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Tracing human papillomavirus in skin and mucosal squamous cell carcinoma: a histopathological retrospective survey

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Abstract

Objective: The annual incidence of squamous cell carcinoma (SCC) has been increasing worldwide. The causative role of human papillomavirus (HPV) in SCC development of cutaneous has been controversial in the literature. In this study, we aimed to assess the presence of the histopathological features of HPV in SCC samples. Materials and Methods: This retrospective study was conducted at a tertiary referral skin center in 2020. Specimens of patients with a definite SCC diagnosis were evaluated for histopathological features of HPV, including koilocytosis, hyperkeratosis, acanthosis, hypergranulosis, parakeratosis, solar elastosis, papillomatosis, as well as the grade of the tumor. All the samples were re-evaluated by two dermatopathologists independently. Results: a total of 331 (male:female ratio= 3.9:1) cases of SCC were analyzed. The mean age was 68.1, with a 15.1 standard deviation. Most lesions were located on the face (40.5%), followed by the scalp (22.7%) and extremities (20.8%). Koilocytes were detected in 50 (15.1%) of lesions. The koilocytosis proportion was significantly higher in lesions on nails (38.1%, *P-value*= 0.007), oral cavity (36.8%, P-value= 0.014), and genitalia lesions (60.0%, *P-value*= 0.026). Although SCCs in-situ were found in 6.6% of our specimens, the highest koilocytosis proportion (64.7%) was detected in in-situ tumors, which was significantly more than other grades (*P-value*< 0.001). Conclusions: The histopathological features of HPV and in specific koilocytes can be frequently seen in SCC pathology. This association is more prominent in nail, oral, and genital lesions and is significantly higher in well-differentiated SCC.

Introduction

Non-melanoma skin cancer (NMSC) is the most common malignancy worldwide, increasing its annual incidence. The main subtypes of NMSC are basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) (Tavakolpour et al., 2017). Although BCC is much more prevalent than SCC, SCC shows more aggressive behavior, which leads to higher rates of local invasion and metastasis (Prieto-Granada and Rodriguez-Waitkus, 2015).

Several risk factors have been proven to have a role in SCC development, summarized as immunosuppression, fair skin, and exposure to ultraviolet (UV) radiation (Gloster and Neal, 2006). UV is known as a definite risk factor for SCC lesions in sun-exposed areas. Exploring the other risk factors of SCC development, some observations have favored the possibility of viral etiology for SCC (Aldabagh et al., 2013). The rates of SCC among organ transplant recipients are reported to be higher up to 100-fold in comparison to the general population (Jensen et al., 1999, Lindelöf et al., 2000). These rates are comparable to Kaposi sarcoma caused by herpesvirus-8 (Arron et al., 2011, Vajdic et al., 2006). Some behavioral patterns of specific types of SCC resemble viral lesions such as viral

warts. In the quest for finding the virus responsible for SCC development, human papillomavirus (HPV) is a highly appropriate candidate.

Koilocytosis is derived from the Greek term "*koilos*", meaning hollow. Koilocytosis is a histopathological feature characterized by the presence of koilocytes, occurring almost exclusively in HPV infection(Koss, 2012). HPV, as a non-enveloped DNA virus, has a well-established history in the pathogenesis of various cancerous and non-cancerous conditions(Gheit, 2019). E6 and E7 proteins transcribed by the DNA of HPV can lead to oncogenic effects on the host cell. The E4 protein is responsible for koilocytes formation by remodeling the cytoskeleton of squamous cells. Furthermore, E6, together with E5 protein, causes perinuclear cavitation and cleared-out appearance in HPV-infected cells. Although most of the infected cells are cleared by the immune system, a persistent HPV infection could cause malignant tumors, such as cervical cancer(Duensing and Münger, 2004, Gheit, 2019, Krawczyk et al., 2008, zur Hausen, 2002).

Contribution of HPV to the development of condyloma acuminata, verruca vulgaris, and SCCs in head and neck or anogenital regions have supported the presumed etiologic role of HPV in the initiation of cutaneous SCC (Cardoso and Calonje, 2011, Syrjänen, 2010). Of note, the role of β-HPV types in SCC occurrence in epidermodysplasia verrciformis (EV) patients has been proven, which gives a key for further research(Harwood et al., 2004). Based on these observations and findings, many studies have been conducted to determine the relationship between HPV and SCC in the general population. These studies have been performed with various study samples, techniques, methods on different HPV types. However, conflicting reports have made the answer to this question more complicated. Some studies have stated that there is no trace of HPV in SCC specimens, while others have reported HPV in some cutaneous SCCs with inconstant degrees of involvement. The detection rates mentioned for HPV in SCC are so diverse in studies. Thus, more studies are needed to clarify this crucial probable association, which can significantly impact the prevention, diagnosis, and treatment of a cluster of cutaneous SCCs.

