The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89, Page 4327- 4331

Genotyping of Human Papilloma Virus Infections

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ABSTRACT

Background: The human papillomavirus (HPV) is a virus that only infects epithelial cells. Skin and mucosal lesions, as well as malignancies, are usually associated with it. In anogenital carcinogenesis, HPV is a key player. Persistent HPV infections have been linked to an increased risk of developing cervical cancer in several studies. The objective of the current study is the detection of HPV-DNA in cutaneous and genital warts by polymerase chain reaction (PCR) and to evaluate their possible association with malignant and non-malignant conditions. **Patients and methods:** This study comprised 24 patients. They are classified into 4 groups (common wart, planter wart, genital wart and cancer cervix groups) according to the clinical and pathological results. Biopsies from lesions were subjected to DNA extraction. Extracted DNA was amplified in the PCR reaction For the purpose of detecting low-risk HPV Samples found to be positive by PCR were then exposed to an additional amplification in order to find high-risk forms of HPV.

Results: revealed that low risk HPV-DNA was detected in 60% among common wart group, 26.6% among genital wart group and 13.3% among cancer cervix group. Meanwhile, it was not detected in planter wart group, with overall detection of HPV-DNA in 62.5 % of the study groups. About 46.7% of the positive cases had high-risk HPV-DNA.

Conclusion: Common wart is the most benign lesions as it rarely converts to malignancy. Genital HPV infection was detected in both malignant and nonmalignant conditions. HPV is a potential risk for cervical neoplasia among Egyptian women.

Keywords: Human papillomavirus, Genital wart, High-risk HPV-DNA, PCR, Al Ahrar Teaching Hospital, Zagazig University.

INTRODUCTION

Infecting the epithelia of the skin or mucosa, the human papilloma viruses (HPV) are a big group of roughly 120 genotypes. It is possible to contract more than 40 different types of genital infections. Asymptomatic or subclinical infection is the most common form of HPV infection. Cervical cancer is caused by HPV types 16 and 18, which are oncogenic or high-risk. However, HPV strains 6 and 11 are more commonly associated with anogenital warts or condylomata acuminate **(1).**

Epidermal infections caused by HPV are widespread and can result in a wide range of clinical symptoms. HPV-infected genital warts (condylomata acuminata) are typically considered to be harmless growths of the anogenital skin and mucosa. Sexual contact can spread genital warts. Infectious genital warts affect approximately two-thirds of those who have sexual contact with an infected partner. For the most part, the incubation period ranges from three weeks to eight months **(2).**

Anogenital cancer can occur despite the benign nature of most HPV-related proliferations. However, specific forms of HPV can increase anogenital cancer risk. These include laryngeal, oral, as well as some pulmonary malignancies **(3).**

Skin cancer research would benefit greatly from more knowledge about papilloma viruses.

The goals of our work were detection of HPV-DNA in cutaneous and genital warts by polymerase chain reaction (PCR) and to evaluate their possible association with malignant and non-malignant conditions.

PATIENTS AND METHODS

This work was carried out at the PCR unit at Clinical Pathology Department. Patients were drawn from the Gynaecology and Dermatology Clinics at Zagazig University Hospitals and Al-Ahrar Teaching Hospital's outpatient clinics for this study.

A thorough history, skin, gynecological, and histological examination were performed on all patients.

The specimens from patients were obtained either by local excision, colposcopic directed biopsy or total specimen after total hysterectomy. In order to use PCR to detect HPV, each biopsy was immediately frozen at -70^{o}C in aluminum foil **(4).** Positive samples were subjected to second amplification another time to detect high-risk HPV typing (16/18/31/33/52b/58).

Polymerase chain reaction technique:

DNA was extracted from the tissue biopsy using Nucleo Spin Nucleic Acid purification Kits CLONTECH Laboratories, Inc. 1020 East Meadow Circle Palo, ALTO, CA94303-4230, USA.

HPV typing fast kit. Supplied by (EXPERTEAM VENEZIA, Italy), done by PCR with L1open reading frame using My09/MY11 consensus primers. The technique involved two amplifications (Nested PCR): (a) The first one for L1 region screening (low risk group). (b) The second one for HPV (high risk groups).

Ethidium bromide staining and UV light transillumination were used to identify the amplified DNA products **(5).**

Received: 02/05/2022
Accepted: 03/07/2022

Epi-Info version 6.02 was used to verify, enter, and analyze the data. For quantitative data, the mean (standard deviation) was used; for qualitative variables, the number and percentage were used. Analyses of results were conducted using ANOVA, t-tests, and chi-squared tests [(6)].

