of squamous cell carcinomas and cervical adenocarcinomas, as well as in precancerous lesions of the cervix (CIN). The most common genotypes involved are 16, 18, 31 and 45, which are found in 49%, 12%, 5% and 8% of cases, respectively. Genotype 16 is more common in squamous cell carcinoma and genotype 18 is more common in cervical adenocarcinoma (22). In the present study, as in the above studies, it was shown that HPV-16 is significantly more common among people with CIN (55.9%). About 50-60% of precancerous lesions such as CIN types 2 and 3 are associated with HPV types 16 and 18 in the United States (23). Also, in this study, the odds ratio evaluation showed that HPV-16 increases the chances of developing precancerous lesions by about 2.67 times, so as in the above study, it can be concluded that precancerous lesions such as CIN type 2 And 3 may be associated with HPV-16. In addition to cervical cancer, HPV infection also causes other genital cancers such as vaginal, penile, rectal, and oropharyngeal cancers (24). Risk factors for HPV infection include: host, environmental and behavioral factors and virus-related factors, the most important of which are age, smoking, prolonged use of hormonal factors, sexual intercourse with different people, *Chlamydia trachomatis* infection, not using condoms and nutritional agents noted. The viral factors that can play a role in the development of cancer include the type of HPV virus and the simultaneous infection with several types of HPV (22). Also, according to various studies, the most important risk factor for infection is having multiple sexual partners during life (23). In the present study, it was shown that out of 261 patients, 113 patients had more than one sexual partner. It can be concluded as in the above study that the presence of multiple sexual partners is a factor for HPV infection and ultimately causes cervical cancer. There is also a comparison of the average age of people at the time of sexual intercourse with high CIN grade. The younger the age of the first sexual intercourse, the higher the risk of precancerous lesions of the cervix, which can be attributed to the greater risk of exposure to risk factors. The results of this study indicate that HPV-16 was the most common type of HPV among patients. Also, during this study, it was shown that the frequency of other types of HPV is related between people with the disease. Also, the odds ratio assessment indicated that in the statistical sample

of the present study, other types of HPV could be a risk factor for precancerous lesions, most of whom were married and had higher education. Also in this study, as in the above study, it was found that the most common type of HPV is HPV-16 and also other types of HPV were also more prevalent among the studied patients. Smoking was also evaluated in precancerous patients, which was found in 12% of patients with precancerous lesions of the cervix. In 2018, 2453 healthy and sexually active Iranian women were studied. This study was using Liquid-based cytology and HPV DNA typing was done by COBAS method. The mean age in this study was 34.18. According to this study, the overall prevalence of HPV in Iranian women is 10.3%. HPV-16 was found in 73 women (3%) and HPV-18 and other high-risk HPV types were found in 16 women (0.7%). The rest were observed in 166 women (8.2%). It was concluded from this study that the prevalence of high-risk HPV in Iranian women has increased in recent years and there is a need for public education, planning to prevent cancer by vaccination and early detection of screening tests. Also, HPV DNA typing and detection of common HPV genotypes in cervical cancer prevention programs in Iran should be considered (26). The results of the above study are consistent with the present study and both studies have shown that the prevalence of high-risk HPV, including HPV-16, was high in women with a high CIN.

Conclusion

Frequency distribution of high-risk HPV types in women with CIN ($P < 0.001$) showed that some types of high-risk HPV were higher, which indicates the association of this type of virus with the pathogenesis of most disease cases and the occurrence of precancerous lesions. After HPV-16, other types of HPV are next in frequency, which indicates the importance of the other types of HPV. In general, the more common types of high-risk HPV in women with CIN may indicate that they may be the cause of high-grade precancerous lesions. In particular, HPV-16 is both the most abundant and the most pathogenic agent identified in precancerous lesions. It should be noted that in the present study, 261 cases of high grade CIN were investigated and there is a limit in terms of number. At the moment, based on the results of this study, the value of other types of HPV in causing precancerous lesions of the cervix is significant. It is recommended to emphasize on the role of other types of HPV similar to positive cases of HPV-16 and conduct additional studies.

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Conflict of Interest

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