In this study, we sought to determine the prevalence of koilocytosis as a marker for HPV infection in SCC lesions classified by organ.

Materials and Methods

This retrospective cross-sectional study was conducted in Razi hospital, Tehran University of Medical Sciences, Tehran, Iran, in 2020. In this study, we reviewed patients with SCC evaluated at the pathology department of the hospital from January 2014 to July 2020. The medical records of patients were retrieved from archives, and patients with underlying immunosuppressive conditions or a history of ultraviolet light therapy were excluded from the study. The paraffin-embedded cutaneous SCC

samples were obtained from the pathology archives. The following histopathological parameters were assessed: hyperkeratosis, acanthosis, hypergranulosis, parakeratosis, solar elastosis, koilocytosis, papillomatosis, vascular/neuronal invasion, and the grade of the tumor. Koilocytosis (Figure 1) was defined as containing nuclear enlargement, a nucleus with irregular membrane and hyperchromasia containing coarse chromatins, and perinuclear cytoplasmic halo (Cho et al., 2005, Lawson et al., 2009). All the samples were re-evaluated by two expert pathologists independently, and a third pathologist was consulted in case of difference.

Statistical analysis was performed with SAS software, version 9.4 (SAS Institute). To provide a descriptive analysis of the data, frequency, percentage, median and interquartile range of parameters were used. Comparison of detection rates of koilocytosis and other features between lesions in different organs were tested by chi-square test, and Mann-Whitney U test was used to compare the age difference between groups. The statistical significance threshold was considered as *P-value* <0.05.

Results

From January 2014 to July 2020, a total of 331 patients with a definite diagnosis of the skin or mucosal SCC were included. Of these cases, 263 (79.5%) were male, and 68 (20.5%) were female, with a male to female ratio of 3.9:1. The mean and median age were 68.1 (SD= 15.1) and 68 (IQR= 23.0), respectively. The age was not significantly different between genders (median age of 65.5 in females vs. 69.5 in males, *P-value*= 0.87). SCC was most prevalent in the face (including lip, nose, ears, and other locations) found in 134 (40.5%) patients. Other anatomic locations were scalp (22.7%), extremities (20.8%), nail (6.3%), oral cavity (5.7%), genitalia (1.5%), and palmar region (1.2%). The details are demonstrated in Table 1.

The age of SCC onset in ear, nose, and scalp was significantly higher compared to other locations (*P-value*= 0.001, <0.001, <0.001, respectively). However, lip, oral cavity, and extremities lesions were significantly detected in younger ages (*P-value*= 0.03, 0.04, <0.001, respectively). A female predominance was seen in patients with nails and oral cavity lesions (*P-value*= 0.004 and 0.006, respectively), whereas scalp involvement was higher in male patients (*P-value*<0.001).

Regarding histological findings, koilocytosis was detected in 50 (15.1%) patients. For parakeratosis, hyperkeratosis, hypergranulosis, and papillomatosis, the percentage was 91.2%, 90.0%, 58.3%, and 35.3%, respectively. We found a significant difference in the presence of koilocytosis in nail, oral cavity, and genitalia lesions (*P-value*= 0.007, 0.014, 0.026, respectively). In other words, koilocytosis was reported in 60% of lesions located in genitalia, 38.1%, and 36.8% of nail and oral cavity locations, respectively. Regarding other histopathological findings, all SCC located in nails had hyperkeratosis,

which is significantly higher than other locations (*P-value*= 0.042). The highest proportion of hypergranulosis was found in palmar lesions (all of the lesions) following extremities (76.8%) (*P-value*=0.144 and 0.042, respectively). However, lesions in the oral cavity (15.8%) and lip (25%) had the lowest percentage of hypergranulosis (*P-value*= 0.021, <001, respectively). Solar elastosis was significantly higher in following locations; scalp (61.2%, *P-value*= 0.002), and face (61.2%, *P-value*= 0.002).

