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Genotyping the human papilloma virus infection in Iranian women referred to ShahidMotahari Hospital, in Urmia, with Real-Time PCR techniques

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Abstract

***Background & Aims*:** Human papilloma virus is inviridae of papilloma virus (HPV) family. These viruses have been classified based on the DNA sequences. At least 120 types of these viruses have been identified. Different types of human papilloma virus genotypes are associated with lower genital tract infection. The viruses in the host cell can create lethal, chronic, latent and transforming infections, and their multiple genotypes are related to human cancers such as cervical cancer.This study aimed to identify the genotypes of human papilloma virus that cause vaginal infections in women with infection.

***Materials and Methods*:** This cross-sectional study was performed on 80 patients with vaginal infection who referred to specialized gynecology unit of Kosar Health Center of ShahidMotahari Hospital of Urmia University of Medical Sciences (Iran). Extracting viral DNA was performed automatically using Mag Core Nucleic Acid Extractor (made in Taiwan) and MagCor®viral nucleic Acid Extration kit (Cartridge code 202) made by MogCore Co. Taiwan, and genotyping the samples was carried out using the Real-Time PCR technique in the Lightcycler 96 system (made in Germany), and Real quality RQ-HPV HR/LR Multiplex Kit made by AB Analitica® Co. Italy.

***Results*:** Among the 80 samples, 30 people (37.5%) were positive for infection with human papillomavirus, evaluating the positive genotypes, it was found that of these, 16 people (53.4%) were infected with Low-Risk genotypes of human papilloma virus (6, 11, 26, 53, 67),7 patients (23.3%) were infected with only High-Risk HPV genotype (16, 31, 58, 18) and 7 people (23.3%) were infected with both low-risk and high-risk genotypes of HPV (co-infection) (6, 33, 53, 39, 68, 70, 52, 35, 26, 51, 16), respectively.

***Conclusion*:** Considering that human papilloma virus infection is asymptomatic, and it has a high prevalence in Iran, according to studies, early diagnosis, and prevention of progressing the infection can prevent the malignancies of the uterus.Molecular techniques, particularly Real-time PCR, are as one of the fast and reliable methods for detecting the human papilloma virus infections even with quite low viral loads.

***Keywords*:** human papilloma virus, vaginal infection, Real-time PCR

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Introduction

Human papilloma viruses (HPVs) with more than 100 genotypes of HPV with worldwide distribution are in the family of HPV viridae with the structure of a protein and double-stranded circular and uncoated genome (1). HPV infection is the most common sexually transmitted infections and is a significant source of mortality caused by cancer (2,3). Many human papilloma virus infections occur dormant, they can be identified on healthy skin, these infections are largely transient and frequently asymptomatic, and in most cases are without clinical results (4). Infection with certain types of human papilloma virusis the main cause for a variety of diseases, and cancers (5). World Health Organization described 12 types of human papilloma virus with 16,18,31,33,35,39,45,51,56,52,58 and 59 genotypes as high-risk types or carcinogenic, and some other subtypes with 68,73,26,30,53,66,67,69,82,85 genotype as those that may be carcinogenic (6). Near 71% of these HPV16 and HPV18, was identified as worldwide cervical cancer cases (7). Other types of human papilloma virus genotypes including genotypes of 6,11,13,40,42, 43,44,54, 61.70, 72, 81.89, mostly are related to genital warts and were classified as a low-risk group. The prevalence of HPV infection was exhibited differently, in different regions and countries and even in different demographic subgroups of a country. However, the global prevalence of HPV was reported, 11.7% (8). Distribution of certain types of HPV infection is different in various geographic areas (9, 10). Some types of HPV in the Asia-Pacific region are more common than in other regions (11).

Several risk factors were identified for development and persistence of HPV infection. Age is an important factor, although significant differences exist in age-HPV curve in different areas (14-16). In some countries, the genital HPV infections were detected in 20% to 40% of sexually active young women that the prevalence of the infection is reduced by increasing the age. Other factors include the existence of sexual partners, the age of starting sexual activity, socioeconomic status, using condoms, using contraceptives, smoking (2, 12, 13), using of public baths (14) and training (15). The number of pregnancies and childbirth were identified as a risk factor for HPV infection (16).

