

Demographical Study of Cutaneous Warts Caused by Human Papillomaviruses

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ABSTRACT

Cutaneous warts are a common viral infection of the skin caused by the human papillomavirus (HPV). The main aim of this study was to investigate the distribution of cutaneous warts among patients and also to identify risk factors associated with the development of cutaneous warts, such as age, gender and residency, occupation and other factors. One hundred and seventy samples of cutaneous warts of different types were collected from patients at the main as well as private clinics during the period from August 2022 to the end of December 2022. A high prevalence of HPV infection (55.30%) was noticed for HPV DNA using the FAP PCR method. The study also revealed that the hand was the most common location for warts, followed by the sole, with varying percentages for other locations. Common warts were more prevalent than plantar warts in all age groups, with a higher prevalence in younger age groups. Patients living in urban areas had a higher prevalence of warts compared to those in rural areas.

Key words: HPV (Human Papillomavirus), cutaneous warts, verruca plantar, *Verruca vulgaris*

INTRODUCTION

Cutaneous warts are a common dermatological condition caused by human papillomavirus (HPV) infection. While cutaneous warts are typically benign, they can cause social stigma and discomfort for affected individuals. The epidemiology of cutaneous warts can vary depending on a range of demographic factors, including age, gender and geographic location. Several studies found that cutaneous warts caused by HPV were more common in children and young adults. A study conducted in Iran found that the highest prevalence of cutaneous warts was among individuals aged 11-20 years (CDC, 2019). Another study conducted in Greece found that the highest incidence of cutaneous warts was among individuals aged 6-15 years (Al-Mubarak *et al.*, 2015). These findings suggest that age is an important demographic factor in the occurrence of cutaneous warts caused by HPV.

While some studies found that the incidence of cutaneous warts was higher in males than females, others had found the opposite. A study conducted in Turkey found that the incidence

of cutaneous warts was significantly higher in females than males (Bristow, 2022). However, a study conducted in Malaysia found that the incidence of cutaneous warts was significantly higher in males than in females (Sterling *et al.*, 2014). These findings suggest that gender may be an important demographic factor in the occurrence of cutaneous warts caused by HPV, but the relationship is complex and may vary across populations.

The epidemiology of cutaneous warts caused by HPV can vary depending on geographic location. A study conducted in Egypt found that the prevalence of cutaneous warts was higher in rural areas than in urban areas (Essa *et al.*, 2019). Another study conducted in Nigeria found that the prevalence of cutaneous warts was higher in urban areas than in rural areas (Arruda *et al.*, 2016). These findings suggest that geographic location may be an important demographic factor in the occurrence of cutaneous warts caused by HPV.

MATERIALS AND METHODS

The study obtained data from 170 patients, with

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ages ranging from 3 to 60 years, and included 73 males and 97 females. To participate in the study, verbal consent was obtained from adult patients or agreement from the family for children. A questionnaire was used to collect information directly from the patients, and it included several key factors such as age, sex and residence in addition to detection of type of warts (common or plantar).

In this study, an excisional biopsy was taken from every patient who was clinically diagnosed with cutaneous warts, including common warts and plantar warts. The samples were collected from the Department of Dermatology in Al-Nassyria Teaching Hospital during the period from August 2022 to the end of December 2022. The samples were collected in a viral transport media tube (VTM tube) and then stored at -20 °C until analysis.

In this study, DNA extraction of the cutaneous wart samples was performed using the FavorPrep Viral DNA/RNA Kit (Favergen Biotech Corporation, Taiwan), according to the protocol provided by the company. Extract viral DNA from the sample using a DNA extraction kit according to the manufacturer's instructions. The purified viral DNA was then eluted from the column and stored at -20°C until analysis.

In this study, the detection of HPV DNA in cutaneous wart samples was performed using PCR amplification with the FAP primer pair, which targets a conserved region of the L1 gene of HPV. The amplified DNA product was then visualized by gel electrophoresis. The primers used in the PCR assay were specific for the β -globin gene (Fap region) as presented in Table 1. An amplicon yield of a 480 bp DNA band using the FAP primer pair was considered as a positive result for HPV DNA as presented in Table 2. The data were statistically analyzed using SPSS version 23 software and the means were compared using T-test and Chi-square under the 0.05 of considered probability level; P value ($P > 0.05$).

