

Genotype distribution of human papillomavirus in anogenital warts in Guilan province, northern Iran

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Background: Anogenital warts are the most prevalent sexually transmitted disease worldwide and are caused by human papillomavirus (HPV). There are more than 100 different types of HPV, of which certain types make the patient more susceptible to malignant neoplasms. This study aimed to determine the frequencies of specific types of HPV in anogenital warts.

Methods: This study included 157 patients diagnosed with anogenital warts that underwent HPV DNA detection using polymerase chain reaction (PCR) with INNO-LiPA genotyping assay.

Results: Of 157 patients with anogenital warts, 122 samples (77.7%) were positive for HPV, with some patients showing mixed HPV subtypes. HPV 6 (n = 99; 68.3%) and HPV 11 (n = 13; 8.9%) were the most prevalent types. 101 patients were male (64.3%). The mean age was 32.02 ± 10.06 years (range: 2 to 72 years). Most patients were infected with the low-risk types (80%); the high-risk HPV type 16 was significantly more common among women (P = 0.0001).

Conclusion: This study showed the higher relative frequency of HPV6 and HPV11 in anogenital warts in Guilan, northern Iran. The frequency of genital HPV infection appears to be higher among men, but the frequency of high-risk types seems to be higher among women.

Keywords: genital warts, human papillomavirus, neoplasms

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INTRODUCTION

Genital warts are a common phenotypic expression of human papillomaviruses (HPV), affecting 1% of the sexually active adults in the United States. With 79 million infected Americans (mostly young adults), HPV is the most common sexually transmitted infection in the United States and it is estimated that about 14 million people become newly infected each year ¹.

Anogenital warts can mimic many skin conditions such as vestibular papillomatosis, pearly penile papules, sebaceous glands of the prepuce and

labia major, molluscum contagiosum, condylomata lata, seborrheic keratoses, melanocytic nevus, Bowenoid papulosis, angiokeratoma, lymphangioma circumscriptum, and localized Darier's disease ²⁻⁴.

More than 150 different types of HPV have been sequenced, including approximately 40 types that can infect mucosal epithelium of the anogenital area and cause genital warts as well as benign or low-grade changes in the cervical cells. On the other hand, some HPV types can cause cervical, anal, and other genital cancers, which is why they are called high-risk types ⁵. The economic burden of HPV infection is very high worldwide; as an

example, the HPV-related annual medical cost burden was estimated to be \$8.0 billion in the US in 2012 ⁶.

Cervical cancer is the second most common cancer in women living in developing countries. As the development of invasive cervical cancer is a long progressive process, cancer screening and early detection of precancerous lesions can reduce morbidity and mortality among a large population. Many studies in different countries have demonstrated HPV types 16 and 18 as the most prevalent types associated with cervical dysplasia and invasive cervical and anal carcinoma. The HPV types detected in genital warts are more diverse ⁷⁻⁹.

Epidemiologic studies investigating the type-specific HPV infections among both men and women in different geographic regions and populations can play an important role in planning public health programs ¹⁰.

METHODS AND MATERIALS

This cross-sectional study was approved by the Research Committee of Guilan University of Medical Sciences (GUMS) with the registration code of 1904. Among the patients who were referred to pathology centers in the Guilan province between the years 2012 and 2015 due to anogenital lesions, 157 patients with anogenital warts were enrolled in the study. The inclusion criteria were all patients who underwent a punch or incisional biopsy for HPV DNA detection and genotyping polymerase chain reaction (PCR).

PCR is a biochemical technique that detects DNA sequences in a biological specimen by amplification of a DNA template to produce particular DNA fragments. The anatomical sites for sampling were the external genital area including the perianal and perineal sites. Patients for whom other methods (e.g., a vaginal smear) were used to evaluate the genotype of anogenital warts were excluded from the study.

Genomic DNA was extracted using the QIAmp Kit (Qiagen). Quantitative DNA was evaluated by spectrophotometry, while the quality of DNA was assessed via the β -globin PCR assay. HPV DNA was extracted from the specimen and reverse hybridization was used for HPV genotyping by the INNO-LIPA kit, which can determine and isolate 32 types of HPV ²¹.

Data about age, sex, and anatomical sites were recorded. HPV strains were categorized into two groups: a high-risk group including HPV 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, and 59, and a low risk group including HPV 6, 11, 40, 42, 43, 44, 53, and 54. Data were gathered and analyzed by SPSS version 20 and P-values below 0.05 were considered significant. The chi-squared test was used to find out the associations between qualitative variables and HPV infection.

RESULTS

A total of 157 patients with anogenital warts were evaluated, including 101 men (64.3%). The mean age of patients was 32.02 ± 10.06 years, ranging from 2 to 72 years. Overall, 122 samples (77.7%) were positive for HPV. There was no significant relationship between age and HPV infection, whereas the two genders differed in terms of the rate of HPV infection ($P = 0.0001$) (Table 1).

The majority of the specimens were from the genital region (86.06%), and the remaining were from the anal and perianal region (13.94%).