Currently, the best way to prevent these infections and related diseases is to perform routine cytological screening and, treatment of precancerous lesions, if necessary (17, 18). However, due to the cost, performance challenges and complexities in preparation for screening and treatment, this method has restrictions in Iran. The HPV vaccine was prepared against the most common types (types 16 and 18) and a vaccine was prepared for types of genital warts (HPV type 6 and 11). Studies have indicated that the vaccine used against genital warts includes the protective effect on the infection of papilloma virus types 16 and 18 (3, 19, 20). In other words, HPV vaccine can prevent cervical cancer potentially, particularly in centers with poor socio-economic status (21). According to the surveys conducted by the researcher, this study is the first epidemiological study, which was designed to determine the prevalence of human papilloma virus infection of the genital tract in Iranian women living in West Azerbaijan, so that if the spread of this viral infection which is the main cause of cervical cancer in the population of women is high, provisions are considered to implement the vaccination and prevention programs against cervical cancer.

Materials and methods

This cross - sectional study was conducted from December 2015 to December 2016.In this study, 80 married women between the ages of 45-25 years, were investigated who had a vaginal infection and referred to the Kosar Women Subspecialty Clinic of Motahari

hospital, in Urmia, for vaginal examination. Sampling was performed by a gynecologist using sterile swabs from the vaginal and secretions and cervical mouth of each patient under sterile conditions. The samples were placed in 2 ml of transport medium (Pasto Cyto Prep) (PCP) (prepared by the Pasteur Institute of Iran) and were transferred to molecular laboratories. The swaps related to vaginal secretions were stored in the transport medium at -20 ° C after stirring with a vortex.

Extraction of DNA:

To perform the molecular test, separating the DNA of human papilloma viruses from vaginal secretions was conducted automatically using Mag Core® Nucleic Acid Extrator (made in Taiwan) and MagCor®viral nucleic Acid Extration kit (Cartridge code 202) made by Magcore Co. Taiwan. To extract DNA from vaginal sample, K proteinase and RNA Carrier and the mentioned kit was used, and the amount of extracted DNA was adjusted to 60 µl. The concentration of extracted DNAs was measured using Nanodrop ND–1000, Thermo Fisher Scientific USA Co.at 260 nm in terms of ng/ml. The extracted DNAs were stored at -20 ° C until implementing the Real Time-PCR reactions.

DNA genotyping using Real-Time PCR technique:

Genotyping the samples through Real- Time PCR technique was performed using Realquality RQ-HPV HR/LR Multiplex Kit made by LightCycler® Co. (Italy) in for typing 22 HPV type including high risk HPV genotypes (16, 18, 31, 35, 39, 45, 51, 52, 56, 59, 66 and 68) and Low- risk HPV genotypes (6, 11, 26, 53, 67, 70, 73, 82) in LightCycler® 96 Roche system (made by Germany) using specific primers of the kit. For genotyping with this technique all 4 channels of the device were used, that

FAM channel: was used to identify High-Risk genotypes (31,33,35,39,45,51,52,56,58,59,66,68) and Low-Risk genotypes (26,53,67,70,73,82),

Hex Channel identifies the High Risk genotype: 16, and Low Risk: 6genotype,

Texas Red channel identifies Internal Control and

Cy5 channel identifies the High Risk genotype: 18, and Low Risk genotype: 11.

The results of Real-Time PCR reactions were read using Light Cycler software which had been designed on the device.

Results

In a study performed on women with vaginal infection who referred to a specialized clinic of Motahhari Hospital, in Urmia,among80 samples, 30 people were infected with human papilloma virus,of these 16 people had Low-Risk genotypes of human papilloma virus (6, 11, 26, 53, 67),7 people had only High-Risk HPV genotype (16, 31, 58, 18),and7 patients had both low-risk and high-risk genotypes of HPV (co-infection), (6, 33, 53, 39, 68, 70, 52, 35, 26, 51, 16). Hence, in terms of frequency, the prevalence of human papilloma virus is 37.5% (Figure 1). Of which 53.4% was with low-risk HPV, 23.3 with high-risk HPV and 23.3% was with co-infection of human papilloma virus, respectively (Figure 2). Among the patients with HPV,18 people (60%)were within the age of 25-30 years, 7 patients (23.3%) were within the age of 35-31 years and 5 patients (16.6%) were within the age of 36-45, respectively.