Participation in the study was subjected to informed consent. All participants had cutaneous warts of papillomaviruses. This

Table 1. The reaction mixture of conventional polymerase chain reaction (25 μ l)

S. No.	Chemical	Amount (μ l)
1.	Master mix	12.5
2.	Forward primer (FAP59)	1.0
3.	Reverse primer (FAP64)	1.0
4.	Nuclease free water	4.5
5.	Template of DNA	6.0

protocol was approved by the Board of the Research Ethics Committee of the Health Office in Thi-Qar Governorate, according to the ethical number (No. 508 on 21/7/2022). It was based on the recommendation and decision of the Research Committee approved by a committee of specialized professors in the Department of Health in Thi-Qar Governorate (No. 2022185 on 21/7/2022). Moreover, informed consent was obtained from all patients and members of the control group. Further, all samples taken from those patients were within the direct supervision of specialist physicians in hospitals.

RESULTS AND DISCUSSION

The purified extracted DNA samples from all cutaneous warts of patients were then amplified by using the FAP degenerate primers. The FAP product for primer used in this study is shown below (Fig. 1).

In the current study, the detection of cutaneous warts caused by human papillomaviruses (HPV) using a PCR-targeted Fab region as what has been followed in different studies in recent years. For example, a study conducted by Arruda *et al.* (2016), the PCR-targeted Fab region was used to detect HPV DNA in cutaneous warts (Arruda *et al.* 2016). The previous study found that the PCR-targeted Fab region had a sensitivity of 91.7% and a specificity of 100% for the detection of HPV DNA in cutaneous warts. Another study by Schmitt *et al.* (2018) compared the PCR-targeted Fab region with other PCR methods for the detection of HPV DNA in cutaneous warts (Schmitt *et al.*, 2018). The study also found that the PCR-targeted Fab region was

Table 2. Primers and sequences used for the conventional PCR in the study

Primer		Sequence (5'-3')	Amplicon size
β -globin gene	F	FAP 59 (5' TAACWGTIGGICAYCCWTATT 3')	480bp
	R	FAP 64 (5' CCWATATCWWHCATITCICCATC 3')	

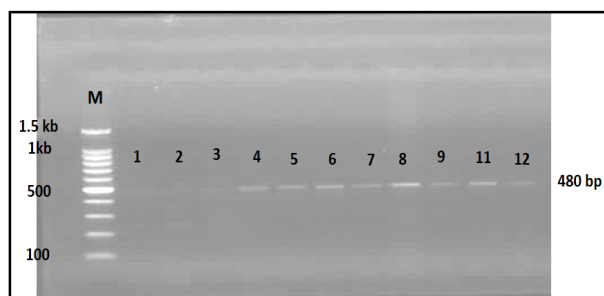


Fig. 1. Gel electrophoresis of FAP PCR products of human papillomaviruses. This figure presents the amplification of the specific DNA gene through the polymerase chain reaction (PCR) using specific forward and reverse primers of the Human Papillomavirus targeted the Fab region within an amplicon size of (480bp). Lane M: DNA ladder (100-1500 bp DNA marker), Lanes 4-12 with positive results for parvovirus DNA genome targeted the β -globin gene (Fab region); while each of Lanes 1-3 negative for amplification.

more sensitive and specific than other PCR methods tested. Another study by Kim *et al.* (2019) used the PCR-targeted Fab region to detect HPV DNA in both common and plane warts. The study found that the PCR-targeted Fab region had a sensitivity of 85.7% and a specificity of 100% for the detection of HPV DNA in both types of warts (Kim *et al.*, 2019). The β -globin gene was a gene that coded for a protein called beta-globin, which was a subunit of hemoglobin. Hemoglobin was an important protein found in red blood cells that was responsible for carrying oxygen throughout the body. The β -globin gene was commonly used as a reference gene in PCR studies as it was presented in all nucleated cells and was expressed at relatively constant levels (Kim *et al.*, 2019).

