RESEARCH COMMUNICATION

Detection of High-risk Human Papillomavirus Types 16 and 18 but not 33 and 52 in External Genital Warts from Iranian Females

Mansooreh Jaberipour[1], Mozhdeh Momtahan[2], Fatemehsadat Najib[2], Sedigheh Amooei[2], Farzaneh Saidifard[2], Abbas Ghaderi[1,3], Mojtaba Habibagahi[3]

Abstract

Background: External genital warts (EGW) are relatively common sexually transmitted diseases. In the majority of cases, low-risk human papilomaviruses (HPV), such as HPV-6 and HPV-11, are responsible but, high-risk types may also be detected and this has a bearing on vaccines for cervical cancer prevention. In this study the incidence of the high-risk HPV types 16, 18, 33 and 52 in EGWs of females from the southwest of Iran was assessed. Methods: Seventy-nine women with EGWs participated in this study. Quantitative real-time PCR with gene specific primers and probes for the E6 gene of HPV-16, 18, 33 and 52, were used for the detection of HPV DNA in the tissue and blood samples. Results: Of the 79 tissue specimens, 13 (16.5%) were HPV positive, only genetic materials of HPV-16 and HPV-18 being detected, twelve patients (15.2%) were positive only for HPV-18 and the coexistence of HPV-16 and HPV-18 was shown in one patient. Only one plasma sample showed evidence of HPV-16 with very low viral load. Conclusion: Our data showed that high-risk HPV types can be found in the tissue specimens of EGW samples obtained from female patients in the Southwest of Iran, with HPV-18 as the most abundant type; however, additional studies with a larger population are required to prove the finding and help to determine the most appropriate type of virus for vaccine design for Iranian women.

Keywords: High-risk HPV - HPV-16 - HPV-18 - external genital warts - Iran

Asian Pacific J Cancer Prev, **12**, 771-774

Introduction

External genital warts (EGW), acuminata condylomata (AC), is a common sexually transmitted viral disease which usually occur among young people (Winer et al., 2005). In 1981, the presence of human papilomaviruses (HPV) in AC was shown for the first time (de Villiers et al., 1981). Generally, HPV is classified into high-risk and low-risk types based on their involvement in the infection of the genital area (Munoz et al., 2006). HPV types such as 16, 18, 33, 52 and 55 are mostly considered as the high-risk subtypes whereas HPV-6 and 11 are the low risk ones. The initial findings of HPV in the warts lesions have been proved by several studies. Potocnik et al. (2007) detected HPV in all 55 genital warts (GW) specimens obtained from male patients in Slovenia.

The study by Brown et al. (1999) also showed the presence of 23 different genotypes of HPV in the GWs samples. In addition to low-risk HPV-6 and HPV-11, which are associated with almost 90% of the cases, high-risk HPV types were also detected in some GW specimens (Dianzani et al., 2004; Insinga et al., 2007). Patients infected with high-risk HPV could be at the risk of developing malignant lesions. The high-risk HPV subtypes were found in various proportions of the lesions from the cases with GW around the world. HPV-16, HPV-52 and HPV-55 were the types most commonly found in the conjunction with low-risk HPV-6 and HPV-11 in GW in Austria, while the high-risk HPV types 16, 18, 58, 52, 33 and 53 were the main subtypes detected in the specimens from other places such as China and Ireland (Dai et al., 2008; Garland et al., 2009; Menton et al., 2009). The multicenter study on HPV genotype distribution among patients with GW in France also showed the presence of high-risk subtypes HPV-16, 18 and 52 with different rates of prevalence (Che et al., 2005; Aubin et al., 2008).

There is limited knowledge about the prevalence of high-risk HPV subtypes in the genital warts from Iranian patients and no published data show such information. Therefore, the objective of this study was to assess the presence and quantify of the high-risk HPV subtypes 16, 18, 33 and 52 in the tissue lesions and blood samples of the EGWs of females from the southwest of Iran by quantitative real-time PCR.

