RESEARCH ARTICLE

Prevalence and Type Distribution of Human Papillomavirus Infection Using the INNo-Lipa Assay, Kerman, Southeast Iran

Reza Malekpour Afshar[1], Hamid Reza Mollaie[2]*, Mehdi Fazlalipour[2], Seyad Alimohammad Arabzadeh[3]

Abstract

The human papilloma virus (HPV) causes skin and mucous membrane infections. It crosses from one person to another by skin-to-skin contact, such as sexual contact. There are more than 100 types of HPV that can influence different parts of the body. Some types of HPV can cause cancer (such as cervical or anal cancer) and others can cause warts (such as genital or plantar warts). HPV infection is one of the most common sexually transmitted infections (STIs) in Iran and around the world. Considerable molecular evidence suggests a role for human papilloma virus (HPV) in the pathogenesis of carcinoma. Epidemiological studies on human papilloma viruses (HPVs) infections in general population are critical for the performing of health policy guidelines for developing the strategies to hinder the primary and secondary different cancer. In different parts of Iran, there is a lack of population-based studies to determine the prevalence of HPV in the general population. The aim of this population-based study was therefore to report the prevalence ratse of HPV types among Iranian patients. To study the risk of human papilloma virus (HPV) infection, we managed a retrospective study in Kerman province, southeast of Iran. For this purpose, 410 patients tested for the presence of HPV DNA using PCR and INNo- Lipa assays. HPV DNA was detected in 108 out of 410 patients (26.34%), while it was not detected in any of the control group samples. Patients included 23 (21.1%) males and 86 (78.8%) females. HPV type 6 was the most common (49%) followed by HPV type 16 (10.1%), and also HPV type11 (9.2%). The prevalence of HPV in Iran is comparable to those reported in other regions of the world. In a similar manner, it seems that HPV types 6, 16 and11 are the most common types in Kerman. Additional studies on larger group of patients, particularly in those with pre-invasive forms of disease, are needed to explain the roles of different HPV types in this location of Iran.

Keywords: Human papilloma virus - subtypes - INNo-lipa assay - Kerman - Iran

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Introduction

In developing countries, clinical and epidemiological studies have shown that Human Papilloma virus (HPV) play a main role in the development of different types of cervical lesions, and are therefore considered as the major infectious agent of genital lesions as well as cancers (Ciarrocca et al., 2013). Papillomavirus with circular double-stranded DNA genome are small non-enveloped viruses with 52 to 55 nm diameter. The length of their genomes is approximately 800 bp and located in icosahedral capsid. Its head is composed of 72 capsomers (Best et al., 2012). The head is formed by two structural proteins L1 and L2. The L1 viral protein makes up about 80% of the total viral proteins.HPV virions are related to receptors such as alpha-integrins and Laminin which are leading HPV to enter in to basal epithelial cells (Norman et al., 2013). It is thought that viral oncogenes E6 and E7 alter cell cycle to remain host keratinocytes differentiated in a state which is suitable for viral genome replication and consequently late gene will be expressed. HPV makes tumors such as warts and Papilloma in its natural hosts (Mohabatkar, 2007). The Lesion may be cutaneous, mucosal squamous epithelium in the larynx, in esophagus or genitals. Infection often produced lesions which are very small and sometimes not even visible with the naked eye (Alsaad et al., 2012). Suppression of immune system in humans and animals causes activation of latent infection or increases the possibility of virus inoculation from active lesions to form bigger lesions (Palefsky., 2006). As a new type of virus is considered when its complete genome sequence be cloned and its L-ORF DNA be different more than 10% with the nearest type of HPV virus. The difference between 2-10% type (Subtype) and less than

*[1]Research Center for Tropical and Infectious Diseases, [4]Department of Medical Virology, Kerman University of Medical Sciences, Kerman, [2]Department of Medical Virology, Iran University of Medical Sciences, Tehran, Iran *For correspondence: hamid2008kmu@gmail.com*

