Gubelin *et al. Infectious Agents and Cancer* (2024) 19:48
https://doi.org/10.1186/s13027-024-00611-5

Infectious Agents and Cancer

RESEARCH Open Access

# Human papillomaviruses in hand squamous cell carcinomas from Chilean patients

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## Abstract

**Introduction** Cutaneous squamous cell carcinoma (SCC) accounts for 20% of all skin cancers and its incidence continues to increase globally. It represents 75% of non-melanoma skin cancer (NMSC) mortality. Risk factors include ultraviolet radiation (UVR) exposure, advanced age, chemical exposure, fair skin types, and immunosuppression. While most human papillomavirus (HPV) infections are associated with the development of warts, a subgroup is potentially implicated in the development of cutaneous SCC. The prevalence of alpha, beta, and gamma-HPV in Chilean patients with hand SCCs has not been previously addressed. The objective of this study was to evaluate the presence of HPV and genotyping in hand SCC from Chilean patients.

**Materials and methods** An observational, cross-sectional, descriptive study was conducted. Alpha (α), beta (β) and gamma (γ)-HPV detection was performed by conventional polymerase chain reaction (PCR) in paraffin-embedded tissue samples from 52 patients diagnosed with hand SCC from Santiago, Chile. HPV genotyping was carried out via direct amplicon sequencing by Sanger method.

**Results** The most frequent carcinoma site was the dorsum of the hands (52.5%). α-HPV was not detected in these specimens, whereas β-HPV and γ-HPV were detected in 25% of the analyzed samples. The most frequent genotypes found were β-HPV 100 (38%) and γ-HPV 178 (15%). Additionally, γ-HPV 101, 162, HPV-mSK_016, HPV-mSK_083, HPV-mSK_213 and HPV-mSK249nr genotypes were detected, none of which had been previously described in cutaneous SCC.

**Conclusion** β-HPV and γ-HPV are detectable in 25% of hand SCCs from Chilean patients. It is important to conduct prospective studies to better elucidate the role of these viruses in the development of this disease.

**Keywords** Cutaneous squamous cell carcinoma, HPV, Skin, Hands

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## Introduction

Cutaneous squamous cell carcinoma (SCC) is the second most common type of skin cancer, responding for roughly one in five skin cancers [1]. While SCC is often treatable by surgery, it remains a serious condition, contributing to most deaths from non-melanoma skin cancer (NMSC) [2]. NMSC is most frequently caused by exposure to ultraviolet radiation (UVR), especially in those with fair skin or a weakened immune system [3]. It is also more common in older adults. Some chemicals encountered at work can increase risk as well. The development of this cancer is thought to be a gradual process, starting with sun damage (actinic keratosis, AK) and accumulating changes in the skin cells over time [4]. While UVR is the undisputed main culprit behind NMSC, research suggests a possible accomplice: human papillomavirus (HPV) types, particularly beta (β)-HPV [5]. The presence of HPV DNA in certain NMSCs has fueled research into its potential role in cancer initiation or progression, especially in immunocompromised individuals. However, the exact mechanisms and the types of HPV definitively linked to this cancer remain under investigation [6]. In addition to the previously mentioned characteristics, β-HPVs also exhibit a specific tropism for the skin, meaning they have a particular affinity for skin cells [7]. This connection between β-HPVs and the skin was further reinforced by the discovery of epidermodysplasia verruciformis (EV), a rare genetic disorder [8]. Subjects with EV develop widespread warts and are at a significantly higher risk of developing cutaneous SCC. The strong association between EV, β-HPV types 5 and 8, and SCC has led to propose that these viruses play a role in the development of skin tumors [9]. Other genotypes besides β-HPVs have also been associated with skin lesions. However, γ-HPVs correspond to the most heterogeneous HPV family, of which many genotypes have been described [10]. These genotypes have mucocutaneous tropism and have been reported to be ubiquitous in normal skin. In immunocompromised patients, they are detected more frequently and in greater quantity, although they have also been detected in hand SCC [11]. To improve our understanding of the presence of and role played by HPV in certain types of SCC, we evaluated the presence of α, β, and γ-HPV in hand SCCs from Chilean patients.