Regarding tumor grading, the majority of the lesions were well-differentiated (43.8%), followed by moderately differentiated (36.0%). Although SCC in-situ was found in 6.6% of our specimens, the highest koilocytosis proportion (64.7%) was detected in in-situ tumors, which was significantly more than other grades (*P-value*< 0.001). The lowest koilocytosis proportion (8.9%) was seen in tumors with poorly differentiated grading (*P-value*= 0.210). Also, poorly differentiated tumors significantly had the lowest papillomatosis percentage, with 11.1 percent (*P-value*< 0.001). The neural invasion was reported in 13 cases (3.9%) and mostly found in the scalp and lip lesions (5 patients in each location). Figure 2 illustrates the most important results of our investigation.

Discussion

In the present study, the histopathological features of HPV were evaluated in 331 pathology samples of SCC. Among the histopathological findings, koilocytes, the most specific histological feature of HPV, were detected in 50 (15.1%) patients. Furthermore, a significantly higher percentage of koilocytes were found in the nail, oral cavity, and genitalia lesions. These findings can be considered as a potential clue for the etiologic role of HPV in SCC, especially in the nail, oral cavity, and genital lesions.

Koilocytosis is known as a pathognomonic histopathological feature of HPV infections. Koilocytes are histologically observed in most HPV-infected lesions. They are large cells with nuclear enlargement and hyperchromasia (a nucleus undergoing pyknosis) beside a clear perinuclear cytoplasmic vacuolization (Okodo et al., 2020, Krawczyk et al., 2008, Reid et al., 1982, Roteli-Martins et al., 2001). Previous studies reported well-established associations between the presence of koilocytes with a variety of HPV-infected tumors such as anogenital cancers (Boon and Kok, 1985), laryngeal papillomatosis (Martins et al., 2008), esophageal carcinoma (Miller et al., 1997), urothelial cancers (Aggarwal et al., 2009), and breast cancers (Lawson et al., 2009).

HPV is a double-stranded DNA virus belonging to the Papillomaviridae family, comprising five major genera, namely α, β, ɣ, mu, and nu (Sias et al., 2019). Alpha families are the main virulent HPVs in humans and have different tissue tropism into the skin and mucosal surfaces (de Villiers et al., 2004). By now, more than 220 types of HPV have been detected and listed on the HPV Reference

Center website (www.hpvcenter.se). HPV serotypes with low risk for SCC include HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89. However, HPV types including HPV16 (most oncogenic), 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 known as HPV with high oncogenic (Bouvard et al., 2009, Halec et al., 2013, Mahmoudi et al., 2018). A global synthetic analysis showed that infections might have a causative role in about 15% of cancers, and HPV contributed to about 30% of infection-related cancers (Plummer et al., 2016). A huge bulk of evidence has supported the etiologic role of HPV in the development of SCC (Ashinoff et al., 1991, Dika et al., 2017, Iorga et al., 2020, Miyahara et al., 2011, Muñoz et al., 2003, Riddel et al., 2011, Sanderson and Ironside, 2002). Studies have shown that HPV was more frequently detected in SCC and benign lesions than in normal skin (Miyahara et al., 2011). A systematic review in 2015 concluded that cutaneous SCC was significantly correlated with HPV compared to normal skin in all patients. Also, immunosuppressed patients showed higher HPV prevalence than immunocompetent subjects (Wang et al., 2014). A study on 349 patients with skin lesions revealed that HPV was positive in 12%, 26%, 22%, 18%, and 26% of healthy skin samples, benign lesions, actinic keratoses, basal cell carcinomas, and squamous cell carcinomas (Forslund et al., 2007).

There is some evidence that HPV may have a more influential role in SCC formation in specific sites. In a study by Dika et al., HPV16 genomes were identified in 15 out of 41 nail SCC specimens (Dika et al., 2017). Moreover, in another study, HPV infection was found in 60% of periungual SCC specimens (Ashinoff et al., 1991). A systematic review of ungual and periungual SCC lesions deduced the critical role of HPV16 in tumor progression in the nails. HPV16 genome was found in about 75% of lesions. The HPV-associated SCCs were seen in males at a rate of 2:1, and nearly half of the lesions were in situ. The majority of HPV-positive SCC lesions were found in persistent and recalcitrant verrucae (Riddel et al., 2011). In our specimens, the presence of koilocytes was significantly higher in these regions than other regions; this suggests an oncogenic role of HPV in ungual areas in accordance with previous reports.

