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SHORT COMMUNICATION

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A non-invasive method for diagnosing plantar warts caused by human papillomavirus (HPV)

Sara García-Oreja[1] | Francisco Javier Álvaro-Afonso[1] | David Sevillano-Fernández[2] | Aroa Tardáguila-García[1] | Mateo López-Moral[1] | José Luís Lázaro-Martínez[1]

[1]Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC), University Podiatric Clinic, Complutense University of Madrid, Madrid, Spain

[2]Microbiology Section, Medical Faculty of the Complutense University of Madrid, Madrid, Spain

Correspondence
Francisco Javier Álvaro-Afonso, Universitary Podiatric Clinic, Complutense University of Madrid. Edificio Facultad de Medicina. Pabellón 1. Avda. Complutense s/n, 28040, Madrid, Spain.
Email: alvaro@ucm.es

Abstract

The methods that are used for the diagnostic confirmation of human papillomavirus (HPV) include excisional biopsy and histopathological studies or polymerase chain reaction (PCR). They are invasive, laborious, and subject to ethical restrictions due to the benign nature of these warts. This study aims to analyse the accuracy of non-invasive swab samples to diagnose plantar warts. Fifty plantar warts were included in the study. Skin swabs and hyperkeratosis skin scales were collected from each wart. Multiplex PCR was performed to detect and type the HPVs. The prevalence of HPV in this study was 90% when the sample was obtained using the wart scraping method and 94% when it was obtained using swabs and the new method. In 45 of the 45 positive samples (sensitivity: 100%), the result between the wart scab and wart swab were almost identical. The genotyping result was identical in all 46 patients who had a positive result using both methods. The swab method appears to be a simple and accurate technique to diagnose plantar warts due to HPV. It is a noninvasive technique that could be performed even by inexperienced professionals and in patients with pain or a fear of needles.

KEYWORDS
cutaneous warts, HPV, human papillomavirus, PCR method, plantar warts

1 | INTRODUCTION

Skin warts are benign skin lesions that are caused by the human papillomavirus (HPV), among which common warts and plantar warts the most common.[1,2] There are over 200 strains of the virus distributed over five genera, and 49 are based on their DNA sequences.[3] HPV 2, 10, 27, and 57 from the alpha genus, HPV 4 from the gamma genus, and HPV 1 from the mu genus are the most prevalent types detected in plantar warts.[2,4]

An estimated 40% of the general population is infected with HPV, of whom 7%–12% will develop a skin wart.[4,5] The annual incidence of plantar warts is 14%.[5,6] Immunosuppression, having a wart before, walking barefoot, and having a partner with a wart are the main risk factors for developing a plantar wart.[4,7,8]

The most frequent clinical signs are the appearance of hyperkeratosis, which is due to the proliferation of mutated cells in the foot, visualisation of thrombosed capillaries, pinpoint bleeding when debriding hyperkeratotic tissue, and the disruption of normal

dermatoglyphics.[6,7] In addition, pain on lateral compression of the wart is common.[9]

The diagnosis is mainly clinical and must be confirmed via a biopsy and histopathologic studies (gold standard) [2,9] using polymerase chain reaction (PCR) results from tissue samples or hyperkeratosis scale samples that were obtained from the wart, which will also provide information on the virus biotype.[1,9,10] Taking a biopsy or scraping the hyperkeratosis to extract the DNA are laborious procedures for clinicians, painful for the patient, and subject to ethical restrictions because of the benign nature of these warts.[1,9]

Noninvasive swab samples have been used in two epidemiological studies of HPV types of cutaneous warts by Bruggink et al.[1,2] In 2011, De Koning et al.[9] compared the results of HPV genotyping from swab samples and wart scabs with those obtained from a biopsy in 25 patients who were being treated for persistent cutaneous warts. Swabs of the overlaying skin of each the wart were taken by firmly rubbing a pre-wetted cotton-tipped stick five times over the lesion surface.[9] These authors found that wart scabs and swabs showed identical viral typing results compared to the biopsies in 24 of 25 patients (sensitivity: 96%).[9]

To the best of our knowledge, no study has been published comparing the swab method and the scraping method of wart hyperkeratosis to diagnose plantar warts. This study aims to analyse the accuracy of swabs for diagnosing plantar warts.