Moreover, in a recent study by Li *et al.* (2021), the PCR-targeted Fab region was used to detect multiple HPV types in cutaneous warts. The study found that the PCR-targeted Fab region had high sensitivity and specificity for the detection of HPV DNA in cutaneous warts caused by multiple HPV types (Li *et al.* (2021). These studies provided strong evidence that the PCR-targeted Fab region was an effective and reliable method for the detection of HPV DNA in cutaneous warts. Accordingly, the use of PCR-targeted Fab region for the detection of cutaneous warts caused by human papillomaviruses (HPV) has become

increasingly popular in recent years, as evidenced by various studies (Kaur *et al.*, 2014). Also, according to the sex distribution of the patients, the results showed that 73 (42.94%) were male in comparison to 97 (57.06%) who were female (Table 3).

Table 3. Comparison between males and females in patients with cutaneous warts

Sex	N	%	P value
Male	73	42.94	0.066
Female	97	57.06	
Total	170		
$X^2 = 3.38$		df = 1	

Accordingly, the difference in wart infection between males and females in the study population was not statistically significant. Moreover, as presented in Table 2, out of 73 males infected with warts about 43 (45.74%) had positive PCR results, while 30 (39.47%) had negative PCR results. In regard to females, 51 (54.25%) out of 97 had positive PCR and the rest 46 (60.52%) had negative PCR results. After comparison between these results, it was found that there was no significant difference between them where the P value was 0.49 (Table 4).

Table 4. Comparison between males and females according to PCR results for HPV

PCR results	N	Male (%)	Female (%)	P value
Positive	94	43 (45.74)	51 (54.25)	0.49
Negative	76	30 (39.47)	46 (60.52)	
Total	170	$X^2 = 0.47$ df = 1		

The results of the current study suggest that most of the skin warts in the study population were *Verruca vulgaris* (foot, hand, neck, leg, face and head), which accounted for 79.41% of the samples as presented in Table 3. These types of warts appeared as nodules or papules with a rough surface and hyperkeratotic border, in addition to black dots on the surface. *Verruca plantar*, plantar warts, was the second most common type of wart in the study population, accounting for 20.59% of the samples. These warts are characterized by keratinous lesions on the plantar surface of the feet. The study also looked at the location of warts in the infected patients. The hand was the most common location for warts, accounting for 35.3% of the cases. The sole was the second most common location, with 20.6% of the cases. Other locations included the face

(15.88%), foot (9.41%), neck (8.23%) and leg (7.64%) as presented in Table 5.

As *V. vulgaris* also known as common wart and was caused by HPV types 2 and 4. They typically occurred on the fingers, hands and nails, but could also appear on other parts of the body, such as the face and feet. A study by Kaur *et al.* (2014) found that common warts were most commonly found on the fingers (58.3%), followed by the face (20.8%) and the feet (16.7%) as also reported by Kaur *et al.* (2014). The second main type detected of cutaneous warts was verruca plantar which was usually found on the soles. It was important to note that the distribution of cutaneous warts varied from person to person, and the above studies represented a general pattern of distribution (Kaur *et al.*, 2014).

Also, results as presented in Table 5 indicated that there were some differences in the distribution of warts among males and females in different sites of skin. Specifically, there was no significant difference in the occurrence of warts on the foot between males and females. However, all patients had warts on their hands, with 20 males (11.76%) and 40 females (23.52%). This difference between males and females was statistically significant. Similarly, for plantar warts on the sole, 10 males (5.88%) out of 35 were affected, compared to 25 females (14.70%). Again, this difference was statistically significant.

