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

Ali Nili,[1,2] Maryam Daneshpazhooh,[2] Hamidreza Mahmoudi,[2] Kambiz Kamyab,[3] Shirin Taraz Jamshidi,[3,4] Saeed Soleiman-Meigooni,[1] Mohammad Darvishi[1,5]

[1]Infectious Diseases and Tropical Medicine Research Center, AJA University of Medical Sciences, Tehran; [2]Autoimmune Bullous Diseases Research Center, Tehran University of Medical Sciences, Tehran; [3]Department of Dermatopathology, Tehran University of Medical Sciences, Tehran; [4]Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad; [5]Department of Aerospace and Subaquatic Medicine, AJA University of Medical Sciences, Tehran, Iran

Abstract

Worldwide, squamous cell carcinoma (SCC) incidence is rising. The literature debates the human papillomavirus (HPV)'s role in cutaneous SCC development. We examined HPV histopathology in SCC samples in this study. Retrospective study at tertiary referral skin center in 2020. Histopathological features of HPV, including koilocytosis, hyperkeratosis, acanthosis, hypergranulosis, parakeratosis, solar elastosis, papillomatosis, and tumor grade, were examined in SCC specimens. Two dermatopathologists independently reevaluated all samples. We examined 331 SCC cases (male:female ratio = 3.9:1). The mean age was 68.1, with 15.1 standard deviation. Lesions were most common on the face (40.5%), scalp (22.7%), and extremities (20.8%). Koilocytes were found in 50 (15.1%) lesions. Nail (38.1%, p=0.007), oral cavity (36.8%, p=0.014), and genitalia (60.0%, p=0.026) lesions had higher koilocytosis rates. SCCs were found in 6.6% of specimens, but *in situ* tumors had the highest koilocytosis (64.7%), significantly higher than other grades (p<0.001). SCC pathology often shows HPV and specific koilocyte histopathology. Well-differentiated SCC has a stronger association with nail, oral, and genital lesions.

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).[1] Although BCC is much more prevalent than SCC, SCC shows more aggressive behavior, which leads to higher rates of local invasion and metastasis.[2]

Several risk factors have been proven to have a role in SCC development, summarized as immunosuppression, fair skin, and exposure to ultraviolet (UV) radiation.[3] 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.[4] The rates of SCC among organ transplant recipients are reported to be higher up to 100-fold in comparison to the general population.[5,6] These rates are comparable to Kaposi sarcoma caused by herpesvirus-8.[7,8] 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.[9] HPV, as a non-enveloped DNA virus, has a well-established history in the pathogenesis of various cancerous and non-cancerous conditions.[10] 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 the 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.[10-13]

The contribution of HPV to the development of condyloma acuminata, verruca vulgaris, and SCCs in head and neck or anogenital regions has supported the presumed etiologic role of

HPV in the initiation of cutaneous SCC.[14,15] Of note, the role of β-HPV types in SCC occurrence in epidermodysplasia verruciformis patients has been proven, which gives a key for further research.[16] 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, and 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 a perinuclear cytoplasmic halo.[17,18] 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 (IQR) 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 $p < 0.05$.

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:female ratio of 3.9:1. The mean and median age were 68.1 (standard deviation =15.1) and 68 (IQR= 23.0), respectively. The age was not significantly different between genders (median
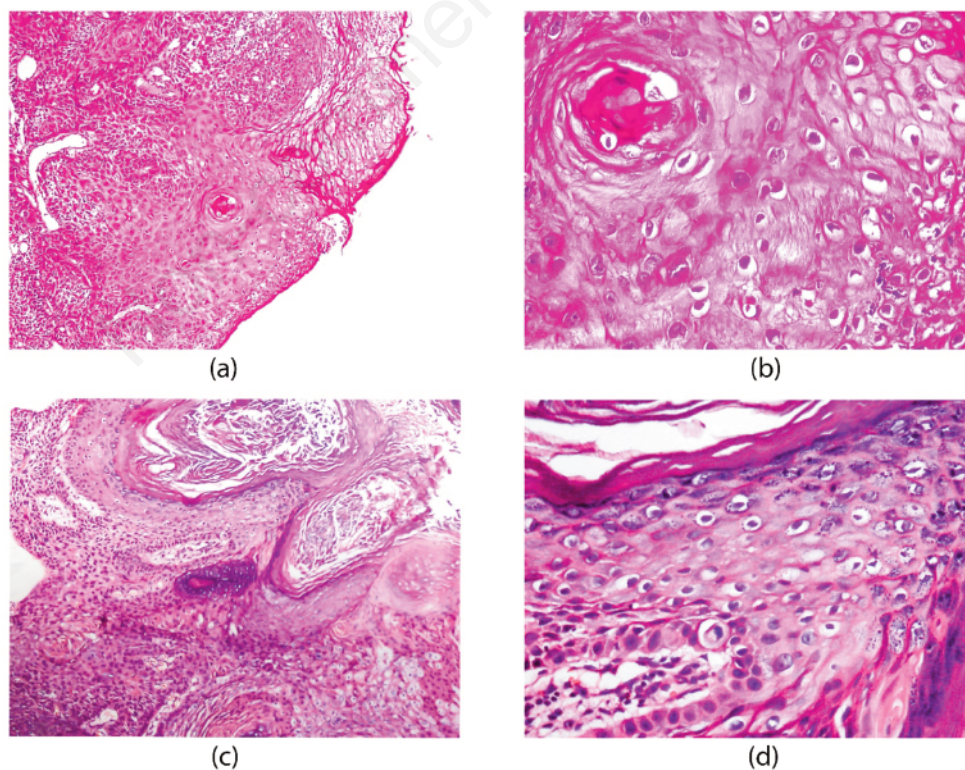