*[1]Shiraz Institute for Cancer Research, [2]Department of Gynecology and Obstetrics, [3]Department of Immunology, Shiraz University of Medical Sciences, Shiraz, Iran *For correspondence: agahim@sums.ac.ir*

Materials and Methods

Patients

Seventy-nine women with external genital warts participated in this study. Tissue biopsy samples from warts lesions and samples from peripheral blood were taken at the Gynecology and Oncology Clinic of Shiraz University of Medical Sciences, Shiraz, Iran in 2010. All the patients provided their informed consent to take part in the study and the study was approved by the Ethics Committee of the University. The participants completed a self-administered questionnaire that included questions on age, economical status, education level, alcohol consumption, smoking habit, age at the first sexual intercourse, lifetime number of sex partners, frequency of sexual intercourse, and condom use. Patients with immunocompromised illnesses or those under the treatment of immune suppressive drugs were excluded from the study.

Tissue processing for viral DNA extraction

The EGW tissue specimens measuring about 4 mm in diameter were taken from all the participants by a surgeon. The biopsy samples were stored at -80°C until the time of DNA extraction. Viral DNA was extracted, using RNA/DNA extraction Kit according to the manufacturer's instruction (Invitek, Germany). After extraction, the DNA was eluted in 50 μl distilled water and stored at -20ºC.

Preparation of DNA from plasma samples

EDTA-anticoagulant blood samples were collected at the time of the interview by venipuncture from the patients. After blood clotting, the blood samples were centrifuged at 750Xg for 10 min, and the plasma fractions were stored at -20°C for the next step. DNA from the plasma samples was extracted, using the QIAmp Ultra Sense Virus Kit according to the manufacturer's instruction (Qiagen, Hilden, Germany).

HPV quantitative real-time PCR

Commercial kits for quantification of human Papillomavirus genomes (Advanced kit version, Primer Design, Southampton, UK), with specific primers and probes for the E6 gene of HPV-16, 18, 33 and 52, were used according to the manufacturer's protocol. The kits were designed to detect 6-12 strains of each HPV subtype. Real-time PCR reactions were set up in a reaction volume of 20 μl, using the TaqMan Universal PCR Master Mix (ABI, Perkin Elmer, USA). DNA amplifications were carried out in a Chromo4 Real-time PCR Detector instrument (Bio-Rad, Foster city, CA, USA). Amplification reactions were done in triplicates for each subtype and each experiment had its own standard curve. The standard curves were also run in parallel with every analysis using positive control template, according to the manufacturer's guidelines. The concentrations of the HPV DNA in the samples were expressed as copies of HPV genome per milliliter of the extracted DNA sample and were calculated from the regression equation using the individual Ct (threshold cycle number) values.

Statistical analysis

The results are shown as median of the three replicate amplifications. Differences in the values were evaluated using Mann–Whitney U test and the student t-test. P-values less than 0.05 were considered as statistically significant in all the analyses. Statistical analysis was performed using the Statistical Package for the Social Sciences Software, version 11.5 (SPSS, Chicago, IL, USA). Graphical presentation was plotted and analyzed, using the Prism 4 software (Graphpad Inc; San Diego CA, USA).

Results

Clinical findings

In this study, we assessed the presence of four subtypes of the high-risk HPV in the total of seventy nine pair samples of the tissue biopsies and peripheral blood from the patients with EGW. The mean age of the participants was 28 years (range between 16 to 51 years); 83% of them were under 30 years of age. The majority of the enrolled women in the study had low education background. Most of the patients (82%) were examined by a gynecologist and donated their sample during the first 6 months after the appearance of the EGW lesions. Analysis of the data showed that six percent of the patients had infected partner, 19% were multi-partner (the median lifetime number of sex partners was 1), 8% divorced and only 17% used condom. Only 4% of the participants were pregnant at the time of the study.

Prevalence of the high-risk HPV subtypes

Commercial quantitative real-time PCR kits with primers and probes specific to the HPV E6 gene of types 16, 18, 33 and 52 were used to detect the HPV DNA in the tissue and plasma samples. Based on the results, only genetic materials of HPV-16 and HPV-18 were found in the tissue sample of the patients, while genetic materials of other studied subtypes were not detectable in the tissue samples. Table-1 lists the corresponding data regarding the HPV infection among the patients. From 79 tissue specimens, 15.2% of the samples were positive only for HPV-18 while HPV-16 was detected only in one case (1.3%) of mixed infection with HPV-18. The median of HPV-18 virus particles (vp) in the unit of the volume of