On the other hand, the distribution of warts in other locations did not show any significant difference between males and females in this study. Overall, these findings in this study indicated that the occurrence of warts on the hand and plantar surfaces may be influenced by gender, while the occurrence of warts in other locations was not significantly different between males and females. And these results in the current study found that both of the afore

mentioned types were found in a higher percentage among females compared to what appeared in males, which were different from what were found by some other similar studies such as the study conducted by Kaur *et al.* (2014) who found that common warts were more common in males (60.7%) than in females (39.3%). However, another study by Cogliandro *et al.* (2018) found no significant difference in the prevalence of common warts between males and females. Similarly, Zubaedi *et al.* (2019) found no significant difference in the prevalence of plantar warts between males and females. It was important to note that the above studies represent a general pattern of distribution and that the distribution of cutaneous warts can vary from person to person. Likewise, these differences may result from the different nature of the work of women and men, the standard of living, and the effort that women make in specific societies, especially their work in the home and kitchen, their standing for long periods of time during work, and their exposure to scratches and wounds in the skin, especially in the area of the feet and hands, according to the daily work that they do.

Also, the results of the current study that focused on the distribution of common and plantar warts among different age groups are summarized in Table 6. The results showed that there was a significant difference in the distribution of warts between different age groups. In the age group of 1-10 years, the majority of patients (13.53%) had common warts, while only a small percentage (1.76%) had plantar warts. In the age group of 11-20 years, a significant number of patients (24.70%) had common warts, while only a few (4.11%) had plantar warts. In the age group of 21-30 years, a similar pattern was observed, with a significant number of patients (26.47%)

Table 5. Distribution of warts (*Verruca vulgaris*) in males and females according to the site of the lesion

Site of lesion	N	Male (%)	Female (%)	X ²	df	P value
Foot	16 (9.41%)	6 (3.52)	10 (5.88)	1	1	0.31
Hand	60 (35.3%)	20 (11.76)	40 (23.52)	6.66	1	0.01
Sole	35 (20.59%)	10 (5.88)	25 (14.70)	6.42	1	0.01
Neck	14 (8.23%)	6 (3.52)	8 (4.70)	0.28	1	0.59
Leg	13 (7.64%)	5 (2.94)	8 (4.70)	0.69	1	0.40
Face	27 (15.88%)	11 (6.47)	16 (9.41)	0.92	1	0.33
Head	5	5 (2.94)	0 (0)			
Total	170	63 (37.05)	107 (62.94)			

Table 6. Distribution of common and plantar warts according to age

Age group (year)	N	Common (%)	Plantar (%)	χ^2	df	P value
1-10	26	23 (13.53)	3 (1.76)	15.38	1	0.00
11-20	49	42 (24.70)	7 (4.11)	25.00	1	0.00
21-30	64	45 (26.47)	19 (11.17)	10.56	1	0.001
31-40	25	19 (11.17)	6 (3.52)	6.76	1	0.009
41-50	5	5 (2.94)	0 (0.00)			
51-60	1	1 (0.58)	0 (0.00)			
61 and above	0	0 (0.00)	0 (0.00)			
Total	170	135	35			

having common warts, and a smaller percentage (11.17%) having plantar warts. For the age group of 31-40 years, a majority of patients (11.17%) had common warts, while a smaller percentage (3.52%) had plantar warts. Interestingly, no plantar warts were seen in the other age groups, including 41-50 years and 51-60 years, and there were no warts of any type in patients above 61 years old. Overall, these results found that common warts were more prevalent than plantar warts in all age groups, and there was a higher prevalence of warts in younger age groups.

Thus, there was a significant difference in the distribution of warts between different age groups. The prevalence of warts was found to be higher in younger age groups. In the age group of 1-10 years, the majority of patients had common warts, while only a small percentage had plantar warts. This finding is consistent with previous studies that have reported a higher prevalence of common warts in children (Silverberg *et al.*, 2018). In the age group of 11-20 years, a significant number of patients had common warts, while only a few had plantar warts. In the age group of 21-30 years, a similar pattern was observed, with a significant number of patients having common warts, and a smaller percentage having plantar warts. This finding is in line with the age distribution of warts reported in previous studies (Silverberg *et al.*, 2018).

For the age group of 31-40 years, a majority of patients had common warts, while a smaller percentage had plantar warts. This finding is consistent with previous studies that have reported a decrease in the prevalence of warts with increasing age (Silverberg *et al.*, 2018). Interestingly, no plantar warts were seen in the other age groups, including 41-50 years and 51-60 years, and there were no warts of

any type in patients above 61 years old. This finding suggested that the risk of developing warts decreased significantly with age.