**Table 1** Clinicopathological features of the patients

| | Total (%) | HPV (+) (%) | HPV (-) (%) | *P*-value |
|---|---|---|---|---|
| **Total** | 52 (100) | 13 (25) | 39 (75) | |
| **Age** | | | | |
| Average | 79 | 78 | 78 | |
| Range | 53–104 | 65–104 | 53–96 | |
| **Sex** | | | | |
| Male | 28 (53.8) | 8 (61.5) | 20 (51.2) | 0.3760[&] |
| Female | 24 (46.2) | 5 (38.5) | 19 (48.7) | |
| **Laterality** | | | | |
| Right | 17 (32.7) | 5 (38.5) | 12 (30.8) | 0.4248[&] |
| Left | 35 (67.3) | 8 (61.5) | 27 (69.2) | |
| **Localization** | | | | |
| Nail Apparatus | 2 (3.8) | 1 (7.7) | 1 (2.6) | |
| Fingers | 2 (3.8) | 1 (7.7) | 1 (2.6) | |
| Back of hand | 27 (52.5) | 7 (53.8) | 20 (51.2) | 0.2475[#] |
| Palm grove | 1 (1.9) | 1 (7.7) | 0 (0) | |
| Not shown | 20 (38.5) | 3 (23.1) | 17 (43.6) | |
| **Histological type** | | | | |
| In situ | 4 (7.7) | 1 (7.7) | 3 (7.7) | |
| Well differentiated | 30 (57.7) | 8 (61.5) | 22 (56.4) | |
| Moderately differentiated | 9 (17.3) | 3 (23.1) | 6 (15.4) | 0.8480[#] |
| Poorly differentiated | 1 (1.9) | 0 (0) | 1 (2.6) | |
| Keratoacanthoma | 8 (15.4) | 1 (7.7) | 7 (17.9) | |
| **Solar elastosis** | | | | |
| Present | 39 (75) | 8 (62) | 31(79) | 0.1763[&] |
| Absent | 13 (15) | 5 (38) | 8 (21) | |

[#]Chi Square test for Table F by C; [&]Fisher's Exact test with mid-P method

## Results

### Patient characteristics

A total of 52 patients were included, 28 males and 24 females. The average age was 79 years, with a range of 53 to 104 years. In terms of lesion laterality, 17 (32.7%) lesions were found on the right hand, while 35 (67.3%) were found on the left hand. The locations of the lesions were: nail apparatus 2 (3.8%), fingers 2 (3.8%), dorsum of the hand 27 (52.5%), palm 1 (1.9%), and in 20 (38.5%) the site of the hand was not specified. There were 4 (7.7%) in situ carcinomas, 30 (57.7%) well differentiated, 9 (17.3%) moderately differentiated, 1 (1.9%) poorly differentiated and 8 (15.4%) keratoacanthoma variant. In terms of solar elastosis, it was present in 36 (69.2%) of the total patients and in 8 (61.5%) of the samples in which HPV was detected. None of the patients had a known history of immunosuppression. The rest of the epidemiological and clinical characteristics of the patients are shown in Table 1.

