

ORIGINAL ARTICLE

Genotyping of Human papilloma virus from warts in Egyptian patients

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ABSTRACT

Key words:

Warts, HPV, genotypes

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Background: Warts are common viral infections of the skin that are caused by human papillomavirus (HPV). There are more than 200 HPV genotypes up till now. **Objective:** To identify HPV genotype distribution among wart patients. **Methodology:** A cross sectional study was conducted on 78 patients with warts. Shave biopsy was taken from wart cases for detection of HPV by conventional PCR. HPV genotyping was performed using real time PCR. **Results:** HPV DNA was detected in 50 (64.1%) of all biopsy samples while 28 (35.9%) of samples were negative. The most frequently detected genotype was HPV-52 (7.1 %) from HPV positive samples. **Conclusion:** obtaining results can increase the knowledge regarding the prevalence of HPV and its type distribution in Egypt. This may contribute to create a national prevention program for HPV.

INTRODUCTION

Warts are the commonest cutaneous manifestations of the HPV. Warts are more common in children and adolescents, although they can appear at any age. They are estimated to affect up to 7-12% of the population. Though not life threatening in immunocompetent patients, they are a source of discomfort, embarrassment and occasionally pain¹.

HPVs are non-enveloped, double stranded DNA viruses that belong to the *Papillomaviridae* family². There are more than 200 strains of HPV that fall into five HPV genera: α -papillomavirus, β -papillomavirus, γ -papillomavirus, μ -papillomavirus and ν -papillomavirus. HPVs classification is based on the nucleotide sequence of the open reading frame (ORF) that codes for the capsid protein L1³.

HPV genotypes are strains within an HPV species. They are designated as HPV followed by a number (e.g. HPV-16). New papillomavirus genotypes are assigned when the completely full-length genome has been identified and the sequence of L1 ORF is verified as differing by more than 10% (less than 90% similarity) from the closest known HPV genotype⁴. Unique HPV genotypes were determined to be disease specific⁵.

Treatment of wart is divided mainly into medications and surgery; medications include: podophyllin, imiquimod, podofilox and trichloroacetic acid while surgery includes: freezing with liquid nitrogen, electrocautery, surgical excision and laser

treatments^{6,7}. Even though the short-term effect of previous treatments is good, no approach could prevent recurrence of warts which was estimated up to 30%⁶. Thus, effective prevention towards HPV transmission and infection is critical. The introduction of the quadrivalent HPV vaccine (Gardasil®) in 2007 and the bivalent HPV vaccine (Cervarix™) in 2008 made a change in the strategy of HPV prevention. The recent arrival of the nonavalent HPV vaccine (Gardasil®9) could significantly impact on HPV-related diseases⁸.

It was found that for effective vaccination; prevalence, genotype distribution of HPV and pre-existing immunity in a certain population are critical⁹.

HPV genotype distribution varies across world regions. HPV genotypes 16, 18, 31, 52, and 58 are consistently found worldwide, although the distribution of certain HPV genotypes still show great regional differences¹⁰. Exploring the HPV genotype distribution characteristics and providing matched vaccine is the prerequisite to ensure the efficiency of vaccination.

The aim of our study is to explore the most common HPV genotypes causing warts in Egyptian patients.

METHODOLOGY

A cross sectional study was carried out in the Medical Microbiology and Immunology as well as the Dermatology Departments, Faculty of Medicine, Zagazig University. The study was approved by the Institutional Reviewer Board (IRB), Faculty of Medicine, Zagazig University.

Patients

The study participants included 78 wart cases recruited from the Outpatient Clinic of Dermatology, Venereology and Andrology Department at Zagazig University Hospitals.

A written informed consent was taken from each participant (before the start of the study) after explaining the nature as well as the purpose of the study.

A shave biopsy was taken as a portion of the lesion above the level of the surrounding skin from wart cases. The biopsy was shaved off using a blade after disinfection of skin by application of 70 % ethyl alcohol then stored in 1 ml of saline solution in storage falcon tube at -20°C until further processing for HPV detection¹¹.

DNA extraction from wart biopsy samples:

DNA Extraction was done using (DNA Technology PREP-NA-PLUS, Russia) following the manufacturer instructions. The extracted DNA was separated on 1.5% agarose gels. Bands were visualized under UV and photographed. Extracted DNA was immediately stored at -20° C until PCR amplification.

Screening PCR

For the detection of HPV DNA presence in the wart samples, before proceeding to genotyping. Extracted DNA was subjected to conventional PCR that amplifies 450 bp product for L1 ORF of HPV¹² using the following primers:

(MY09): 5' CGTCCMARRGGAWACTGATC 3'

and (MY11): 5' GCMCAGGGWCATAAYAATGG 3'.