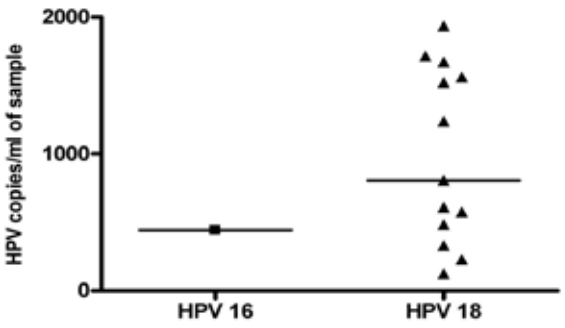

Figure 1. Comparison of HPV-18 and HPV-16 DNA Copy Numbers. Determined by quantitative real-time PCR using specific primers and probes to E6 gene of each type.

the extracted DNA, proportional to the viral DNA copy number, was 810 vp/ml. The viral load of HPV-16 in the tissue sample from only one infected tissue sample was 443 vp/ml while the HPV-18 viral load in the same sample was 611 vp/ml (Figure 1). This patient with double infection of HPV-16 and HPV-18 was one of the pregnant women participating in the study while the tissue sample from another pregnant patient contained only HPV-18 and the sample from the third pregnant patient was negative for the tested HPV subtypes.

Only one plasma sample (1.3%) was HPV-16 positive with very low virus particle (9 Vp/ml). However, neither of tested HPV subtypes could be detected in her GW tissue sample. The Pap test for this case showed abnormal result. Out of 13 patients who were positive for the tested high-risk HPVs, the majority (85%) were in the age group of 25-35 years. No significant association was observed between the detected HPV or the viral load and the variables such as education level, smoking habit, lifetime number of partners and using condom.

Discussion

HPV-6 and HPV-11 have been detected in almost 90% of the cases with external genital warts; however, growing amounts of results provide evidence that high-risk subtypes of the HPV such as HPV-16, 18, 33 and 52 can also be associated with EGW (Menton et al.2009). In this study, 79 women from the southwest of Iran were tested for the presence of genetic materials of some high-risk HPV (16, 18, 33 and 52) by a sensitive quantitative real-time PCR assay. We found different loads of HPV-18 in almost 15% of the EGW specimens (N=12) whilst only one sample was positive for HPV-16 (in addition to the co-infection with HPV-18). Other tested HPV subtypes could not be detected in this study. Despite such findings, the review of literature shows that HPV-16 is the most frequent HPV subtype in the majority of the infected women worldwide and is about 2–3 times more common than other high-risk subtypes in many studies (Clifford et al., 2005; de et al., 2007). In the study by Garland et al., the prevalence of fourteen HPV genotypes in the biopsy-confirmed GW samples was investigated. In that study, 31% of the lesions were infected with the high-risk subtypes, most commonly HPV-16, HPV-52 and HPV-55 (Garland et al., 2009). Beside this, some other recent studies in Hong Kong, China and Taiwan show the widespread presence of the subtypes 18, 58, 52, and 33 in conjunction with HPV-16 in those regions (Chan et al., 2006; Wu et al., 2007; Smith et al., 2007; Lin et al., 2006). Likewise, using real-time PCR, Che et al. showed the presence of both HPV-16 and HPV-18 in 11.3% of the patients in China (Aubin et al., 2008).

Similarly, the report from multicenter studies in France shows the existence of high-risk HPV 16, 18 and 52 in 9%, 3% and 7%, of cases of GW, respectively (Menton et al., 2009). Also, Wong et al. showed that HPV DNA can be detected in the menstrual blood from patients with condyloma acuminatum; however, it disappears after the remission (Wong et al., 2010).