2 | MATERIALS AND METHODS

2.1 | Patients and samples

We prospectively collected data from all patients who attended the University Podiatry Clinic of the Complutense University of Madrid between December 2019 and December 2020. Patients older than 5 years of age who presented clinical signs of a plantar wart (hyperkeratosis, pinpoint bleeding when debriding hyperkeratotic tissue, disruption of normal dermatoglyphics or pain on lateral compression of the wart)[6,7,9] (Figure 1A) were included in the study. Patients with an allergy or hypersensitivity to cotton and patients with a wound or bleeding in the wart area at the time of microbiological sampling were excluded from the study.

Wart samples were obtained using swabs and scraping by a single experienced researcher. After the wart was cleansed with sterile saline, a sterile cotton swab was gently rotated five times over the lesion surface (Figure 1B). The swab stick was placed into the transport tube without preservation medium, and it was stored at 5–8°C until use. Later, the scab from the warts was obtained using a scalpel by scraping on the hyperkeratotic surface of the lesion into an Eppendorf tube (Figure 1C), and the sample was stored.

2.2 | HPV identification

All of the samples were processed within 24 h after collection.

Genomic DNA was extracted from hyperkeratosis scales and swabs using the NZY Tissue gDNA Isolation kit (NZYTech), according to the manufacturer's instructions, after 5 h of sample pre-lysis at 56°C. For swab samples, the protocol was slightly modified. Before pre-lysis incubation, the swabs were resuspended in the pre-lysis solution (acid buffer NT1 and 25 µl Proteinase K) by vortexing for 1 min at 3500 rpm. Subsequently, the pre-lysis solution that was retained on the cotton was drained by pressing the swab over the walls of the tube.

DNA concentration was quantified after extraction at 260 nm using a Nanodrop 2000 spectrophotometer. The purity was determined by absorbance ratio 260 nm/280 nm.

HPV DNA amplification (one hundred ng of DNA) was performed using a multiplex PCR reaction or by nested PCR (Mastercycler® Nexus GSX1) using the NZYTaq II 2x Green Master Mix (NZYTech). Multiplex PCR was performed for detecting and typing HPVs that are related to verrucae vulgaris (HPV-1, −2, −27, and −57) using the specific primers and conditions that were proposed by Lei et al.[11] The nested PCR was performed using the primers and conditions that were previously described by Forslund et al.[12] for DNA samples that were not amplified in the multiplex PCR followed by sequencing for HPV typing. Positive and negative controls were included in every amplification cycle. Amplification of the β-globin gene using primers PC04 and GH20 was used as internal control to assess the integrity of human DNA. An amplicon of 286 bp was considered positive and adequate the sample amount for HPV detection. All samples, including HPV negative samples, resulted positive for β-globin test. In

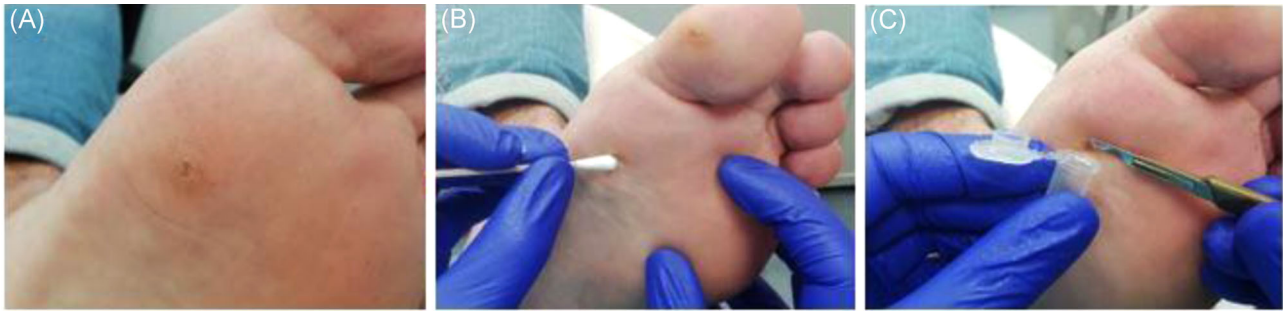

FIGURE 1 (A) Clinical signs of plantar warts. (B) Sampling using the swab method. (C) Sampling using the scraping method

any case, DNA was extracted again, and the amplification was repeated for HPV negative samples and samples with a nontypable HPV.

The PCR products were purified using a NZYGelpure PCR purification kit (NZYTech) and sequenced with the Sanger method with a BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems) using the nested PCR primers and an ABI 3730 XL genetic analyser (Applied Biosystems). Sequences were analysed using Sequencing Analysis software v.5.1 (Applied Biosystems) and the HPV sequence alignment was determined through comparison with known sequences in the GenBank database using BLASTn software (https://blast.ncbi.nlm.nih.gov/).