2% a variant (Variant) are measured. Approximately 140 HPV types have been identified so far. Some types of HPV can make warts or Verrucae which they do not cause cancer and other types which cause cancer, they do not produce warts(Nirchio et al., 2008). Other types have no symptoms and they are harmless. Generally, about 30-40 types of HPV are transmitted through sexual contact and infected genital areas. Some types of HPV are transmitted through sexual intercourse may cause genital warts (Babiker et al., 2013). Persistent infection with High-risk HPV types opposing of warts may lead to Precancerous lesions and invasive cancers. However, most infections with these types cannot create disease (Gold et al., 2013). Seventy percent of women can get HPV in first year and 90% of them get it in 2 years. Pap test is used to detect abnormal cells that may lead to cancer progression. Although the Pap test for cervical cancer decreased the incidence and case fatality in developed countries, 11,000 cases and 3,900 deaths in 2008 occurred in America and this rate in the world is 490,000 cases and 270,000 deaths (Grace et al., 2011; Huang et al., 2013). Due to extensive changes in epidemiological studies of various cancers and new information about the prevalence and HPV types in different countries and also non-uniform prevalence, it is concluded that Iran is one of the countries where the incidence of cancer and the HPV prevalence is high. Iran, which is creating a fair system for death registration, it is predicted that nearly 30 thousand people annually die from their cancer. According to the study which is done by International Agency for Research on Cancer (IARC), the cancer incidence in Iran is located in the East Mediterranean, about 140 thousand cases, but according to other studies, this figure is estimated to be 250-200 thousand patients (Karimi et al., 2009). In developed countries, 7% of risk factors for cancer are related to the infection, while in the developing countries like the Eastern Mediterranean countries include 26%. According to research, now in western countries 40% of cancer cases are preventable and in third world countries the possibility of preventing cancer is 70%. Thus, the incidence of cancer factors should be considered much more serious (Chimeddorj et al., 2008; Aljunid et al., 2010; Diamantopoulou et al., 2013). The most common infectious agents for cancer in Iran are HPV, Hepatitis B virus and Hepatitis C virus, and Helicobacter pylori infection which are including 85% of adult population. Therefore, regarding to incidence and high prevalence of HPV in Iran, the prevalence of HPV in patients with risk factors as well as common genotypes in patients could be documented in the report of the ordinary genotypes of HPV in Iran. In the future to prevent this virus as well as comprehensive supportive program for cancer control and fighting with cancer in the country will be important. INNO-LiPA HPV detection/genotyping assay kit could be used principally for screening purposes. However, no data are currently available on the performance and value of the assay in a clinical setting.The purpose of this study was to determination HPV genotypes in several specimens from Kerman ,Southeast of IRAN using the INNO-LiPA HPV detection/genotyping assay. The INNO-LiPA HPV detection/genotyping assay is capable of detecting and genotyping 25 different HPV types simultaneously and has proved to be sensitive, specific, simple, and rapid in the assessment of HPV.

Materials and Methods

Study population and samples

In a retrospective study all samples were collected and received from different cities of Iran in Besat clinic in Kerman during 2008-2012 and the INNO-LiPA test was used to verify the positive result and their specific types. Specimens were obtained from 410 Women and Men. HPV was confirmed in 108 samples using PCR in Center lab. Source of samples were Paraffin embedded block, vaginal scrape, genital, biopsy, serum specimens. Paraffin embedded blocks were processed using xylen for remove of paraffin that possibility of isolating DNA for HPV detection assays. This method has received approval for clinical use from the U.S. Food and Drug Administration .Cytological classification was performed by an experienced pathologist.

Specimen preparation

For isolation of nucleic acid from specimens in first step using xylen to remove paraffin from blocks in three steps and get rid of it by centrifuge, then add 200 µl of tissue lysis buffer and proteinase K for 24 hours at room temperature then material was isolated using the Total Nucleic Acid isolation kit (Roche Applied Science) as described by the manufacturer. Nucleic acid was resuspended in a final volume of 100 µl; 10 µl was used for PCR analysis.