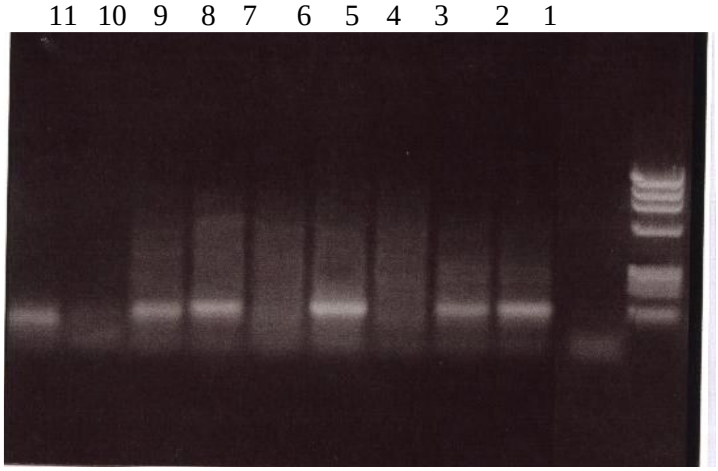

Figure (1): PCR products of HPV after gel electrophoresis and ethidium bromide staining in 1st amplification.
1=Marker. 2=Negative control. 5,7,10=Negative samples. 3,4,6,8,9,11=Positive samples for low-risk HPV at 150bp.

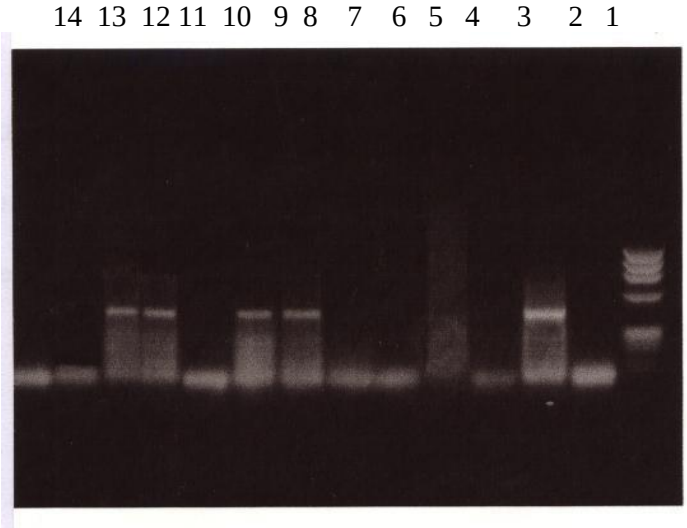

Figure (2): PCR products of HPV after gel electrophoresis and ethidium bromide staining in 2nd amplification.

1=Marker. 2=Negative control.
4,5,6,7,10,13,14=Negative samples.
3,8,9,11,12=Positive samples for high-risk HPV at 233-268bp.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 22 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro-Walk test. Qualitative data were represented as frequencies and relative percentages. Chi-square test ($\chi 2$) was done to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P-value ≤ 0.05 was considered significant.

RESULTS

This study comprised 24 patients (16 females and 8 males) their ages ranged between 8-54 years with a mean of 23.12 (SD 7.28). They were classified according to the clinical and histopathological results of the lesions into 4 groups: Group I: Common warts (11 patients), Group II: Planter warts (3 patients), Group III: Genital warts (6 patients) and Group IV: Cancer cervix (4 patients).

According to the clinical features of patient's groups, an age-related rise in HPV infection rates was shown to be highly significant (P-value <0.001), Women had 66.7 percent of HPV, whilst men had only 13.6 percent. (P-value <0.05) (Table 1). The duration of the disease was found to be longer in high-risk HPV infection. The difference was highly significant in high-risk HPV (P-value <0.001), while it was not significant in low-risk HPV (Table 2).

Table (1): Demographic data of patient groups.

Patient Group	**I (N=12)**		**II (N=6)**		**III (N=3)**		**IV (N=2)**		**V (N=2)**		**Sig. test**	**P-value**
Age θ ± SD Range	15.7±5.3 (7-23)		27.5±3.6 (22-32)		49.3±3 (46-52)		27±1.4 (26-28)		34±5.6 (30-38)		F test 34.97	0.001 (HS)
	No	%	No	%	No	%	No	%	No	%		
Sex:												
M	6	50	0	0	0	0	2	100	0	0	X^2 11.21	0.05
F	6	50	6	100	3	100	0	0	2	100		(S)

Group I= Common wart. Group II= Genital warts. Group III= Cancer cervix. Group IV= Planter warts. Group V= Cervical polyp.

Table (2): Relation of duration by months with results of low and high-risk HPV.