Twenty-five microliters of reaction solutions were prepared (12.5 µL Dream Taq Green PCR Master Mix ,2µg DNA template, 1µl of each primer) and 8.5 µL nuclease-free). The amplification was carried out in a DNA thermal cycler (Biometra, Germany). PCR was performed as follows: Initial denaturation at 95°C for 3 min followed by 40 cycles of denaturation at 95°C for 30 sec, annealing for 30 sec at 45°C, extension at 72°C for one min. The final extension step was extended to 10 min at 72°C. Amplified products were visualized on 2% agarose gel under UV light then stored at -20°C.

Genotyping of HPV:

HPV positive samples were genotyped using quantitative REAL-TIME PCR Kit (DNA Technology-HPV QUANT-21).This kit is intended for identification and quantification of 21 HPV types including HPV 6, 11, 44 ,16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82 in human biological samples. The strips were loaded by adding 10 µL of MAX Taq-polymerase solution then 20 µl of mineral oil and finally 5 µL DNA. The strips were setted to real time PCR instrument (DNA Technology, Russia) and the program was started. Real-time PCR software "HPV_quant-21" was downloaded from «HPV_QUANT_en.ini».

Statistical analysis:

Data were analyzed using SPSS, version 25 (SPSS Inc., Chicago, IL, USA). Continuous data were presented as mean ± standard deviation (SD). Categorical data were presented by the frequency and percentage.

RESULTS

This study was performed on seventy - eight patients (32 males and 46 females), with ages ranging from 5-52 years. Different types of warts were included in the study. Plantar wart was the most prevalent type. Multiple warts were present in 67.9% of the studied cases. The wart characteristics of the studied group are shown in table 1.

Table 1: Wart characteristics of studied group.

Variable	Cases (n=78)	
	No	%
Size:		
< 1 cm	30	38.5
> 1 cm	48	61.5
Site:		
Face	9	11.5
Dorsum of the hands	10	12.8
Palms	13	16.7
Dorsum of the foot	13	16.7
Sole	29	37.2
Genital	4	5.1
Number of warts		
Single	25	32.1
Multiple	53	67.9

HPV DNA was detected in 50 (64.1%) of all biopsy samples while 28 (35.9%) of samples were negative (figure 1,2).

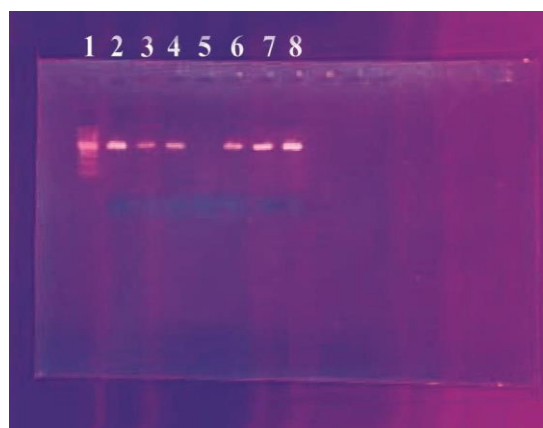


Fig. 1: Agarose gel electrophoresis showing PCR detection of HPV DNA in biopsy samples from warts. Lane 1: 100 bp DNA ladder. Lane 2, 3,4,6,7and 8: 450 bp band (+ve). Lane 5: No band (-ve).

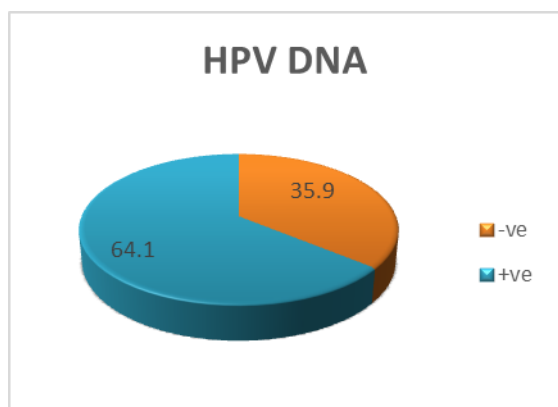


Fig. 2: Frequency of detection of HPV DNA in biopsy samples from warts by conventional PCR.

The most commonly detected HPV genotype among HPV positive samples as shown in table 2 and figure 3 was genotype HPV -52 (14 % of cases).

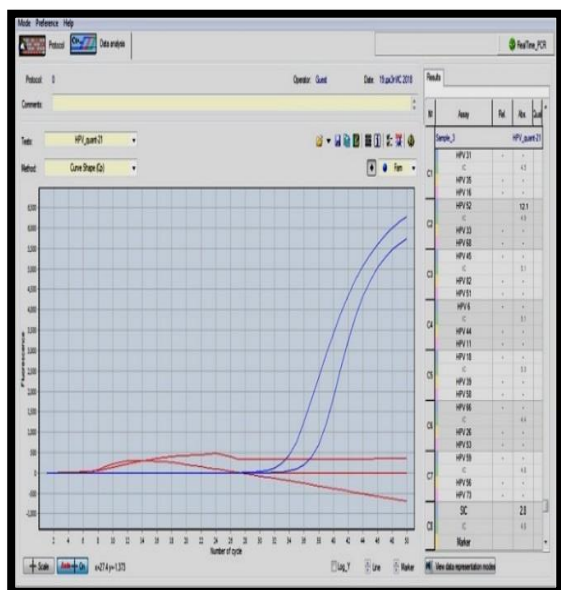


Fig. 3: Amplification plot of real time PCR for HPV genotyping showing a specimen positive for genotype 52.