### HPV detection

HPV was detected in 13/52 (25%) hand SCCs, 8 males and 6 females. No coinfection of different HPV genotypes was detected in the same lesion. The average age was 78 years, with a range of 65–104 years. The locations of the lesions were: nail apparatus 1 (7.7%), fingers 1 (7.7%), dorsum of the hand 7 (53.8%), palm 1 (7.7%), and not indicated in 3 (23.1%). According to histological grade, we found 1 (7.7%) in situ carcinoma; 8 (61.5%) well differentiated carcinomas; 3 moderately differentiated carcinomas (23.1%); poorly differentiated 0 (0%); and keratoacanthoma variant 1 (7.7%) (Table 2). According to

**Table 2** Clinicopathological features of HPV (+) hand SCCs

| Case Nº | Age | Sex | Location | Solar elastosis | Histological grade | Genus | Species | Genotype |
|---|---|---|---|---|---|---|---|---|
| 1 | 86 | Male | Back of right hand | (+) | Moderately differentiated | Betapapillomavirus | Betapapillomavirus 2 | Human papillomavirus 100 |
| 2 | 68 | Female | Back of left hand | (-) | In situ | Gammapapillomavirus | Gammapapillomavirus 6 | Human papillomavirus 101 |
| 3 | 69 | Male | Left palm | (+) | Well differentiated | Gammapapillomavirus | Gammapapillomavirus 24 | Human papillomavirus 178 |
| 4 | 68 | Male | Right hand, not specified | (-) | Moderately differentiated | Gammapapillomavirus | Gammapapillomavirus 19 | human papillomavirus 162 |
| 5 | 65 | Female | Back of left hand | (+) | Well differentiated | Gammapapillomavirus | Unclassified Gammapapillomavirus | Human Papillomavirus mSK_249 |
| 6 | 75 | Male | Nail apparatus right hand | (-) | Well differentiated | Gammapapillomavirus | Unclassified Gammapapillomavirus | Human Papillomavirus mSK_083 |
| 7 | 104 | Male | Back of left hand | (+) | Well differentiated | Gammapapillomavirus | Unclassified Gammapapillomavirus | Human Papillomavirus mSK_213 |
| 8 | 87 | Male | Left hand, not specified | (+) | Well differentiated | Betapapillomavirus | Betapapillomavirus 2 | Human papillomavirus 100 |
| 9 | 84 | Male | Left hand, not specified | (-) | Keratoacantho ma | Gammapapillomavirus | Unclassified Gammapapillomavirus | Human Papillomavirus mSK_016 |
| 10 | 78 | Female | Left hand fingers | (+) | Well differentiated | Betapapillomavirus | Betapapillomavirus 2 | Human papillomavirus 100 |
| 11 | 77 | Male | Back of left hand | (-) | Well differentiated | Betapapillomavirus | Betapapillomavirus 2 | Human papillomavirus 100 |
| 12 | 71 | Female | Back of right hand | (+) | Well differentiated | Gammapapillomavirus | Gammapapillomavirus 24 | Human papillomavirus 178 |
| 13 | 80 | Female | Back of right hand | (+) | Moderately differentiated | Betapapillomavirus | Betapapillomavirus 2 | Human papillomavirus 100 |