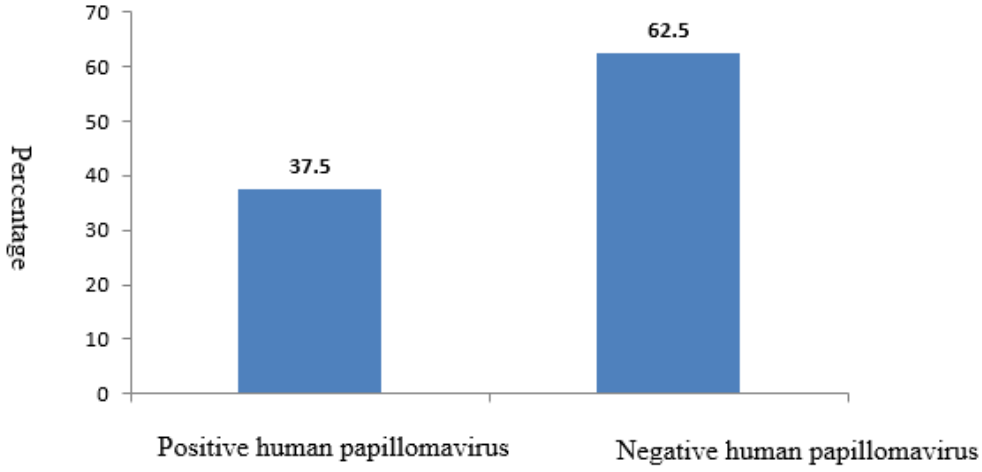

Figure 1: Percentage of positive or negative frequencies of human papilloma virus in the samples.

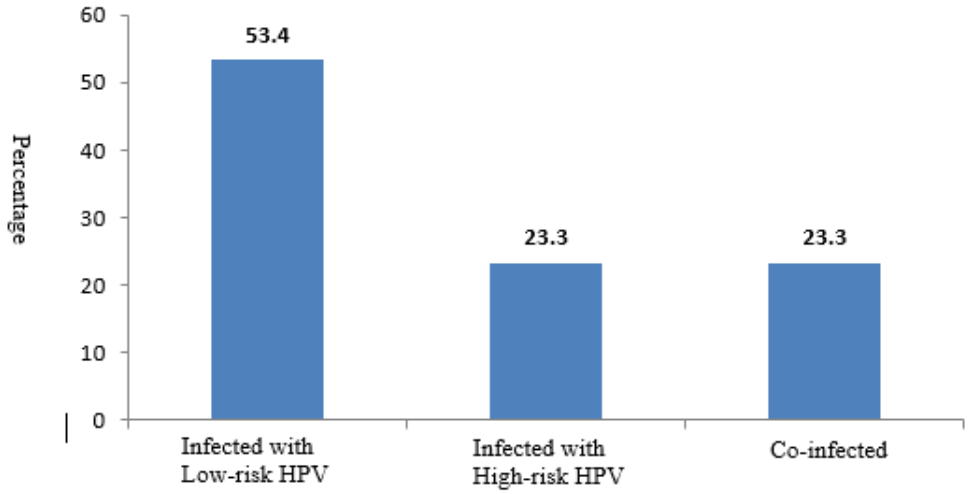

Figure 2: The percentage of low-risk HPV, high-risk HPV, and co-infection of human papilloma virus in samples.

Discussion

More than 450 thousand cases of cervical cancer occur in the world annually. Although the mortality rate has been decreased in the last 30 years compared to the past, each year almost 200 thousand deaths caused by cervical cancer occur that this rate is higher than 12,000 new cases of cervical cancer in developed countries annually. Of this rate, 4,000 deaths are associated with this disease. Human papilloma virus is certainly considered as the main cause of uterine malignancies and progressive cancer of the uterus.

In the study of Mahmudi et.al which was conducted to determine the human papillomavirus genotypes in cervical cancer samples, the certain types of human papilloma virus (HPV) and their correlation with cervical malignancies in Yazd province were detected. In this study, in 70%of the samples,HPV genome was detected that the 16 and 18 types with the frequency of 70% and 16.7%, respectively, were diagnosed as the