Regarding the role of HPV in oral SCCs, the findings presented herein reveal a high rate of koilocytosis detection and a higher female proportion. Furthermore, we found that the age of patients with oral SCCs at diagnosis is significantly lower. Previous reports showed HPV-positive tumors include 25.9% to 34.5% of the oral SCCs, and the detection rate of HPV was 6-fold more than other head and neck malignancies. HPV-associated SCCs were mostly diagnosed in younger patients (about 80% of patients were under 60-year). Furthermore, HPV infection in the oral SCC is associated with a better survival and treatment response rate (Gillison et al., 2000, Kreimer et al., 2005, Weinberger et al., 2006).

In the present study, koilocytosis was detected in 60% of genital SCCs, mostly similar to former reports. The rate of HPV detection in penile SCCs is nearly 46-48%, with HPV16 and 18 being the predominant subtypes. The highest frequency of HPV was seen in SCC lesions with warty and basaloid histological features (Iorga et al., 2020). Previously, HPV DNA was found in about 60% of genital malignancy in women (Muñoz et al., 2003).

Another important finding was the higher frequency of koilocytosis in well-differentiated tumors, matching those observed in earlier studies. Published data showed a better prognosis and survival rate for head and neck, and genitalia SCC cases with HPV-positive lesions regardless of applied treatment (Lassen et al., 2009, Licitra et al., 2006, Lindquist et al., 2007). For instance, the 5-year survival rate for patients with HPV16 E6 seropositive oropharyngeal tumor was about 30% higher than those who were seronegative (Kreimer et al., 2013). A study conducted by Ang et al. among patients with oropharyngeal SCC revealed a favorable response to the treatments and a higher sensitivity to radiotherapy in HPV-positive lesions (Ang et al., 2010). The better prognosis could probably be due to less genomic instability and comparatively less aneuploidy and chromosomal aberrations (Dahlstrand et al., 2004).

Some limitations are attributable to the present study. First, the retrospective nature of studies could make our study vulnerable to selection bias. Second, although observation of koilocytosis is characteristic of HPV infection, polymerase chain reaction (PCR) is the most reliable and accurate test to evaluate the presence of HPV in histology. In the present study, we only assessed histopathological features of HPV in SCC sections which is proportionately less specific than PCR (Abadi et al., 1998). A prospective investigation with biological assessments besides histopathological examination could provide more precise results with more validity for evaluating HPV role in SCC progression.

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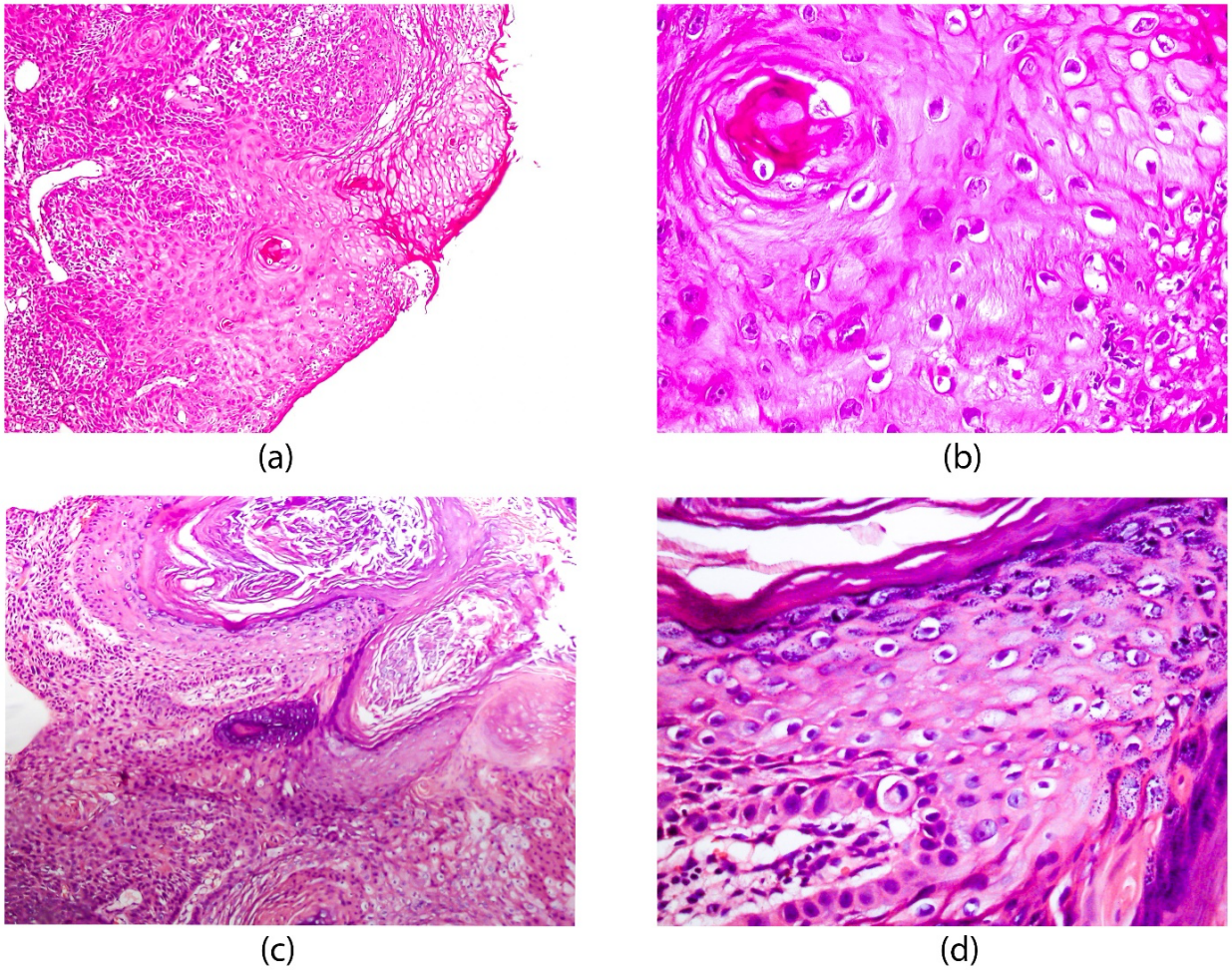