After isolation of DNA, samples were tested for the presence of HPV by the INNO-LiPA HPV detection/genotyping assay kit. General detection assays, with a broad spectrum of specificity for HPV, are now widely used for the detection of HPV in clinical specimens although the Hybrid Capture II assay (Digene) is the only commercially available HPV screening test on the market. The Hybrid Capture II assay for HPV DNA detection is a liquid-based hybridization assay capable of detecting 13 HR HPV genotypes simultaneously. In 2003, a newly developed PCR-based technique, the Roche AMPLICOR HPV Test, was launched. This test is also capable of detecting 13 HR HPV types, with simultaneous assessment of the presence of the human β-globin gene as a positive control.

INNO-LiPA HPV detection and genotyping

A) PCR amplification of HPV DNA: Broad-spectrum HPV DNA amplification was performed using a short PCR fragment assay (INNO-LiPA HPV detection/genotyping assay, SPF_{10} system version 1, manufactured by Labo Biomedical Products bv, Rijswijk, The Netherlands). This assay amplifies a 65-bp fragment of the L1 open reading frame and allows detection of at least 43 different HPV types. The SPF_{10} PCR was performed with a final reaction volume of 50 µl containing 10 µl of the isolated DNA sample, 10 mmol/liter Tris-HCl (pH 9.0), 50 mmol/liter KCl, 2.0 mmol/liter $MgCl_2$, 0.1% Triton X-100, 0.01% gelatin, 200 µmol/liter of each deoxynucleoside

triphosphate, 15 pmol each of the forward and reverse primers tagged with biotin at the 5'end, and 1.5 U of AmpliTaq Gold (Perkin-Elmer). The mixture was incubated for 9 min at 94°C, 40 cycles of 45s at 45°C, and 40 cycles of 45s at 72°C, with a final extension of 5 min at 72°C. Each experiment was performed with separate positive and negative PCR controls.The presence of HPV DNA was determined by hybridization of SPF10 amplimers to a mixture of general HPV probes recognizing a broad range of HPV genotypes, in a microtiter plate format, as described previously.

B) HPV genotyping by reverse hybridization using the INNO-LiPA HPV genotyping system: A poly(dT) tail was enzymatically added to the 3' end of each of 25 oligonucleotides specific for 25 different types, namely, types 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74. The tailed probes were applied as horizontal lines to membrane strips (manufactured by Labo Biomedical Products bv, Rijswijk, The Netherlands). The HPV genotyping assay was performed as described previously. Briefly, equal volumes (10 µl each) of the biotinylated PCR products and denaturation solution (400 mmol/liter NaOH, 10 mmol/liter EDTA) were mixed in test troughs and incubated at room temperature for 5 min, after which 1 ml of prewarmed (37°C) hybridization solution was added, followed by the addition of one strip per trough. Hybridization was performed for 1 h at 50±0.5°C in a closed water bath with back-and-forth shaking. The strips were then washed twice with 1 ml of wash solution at room temperature for 20 s and once at 50°C for 30 min. Following this stringent washing, strips were rinsed twice with 1 ml of a standard rinse solution. Strips were then incubated on a rotating platform with an alkaline phosphatase-labeled streptavidin conjugate diluted in a standard conjugate solution, at 20 to 25°C for 30 min, after which strips were washed twice with 1 ml of rinse solution and once with standard substrate buffer; color development was initiated by the addition of 5-bromo-4-chloro-3-indolylphosphate and nitroblue tetrazolium to 1 ml of substrate buffer. After 30 min of incubation at room temperature, the color reaction was stopped by aspiration of the substrate buffer and addition of distilled water. After drying, the strips were visually interpreted using a grid.

Statistical analysis

Chi square and Fisher's exact Tests were used to analyze the data obtained by SPSS 11.5 software (SPSS Inc, Chicago; USA). The differences or association with $p<0.05$ were considered statistically significant.