There are only few published reports regarding the HPV subtypes in the genital warts from Iranian patients. However, some studies have investigated the type of the virus in the samples from patients with cervical cancer. Such results show that in most regions of Iran, HPV-16 is the foremost subtype (Hamkar et al., 2002; Ghaffari et al., 2006a; Esmaeili et al., 2008). In the study by Ghaffari et al.,HPV-16 was found to be significantly more frequent in patients with cervical cancer than normal group (Ghaffari et al., 2006). In their report, however, they showed the high prevalence of HPV-18 in the patients compared to the healthy individuals. The difference in the result of these studies and our finding could be due to the type of patients and sample size, regional variations and the method of detection. Consistent with the findings for genital warts, we also found that HPV-18 is more prevalent than other types in blood samples from patients with cervical cancer (Jaberipour et al., 2011). Studies demonstrate that HPV DNA could be detected in pregnant women in higher rates than un-pregnant women (Medeiros et al., 2005; Nobbenhuis et al., 2002). In the current study, three pregnant women were examined, two of whom revealed to be positive for the presence of the high-risk HPV subtypes. Similarly, Aydin et al. showed the higher rates of the infection with the high-risk HPV genotypes in the Turkish pregnant women compared to the non-pregnant ones (Aydin et al., 2010). It seems that the hormonal and immunological changes during the pregnancy could modulate the rate of the HPV infection and clearance in women (Sethi et al., 1998).

We found that the majority of HPV-18 positive patients were in the group of 25-35 years of age. In the report by Chan et al., the prevalence of HPV-18 also peaked at the ages older than 26 years (Chan et al., 2009). Such findings might point to the specific pattern of age specific prevalence for the HPV infection.

In our study, patients' plasma samples were also examined for the presence of HPV genome; however, only one sample showed positive for the presence of HPV-16 with very low viral burden. Neither of the HPV subtypes was detectable in her EGW tissue sample.

Many studies reported that high-risk HPV might be co-infected with other HPV subtypes in the genital warts (Dai et al., 2008; Menton et al., 2009; Aubin et al., 2008). Of a selected panel of HPV subtypes (16, 18, 33 and 52) that were examined in this study, however, only one patient had simultaneous infection of HPV-16 and HPV-18. We did not investigate the presence of other high risk subtypes; therefore, it might be possible that HPV-infected patients have co-infection with other subtypes. The presence of the two main low-risk genotypes (HPV-6 and HPV-11) was not investigated either. Based on such results, we cannot suggest that high-risk HPV could act as the causative infection of GW in these patients although they may assist further pathogenesis towards malignancies.

In conclusion, our data showed that high-risk HPV can be found in the tissue specimens of the external genital warts from female patients in the southwest of Iran. We also found that HPV-18 was the most frequent HPV infection in this group; however, we suggest that larger population from multiple centers is required to prove such findings and to determine the most appropriate viral subtype for

the HPV vaccine in Iranian women.

Acknowledgements

This work was funded by grants from Shiraz University of Medical Sciences (No. 4565 and No. 4965), Iranian Cancer Network, and Shiraz Institute for Cancer Research (No. ICR-87-505). We are also grateful of those women who participated in this project. Authors of the manuscript declare that they have no conflict of interest.

References

Aubin F, Pretet JL, Jacquard AC, et al (2008). Human papillomavirus genotype distribution in external acuminata condylomata: a Large French National Study (EDiTH IV). *Clin Infect Dis*, **47**, 610-5.

Aydin Y, Atis A, Tutuman T, Goker N (2010). Prevalence of human papilloma virus infection in pregnant Turkish women compared with non-pregnant women. *Eur J Gynaecol Oncol*, **31**, 72-4.

Brown DR, Schroeder JM, Bryan JT, Stoler MH, Fife KH (1999). Detection of multiple human papillomavirus types in Condylomata acuminata lesions from otherwise healthy and immunosuppressed patients. *J Clin Microbiol*, **37**, 3316-22.

Chan PK, Cheung TH, Tam AO, et al (2006). Biases in human papillomavirus genotype prevalence assessment associated with commonly used consensus primers. *Int J Cancer*, **118**, 243-5.

Chan PK, Ho WC, Wong MC, et al (2009). Epidemiologic risk profile of infection with different groups of human papillomaviruses. *J Med Virol*, **81**, 1635-44.

Che YM, Wang JB, Liu YH (2005). Correlation between deoxyribonucleic acid loads of human papillomavirus and recurrence of condylomata acuminata. *Int J STD AIDS*, 16, 605-7.

Clifford GM, Gallus S, Herrero R, et al (2005). Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *Lancet*, **366**, 991-8.

