Thi-Qar Medical Journal (TQMJ):Vol.(25),No.(1),2023

Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq

ISSN (Print):1992-9218, ISSN (Online):1992-9218

Molecular Detection of Cutaneous Warts Among Male and Female Patients in Thi-Qar Province

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Abstract:

Background:

Cutaneous warts are a common viral infection of the skin caused by the Human Papillomavirus (HPV). These warts are characterized by raised, rough, and sometimes painful growths on the skin. Cutaneous warts can occur in various parts of the body, including the hands, feet, and face (1). While cutaneous warts can affect individuals of any age and sex, there is evidence that the prevalence and incidence of HPV infection and cutaneous warts may differ between males and females (2). The molecular detection of cutaneous warts among male and female patients in Thi-Qar Province aims to investigate the prevalence and distribution of HPV strains associated with cutaneous warts in this population (3). Cutaneous warts are caused by infection with Human Papillomavirus (HPV), a DNA virus that is transmitted by direct contact with infected skin or fomites. There are over 100 types of HPV, and certain types are more commonly associated with cutaneous warts than others. The most common types of HPV associated with cutaneous warts are HPV 1, 2, 3, 4, 27, and 57. HPV infects the basal cells of the epidermis, and viral replication results in the formation of hyperkeratotic, acanthotic lesions (3). This information can inform strategies for the prevention and treatment of cutaneous warts in this region (4).

Aim:

The main aim of this study is the molecular detection of cutaneous warts among male and female patients in Thi-Qar Province and to investigate the prevalence and distribution of Human Papillomavirus (HPV) strains associated with cutaneous warts in this population.

Methods:

This study is using molecular techniques, such as polymerase chain reaction (PCR), to detect the virus in skin samples (170 samples of skin warts) collected from male and female patients with cutaneous warts. The objective of detecting serotypes of Human Papillomaviruses from patients with non-genital cutaneous warts can be achieved by collecting swab samples from warts and performing polymerase chain reaction (PCR) tests. The PCR tests can detect the DNA of the virus and identify the specific serotype. This information can help in understanding the prevalence of different serotypes and their association with non-genital warts. The study aimed to investigate the presence of human parvoviruses in cutaneous warts of infected patients. To achieve this, purified extracted DNA samples from all cutaneous warts were used, and the FAP degenerate primers were employed to amplify the DNA.

The amplification products were analyzed by gel electrophoresis to confirm the presence of the specific DNA Human Papillomavirus. The results showed that 8 out of 20 samples tested positive for parvovirus DNA. The sequencing of the amplified products confirmed the presence of Human Papillomavirus in all positive samples. And also this study is focusing on the comparison between males and females in relation to the distribution of all types of cutaneous warts detected in patients and then compares the distribution of various types of these cutaneous warts between males and females

Results:

The study used the FAP primer pair to amplify the conserved region of the L1 gene, which is a commonly used target for HPV detection. An amplicon yield of a 480 bp DNA band using this primer pair was considered a positive result for the presence of HPV DNA in the samples. This size corresponds to the expected size of the PCR product amplified from the conserved region of the L1 gene. Also, according to the sex distribution of the patients, the results showed that 73 (42.94%) were male in comparison to 97 (57.06%). Based on the PCR results presented in Table 2, it appears that there is no significant difference in the distribution of HPV DNA between males and females infected with warts, as the *P*-value is greater than 0.05. Out of the 73 male patients, 43 (45.74%) tested positive for HPV DNA by PCR, while 30 (39.47%) tested negative. Among the 97 female patients, 51 (54.25%) tested positive for HPV DNA, while 46 (60.52%) tested negative. The results of the current project 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. These types of warts appear as nodules or papules with a rough surface and hyperkeratotic border, in addition to black dots on the surface. Verruca plantar, which are 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%).