Other detected genotypes were HPV-6, HPV-18, HPV-26, HPV-66 (6%), HPV-11 (4%), HPV-16, HPV-31, HPV-39, HPV-53 and HPV-59 (8%) while no genotype was detectable in 9 cases (18%).

Table 2: Frequency of different HPV genotypes detected by real time PCR among HPV DNA positive samples.

Variable	Cases (n=50)	
	No	%
HPV genotypes:		
HPV-6	3	6
HPV-11	2	4
HPV-16	4	8
HPV-18	3	6
HPV-26	3	6
HPV-31	4	8
HPV-39	4	8
HPV-52	7	14
HPV-53	4	8
HPV-59	4	8
HPV-66	3	6
ND	9	18

DISCUSSION

Warts are benign papillomas, caused by infection of mucosal or epidermal cells with HPV. Although they seem harmless, warts cause quite a lot of morbidity¹. Significant improvement concerning HPV control and prevention had been made in the recent years however, HPV vaccines is still the best method at all¹³. Thus, surveillance of HPV genotype distribution has the same importance as vaccine development¹⁴. It was found that the diversity of results in published data may result from variations in the prevalent HPV types in warts, differences within the populations studied and the different methods used for HPV detection¹⁵.

In view of the above, this study tried to explore the HPV genotype distribution in different types of warts.

In the present study, HPV DNA was found in the biopsy samples of 50 patients (64.1%) using the primer MY09-11 to detect L1 ORF. This result agreed with the study carried out by Giannaki et al.¹⁵ where HPV DNA was found in 75% of the wart tissue analyzed, using the same primer, Chen et al.,¹⁶ who found HPV DNA in 47.8% in cases of genital warts and Shaheen et al.,¹⁷ who detected HPV DNA in 46.4% among 57 wart patients. On the other hand, Nobre et al.,¹⁸ Shaltout et al.,¹⁹ and Youssef et al.,¹² found HPV DNA only in 25%, 16.7%, 10.4% and 40.8% of cases, respectively, using the same primer but in cervical samples using cervical brush. These results clearly indicate the sensitivity of MY09-11 primer differs with the different tissue specimens.

The relatively high prevalence of negative samples for HPV DNA (35.9%) could be partially explained by the inadequate superficial sampling of the wart (while virus is being in the basal layers) or the possibility that

the HPV DNA load was lower than the cutoff detection threshold of the used techniques.

Our results of genotyping revealed that the most commonly detected HPV genotype among HPV positive samples was genotype HPV -52 (14 % of cases). Other detected genotypes were HPV-6, HPV-18, HPV-26, HPV-66 (6%), HPV-11 (4%), HPV-16, HPV-31, HPV-39, HPV-53 and HPV-59 (8%) while no genotype was detectable in 9 cases (18%).

Different HPV genotypes have been reported in different previous studies. HPV-27 was the most frequent type identified by Sasagawa & Mitsuishi,²⁰ which was detected in 44% of cutaneous warts samples from Japanese patients while Giannaki et al.¹⁵ reported HPV- 57 as the most frequently observed type (43.1%) in cutaneous warts of Greek children. Horn et al.²¹ found that among 146 subjects with warts, types 2, 27, or 57 were the most frequent genotypes being found in 120 American subjects. This strongly points to the effects of different geographical localities on different HPV genotypes distribution.

Boda et al.,²² found that HPV 16, followed by strains 52, 51 and 18 were the predominant types among 713 females with genital warts.

Murtiastutik et al.,²³ found that HPV 6, 11 were the predominant low risk genotypes in a study conducted on 12 males with anogenital warts.

Prevalence studies of the types of HPV have been conducted more frequently in cervical lesions patients. Shaltout et al.,¹⁹ found that the most prevalent genotypes among HPV-positive women were HPV-16 (19.6%) HPV-31 and HPV-51 (15.2% each), HPV-18 (6.5%), HPV-62 (17.4%) and HPV-84 (10.9%). Also, Youssef et al. .,¹¹ revealed that HPV 16, 18, 31, 6 and 62 were the most prevalent HPV types, constituting 41.9, 29.03, 12.9, 11.3 and 9.7 % respectively.

CONCLUSION

Further studies that focus on the prevalence and type distribution of HPV in Egypt should be carried out so that a national HPV prevention program can be achieved.

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Conflicts of interest:

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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