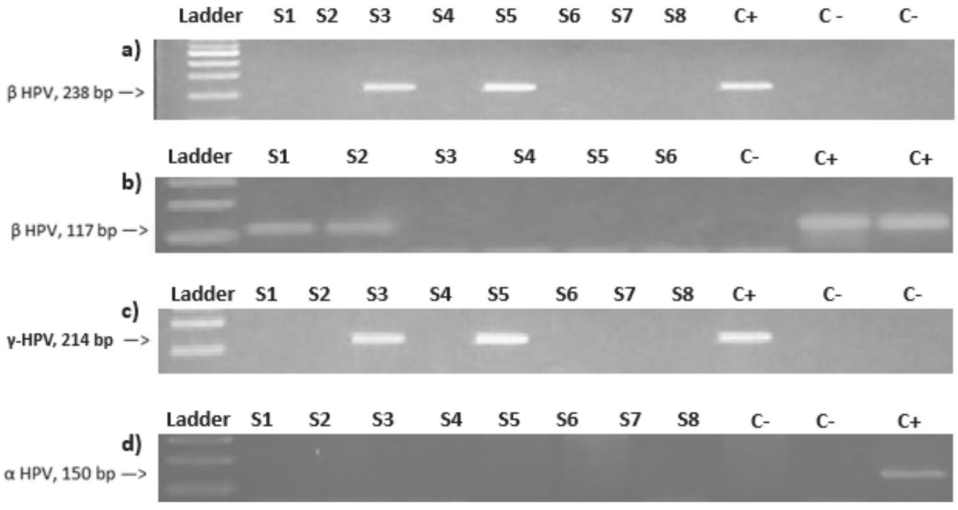


**Fig. 1** HPV detection in hand SCCs from Chilean patients. **(A)** Beta HPV band of 238 bp. **(B)** Beta HPV band of 117 bp. **(C)** Gamma HPV band of 214 bp. **(D)** Alpha HPV band of 150 bp

histological grade and location, the positivity of HPV was 25% of SCCs in situ, 26.7% of well differentiated SCCs, 33% of moderately differentiated SCCs, and 12.5% of keratoacanthoma. HPV was not detected in any poorly differentiated SCCs.

### HPV genotypes

β-HPV genotype 2 was detected in 5/13 (38%) patients, with 3 cutaneous SCCs located on the dorsum of the hand, 1 on the fingers, and in the case of one patient the location of the lesion removal was not specified. Of these HPV cases, 3 were well differentiated and 2 were moderately differentiated (Fig. 1a, b). In the other 8 patients, genotypes belonging to γ-HPV were detected (Fig. 1c). These genotypes were γ-HPV 101, 162, 178, HPV-mSK_016, HPV-mSK_083, HPV-mSK_213, and HPV-mSK249nr. The HPV-mSK_016 genotype was detected in the keratoacanthoma sample, while the HPV-mSK_083 genotype was detected in the nail apparatus carcinoma. The details of each patient can be seen in Table 2. No α-HPV was found (Fig. 1d).

## Methods

### Patients

A cross-sectional, observational, descriptive study was conducted. All patients with a histopathological diagnosis of SCC of the hands were included. Patients were diagnosed between 2022 and 2023 at the Citolab® pathology laboratory, Santiago, Chile. A total of 52 patients were included. Both male and female patients with a

diagnosis of primary cutaneous SCC of the hands were included. Patients with small tissue samples that were insufficient for molecular biology studies were excluded. This study was approved by the ethics committee of the Servicio Metropolitano Norte, Santiago, Chile. (Folio N° 053/2023; Carta N°028/2024).

### DNA extraction

DNA was extracted from paraffin-embedded tissues using 10 µm sections by a previously reported protocol. Briefly, the specimens were incubated with digestion buffer (10 mM Tris-HCl pH 7.4, 0.5 mg/mL proteinase K, and 0.4% Tween 20) for 8 h at 56 °C with stirring. Subsequently, the samples were incubated at 100°C for 10 min and rapidly centrifugated. DNA purity was analyzed by spectrophotometry using a NanoDrop 2000 (Thermo Fisher Scientific Inc, Wilmington, DE) instrument. Purity was determined by the 260/280 absorbance ratio, which was equal to or close to 1.8.

### Polymerase chain reaction and HPV genotyping

To verify the quality of the samples, a 110 bp fragment of the β-globin gene was amplified using the primers PCO3; 5'-ACACA ACTGT GTTCA CTAGC-3' and PCO4; 5'-CAACT TCATC CACGT TCACC-3'. The conventional PCR reaction was performed using Green GoTaq 1X buffer and each primer. Amplification was carried out in a Swift TM Mini-Pro Thermal Cycler according to the manufacturer's recommended protocols. The amplified product was characterized by electrophoresis on a 2.5% agarose gel and visualized using Syber Safe intercalating agent and UV exposure under a transilluminator (Vilber Lourmat). HPV-α amplification was performed using the primers GP5+5'-TTTGTTACTGTGGTAGATACT AC-3' and GP6+5'-GAAAATAAACTGTAAATCATAT TC-3' (150pb), for HPV-β, HPV- γ, HPV- α the primers were FAP59 (5′-TAACWGTIGGICAYCCWTATT-3′) and FAP64 (5′-CCWATATCWVHCATITCICCATC-3′) and PM-A 5'-ACTGACCAAAGCTGGAAATC-3' and PM-B 5' TCTTGCAGAGCATTGAAACG-3' (117pb), and for HPV- α the primers SKF 5' -AAATATCCAGAT-TATCTRAARATG-3' and SKR 5'-ATACCATAGAYC-CACTRGG-3' [12–15] Genotyping was performed on HPV-positive samples by direct sequencing of the respective amplified fragment using the Sanger dideoxy method. The sequences were aligned to a reference sequence of the respective gene using MEGA11. The sequences were then entered into the pave.niaid.nih.gov database for genotyping. The following HPV genotypes were used as reference: NC_001526.4, NC_001531., NC_014955.11.