Dai Y, Huang YS, Tang M, et al (2008). Distribution and clinical significance of human papillomavirus subtypes in Shenzhen city, People's Republic of China. Int. J. Gynecol. Cancer, 18, 295-299.

de Villiers EM, Gissmann L, zur HH (1981). Molecular cloning of viral DNA from human genital warts. *J Virol*, **40**, 932-5.

de Sanjosé S, Diaz M, Castellsague X, et al (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis*, **7**, 453-9.

Dianzani C, Calvieri S, Pierangeli A, Degener AM (2004). Identification of human papilloma viruses in male dysplastic genital lesions. *New Microbiol*, **27**, 65-9.

Esmaeili M, Bonyadi M, Dastranj A, et al (2008). HPV typing in women with cervical precancerous and cancerous lesions in northwestern Iran. *Gynecol Obstet Invest*, **66**, 68-72.

Garland SM, Steben M, Sings HL, et al (2009). Natural history of genital warts: analysis of the placebo arm of 2 randomized phase III trials of a quadrivalent human papillomavirus (types 6, 11, 16, and 18) vaccine. *J Infect Dis*, **199**, 805-14.

Ghaffari SR, Sabokbar T, Mollahajian H, et al (2006). Prevalence of human papillomavirus genotypes in women with normal and abnormal cervical cytology in Iran. *Asian Pac J Cancer Prev*, **7**, 529-32.

Hamkar R, Azad TM, Mahmoodi M, et al (2002). Prevalence of human papillomavirus in Mazandaran Province, Islamic Republic of Iran. *East Mediterr Health J*, **8**, 805-811.

Insinga RP, Dasbach EJ, Elbasha EH, Liaw KL, BarrE (2007). Incidence and duration of cervical human papillomavirus 6, 11, 16, and 18 infections in young women: an evaluation from multiple analytic perspectives. *Cancer Epidemiol Biomarkers Prev*, **16**, 709-15.

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.

Lin H, Ma YY, Moh JS, et al (2006). High prevalence of genital human papillomavirus type 52 and 58 infection in women attending gynecologic practitioners in South Taiwan. *Gynecol Oncol*, **101**, 40-5.

Medeiros LR, Ethur AB, Hilgert JB, et al (2005). Vertical transmission of the human papillomavirus: a systematic quantitative review. *Cad Saude Publica*, **21**, 1006-1015.

Menton JF, Cremin SM, Canier L, Horgan M, Fanning LJ (2009). Molecular epidemiology of sexually transmitted human papillomavirus in a self referred group of women in Ireland. *Virol J*, **6**, 112.

Munoz N, Castellsague X, de Gonzalez AB, Gissmann L (2006). HPV in the etiology of human cancer. *Vaccine*, **24 Suppl 3**, S301-S10.

Nobbenhuis MA, Helmerhorst TJ, van den Brule AJ, et al (2002). High-risk human papillomavirus clearance in pregnant women: trends for lower clearance during pregnancy with a catch-up postpartum. *Br J Cancer*, **87**, 75-80.

Potocnik M, Kocjan BJ, Seme K, Poljak M (2007). Distribution of human papillomavirus (HPV) genotypes in genital warts from males in Slovenia. *Acta Dermatovenerol Alp Panonica. Adriat*, **16**, 91-6, 98.

Sethi S, Muller M, Schneider A, et al (1998). Serologic response to the E4, E6, and E7 proteins of human papillomavirus type 16 in pregnant women. *Am J Obstet Gynecol*, **178**, 360-4.

Smith JS, Lindsay L, Hoots B, et al (2007). Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. *Int J Cancer*, **121**, 621-32.

Winer RL, Kiviat NB, Hughes JP, et al (2005). Development and duration of human papillomavirus lesions, after initial infection. *J Infect Dis*, **191**, 731-8.

Wong SC, Au TC, Chan SC, et al (2010). Human papillomavirus DNA detection in menstrual blood from patients with cervical intraepithelial neoplasia and condyloma acuminatum. *J Clin Microbiol*, **48**, 709-13.

Wu RF, Dai M, Qiao YL, et al (2007). Human papillomavirus infection in women in Shenzhen City, People's Republic of China, a population typical of recent Chinese urbanisation. *Int J Cancer*, **121**, 1306-11.