Conclusions

1. The PCR-targeted Fab region shows promise as a valuable tool for the detection of cutaneous warts caused by HPV.
2. The high prevalence of Verruca vulgaris and Verruca plantar in the study population highlights the need for effective diagnosis and management of these types of warts.
3. The findings also confirm that Verruca vulgaris and Verruca plantar are the most common types of warts, with the hand being the most common location for warts.
4. In general, there is no effect of the patient's sex on the appearance of skin warts caused by a human papillomavirus
5. The occurrence of warts on the foot was not significantly different between males and females, but there were significant differences in the occurrence of warts on the hand and sole.

Keywords: HPV (human papillomavirus); FAP primer pair; Verruca vulgaris; Verruca plantar

Introduction

Human Papillomaviruses (HPVs) are a group of small, non-enveloped, double-stranded DNA viruses that infect epithelial tissues. HPV genomes are circular double-stranded DNA molecules of approximately 8,000 base pairs. They encode early (E1-E7) and late (L1 and L2) genes (3). HPV infects keratinocytes, the main cell type in the skin and mucosal epithelium. Different HPV types have different tissue tropisms, with some types causing skin warts (cutaneous HPV types), and others causing genital warts or cervical cancer (mucosal HPV types) (5). Human Papillomaviruses are most commonly inducing benign papillomas or warts distributed in different parts of the body (6). Cutaneous warts are a common skin condition caused by infection with Human Papillomavirus (HPV). While the diagnosis of cutaneous warts can typically be made based on clinical appearance, molecular detection methods can provide additional information on the specific HPV types present in the lesion (7). Several studies have investigated the distribution of HPV types in cutaneous warts among male and female patients. For example, a study conducted in China found that the most common HPV types detected in cutaneous warts were HPV-57 and HPV-27 in males and HPV-57 and HPV-1 in females (8). Another study conducted in Iran found that the most common HPV types detected in cutaneous warts were HPV-57 and HPV-27 in both males and females (9). A study conducted in Italy found that larger cutaneous warts were more likely to be positive for high-risk HPV types, including HPV-16 and HPV-18 (10). Another study conducted in Brazil found that larger cutaneous warts were more likely to be positive for low-risk HPV types, including HPV-6 and HPV-11 (11).

A study conducted in the United States found that HPV-2 was the most common type detected in plantar warts in both males and females, followed by HPV-1, HPV-4, and HPV-63 (12; 13).

Several molecular detection methods have been used to identify HPV types in cutaneous warts. These include polymerase chain reaction (PCR), in situ hybridization (ISH), and DNA sequencing.

PCR is a sensitive and specific method that can amplify specific HPV DNA sequences from tissue samples (14; 15). One study conducted in Baghdad, Iraq, found that the prevalence of cutaneous warts was higher in males (62%) compared to females (38%) (16). Another study conducted in Basrah, another province in southern Iraq, reported a higher prevalence of cutaneous warts in females (56.1%) compared to males (43.9%) (17). Another study conducted in Kuwait found that the prevalence of cutaneous warts was higher in females (57.5%) compared to males (42.5%) (18). In Saudi Arabia, another neighboring country, the prevalence of cutaneous warts was reported to be higher in males (64.6%) compared to females (35.4%) (19).

Patients and Methods

This study was excisional biopsies (170 samples) taken from patients with cutaneous warts (specifically, common warts and plantar warts) at the Department of Dermatology in Al-Nassyria Teaching Hospital. The biopsies were collected during a specific time period (August 2022 - December 2022) and were stored in a viral transport media tube (VTM tube) at -20°C until analysis. The main purpose of this project is to characterize the clinical and biological features of cutaneous warts in a specific population of patients. All these samples are then tested using PCR method for the presence of human papillomavirus (HPV). DNA was extracted from samples using a kit from a company called FavorPrep Biotech Corporation, and the extracted DNA was then used as a template in a PCR reaction with primers specific for the β-globin gene (as described in the leaflet of company protocol). Once DNA has been extracted using the kit, it can be used in a PCR reaction. PCR, or polymerase chain reaction, is a technique for amplifying specific DNA sequences using DNA polymerase enzyme, primers that bind to the target sequence. The primers used were specific for the β-globin gene targeting the (FAP region: within an amplicon size of 840 base pair (bp) as presented in Table 1. By amplifying the β-globin gene using PCR, it could potentially use this technique to detect the presence of the gene in these samples. This information was important for the study in order to analyze the prevalence and characteristics of warts caused by human papillomavirus (HPV) in the study population, as well as identify any association of sex factors in males and females with the development of warts.