most common genotypes. Other identified types were 45.33 and 73 (22). In another study in this field which was conducted in Isfahan, the prevalence of HPV in married women with the age of 18-60 years old with normal Pap smear was investigated who referred to gynecology clinics affiliated with University of Medical Sciences of Isfahan. In this study of 46 positive HPVs, 15.21% was genotype 16 (7 samples), 13.04% was genotype 18 (6 samples), and 21.74% was genotype 11 or 6 (23). A study conducted in Kerman on 20000genotypingPap smear samples, in which in 93.75% of the people, positive HPV type 16 was identified, and type 18 was identified in just 6.25 (24). In the study of Jafari et.al (2006) the prevalence of HPV genotypes in Iranian women was evaluated to determine the important role of human papilloma virus infection in progressing the uterus cancer. This study aimed to determine the genotype of the common HPV in women with normal and abnormal cervical cytology. Genotyping was performed by PCR and the results showed that the infection with HPV type 16 in patients was significantly higher compared to the normal group (42% vs 11.6%). Similarly, HPV genotype type 18was more common in the abnormal group. In this study, a significant correlation was found between genotypes of HPV and cervical pathological changes (25). Other study in this regard conducted by Khodakarami et al (2008) regarding the prevalence of human papilloma virus infection in women living in the areas covered by health regions of ShahidBeheshti University of Medical Sciences, Tehran, aimed to determine the prevalence of human papilloma virus infection and its role in the incidence of cervical cancer in Iran. The genotyping of HPV in this study was performed by PCR; and the results showed that although the prevalence of HPV infection in patients under 25 years was higher than other groups slightly, it was not significant statistically, and the prevalence of infection in the samples was 7.8%, of which 5.1% was high-risk, and the most common HPV was type 16 as well (26). In other study performed by Sohrabi et al (2015) based on evaluating of HPV as the primary cause of cervical cancer, the different genotypes of human papilloma virus especially high-risk genotypes were the main causes of cancer. In this study, the documentation of about 100 papers was reviewed in scientific bases and molecular diagnostic methods such as PCR and hybridization were introduced as valid methods for detecting and identifying the genotypes. The results of this study showed that increasing the genital infections, sexually transmitted diseases and cancers caused by microorganisms, especially HPV, demands cooperation with the international community to perform more comprehensive studies, particularly in developing countries (27).

In a population-based study performed by Kvamrvn Naharet al (2014) regarding the genital infection with human papilloma virus in women in Bangladesh, aimed to determine the prevalence of HPV genotypes in an urban and a rural area in Bangladesh, HPV genotyping was performed by PCR techniques. In this study, the prevalence of HPV was 7.7% and there was no significant difference between rural and urban women. The most common high-risk genotypes were HPV31,40,18,66,16,53.The maid urban women were at high risk for HPV infection compared to housewives or garment workers. Rural women whose husbands were living abroad were almost two times more likely to develop HPV in comparison with the rest. The results of this study showed that the prevalence of infection in Bangladeshi women was similar to that in other Asian regions. However, they were different in terms of HPV genotypes (28). The study of Kuzaran et al. (2015) was performed based on HPV infection in the cervix and its continuation in 300 women in public health care clinics in a region of Brazil, and the genotyping was performed

based on PCR. Cytology analysis was performed by Pap smear test, and HPV infection was diagnosed in 47 women. The results of this study showed that HPV infection is more common in young women aged less than 30 years and in low-income individuals and the persistence of viral infection in this group was lead to precancerous lesions in the cervix. High-risk HPV genotypes were found in precancerous lesions (29).

Fio-X et al(2003)studied the incidence of certain types of human papilloma virus infections in squamous intraepithelial lesions of the cervix tissue in the women over 35 years old in West Africa. In this study, the women over 35 years old and older were tested for cytologic abnormalities of the cervix using Pap smear and PCR, respectively.HPV infection was found in 18% of women with negative cytologic abnormalities. The prevalence of high and low-risk HPVs increases with the age.HPV16 and HPV58 were predominant types in this study. The results showed a significant association between HPV types and severity of cervical tissue lesions(30). In other study performed by Clifford et al (2005) based on the distribution of genotypes of human papilloma virus in low-intensity lesions in the cervix (LSIL) and comparison of different geographical position and their correlation with cervical cancer. The prevalence of HPV in cervical lesions with low intensity (LSIL) in women in North America, was 80%, and it was less than 70% in other areas. This reflects the regional differences in diagnosing LSIL. Among the 5910 HPV positive samples the most prevalent genotype was for HPV type 16,and then the most common genotypes were HPV31 (11.5%), HPV51 (10.6%) and HPV53 (10.2%), respectively. The positive HPV type 16 in African women was 2 times less than infected women in Europe, and in North America, papillomavirus type 18 infection was higher than in Europe, South and Central America (31).

Conclusion

Despite the fact that Iran, as a developing country is exposed to prevalence and incidence of malignancies caused by human papilloma virus infection and resulted deaths, comprehensive statistics regarding the distribution of HPV types in the country and regional distribution in the city of Urmia has not been published. Hence, the need for determining a regional genotyping is felt to prevent the infection more accurately and cost-effectively. According to studies, this study is the first study which was conducted in West Azerbaijan. So that if the spread of the infection is high which is the main cause of cervical cancer, appropriate provision should be considered regarding the preventing programs, including training, screening, immunizing and treatment.

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