In conclusion, this study provided further evidence that common warts were more prevalent than plantar warts in all age groups, and there was a higher prevalence of warts in younger age groups. These findings can help/guide the development of targeted prevention and treatment strategies for warts, particularly for children and young adults.

In regard to the role of residency of patients in the detection of papillomavirus-caused cutaneous warts, the results of the current study found that there was a significant difference in the prevalence of warts between patients who lived in urban and those who lived in rural areas. Specifically, the study found that wart prevalence was higher among patients who lived in urban regions compared to those who lived in rural regions. The data from Table 5 showed that 64.70% of patients living in urban areas had warts, while only 35.30% of patients living in rural areas had warts. The difference between the two groups was statistically significant (Table 7). These findings suggest that there may be environmental or lifestyle factors that contribute to the higher prevalence of warts in urban areas. Further research is needed to explore these factors and develop targeted interventions to reduce the incidence of warts in both urban and rural areas.

Table 7. Relationship between wart and residency

Residency	No.	%	χ^2	df	P value
Urban	110	64.70	14.70	1	0.00
Rural	60	35.30			

The results of the current study provided important insights into the distribution of warts

in urban and rural areas, and suggested that there was a significant difference in the prevalence of warts between these two groups. The finding that wart prevalence was higher among patients who lived in urban regions compared to those who lived in rural regions was significant, as it highlighted the need for further research to explore the potential environmental and lifestyle factors that may be contributing to this difference. The higher prevalence of warts in urban areas could potentially be explained by several factors. For example, urban areas are often characterized by higher population density, which can increase the likelihood of person-to-person transmission of HPV, the virus that causes warts. Additionally, urban areas may have a greater prevalence of risk factors associated with HPV transmissions, such as sexual activity and sharing of personal hygiene items. Other potential contributing factors could include differences in access to healthcare or differences in the prevalence of other infections or underlying medical conditions that may increase the risk of developing warts. The prevalence of cutaneous warts caused by papillomaviruses has been reported to be higher in urban areas compared to rural areas. Hashemi *et al.* (2014) in Iran found that the prevalence of warts was significantly higher in urban areas (33.5%) compared to rural areas (16.6%). Another study conducted in China by Senapati *et al.* (2017) also found that the prevalence of warts was significantly higher in urban areas (24.3%) compared to rural areas (14.9%). A study conducted in India found that the prevalence of HPV infection was significantly higher among women living in urban areas compared to those in rural areas (Sreedevi *et al.*, 2017). Similarly, a study in Iran found that the prevalence of genital warts was significantly higher among women living in urban areas compared to those in rural areas (Hosseini *et al.*, 2015).

CONCLUSION

PCR-targeted Fab region was an effective method for detecting cutaneous warts caused by human papillomaviruses (HPV). There was no significant difference between the rates of HPV infection in males and females. The most common type of skin warts in the study population was *Verruca vulgaris*, followed by

Verruca plantar. There was a significant difference in the distribution of common and plantar warts among different age groups. Common warts were found to be more prevalent than plantar warts in all age groups, with a higher prevalence of warts in younger age groups. There was a significant difference in the prevalence of papillomavirus-caused cutaneous warts between patients who lived in urban and rural areas. The study found that wart prevalence was higher among patients who lived in urban regions compared to those who lived in rural regions.

RECOMMENDATIONS

PCR-targeted Fab region should be used as a preferred method for detecting cutaneous warts caused by human papillomaviruses (HPV) due to its high effectiveness. Further studies should be conducted to investigate the factors that might explain the lack of significant difference in the rates of HPV infection between males and females. Health professionals and practitioners should pay more attention to *Verruca vulgaris*, as it was the most common type of skin wart among the study population. Public health interventions and education programs should be targeted towards younger age groups to raise awareness about the prevalence of warts and the importance of early detection. Health authorities should also focus on developing targeted interventions to reduce the incidence of warts in both urban and rural areas. This included creating public health campaigns to promote healthy lifestyles, hygiene and sanitation practices.

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