This project followed a cross-sectional study involving individuals with cutaneous warts caused by HPV. Participants were recruited from dermatology clinics and hospitals in various geographic locations. Data were collected using a structured questionnaire that will collect information on socio-demographic characteristics and details of cutaneous warts. Data on the location of warts are recorded. The data were analyzed using statistical analysis to identify any significant associations between sex parameters, and the occurrence of cutaneous warts in the group of patients.

Table 1: Primers sequences and amplicon Size for Detection of Cutaneous Wart-causing Human Papillomaviruses targeting the FAP region of the β-globin gene.

This table shows the specific primers targeting a region of the β-globin gene known as the FAP region to detect human papillomaviruses that cause cutaneous warts. The amplicon size for this region was 840 base pairs (bp).

Primer	Sequence (5' – 3')		Amplicon Size	Reference
B-Globin Gene	F	Fap 59 (5' Taacwgtiggicayccwtatt 3')	480bp	Kim, D., Et Al., 2019
	R	FAP 64 (5' CCWATATCWVHCATITCICCATC 3')		

Statistical Analysis:

The data were statistically analyzed using SPSS version 23 software and the means were compared using T-test and Chi-square "chi ^{2}"under the 0.05 of considered probability level; *P* value (*P* > 0.05).

Ethical Consideration:

Participation in the study was subjected to informed consent. All participants had cutaneous warts of papillomaviruses. This 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). Also, 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. And all samples taken from those patients are within the direct supervision of specialist physicians in hospitals. The molecular detection of cutaneous warts among male and female patients in Thi-Qar Province raises several ethical considerations that were taken into account to ensure the protection and welfare of study participants and respect for their rights and dignity. All study participants were provided informed consent before enrolling in the study. As they were fully informed about the study's purpose, procedures, potential risks and benefits, and their right to withdraw from the study at any time without consequences. The study is also keen on the mind to ensure that all personal information and data collected from study participants are kept confidential.

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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 in Figure 1.

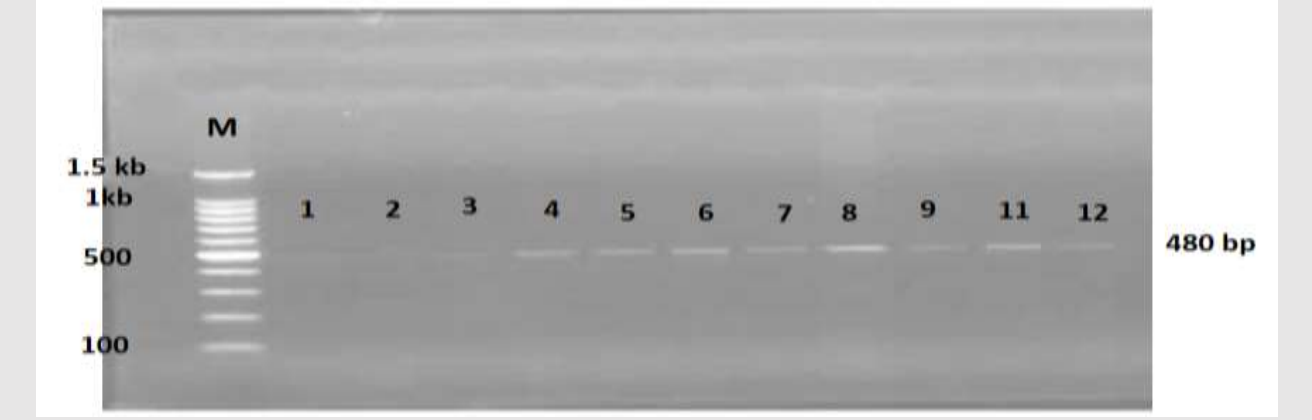


Figure 1: Gel electrophoresis of FAP PCR products of human papillomaviruses. The figure showed a gel electrophoresis image of PCR products of human papillomaviruses targeting the FAP region of the β-globin gene. Lane M represents a DNA ladder marker with fragments ranging from 100 to 1500 base pairs (bp). Lanes 4-12 display positive results for amplification, with an amplicon size of 480 bp.