Duration (months)	Mean ± SD	t -test	P-value
Low risk -ve +ve	 8.1 ± 1.7 (4-18 M) 9.7 ± 2.2 (3-24M)	0.758	(NS)
High risk -ve +ve	 6.2 ± 1.3 (3-10M) 14.1 ± 3.2 (9-24M)	4.24	0.001(HS)

Figures 1 and 2 show the results of PCR in low and high-risk HPV infections. Positive samples for low-risk HPV were detected on gel electrophoresis at 150 bp. The positive samples for high-risk HPV were detected at 233-268 bp.
The PCR reveals a positivity of 62.5% (15 out of 24) for low-risk HPV. The positive cases were 9 cases of common warts, 4 cases of genital warts and 2 cases of cancer cervix (Table 3). No significant difference was observed in different groups.

Table (3): Results of (PCR) in low-risk HPV

Patient group	-ve (n= 9)		+ve (n=16)		X^2	P-value
	No	%	No	%		
I. Common wart (n=12)	2	22.2	10	62.5	3.74	NS
II. Genital warts (n=6)	2	22.2	4	25	0.02	NS
III. Cancer cervix (n=3)	1	11.1	2	12.5	0.01	NS
IV. Planter warts (n=2)	2	22.2	0	0	3.71	NS
V. Cervical polyp (n=2)	2	22.2	0	0	3.71	NS

For high-risk HPV, PCR was positive in 7 out of 15 (46.7 %) (1 case of common warts, 4 cases of genital warts and 2 cases of cancer cervix) (Table 4). There was a substantial difference between common and genital warts, but not in cancer cervix.

Table (4): Results of (PCR) in high-risk HPV

Patient group	-ve (n= 9)		+ve (n=7)		X^2	P-value
	No	%	No	%		
I. Common wart (n=10)	9	100	1	14.3	12.34	0.001(HS)
II. Genital warts (n=4)	0	0	4	57.1	6.86	0.05 (S)
III. Cancer cervix (n=2)	0	0	2	28.6	2.94	NS

DISCUSSION

Infecting the epithelia of the skin or mucosa, the Human Papilloma Viruses are a big group of roughly 120 genotypes. Infecting the vaginal region is possible with more than 40 of these organisms [(7)].

Anogenital warts are noncancerous lesions of the epithelium that commonly appear in areas of the genital area that are particularly prone to abrasion or trauma during sexual activity. It is common for anal warts to appear in people who have engaged in receptive anal intercourse; however, they may also appear in men and women who have never engaged in such activity. In many cases, anogenital warts go unnoticed until they cause pain, itchiness, or bleeding [(8)]. The goal of this study was to detect HPV infection in warts and its correlation with malignant and nonmalignant situations by PCR. **Boxer** [(9)] stated the When it comes to DNA sequencing, PCR is the most important technique. This very basic method provides an astonishing level of precision. To achieve this aim, 24 patients with different types of warts and cervical lesions were classified clinically and histopathologically into 4 groups: common wart, planter wart, genital wart and cancer cervix. In this study, HPV infection rates increased significantly in direct proportion to an individual's age (p-value <0.001).

This result is in accordance to the study performed by **Hildesheim *et al.*** [(10)], who stated that, In young women, HPV infection is a temporary phenomenon, either because the virus is eliminated by the host or because viral shedding falls below the detection level of PCR. They stated that, the immune response mounted by older women is less effective suppressing the virus. Also, **Tiggelaar *et al.*** [(11)] compared to women aged 19-26, girls aged 9-18 exhibited lower levels of oncogenic HPV seroprevalence.

In contrast to our result, **Kiviat *et al.*** [(12)] HPV was more prevalent in younger individuals due to the in situ hydridization approach utilised in the study, according to the authors' findings. The same result was reported by **Guzick *et al.*** [(13)] and **Adam *et al.*** [(14)] the higher prevalence of HPV infection among the younger women is attributed to the fact that all their specimens were obtained from patients suffered from cervical intraepithelial neoplasia.

In terms of their ability to cause cancer, genital HPV genotypes are categorized into high- and low-risk groups. As a general rule, the HPV genotypes that

pose the most danger include 16, 18, 31, 35; 39; 45; 51; 52; 56; 58,59; 59; 68; 73; and 82; while the low-risk HPV genotypes include 6, 11, 40 [15, 16].

Patients with genital warts have been shown to carry HPV genotypes that pose a significant risk to their health [17]. A significant rate of transfer from one partner to another has been found in epidemiological investigations. The HPV high-risk genotype impacts not only the person who has it, but also the person's partner [18].

HPV-16, 31, 35, and 51 persistent skin infections have recently been linked to an increased risk of cervical cancer [19].