Results

Of 108 samples 86 was female (78.8%), mean of age 30.44±11.2, the age range was 1-54 years old and 23 was Male (21.1%), mean of age 31.7±9.4, the age range was 3-42 years old (Figure 1). A total samples was enrolled to our study, which is divided in biopsy 15.5%, paraffin embedded block 2.7%, eye 0.9%, genital 71.6% and serum 8.2%. Twenty-two genotypes among samples were detected. In six samples HPV genotypes was undetectable.

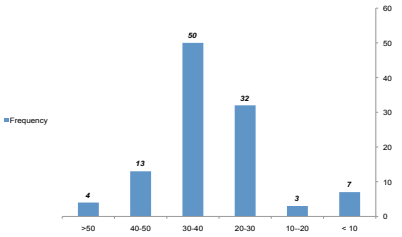

Figure 1. Frequency of Patients by Age Group

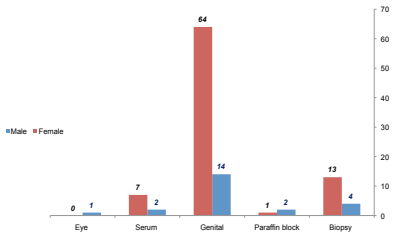

Figure 2. Frequency Source of Samples by Sex

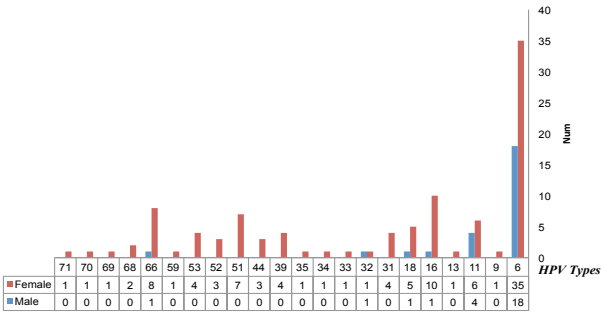

Figure 3. Frequency of HPV Types in Two Gender

The most common HPV types in this study were HPV genotype 6 that was in female 35 (32.4%) and male 18 (16.6%) that source of this samples was genital. According to our study results, of 108 patients who were positive in human papilloma viruses, based on gender, 82 cases (75.9%) female and 26 cases (24%) male were positive (Figure 2). From this 108 positive list, 53 cases (49%) were positive for HPV-6 and showed that 35 (32.4%) female and 18 (16.6%) male were positive. For studying about rare types such as HPV-9, 13, 33, 34, 35, 59, 69, 70, 71 just one time these types were positive and that in all of types were female (0.9%) and also from total 10 positive cases (9.2%) for HPV-11, we reported 6 (5.5%) female and 4 (3.7%) male. As we predicted, In HPV-16 we had total 11 (10.1%) positive with 10 (9.2%) female and 1 (0.9%) male, showing this type of HPV is prevalent in women. Interestingly, 6 cases were positive (5.5%) for HPV-18 and from which 5 (4.6%) female and 1 (0.9%) male were shown and also total 4 cases (3.7%) of HPV-31, 39, 53 were positive and all of them were female. Moreover, in HPV-32, just 2 positive (1.8%) detected and in which 1 (0.9%) female and 1 (0.9%) male shown and for HPV-44, 52 only 3 cases (2.7%) and for HPV-51 only 7 cases (6.4%) female were shown but no positive male detected. Eventually, from total 9 cases (8.3%) in HPV-66, female 8 (7.4%) and male 1 (0.9%) reported and for HPV-68 only 2 cases (1.8%) were positive and all were female. Generally, we were not able to detect HPV-9, 13, 33, 34, 35, 59, 69, 70, 71, 31, 39, 53, 44, 52, 51, 68 types in our male population (Figure 3).