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. 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 (20). 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. The study also found that the PCR-targeted Fab region was more sensitive and specific than other PCR methods tested (21).
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 (22).

In a recent study conducted 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 (23). These studies provide strong evidence that the PCR-targeted Fab region is an effective and reliable method for the detection of HPV DNA in cutaneous warts. The PCR-targeted Fab region is a highly specific and sensitive method that can detect multiple HPV types in cutaneous warts, making it a valuable tool for the diagnosis and treatment of HPV-associated

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 (20). As this technique allows for sensitive and specific detection of HPV in clinical specimens and can help in the diagnosis and management of cutaneous warts. Further research is needed to evaluate the effectiveness of this technique in different populations and settings and to compare its performance with other detection methods. 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 as presented in Figure 2.

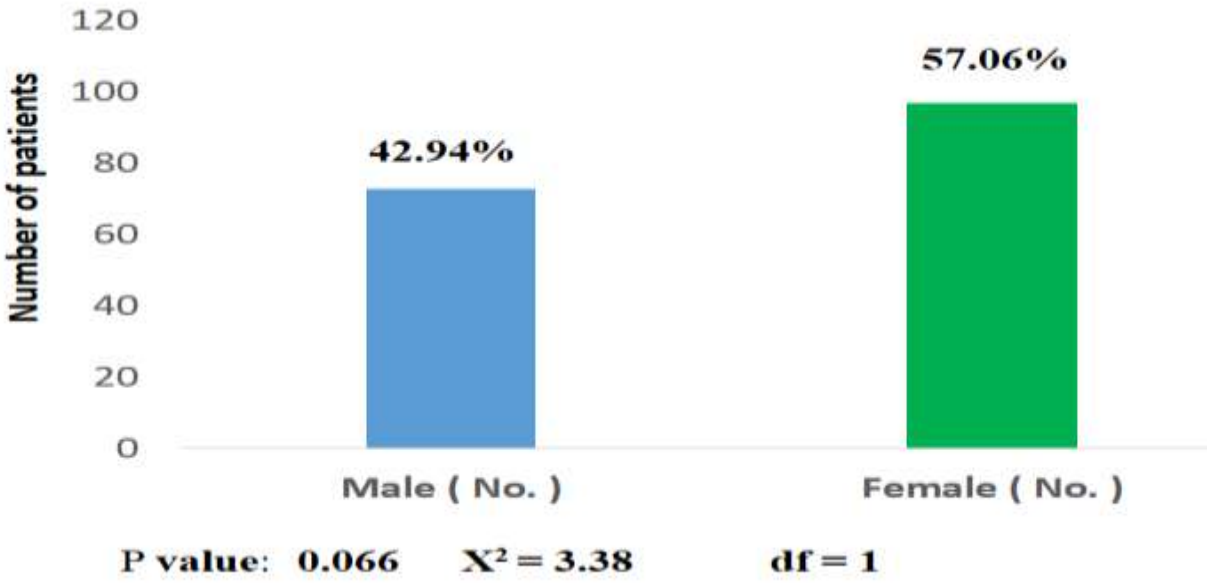
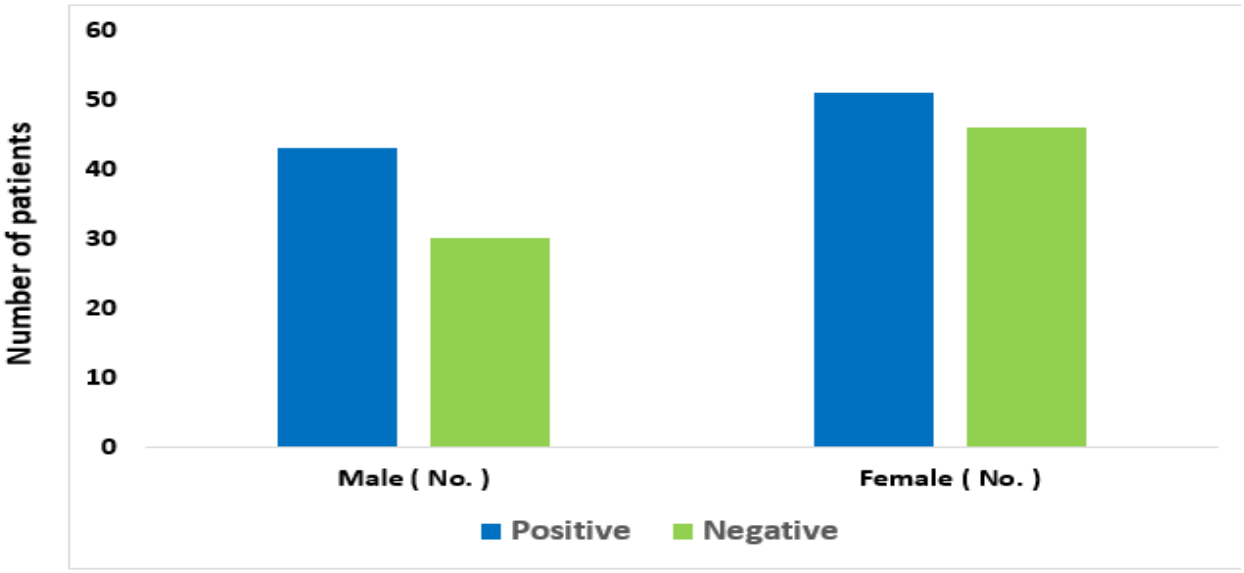