Figure 1. Histopathological features of two cutaneous skin lesion a, b) Acanthotic squamous epithelium with nuclear atypia and dyskeratotic cells, atypical squamous eddies, solar elastosis, and lymphocytic infiltration (H and E, ×40). c, d) A few clear cells with nuclear atypia in the outer layer of epidermis-Koilocytes (H and E, ×100).

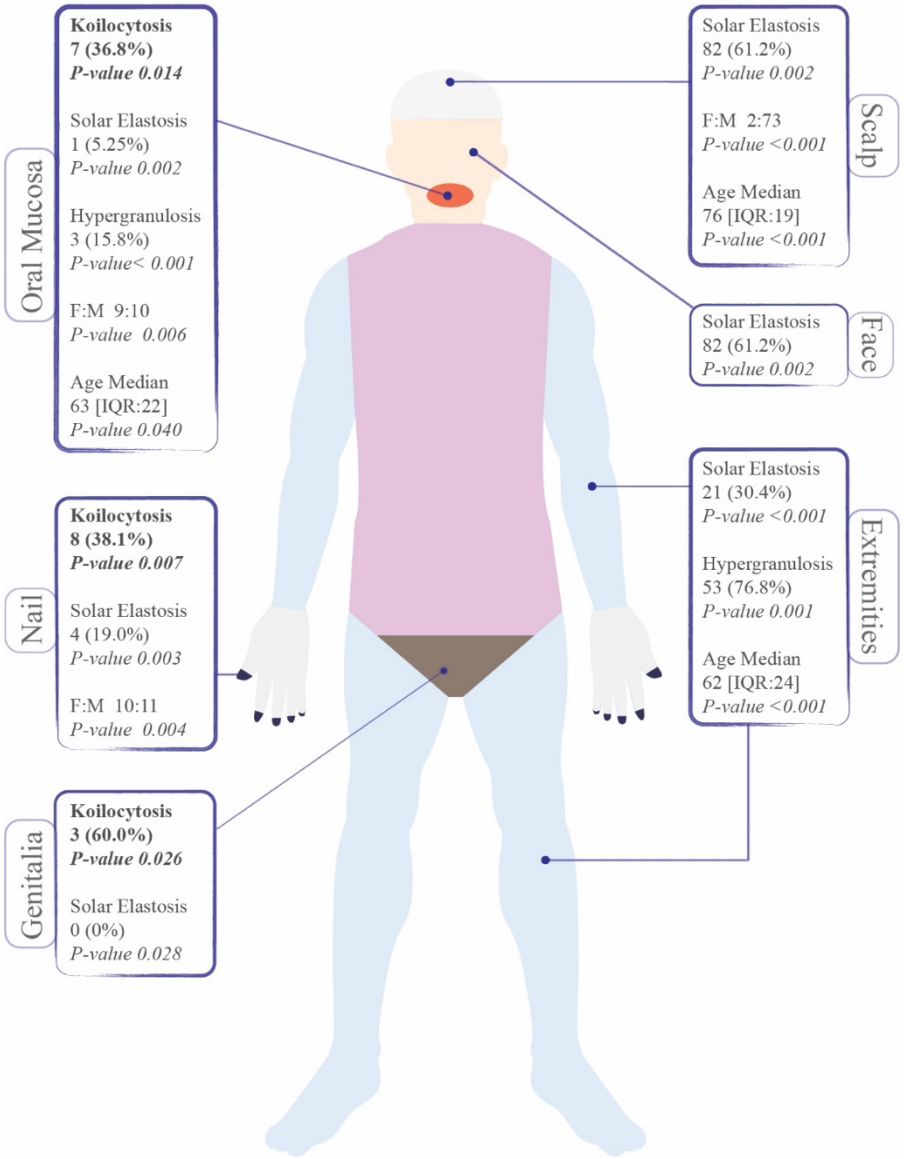

Figure 2. A schematic scheme showing the distribution and characteristics of SCC and histopathological features of HPV.

Table 1. Characteristics of the patients at diagnosis and histopathological features of their lesions.

Location	Total case, N	Age, Median [IQR]	Female: Male, N	Koilocytosis, N (%)	Papillomatosis, N (%)	Hypergranulosis, N (%)	Hyperkeratosis, N (%)	Parakeratosis, N (%)	Tumor grade, N (%)			
									In situ	I	II	III
Face	134	71.0 [22]	31: 103	17 (12.7)	44 (32.8)	73 (54.5)	120 (89.6)	123 (91.8)	8 (6.0)	57 (42.5)	50 (37.3)	19 (14.2)
Lip	60	64.0 [22]	11: 49	7 (11.7)	20 (33.3)	27 (45.0)	55 (91.7)	56 (93.3)	2 (3.3)	27 (45.0)	23 (38.3)	8 (13.3)
Nose	17	74.0 [20]	6: 11	2 (11.8)	7 (41.2)	13 (76.5)	14 (82.3)	16 (94.1)	1 (5.9)	6 (35.3)	8 (47.1)	2 (11.8)
