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Prevalence of Human Papillomavirus Genotypes in Patients with Genital Warts in Gorgan, Iran

Majid Mehri¹, Saied Hosseinzadeh Kakroudi¹, Fatemeh Sana Askari², Alireza Mohebbi²,
Alijan Tabarraei^{*2,3} 

1. Department of Internal Medicine, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

2. Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

3. Infectious Disease Research Center, Golestan University of Medical Sciences, Gorgan, Iran

* **Correspondence:** Alijan Tabarraei, Department of Microbiology, School of Medicine, Golestan University of Medical Sciences, Gorgan, Iran

Tel: +989112733321

Email: Tabarraei@goums.ac.ir

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Abstract

Background and objectives: Low-risk and high-risk human papillomavirus (HPV) genotypes are the main cause of anogenital warts. The present study aimed to determine prevalence of HPV genotypes in patients with anogenital warts in Gorgan, northeast of Iran.

Methods: In this cross-sectional study, 40 biopsy samples were taken from patients with anogenital warts in Gorgan, Iran. After DNA extraction, multiplex polymerase chain reaction was carried out for detecting HPV genotypes 54, 18, 16 and 6. Demographic characteristics of subjects including gender, age, education level, marital status, smoking and method of contraception were also collected. Data were analyzed in SPSS 16 software at statistical significance of 0.05.

Results: The mean age of male and female patients was 31.81 ± 6.9 and 27.95 ± 6.92 years, respectively. The frequency of HPV-6, HPV-16 and HPV-54 was 77%, 15% and 7.5%, respectively. In addition, HPV-18 was not detected in the collected specimens. Co-infection of HPV-54 with HPV-6 and HPV-16 was also observed in some cases. No significant association was found between HPV infection and age, gender, smoking, contraceptive method and education level.

Conclusions: Similar to previous studies in Iran and other countries, HPV type 6 is the predominant cause of genital warts in Gorgan, Iran. Further studies with a larger study population are needed to explore the role of other contributors to HPV-induced genital warts.

Keywords: Genital wart; Condylomata acuminata; Human Papillomavirus

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INTRODUCTION

Human Papillomavirus (HPV) is the leading cause of cervical cancer, the fourth most common cancer among women worldwide (1).

It is also involved in progression of anogenital tumors and warts. The virus also has an established role in the etiology of oropharyngeal cancers (2). Until now, more than 200 HPV genotypes have been identified, while only one fifth of them are associated with anogenital infections. Genital HPV genotypes are divided into high-risk and low-risk groups based on the carcinogenicity potential (3,4). The high-risk HPV genotypes include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, while, HPV types 6, -11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 89 are considered low-risk genotypes (5-7). HPV-16, -18 and -51 are the predominant oncogenic genotypes involved in the development of the majority of cervical cancer cases (8,9). Almost 4.5% of all cases of head and neck cancer as well as anogenital cancers are caused by HPV (10). Moreover, high-risk HPV types 16 and 18 are the predominant genotypes in Iran with frequency of 77.5 % and 32.4% in cervical cancer and head and neck cancer cases, respectively (11). Low-risk HPV types are commonly associated with benign anogenital warts (12). Although most genital warts arise from infections caused by HPV types 6 or 11, high-risk genotypes have been also isolated from patients with genital warts (13,14). Alongside HPV-11 and -6 genotypes, HPV-54 is also isolated from clinical samples of anogenital warts (15,16). Incidence of HPV genotypes (77.5%) in Iran is almost identical to other parts of the world (11). Low-risk HPV genotypes, including types 6, 11 and 54 are more prevalent among Iranian men (17). A similar distribution of HPV genotypes 18, 16, 11 and 6 exists among Iranian women (18,19).

Therapeutic approaches for the treatment of genital warts include podophyllin, trichloroacetic acid, cryotherapy, electrocautery, imiquimod, carbon dioxide

laser and surgery. Despite treatment, recurrence rate of genital warts ranges between 40% and 90% (13). In this study, we investigate the frequency of HPV infection in patients with genital warts in Gorgan, North of Iran.