Figure 2: Comparison between males and females in patients with cutaneous warts.
This figure presented that 73 (42.94%) positive detected samples of cutaneous warts for papillomavirus were male in comparison to 97 (57.06%) for females. The study concluded that there was no significant difference between the PCR results of males and females, with a p-value of 0.066.

Accordingly, the difference in wart infection between males and females in the study population is not statistically significant. Moreover, as presented table 2 reveals that out of 73 males infected with warts about 43 (45.74%) have positive PCR results while 30 (39.47%) have negative PCR results. In regard to females, 51 (54.25%) out of 97 have positive PCR and the rest 46 (60.52%) have negative PCR results after comparison between these results, it is found that there is no significant difference between them where the P value was equal to 0.49 ($P > 0.05$).



P value (0.49); X2 = 0.47; df = 1

Figure 3: Comparison between males and females according to PCR results for HPV.

This figure presented the presence of cutaneous warts in males and females and compared the PCR results between the two groups. The study found that out of 73 males, 43 had positive PCR results and 30 had negative PCR results. Out of 97 females, 51 had positive PCR results and 46 had negative PCR results. The study concluded that there was no significant difference between the PCR results of males and females, with a *P*-value of 0.49.

The results of the current project 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 appear as nodules or papules with a rough surface and hyperkeratotic border, in addition to black dots on the surface. Verruca plantar, which are 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 Figure 3.

The results of this study are somewhat different from the results of some previous studies, which found a completely different distribution for this study according to the environment in which the study was conducted. As Verruca vulgaris also known as a common wart and are typically occurs on the fingers, hands, and nails, but can 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%) (23). The second main type detected of cutaneous warts is Verruca plantar is usually found on the soles. A study by Telfer et al.

(1992) found that plantar warts were most commonly found on the ball of the foot (50%), followed by the heel (30%) and the arch (20%) (24). It is important to note that the distribution of cutaneous warts can vary from person to person, and the above studies represent a general pattern of distribution. Also, results as presented in Figure 3 indicated that there are 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%) affected. This difference between males and females is 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 is statistically significant, with a P-value of 0.01.