Among 122 HPV-positive samples, 145 HPV genotypes were detected, with some specimens showing more than one genotype. Among the detected HPV genotypes, HPV 6 ($n = 99$; 68.3%) and HPV 11 ($n = 13$; 8.9%) were the most prevalent types (Table 2). The frequency of HPV 6 was significantly higher in men ($n = 74$; 74.7%) than ($n = 25$; 25.3%) women ($P = 0.0001$). Also, HPV 16 was observed in 5 women and 1 man ($P = 0.022$).

According to the risk of oncogenicity, most HPV genotypes were in the low-risk group (83.4%), while only 16.6% were in the high-risk group.

Interestingly, HPV 16 was more frequently detected in women and HPV 6 was more prevalent

Table 1. Association between HPV infection of anogenital warts with age and sex

Variable	HPV infection		P-value
	Positive n (%)	Negative n (%)	
Age group (yrs)			
<30	62 (50.8)	17 (48.6)	NS †
30-40	42 (34.4)	12 (34.3)	
>40	18 (14.8)	6 (17.1)	
Sex			
Male	88 (72.1)	13 (37.1)	0.0001
Female	34 (27.9)	22 (62.9)	

† NS: not significant

Table 2. Frequency distribution of HPV genotypes in study specimens

HPV genotype	Number	Percent
6	99	68.3
11	13	9
16	6	4.1
31	3	2.1
44	3	2.1
52	3	2.1
40	3	2.1
45	2	1.4
51	2	1.4
66	2	1.4
68	2	1.4
Others	7	4.6

Table 3. Association between HPV genotypes' oncogenicity with age and sex

Variable	HPV genotypes' risk groups		P-value
	Low n (%)	High n (%)	
Age group (year)			
<30	57 (49.1)	12 (52.2)	NS †
30-40	41 (35.3)	9 (39.1)	
>40	18 (15.5)	2 (8.7)	
Sex			
Male	87 (75)	8 (34.8)	0.0001
Female	29 (25)	15 (65.2)	

† NS: not significant

among men. There was no other significant association between HPV genotype groups and sex or age (Table 3).

DISCUSSION

Recent epidemiological studies have shown a global increasing trend of sexually transmitted diseases including genital warts among young adults. HPVs are a large group of DNA viruses with a wide range of clinical features, ranging from latent and subclinical infections to clinically apparent disease as well as premalignant and malignant lesions. The frequencies of HPV genotypes vary by country, population, and even method of genotyping and sampling. Most HPV studies are based on cervical specimen due to the high importance of cervical cancer ^{11,12}. In order of prevalence, a significant proportion of anal, vaginal, penile, and vulvar cancer are associated with high-risk HPV types. High-risk HPVs can persist in the host cell and reactivate many years later.

All of these data highlight the importance of

disease prevention and early diagnosis of HPV infections.

One of the best strategies for the prevention of HPV infections is vaccination. However, as race and ethnicity play major roles in HPV infection, before the implementation of this strategy, epidemiological studies demonstrating the distribution pattern of this virus in different geographic areas seem necessary.

In this study, HPV DNA was identified in 77.7% of the samples. In previous studies of the Iranian population, HPV DNA was detected in between 49.5-92% of subjects with various sampling methods ^{10,12-13}. The wide range of detection rates could be related to the differences in the anatomic sites of the specimens, clinical or subclinical stages of the disease course, sampling methods, laboratory methods, as well as underlying diseases.

In our investigation, the predominant HPV genotypes were HPV 6 and HPV 11. As a result, the majority of subjects were infected with low-risk HPV, which is consistent with most of the previous research even though some studies illustrated different results ¹⁰⁻¹⁷.

This study showed that although the frequency of anogenital HPV infection appears to be higher among men, the frequency of HPV type 16 seems to be higher among women, making them more susceptible to cervical cancer. The higher frequency of HPV in men can be related to the earlier onset and higher years of sexual activity ¹⁸. It seems that in addition to behavioral differences, the immune response to HPV is different in men and women. For instance, viral clearance is faster in males (average 7.5 months) than females (90% clearance within two years) ¹⁸.

However, a study published in 2017 by the Centers for Disease Control and Prevention (CDC) reported that between 2013 and 2014, the prevalence of genital HPV including high-risk genital HPV infection was higher among American men ¹⁹. Cultural and host factors such as immune responses and sex hormones may contribute to the difference in the gender-specific epidemiology of HPV infection.

Some studies suggest that the prevalence of high-risk HPV infections seems to be lower among circumcised men. This may explain our findings as based on religious and ethnic reasons, all of the male patients enrolled in our study were circumcised.

Another possible reason is that the prevalence of high-risk HPV infections among men who have same-gender sex partners is significantly higher²⁰.

There are two limitations to this study. Firstly, the sample size was relatively small. A larger sample size, as well as the use of a single method of sampling and laboratory evaluation, can give us more reliable results. Another limitation is that although PCR is a standard and accurate tool in HPV detection, most available kits are not able to identify all HPV types²¹.

CONCLUSION

This study showed a higher frequency of HPV 6 and HPV 11 than other HPV genotypes in anogenital warts in the Guilan province of Iran. Also, we obtained a relationship between HPV genotypes and gender. Further molecular studies at the cellular and immunologic levels are needed to understand the exact relationship between gender and HPV genotypes.

Conflict of interest: None declared.

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