Discussion

Despite the numerous methods for detecting HPV viral infection, we have been used INNO-Lippa in this study. The INNO-LiPA HPV genotyping assay is capable of detecting and genotyping 25 different HPV types at the same time and has proven to be sensitive, specific, simple, and rapid in the determination of HPV (Chinchai et al., 2011; Barbieri et al., 2012). Detection of HPV is dependent on the methods employed it cannot be cultured *in vitro* and serological assays lack sensitivity and specificity. Therefore detection is entirely based on molecular methods such as the INNO-LiPA HPV (Didelot-Rousseau et al., 2006).

In many developed countries and some developing countries many researches in this field have been done. In all these studies which have been done by different molecular techniques such as PCR and In situ hybridization been used, HPV as the main cause of cervical cancer is introduced. Papillomavirus (HPV) type 16 and type 18 in the first and type 31, 33 and 45 in the second position are observed in cancer samples. Some types, like 51, 52 and 54 in some areas are identified as the cause of cervical cancer. (2, 3, 4, 5, 9, 17, 18, 28, ... and 116) (Galan-Sanchez et al., 2011; Micalessi et al., 2013).

In a research on samples of cervical cancer have been conducted in Croatia, in 61.1% of samples HPV DNA has been detected and the most common types in this study were HPV-16, HPV-18, HPV-31 and HPV-33 respectively. The samples with Dysplasia, HPV-6 and HPV-11 have been identified. The few reported cases with more than one type of papillomavirus (HPV) are observed in this study (Grce et al., 2007). A case-control study in Philippines, from all samples, 93/8% of cervical cancers was HPV DNA positive while in the control group only 9.2% of the cases have HPV genome (Young et al., 2010). In Thailand, 100 samples with cervical cancer were analyzed by PCR and in 82% of cases HPV has been detected. In this study, type 16 (42%), type 18 (21%) and type 33 (4%) have been identified (Chinchai et al., 2011).

In a study on patients with invasive cervical cancer which have been conducted in Russia, HPV DNA has been detected in all cases. The prevalence of different HPV types were HPV-16 (64/8%), HPV-18 (10/7%) and HPV-45 (8/2%) respectively. However 3/8% of cases has not been determined by this method (Samoylova et al., 1995). In 2012 another study was done in Iran and cervical specimens were obtained from women aged 18-59 years and from locally diagnosed invasive cervical cancers (ICC). HPV was detected and genotyped using PCR-based assay. HPV prevalence in the general population was 7.8% (95% confidence interval: 6.0-9.8) (5.1% of high-risk types), with no significant variation by age. HPV16/18 accounted for 30 and 82.2% of HPV-positive women in the general population and ICC, respectively (Zandi et al., 2010).