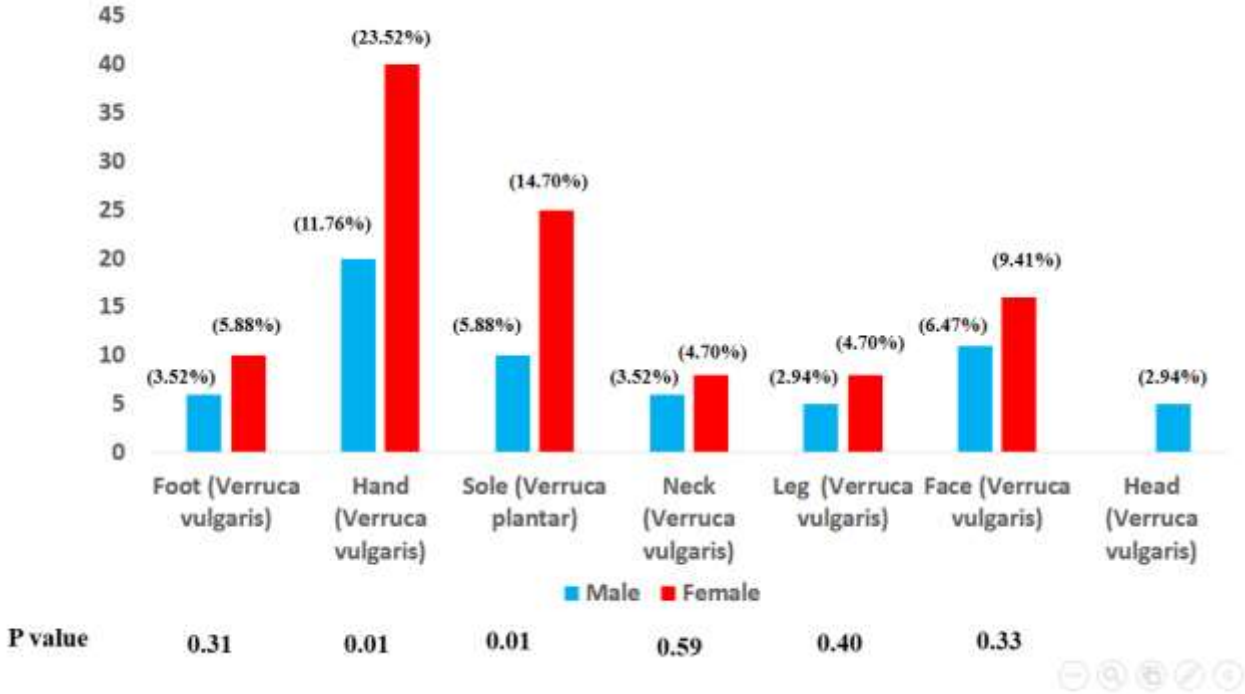


Figure 3: Distribution of warts in males and females according to the site of the lesion.

The figure presented the distribution of warts according to site and gender was analyzed in this study, where Verruca vulgaris was found to be the most common type of wart, accounting for almost 80% of the samples. Verruca plantar, which are plantar warts found on the soles of the feet, were the second most common type of wart, present in 20% of the samples. The analysis of wart distribution by location revealed that the hand was the most common site of occurrence, followed by the sole of the foot, face, foot, neck, and leg.

On the other hand, the distribution of warts in other locations did not show any significant difference between males and females in this study, as indicated by the statistical analysis.

And these results in the current study found that both of the aforementioned types were found in a higher percentage among females compared to what appeared in males, which is different from what was found by some other similar studies such as the study conducted by Kaur et al. (23) 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 (10). Also, another study by Telfer et al. found that plantar warts were more common in males (62%) than in females (38%) (24). However, another study by Zubaedi et al. (2019) found no significant difference in the prevalence of plantar warts between males and females (25). It is 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 our 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.

Recommendations

1- Researchers should consider using PCR-targeted Fab region as a diagnostic tool for cutaneous warts caused by HPV.

2- There is a need for effective management strategies for the most common types of warts, Verruca vulgaris and Verruca plantar.

3- It is recommended to educate patients on the importance of maintaining good hygiene practices to reduce the risk of contracting and spreading HPV infections that lead to skin warts.

4- Further research is needed to confirm the generalizability of these findings to other populations and to investigate the underlying factors that contribute to the distribution and prevalence of different types of warts in various locations on the body.

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