2.3 | Statistical analyses

Statistical analysis was performed using SPSS v.22 (IBM Corp.). Frequency and descriptive analyses were performed. We used the χ^2 test to compare the qualitative variables and a Student's *t*-test for the association of qualitative variables with quantitative variables.

The sensitivity, specificity, positive and negative predictive values, and positive and negative likelihood ratios for the swab procedure in diagnosing HPV were determined by comparing them with the HPV results that were generated from the wart scabs. For this, we used Epidat v.3.1 (Galicia, Spain).

The sample size was estimated to be 46 samples with a statistical power of 0.80 and an alpha of 0.05, using the GRANMO Sample Size Calculator version 7.12 Online (Institut Municipal d'Investigació Mèdica, Barcelona, Spain).

3 | RESULTS

A total of 50 samples from 46 patients were analysed. Four patients had 2 warts and in these cases 2 samples were taken and analysed, one for each wart. The mean age of the people studied was 49.46 ± 20.41 years with a normal distribution. Eighteen participants (36%) were men and 32 (64%) were women. Twenty-seven (54%) lesions were in the plantar area of the forefoot, 16 (32%) were on the heel, six (12%) were in the ball of the fingers, and one (2%) was periungual. Thirty-six (72%) lesions were considered to be recalcitrant because they had an evolution time greater than 6 months,[13] and 14 (28%) were considered to be non-recalcitrant. The most frequent clinical signs were as follows: 48 (96%) warts presented hyperkeratosis, 47 (94%) showed disruption of normal dermatoglyphics, 29 (58%) had pinpoint bleeding, and 48 (96%) had pain on lateral compression.

The prevalence of HPV in this study was 90% when the sample was obtained using the wart scraping method and 94% when it was obtained using the new swab method (Table 1). Discordant results were obtained between methods for only two samples (Table 1). In these two samples who had positive swab results, the scraping results were negative.

TABLE 1 HPV prevalence data and PCR results by sampling method

	Wart scab		
	HPV negative	HPV positive	Total
Swabs			
HPV negative	3	0	3 (6%)
HPV positive	2	45	47 (94%)
Total	5 (10%)	45 (90%)	50

Abbreviation: HPV, human papillomavirus; PCR, polymerase chain reaction.

One of the negative swabs was taken from a wart located on the plantar area of the forefoot and the other 2 negative swabs were taken from warts located on the heel ($p = 0.594$). The three swabs with a negative result were taken from recalcitrant lesions ($p = 0.550$) and one was taken from a lesion that had been previously treated with a keratolytic ($p = 0.424$).

The genotyping results were identical in the 45 samples, for whom both methods had a positive result. The most prevalent HPV biotype was 2, which was present in 26.7% (12/45) of samples. Seven samples were positive for HPV57 (15.6%), seven for HPV19 (16.6%), six for HPV5 (13.3%), four for HPV27 (8.9%), three for HPV1 (6.7%), two for HPV20 (4.4%), one for HPV14 (2.2%), one for HPV50 (2.2%), one for HPV65 (2.2%), and one was nontypeable HPV (2.2%). In the two samples in whom there was a discordant result, swab genotyping showed HPV65. No sample had a result of more than one biotype in the multiplex PCR. DNA was extracted again, and the amplification was repeated for the sample with a non-typeable HPV, and the results were identical.

Diagnostic accuracy data (with 95% confidence intervals) for the swab versus the scraping method were as follows: sensitivity of 1 (0.99–1), specificity of 0.6 (0.7–1), positive predictive value of 0.96 (0.89–1), negative predictive value of 1 (0.83–1) and positive likelihood ratio of 2.5 (0.85–7.21).

4 | DISCUSSION

In this study, we observed that in 45 of the 45 positive samples (sensitivity: 100%), the result between wart scab and the wart swab were identical. Therefore, using swabs is a sensitive test to confirmation an HPV diagnosis, and it has the advantage of being less invasive for the patient as well as simpler, easier, and faster to perform for the clinicians. De Koning et al.[9] observed a similar sensitivity (96%) for swabs and scraping compared to the gold standard test (biopsy) in a sample of 25 patients. However, we observed that the swab method has a specificity of 60% compared with scraping method. Previous studies have not calculated the specificity of swabs because they did not include lesions that were not clinically diagnosed as skin warts.[9] In our study, only patients with clinical signs of a plantar wart were included, which could lead to a bias when