### Statistical analysis

A database was created containing the following variables: age, sex, location, HPV (YES/NO), HPV genotypes present, location (nail apparatus, finger, dorsum of hand, and palm), and histological grade. Descriptive analysis was performed using the Excel® program.

## Discussion

Cutaneous SCC accounts for approximately 20% of all NMSCs and its incidence is steadily increasing worldwide [1]. There is a growing body of evidence suggesting that HPV may play a role in the pathogenesis of cutaneous SCC [16]. The hands are identified as one of the sites with the highest risk of HPV detection due to constant contact with the mouth, the anogenital area, surfaces, and other people [17]. Previous reports suggest that β- and γ-HPV genotypes exhibit a greater tropism for keratinocytic lesions than α-HPV, which is consistent with the results obtained in this study [18]. In the present study, nine distinct HPV genotypes were detected. β-HPV 100 and γ-HPV 178 had been previously reported in SCC [19, 20]. In contrast, γ-HPV 101, 162, HPV-mSK_016, HPV-mSK_083, HPV-mSK_213 and HPV-mSK249nr had not been previously reported in SCC in the literature. No α-HPV presence was detected in our study. HPV was only detected in one sample in the periungual apparatus, where α-HPV is usually described to be more frequent [21]. It is noteworthy that a previously unreported γ-HPV, HPV-mSK_083, was found in this area. In this study, HPV positivity was observed in 25% (1/4) of SCCs in situ, 26.7% (8/30) of well differentiated SCCs, 33% (3/9) of moderately differentiated SCCs, 0% (0) of poorly differentiated SCCs and 12.5% (1/8) of keratoacanthomas. Since there is a higher proportion of well differentiated SCCs, it is not possible to confirm that the presence of HPV decreases in lesions of higher grade, as has been suggested in other studies. While we unfortunately do not have the clinical assessment of sun exposure in patients, the presence of solar elastosis was reported in 69.2% of the total samples and 61.5% of the HPV (+) samples. In addition, there is a higher proportion of lesions on the left hand (67.3% vs. 32.7%), which could be due to UVR exposure during activities such as driving. UVR is the main recognized risk factor for the development of SCC. The damage caused by this non-ionizing radiation is cumulative over time. The hands are a site that is habitually exposed to UVR, and in the literature, a higher proportion of β-HPV has been reported in photo exposed skin [22]. This could be due to UV-induced immunosuppression, which implies a reduced capacity for antigen presentation, thus perpetuating HPV presence [23].