MATERIALS AND METHODS

Patient recruitment and sample collection From February 2018 to July 2018, this cross-sectional study was carried out on anogenital wart samples. The study was approved (approval code of IR.GOUMS.REC.1396.120) by the Ethical Committee of the Golestan University of Medical Sciences, Gorgan, Iran. A written informed consent was obtained from all subjects. The samples were collected from 40 patients (21 men and 29 women) with mean age of 29.98 ± 7.084 years (age range: 18 to 48 years) who voluntarily participated in the study. Initial diagnosis of genital warts was made by a dermatologist. One representative biopsy sample was collected from each patient. Demographic characteristics including marital status, smoking, contraceptive method, educational level and medical history were collected.

DNA extraction and HPV genotyping

A Genomic DNA extraction kit (Macherey-Nagel, Germany) was used For DNA extraction from sections of anogenital arts. A multiplex polymerase chain reaction (PCR) experiment was designed to investigate presence of low-risk (types 6 and 54) and high-risk (types 16 and 18) HPV genotypes. HPV types 16 and 18 are the predominant high-risk genotypes in Iran, while HPV-6 is the most common low-risk HPV genotype in anogenital specimens. However, the prevalence of the low-risk HPV genotype 54 is not clear. Four primer pairs encompassing the HPV L1 coding sequence were designed (Table 1). PCR was performed as follows: initial denaturation at

94 °C for 5 minutes, followed by 35 cycles at 94 °C for 30 seconds, at 58 °C for 30 seconds and at 72 °C for 40 seconds. PCR products were electrophoresed on 1% agarose gel. A HPV-positive specimen and a specimen-free sample were used as the positive and negative controls, respectively.

Statistical analysis

Descriptive analysis was performed using SPSS 16.0 software package. Cross-tabulation and Chi-square test were performed to evaluate association of HPV infection with the demographical factors. Results are reported as frequency percentage among patients with HPV-positive genital warts. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of men and women was 31.81 ± 6.9 and 27.95 ± 6.92 years, respectively. The frequency of HPV genotypes among patients with HPV-positive genital warts is shown in table 2. The highest and lowest genotype frequency was related to HPV-6 (77.5%) and HPV-54 (7.5%), respectively. In addition, HPV-18 was not detected in any of the samples. Co-infection with two or three HPV genotypes was also observed. Accordingly, co-infection of HPV-6 with HPV-54 and HPV-16 with HPV-54 was detected in two patients. In addition, a co-infection of HPV-6, HPV-16 and HPV-54 was observed in one married woman. As shown in table 2, HPV-6 was more prevalent in men than in women ($P > 0.05$). On the contrary, HPV-16 was only detected among women. The prevalence of HPV-16 was 60.7% among patients aged 20 to 30 years. Overall, 29 patients were married and 13 patients were cigarette smokers. Of 40 patients, five had primary education, 15 had high school diploma, 18 had bachelor's degree and four had post-graduate education. Two patients had hypertension and high blood low-

density lipoprotein level. Condom use ($n=38$) was the preferred contraceptive method, followed by tubectomy ($n=1$) and contraceptive pills ($n=1$). There was no significant relationship between HPV infection and demographic characteristics.

DISCUSSION

Genital warts are benign epithelial cell growths caused by sexually transmitted HPV infection (21,22). Genotyping of genital HPV is of great clinical significance in terms of developing treatment plans as well as follow-up and prevention strategies (23). Low-risk HPVs are frequently associated with anogenital warts or condylomata acuminata. Based on test sensitivity, HPV-6 can be detected in more than 90% of clinical samples of genital warts (24). In this study, 77.5% of HPV-positive samples were infected with HPV-6. In a previous study on 100 tissue specimens from women with HPV genital warts in Iran, HPV types 6 and 11 were found in 49% and 67% of patients, respectively. The mentioned study also found no significant association between marriage and HPV-11 (14). These reports demonstrate the high prevalence of low risk HPV types 6 and 11 in anogenital warts. In recent reports, 20–50% of patients with genital warts harbored high-risk HPV genotypes. However, we found no HPV-18 and seven (15%) HPV-16-positive samples among patients with anogenital warts. Following low-risk HPV types 6 and 11, HPV-16 is reported to be the most frequent HPV type isolated from anogenital warts. Consistent with our results, Ma et al. found HPV-16 in 10.6% of patients with genital warts (25). Similar to our results, Jalilian et al. found a high frequency of HPV-6 (64.8%) and HPV-16 (9.2%) in patients with genital warts in West of Iran (18). In the present study, co-infection with HPV types 6, 16 and 54 was observed in some cases. Another study also reported a high rate of HPV-6 and -11 co-