In this study, low-risk HPV-DNA was detected in 9 out of 15 (60%) among common wart group, and 4 out of 15 (26.6%) among genital wart group and 2 out of 15 (13.3%) among cancer cervix group while it was not detected in cases of planter warts, with over all detection of HPV-DNA in 15 out of 24 (62.5%) of the study groups. On second amplification for positive cases, the high-risk HPV-DNA was detected in 1 out of 9 among common wart group, the 4 cases of genital warts were positive and the 2 cases of cancer cervix were positive with over all incidence of 7 out of 15 (46.7%).

In low-risk HPV, our findings agree with the findings of **Adam *et al.*** [14] who reported an incidence of 65%. On the other hand, a higher result 70.7% in high-risk HPV of the study groups was reported. This was attributed to the preliminary papanicolaou stain for their cases which lacked the sensitivity for detecting HPV and revealed a high grade squamous intraepithelial lesion.

In the current study, as regard common warts low-risk HPV was detected in 9 out of 11 cases. In planter warts the three cases were negative for HPV. **Porro *et al.*** [20] found that about 79% of all cutaneous warts tested positive for HPV DNA, according to a study looking at different forms of HPV found in the skin. HPV 2/27/57 predominated in the lesion. HPV DNA was found in 90.9 percent of benign warts in another investigation [21]. Also, in high-risk HPV, we detected 1 out of 9 cases. These results of common warts indicated that the opinion of benign behavior of the HPV must be changed. Until now no studies on high-risk HPV of common warts are available for us. So, further studies must be done.

We found that in low-risk HPV, the genital wart was 26.6% and cancer cervix was 13.3% of all cases. In high-risk HPV the genital wart was 57.1% and cancer cervix was 28.6% of positive cases.

In agreement with our results, **Mathews-Greer *et al.*** [22] HPV cervical cancer rates were reported to be 12 percent and 28 percent for low and high risk HPV, respectively. Also, **Ozaydin-Yavuz *et al.*** [17] reported with a prevalence of 62.1% (42/66), low-risk genotypes predominated in anogenital warts. HPV-6 (47 percent) and HPV-11 (11 percent) were the most common genotypes (13.6 percent). In addition, HPV-18 and HPV-3 were found.

In many studies,[23-25] infection with genital HPV has been linked to an increased risk of developing cervical cancer. Cervical malignancies with high-risk HPV genotypes have been found in nearly all cases, and part of the process of HPV-mediated carcinogenesis has been elucidated. [23].

This incidence of HPV in genital wart and cancer cervix may be attributed to the fact that the malignant conversion of HPV induced tumors is facilitated by physical and chemical carcinogens, which induce mutations, recombination and selective DNA amplification [24]. Furthermore, **Shen *et al.*** [25] attributed the progression of HPV infection to invasive cancer is the concomitant infection with other virus as CMV or HSV type II. Another explanation for the malignant transformation is that low risk HPV may produce cancer in immunocompromised host [26-28]. Furthermore, **Turazza *et al.*** [29] analyzed biopsies from genital cancer and found that two cases of cervical carcinoma harbored HV-11 DNA. These previous reports clarify the association of low-risk HPV to malignant tumors.

In accordance to our results, **Bauer *et al.*** [30] a whopping 69% of sexually active females tested positive for the HPV virus. Women who are sexually active for the first time have a 50 percent chance of contracting a genital HPV infection within two years. Fewer than 1% of women infected with HPV will develop cervical cancer in their lifetimes [23,31].

The duration of the disease differed significantly between the high-risk and low-risk groups in this study (p-value <0.001) and this agree with the study performed by **Hidesheim *et al.*** [10]. This significant difference is attributed to the accumulation of HPV infection over the time. Cervical cancer is closely linked to the persistence of genital HPV infections. [24]. The inhibition of virus replication should prevent tumor development.

William *et al.* [32] stated that HPV is the most common sexually transmitted disease in women and is normally removed without therapy, but the persistence of high-risk HPV varieties can lead to aberrant cervical cellular alterations if they are not treated promptly. The recurrence of genital warts after therapy is common. In some cases, reactivation of HPV that has lain dormant in hair follicles or reinfection may be to blame [27,33,34].

CONCLUSION

It could be concluded that common wart is the most benign lesion as it rarely converts to malignancy. Genital HPV infection was identified in malignant and nonmalignant conditions. HPV is a potential risk for cervical neoplasia among Egyptian women.

PCR method is simple to perform, easy to interpret and could be included with cytology in routine HPV screening programs.

Many studies have examined the clinical importance of HPV genome type. We feel that this study will throw some light on vaccination initiatives

in the future. A reduction in the incidence of genital warts may be achieved by include HPV genotypes 6 and 11 in vaccination programmes, in addition to carcinogenic HPV genotypes.

Conflict of interest: The authors declare no conflict of interest.

Sources of funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contribution: Authors contributed equally in the study.

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