Ear	13	83.0 [17]	3: 10	1 (7.7)	3 (23.1)	6 (46.2)	11 (84.6)	12 (92.3)	1 (7.7)	8 (61.5)	2 (15.4)	2 (15.4)
Other facial subsites	44	74.0 [21]	11: 33	7 (15.9)	14 (31.8)	27 (61.4)	37 (84.1)	39 (88.6)	4 (9.1)	16 (36.4)	17 (38.6)	7 (15.9)
Oral cavity mucosa	19	63.0 [22]	9: 10	7 (36.8)	7 (36.8)	3 (15.8)	18 (94.7)	18 (94.7)	4 (21.1)	9 (47.4)	3 (15.8)	3 (15.8)
Scalp	75	76.0 [19]	2: 73	7 (9.3)	24 (32.0)	44 (58.7)	62 (82.7)	65 (86.7)	1 (1.3)	26 (34.7)	37 (49.3)	11 (14.7)
Extremities	69	62.0 [24]	11: 58	7 (10.1)	26 (37.7)	53 (76.8)	65 (94.2)	64 (92.8)	1 (1.4)	42 (60.9)	20 (29.0)	6 (8.7)

Genitalia	5	55.5 [24]	2: 3	3 (60.0)	3 (60.0)	3 (60.0)	4 (80.0)	5 (100)	4 (80.0)	0 (0)	1 (20.0)	0 (0)
Nail	21	70.0 [18]	10: 11	8 (38.1)	11 (52.4)	12 (57.1)	21 (100)	19 (90.5)	5 (23.8)	3 (14.3)	8 (38.1)	5 (23.8)
Palmar	4	62.0 [22]	2: 2	0 (0)	0 (0)	4 (100)	3 (75.0)	3 (75.0)	0 (0)	3 (75.0)	0 (0)	1 (25.0)
Trunk	4	52.5 [13]	3: 1	1 (25.0)	2 (50.0)	1 (25.0)	4 (100)	4 (100)	1 (25.0)	3 (75.0)	0 (0)	0 (0)
All body parts	331	68.0 [23]	68: 263	50 (15.1)	117 (35.3)	193 (58.3)	298 (90.0)	302 (91.2)	24 (7.3)	143 (43.8)	119 (36.0)	45 (13.6)