In an investigation in IRAN in 2012, the prevalence of HPV DNA Infection in Patients with Esophageal Squamous Cell Carcinoma from the Caspian Sea Area was 28.3% of upper, 29% of middle and 25.8% of lower third of ESCC samples were positive for HPV DNA. 13.6% for males and 14.1% for females were HPV positive in all samples. In other study on same samples, positive specimens were evaluated by Real-time PCR to determine HPV genotypes. From the 49 HPV positive cases, of ESCC patients, 5 (23.1%), 11 (55 %) and 9 (56.3%) of upper, middle and lower third of ESCC specimens, respectively were positive by at least one high and one low-risk HPV genotypes (Yahyapour et al., 2012). In the same study in Iran in 2012, the prevalence of high-risk human papillomavirus Types 16 and 18 in cervical samples from healthy women with normal Pap smears was tested by polymerase chain reaction. The prevalence of positive HPV findings was 5.5%; high-risk HPV human papillomavirus Type 16 prevalence was 2% and no HPV-18 was reported. The prevalence of HPV was 4.5% in younger age group and gradually increased to 20% in next decade (Zandi et al., 2010; Shahramian et al., 2011). For HPV genotyping in Iran in 2011, patients with cervical neoplastic and invasive carcinomas were examined by real-time PCR and subsequently PCR products were sequenced. The overall genotyping results of phylogenetic analysis and hybridization methods were as follows: HPV 16: 75%; HPV 18: 3%; HPV 31: 1%; HPV 45: 1%. High frequency of HPV 16 and low frequency of HPV 18 were found (Jaberipour et al., 2011). In our study of 410 patients that referred to Cancer center lab, 108 patients were positive for HPV DNA, this test was performed using Conventional PCR in center lab, that prevalence of HPV in Kerman province was 26.3%. in compared with other study in Iran, in a study in 2013, the prevalence of HPV infection was 76% in cervical cancer patients and 7% among healthy Iranian women. Of the HPV types isolated, HPV 16 (54%), 18 (14%), and 31 (6%) were the most commonly detected in Iranian cervical cancer patients. In an investigation in Shiraz in 2010, the prevalence of positive HPV findings was 5.5%; high-risk HPV Type 16 prevalence was 2% and no patient harbored HPV-18. The prevalence of HPV was 4.5% in younger age group and gradually increased to 20% in the 4th decade (Safaei et al., 2010). According to our study results, of 108 patients who were positive in human papilloma viruses, based on gender, 82 cases (75.9%) female and 26 cases (24%) male were positive. From this 108 positive list, 53 cases (49%) were positive for HPV-6 and showed that 35 (32.4%) female and 18 (16.6%) male were positive. The most common HPV types in this study were HPV genotype 6 that was in female 35 (32.4%) and male 18 (16.6%) that source of this samples was genital. We recommended the researchers do further studies on the prevalence of HPV infection in Kerman and to inform people of different ways to expand it. In addition to periodic examinations and Pap smears for women to be given the necessary recommendations.

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References

Aljunid S, Zafar A, Saperi S, et al (2010). Burden of disease associated with cervical cancer in malaysia and potential costs and consequences of HPV vaccination. *Asian Pac J Cancer Prev*, **11**, 1551-9.

Alsaad M, Shamsuddin K, Fadzil F (2012). Knowledge towards HPV infection and HPV vaccines among Syrian mothers. *Asian Pac J Cancer Prev*, **13**, 879-83.

Babiker A, Eltom F, Abdalaziz A, et al (2013). Screening for high risk human papilloma virus subtypes, among Sudanese patients with oral lesions. *Int J Clin Exp Med*, **6**, 275-81.

Barbieri D, Nocera M, Gallinella G, et al (2012). Comparison of HPV sign genotyping test with INNO-LiPA HPV genotyping extra assay on histologic and cytologic cervical specimens. *Diagn Microbiol Infect Dis*, **74**, 43-8.

Best S, Niparko K, Pai S (2012). Biology of human papillomavirus infection and immune therapy for HPV-related head and neck cancers. *Otolaryngol Clin North Am*, **45**, 807-22.

Chimeddorj B, Pak C, Damdin A, et al (2008). Distribution of HPV-16 intratypic variants among women with cervical intraepithelial neoplasia and invasive cervical cancer in Mongolia. *Asian Pac J Cancer Prev*, **9**, 563-8.

Chinchai T, Chansaenroj J, Junyangdikul P, et al (2011). Comparison between direct sequencing and INNO-LiPA methods for HPV detection and genotyping in Thai Women. *Asian Pac J Cancer Prev*, **12**, 989-94.

Ciarrocca K, Jackson L, Rossi S (2013). Human papillomavirus: the fundamentals of HPV for oral health care providers. *J Calif Dent Assoc*, **41**, 349-55.

Diamantopoulou S, Spathis A, Chranioti A, et al (2013). Liquid based cytology and HPV DNA testing in a Greek population compared to colposcopy and histology. *Clin Exp Obstet Gynecol*, **40**, 131-6.

Didelot-Rousseau M, Courgnaud V, Nagot N, et al (2006). Comparison of INNO-LiPA HPV genotyping v2 with PCR product subcloning and sequencing for identification of genital human papillomavirus genotypes in African women. *J Virol Methods*, **135**, 181-5.

