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Frequency of Human Papillomavirus Types in Women with Genital Warts by Probe-based Real-time PCR

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Abstract

The second most common cancer and the fourth leading cause of death in women is cervical cancer. In 90% of cases of human papillomavirus infections, HPV is the cause of this cancer. More than 200 different types of the virus have been identified, including low-risk and high-risk variants. According to statistics, infections from types 16 and 18 of the virus account for 70% of cervical cancers. This cancer is preventable, and one of the ways to prevent it is to have regular screenings. Probe-based real-time PCR methods are more accurate and sensitive methods for detection. In this study, we performed HPV typing with probed base real-time PCR in 50 genital wart samples and checking the relationship between questionnaire information.

INTRODUCTION

The second most common cancer and the fourth leading cause of death in women is cervical cancer. Studies show that half a million people worldwide get cancer every year, most of them in developing countries. This cancer is one of the preventable cancers (1). Cervical cancer usually develops from small, slow-moving lesions. It can be cured if it is diagnosed in the early stages. Therefore, if you see unusual changes and symptoms in your sexual and reproductive organs, you should see a specialist physician immediately because the chances of treatment in the early stages are much higher than in the more advanced stages of the disease (2). Cancer can be caused by a variety of causes, such as smoking, frequent use of multiple birth control pills. But in 90% of cases of human papillomavirus infections, HPV is the cause of this cancer (3). Human papillomavirus has double-stranded DNA genome of 8000 bp, and no envelope, The genome of the virus is divided into two parts, which include the Early region and the Late region, The early area contains 6 ORFs (E1, E2, E4, E5, E6, E7) that are expressed immediately after entry into the host cell, and the late region contains two major capsid protein L1 and minor capsid protein L2 genes that The following are expressed (4). More than 200 different types of the virus have been identified, including low-risk and high-risk variants. The most common low-risk genotypes of the virus, which are also common, are the 6 and 11 variants, which in many cases cause benign lesions such as genital warts and low-grade precancerous lesions. The most dangerous types of the virus include genotypes 16, 18, 31, and 45, which are the most common causes of cervical cancer (5). According to statistics, infections from types 16 and 18 of the virus account for 70% of cervical cancers, 80% of vaginal cancers, and 92% of rectal cancers worldwide (6, 7). As mentioned, this cancer is preventable, and one of the ways to prevent it is to have regular screenings, Pap smears, and HPV diagnostic tests. In recent years, HPV-based PCR has been replaced by Pap smear cytology tests, one of the reasons for this decision by health authorities to be more accurate. There are various PCR-based diagnostic methods for identifying different HPV types, including conventional PCR, types of hybridization, and Real-time PCR. Probe-based real-time PCR methods are more accurate and sensitive than other methods (8). The study was performed on 50 women referred to the laboratory with genital wart lesions who participated in this study after obtaining consent and inserting information. The HPV HR NovinGene kit was used to identify 14 high-risk and two low-risk types. Results were compared with patient questionnaire information and HPV infection.

MATERIALS AND METHODS

The study population consisted of 50 women with a mean age of 38.6 who had genital wart lesions. The questionnaire included items such as age, height, weight, education, marital status, having children, having cancer

in first degree relatives, smoking history, and patient consent. Genital warts were transported to the laboratory after being sampled by the physician in a transport medium and kept at refrigerated temperature until DNA extraction. The DNA extraction was performed with the BioBasic one4all extraction kit. After removal, 16 HPV types were evaluated with HPV HR16 Novin Gene kit. These 16 types include (6/11, 16, 18, 31/33, 35/45, 39, 5258, 51, 59, 56/66 and 68), in this kit, the Albumin gene was used as an internal control for the accuracy of extraction. The rest was done according to the kit instructions. Statistical analysis was performed using SPSS 19 software, and $P \leq 0.05$ was considered significant.

RESULTS

All 50 specimens used in this study were genital warts with a mean age of 38.6 years.

Table 1. HPV Type Frequency

Group	Frequency (%)
Total	50
HPV positive	41 (81)
HPV positive only 6.11 type	19 (38)
One of the high risk positive	9 (18)
Positive high risk & 6.11	22 (44)
Positive for 16 Or 18	11 (22)
Positive for 31.33	2 (4)
Positive for 51.58	1 (2)
Positive for 34.35	1 (2)
Positive for 16+31.33	12 (4)
Positive for 18+31.33	1 (2)
Positive for 51+31.33	1 (2)
Positive for 18+59	1 (2)
Positive for 16+51.58	2 (4)

Table 2. Relationship between Questionnaire Information and HPV Infection

Factors	Frequency (%)	P-value
Age, mean		0.47
Total	38.6	
HPV positive	29.4	
Height, mean		0.31
Total	165	
HPV positive	163	
Weight, mean		0.07
Total	61.2	
HPV positive	68.4	
Education level of HPV positive		0.14
Diploma	8 (16)	
BS	29 (58)	
Masters and higher	4 (8)	
Marital status		N.A
Single	0	
Married	100%	
Having a child		0.64
HPV positive	7 (14)	
Total	23	
History of smoking		0.84
HPV positive	1 (2)	
Total	9	
Family history of breast and cervical cancer		N.A
HPV positive	0	
Total	2	

In 41 cases (81%), HPV positive that has one of the 16 types were studied, and nine circumstances (18%) were not favorable for any class (Table 1). In these cases, either the person has no HPV infection or other HPV types other than the 16 types examined. The mean age of the patients was 29.4, and all were married. The relationship between the characteristics of the questionnaire and the types of HPV can be seen in Table 2. In 34 cases (68%) the individual had one of the 6 or 11 samples. In 12 cases (24%), the person had co-infection with 11/6 and one of the high-risk types. Seven cases (14%) had only a kind of high-risk type. The results of checking the relationship between questionnaire information and HPV infection are also presented in Table 2. Based on these results, no significant correlation was found between HPV infection and these characteristics.

DISCUSSION

Human papillomavirus infections account for 90% of cervical cancers. In recent years we have seen an increase in the number of people infected with the virus in the country. Given the importance of timely diagnosis and selection of appropriate therapeutic approaches in the prevention of cervical cancer, periodic HPV screening tests with precise methods such as Probe-based Real-time PCR have been recommended by health authorities. In this study, 81% of the target population who had vaginal lesions were reported to be HPV positive, of which 44% were at high risk for the virus. Because cervical cancer is one of the most preventable cancers, accurate identification of the type of virus (high risk or low risk) is of great importance. Probed based real-time PCR methods are capable of detecting tiny copies of the virus in the patient sample, The NovinGene kit is capable of detecting 50 copies of the virus in 1 microliter of the patient sample, and in 95% of cases where the virus titer in the example is higher than this, the kit is capable of detecting viral infection. Other studies in Iran have reported a prevalence of HPV between 0.6 and 33% (9). The high incidence in our study could be due to the small population under study, careful selection of individuals (only those with genital warts), in other studies, individuals with normal cervical cytology were also included in the study population. In other study prevalence rates of 11/6were between 3% and 6%, and high-risk types 18/16 were between 24-2%. The two low-risk types 6 and 11 are the leading cause of non-cancerous genital warts (10). Since all of the specimens used in this test were warts, the high prevalence of these two types (68%) is justified. The rapid detection of high-risk types of the virus is essential in the prevention of cervical cancer. Using accurate screening and genotyping techniques can reduce the incidence of cervical cancer and reduce the cost of treatment time. One of the most accurate diagnostic methods used in this study. In future studies, we

compare PCR-based diagnostic methods in larger populations.

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