Although none of the patients in our series had a history of known immunosuppression, advanced age—with

an average of 79 years (range 53–104 years)—is a factor to consider due to immuno-senescence. Changes in the immune system that imply a reduced capacity for immune surveillance and antitumor response, among other alterations, explain the increased predisposition to infections and the development of certain pathologies such as cancer in the elderly population [24]. Thus, immuno-senescence could be a factor that facilitates the persistence of HPV presence and its effects on the initiation of oncogenesis. γ-HPV is the most numerous HPV genus. It has been recognized that this family has a tropism for skin and mucous membranes, but more frequently for hands and the genital area [25]. These findings coincide with the present study, in which a high positivity for γ-HPV was found in SCC of the hands. Better detection techniques have allowed γ-HPV to be identified more frequently in benign and malignant skin lesions [26]. In the present study, γ-HPV 101, 162, 178 and HPV-mSK_016, HPV-mSK_083, HPV-mSK_213 and HPV-mSK249nr were detected. Of these, γ-HPV 101, 162, HPV-mSK_016, HPV-mSK_083, HPV-mSK_213 and HPV-mSK249nr had not been previously reported in SCC in the literature. Arroyo et al. reported that γ-HPV 178 [location of this data] could be the genotype most frequently found in SCC, which coincides with the results of this study [20]. In fact, some authors suggest that the γ-HPV family is up to 10 times more frequent in lesions such as AK and SCC [27]. Further evidence is needed to attribute an oncogenic role to γ-HPV, indicating that more research will be required to fully establish its role in this pathology.

The "hit and run" hypothesis offers a possible explanation for how β-HPVs contribute to skin cancer. This theory suggests that β-HPVs presence in skin cells, interfering with their normal repair mechanisms. This allows mutations caused by UVR to accumulate, eventually leading to the development of cancer. However, the β-HPVs themselves may not be present in the later stages of the tumor [28]. Further knowledge on the pathogenesis of SCC by HPV could provide new tools not only for its prevention, but also for its risk stratification and treatment. For example, determining a patient's presence status could have an impact on their prognosis, as seen in cases of HPV presence in head and neck cancers [29]. According to the literature, early lesions such as QA and SCC in situ would present a higher HPV load, and thus contribute to predicting the risk of tumor invasion or response to immunotherapy [23]. In addition, deeper knowledge of the importance of HPV in the oncogenesis of skin tumors could change the paradigm on the treatment of warts and their prognosis. We must acknowledge the limitations of an observational model, which cannot establish causality due to the absence of complete clinical data on the patients as well as other risk factors, habits, and comorbidities. In addition, there is evidence in the literature that the detection of β-HPV in fresh or frozen tissue is more efficient than in paraffin, given the fragmentation of cellular and viral genetic material in the latter due to formalin fixation [21, 30]. This could affect the sensitivity of viral sequence detection.

## Conclusion

Cutaneous SCC is a pathology that causes high morbidity in Chile and worldwide and places a great burden on health systems. This is the first study carried out in Chile with these characteristics. HPV was detected in 25% of the SCCs, mainly evidencing β- and γ-HPV genotypes. HPV presence was primarily detected on the dorsum of the hands in patients with SCC. Evaluation of the role played by the new HPV genotypes detected in SCC remains a future challenge. This study contributes to evidencing the importance of knowing the HPV presence status in skin SCCs and of developing prevention strategies, such as vaccines. Prospective studies should be carried out to better clarify the role of HPV in the development of this disease.

#### Author contributions

Conceptualization: H.G., J.C.O., F.A., W.G. and A.G.; Formal analysis: J.C.O.; Funding acquisition: F.A. and J.C.O.; Investigation: H.G., J.C.O., F.A., W.G. and A.G.; Methodology: J.C.O. and F.A.; Supervision: F.A., and W.G.; writing original draft: H.G., J.C.O., F.A., W.G and A.G.; writing-review and editing: H.G., J.C.O., F.A., W.G and A.G.

#### Funding

This study was funded by Fondecyt Grant 1221033 (F.A.); Fondecyt postdoc 3220486 (J.C.O.).

#### Data availability

No datasets were generated or analysed during the current study.

## Declarations

#### Ethics approval and consent to participate

This study was approved by the Ethics Committee Board of the Servicio de Salud Metropolitano Norte" (Folio N° 053/2023; Carta N°028/2024).

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

Received: 12 June 2024 / Accepted: 16 September 2024
Published online: 01 October 2024

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## Publisher's note