Galan-Sanchez F, Hernandez M, Hernandez D, et al (2011). Performance of the new INNO-LiPA HPV extra to genotype human papillomavirus in cervical cell specimens. *Acta Cytol*, **55**, 341-3.

Gold M, Thomas M, Huh W, et al (2013). High-risk human papillomavirus detection in women with low-grade squamous intraepithelial lesions or higher-grade cytology using the Cervista HPV HR test. *J Low Genit Tract Dis*, **17**, 51-7.

Grace J, Narendhirakannan R (2011). Detection and genotyping of high-risk HPV and evaluation of anti-oxidant status in cervical carcinoma patients in Tamil Nadu State, India. *Asian Pac J Cancer Prev*, **12**, 2689-95.

Grce M, Grahovac B, Rukavina T, et al (2007). HPV testing for cervical cancer screening in Croatia. *Coll Antropol*, **31**, 67-71.

Huang Y, Lin M, Luo Z, et al (2013). Low prevalence of HPV in male sexual partners of HR-HPV infected females and low concordance of viral types in couples in Eastern Guangdong. *Asian Pac J Cancer Prev*, **14**, 1755-60.

Jaberipour M, Samsami A, Sahraiian F, et al (2011). Elevation of HPV-18 and HPV-16 DNA in the plasma of patients with advanced cervical cancer. *Asian Pac J Cancer Prev*, **12**, 163-7.

Karimi M, Behtash N, Chiti Z, et al (2009). Cervical cancer and HPV vaccines in developing countries. *Asian Pac J Cancer Prev*, **10**, 969-74.

Micalessi M, Boulet G, Vorsters A, et al (2013). A real-time PCR approach based on SPF10 primers and the INNO-LiPA HPV genotyping extra assay for the detection and typing of human papillomavirus. *J Virol Methods*, **187**, 166-71.

Mohabatkar H (2007). Prediction of epitopes and structural properties of Iranian HPV-16 E6 by bioinformatics methods. *Asian Pac J Cancer Prev*, **8**, 602-6.

Nirchio V, Lipsi R, Fusilli S, et al (2008). HPV infection: comparison between morphological studies and molecular biology. *Pathologica*, **100**, 149-55.

Norman I, Hjerpe A, Andersson S (2013). High-risk HPV L1 capsid protein as a marker of cervical intraepithelial neoplasia in high-risk HPV positive women with minor cytological abnormalities. *Oncol Rep*, **30**, 695-700.

Palefsky J (2006). Biology of HPV in HIV infection. *Adv Dent Res*, **19**, 99-105.

Safaei A, Khanlari M, Momtahen M, et al (2010). Prevalence of high risk human papillomavirus types 16 and 18 in healthy women with cytologically negative pap smear in Iran. *Indian J Pathol Microbiol*, **53**, 681-5.

Samoylova E , Shaikhaiev G, Petrov S, et al (1995). HPV infection in cervical-cancer cases in Russia. *Int J Cancer*, **61**, 337-41.

Shahramian I, Heidari Z, Mahmoudzadeh H, et al (2011). Prevalence of HPV infection and high risk HPV genotypes (16, 18) among monogamous and polygamous women, In Zabol, Iran. *Iran J Public Health*, **40**, 113-21.

Yahyapour Y, Shamsi-Shahrabadi M, Mahmoudi M, et al (2012). High risk and low risk human papillomavirus in esophageal squamous cell carcinoma at Mazandaran northern Iran. *Pathol Oncol Res*, **21**, 54-8.

Young A, Crosby R, Jagger K, et al (2010). HPV vaccine acceptability among women in the philippines. *Asian Pac J Cancer Prev*, **11**, 1781-7.

Zandi K, Eghbali S, Hamkar R, et al (2010). Prevalence of various human papillomavirus genotypes among women who subjected to routine Pap smear test in Bushehr city. *Virol J*, **7**, 65.