calculating the specificity. In the present study, the swab method showed a positive predictive value of 0.96, a negative predictive value of 1, and a positive likelihood ratio of 2.5 compared with scraping method. The positive predictive value of 0.96 for our study population is not surprising because our HPV prevalence was 90% when the sample was obtained using the wart scraping method (which we consider to be the gold standard of noninvasive methods). De Koning et al.[9] had a prevalence of 96% in their study using a biopsy and a similar prevalence between scraping and swab methods (92%). A randomized controlled trial that was conducted by Bruggink et al.[1] used the swab method and PCR as a diagnostic method. They reported only 7% negatives in a sample of 250 participants with 391 common warts and 379 plantar warts. In the present study, HPV prevalence was also 94% using the swab method. To the best of our knowledge, other diagnostic precision calculations, such as positive and negative predictive values and positive and negative likelihood ratios, have not been reported in previous studies.

There was no statistically significant association between negative swab results and wart location, recalcitrant or nonrecalcitrant wart and whether the patient had previously received treatment, which in our opinion may be due to the lower number of negative cases.

For genotyping, all positive results were identical between the sampling methods. Genotyping results that were obtained from scabs and swabs of the warts in the study by De Koning et al.[9] were identical to the results from the deeper wart portions (wart biopsy) in 24 of the 25 (96%) patients. In two patients, the result between the wart biopsy and the wart scab or wart swab were not identical, with the scab and wart swab showing negative results.[9] Both sample collection methods yielded identical results in the sample where HPV genotyping was not possible (HPV nontypeable). In our opinion, assuming that the amount of DNA was optimal, the nontypeable genotypes could indicate an overlapping sequence of more than one HPV in this sample.

In the present study there were two samples in whom the swab had a positive result, but the results of scraping (which is considered to be the gold standard of noninvasive tests) were negative. Both patients had a HPV65 genotype, which is a type of Gammapapillomavirus (GammaPV) that is frequently isolated in common and plantar warts.[10,14–16] De Koning et al.[17] recently showed the presence of HPV in up to 80% of swab samples of clinically normal skin, thus raising the question of whether HPV types detected on wart surfaces represent colonization or a latent or productive HPV infection.[16] We consider that the patients who had a positive swab result had an active HPV infection because these patients had clinical signs of a plantar wart (hyperkeratosis, pinpoint bleeding when debriding hyperkeratotic tissue, disruption of normal dermatoglyphics, or pain on lateral compression of the wart).[6,7,9]

This study has some limitations. The results of the swab method were not compared with the biopsy, which is considered to be the gold standard for diagnosing HPV. However, this test is considered to be a very invasive test. Additionally, patients without clinical signs of a wart were not included, which could lead to a bias in calculating the specificity. Future studies could use the swab method in patients with healthy skin, but a comparison with the biopsy or scraping would be difficult because they are invasive methods for the patient. On the other hand, in future studies it would be advisable to sample the same wart by different practitioners to evaluate the interobserver realiability of this test.

In conclusion, the swab method appears to be a simple and accurate technique to diagnose plantar warts that are caused by HPV. It is a noninvasive technique that could be performed by even inexperienced professionals and in patients with pain or a fear of needles.

FUNDING INFORMATION

Funding information is not available.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Sara García Oreja, Francisco Javier Álvaro Afonso, and José Luís Lázaro Martínez: had full access to all data in the study and were responsible for their integrity. **Sara García Oreja**: provided the study concept and design. **Sara García Oreja and Francisco Javier Álvaro Afonso**: obtained the data. **David Sevillano-Fernández**: processed and analysed the samples in the microbiology laboratory. **Aroa Tardáguila-García and Mateo López-Moral**: statistically analysed the data. All authors and interpreted the data, drafted the manuscript, and critically revised the manuscript.

ETHICS AND CONSENT STATEMENT

The protocol was approved by the medical ethics committee of the *Instituto de Investigación Sanitaria del Hospital Clínico San Carlos (IdISSC)* (protocol number 20/013-E). All patients voluntarily signed consent statement for the use of their image and publication of their case details.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Sara García-Oreja http://orcid.org/0000-0002-2937-7122
Francisco Javier Álvaro-Afonso http://orcid.org/0000-0003-4674-3822
Aroa Tardáguila-García http://orcid.org/0000-0001-5650-7351
Mateo López-Moral http://orcid.org/0000-0001-7342-059X
José Luís Lázaro-Martínez http://orcid.org/0000-0001-6110-0265

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