infection in genital warts (17). HPV-54 is mainly isolated from genital tumors (26). Similarly, the prevalence of HPV-18 is not high in anogenital warts. In this regard, Ozaydin-Yavuz et al. detected one (1.5%) case of HPV-18 infection among 66 patients with genital warts (13). Furthermore, Park et al. observed co-infection with HPV-18 in three cases (27). This could be due to the fact that HPV-18 poses a greater risk for development of glandular lesions rather than squamous lesions (28). Several contributing risk factors have been suggested for anogenital HPV infections.

Based on our results, young adults (20-30 years old) were at a higher risk of HPV infection. Boda et al. also reported a higher incidence rate for high-risk HPV infection in patients under 35 years of age (29). According to Sohrabi et al., the frequency of HPV genotypes in cervical lesions and genital warts was higher among patients aged 30-39 years (20). In a study by Chen et al., a large proportion of low-risk HPV genotypes was detected in patients under 20 years of age (30).

We found no association between HPV infection and gender, smoking, condom use or education level. Further studies with a larger study population could help clarify the role of environmental factors in the incidence of HPV infection. In the present study, HPV-6 was more prevalent in men than in women. In a study on 66 patients with anogenital warts, Ozaydin-Yavuz et al. found a relationship between HPV type distribution and age, gender, place of residence and number of sexual partners. Similar to our findings, they detected a higher frequency of HPV genotypes in men with genital warts (13). Another study in Iran also reported a higher frequency of HPV-6 in HPV-positive genital specimen from men (17).

However, geographical factors can affect the distributions of HPV infection in men and women.

CONCLUSION

Based on the results, HPV-6 is the predominant cause of anogenital warts in Gorgan, northeastern Iran. In addition, the rate of HPV infection was slightly higher in patients under 30 years of age. Further studies with a larger study population are needed to explore the role of other contributors to HPV-induced genital warts.

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DECLARATIONS

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Ethics approvals and consent to participate
The study was approved (approval code of IR.GOUMS.REC.1396.120) by the Ethical Committee of the Golestan University of Medical Sciences, Gorgan, Iran. A written informed consent was obtained from all subjects

Conflict of interest

The author declares that there is no conflict of interest regarding publication of this article.

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Table1. Sequences of the primers used in the multiplex-PCR experiment

HPV genotypes	Primers (5' to 3')	Size (bp)
HPV-54	Forward: TTGCATCCACGCAGGATAGC	386
	Reverse: ACGTAGGCCAGCCTGTAGTA	
HPV-18	Forward: CGTGGTCAGCCTTTAGGTGT	95
	Reverse: GAAACATTAGACGTGGCGGC	
HPV-16	Forward: CGTGGTCAGCCATTAGGTGT	643
	Reverse: TGCCATTATTGTGGCCCTGT	
HPV-6	Forward: AAAGTTGTTGCCACGGATGC	204
	Reverse: AGACGAGTCAGGCAATGCAA	

Table2. Frequency distribution of HPV genotypes among patients with anogenital warts

HPV genotypes	Number (%) of positive patients		Total
	Female	Male	
HPV-54	2 (10.53%)	1 (4.76%)	3 (7.5%)
HPV-18	0 (0%)	0 (0%)	0 (0%)
HPV-16	6 (31.58%)	0 (0%)	6 (15%)
HPV-6	11(57.9%)	20 (95.24%)	31 (77.5%)
Total (%)	19 (47.5%)	21 (52.5%)	40 (100%)