Figure 1. Histopathological features of two cutaneous skin lesions. **a, b**) Acanthotic squamous epithelium with nuclear atypia and dyskeratotic cells, atypical squamous eddies, solar elastosis, and lymphocytic infiltration (H and E, ×40); c, d) a few clear cells with nuclear atypia in the outer layer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

age of 65.5 in females *vs*. 69.5 in males, p=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 shown in Table 1. The age of SCC onset in ear, nose, and scalp was significantly higher compared to other locations (p=0.001, p<0.001, p<0.001, respectively). However, lip, oral cavity, and extremities lesions were significantly detected in younger ages (p=0.03, p=0.04, p<0.001, respectively). A female predominance was seen in patients with nails and oral cavity lesions (p=0.004 and p=0.006, respectively), whereas scalp involvement was higher in male patients (p<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 nails, oral cavity, and genitalia lesions (p=0.007, p=0.014, p=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 in other locations (p=0.042). The highest proportion of hypergranulosis was found in palmar lesions (all of the lesions) following

extremities (76.8%) (p=0.144 and p=0.042, respectively). However, lesions in the oral cavity (15.8%) and lip (25%) had the lowest percentage of hypergranulosis (p=0.021, p<001, respectively). Solar elastosis was significantly higher in the following locations; scalp (61.2%, p=0.002), and face (61.2%, p= 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 and was significantly higher than in other grades (p<0.001). The lowest koilocytosis proportion (8.9%) was seen in tumors with poorly differentiated grading (p=0.210). Also, poorly differentiated tumors significantly had the lowest papillomatosis percentage, with 11.1 percent (p<0.001). The neural invasion was reported in 13 cases (3.9%) and was 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 histolog-
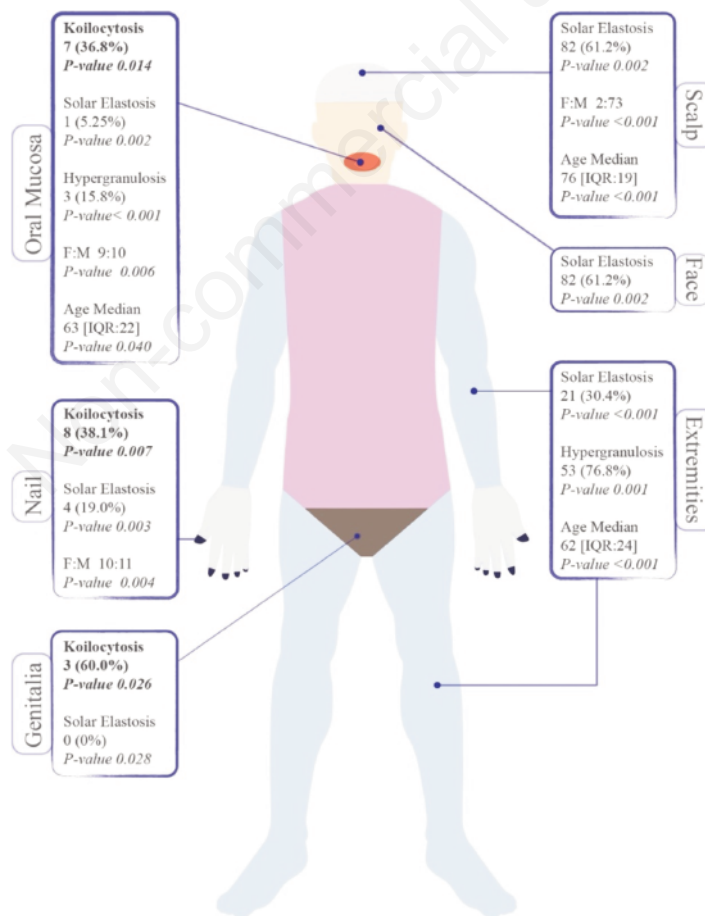

Figure 2. An illustrative scheme showing the distribution and characteristics of squamous cell carcinoma and histopathological features of human papillomavirus. F:M, female:male; IQR, interquartile range.

ical 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.[12,19-21] Previous studies reported well-established associations between the presence of koilocytes with a variety of HPV-infected tumors such as anogenital cancers,[22] laryngeal papillomatosis,[23] esophageal carcinoma,[24] urothelial cancers,[25] and breast cancers.[18]

HPV is a double-stranded DNA virus belonging to the Papillomaviridae family, comprising five major genera, namely α, β, ɣ, mu, and nu.[26] Alpha families are the main virulent HPVs in humans and have different tissue tropism into the skin and mucosal surfaces.[27] By now, more than 220 types of HPV have been detected and listed on the HPV Reference Center website. 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.[28-30] 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.[31] A huge bulk of evidence has supported the etiologic role of HPV in the development of SCC.[32-38] Studies have shown that HPV was more frequently detected in SCC and benign lesions than in normal skin.[35] 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.[39] 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.[40]

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.*,[33] HPV16 genomes were identified in 15 out of 41 nail SCC specimens. Moreover, in another study, HPV infection was found in 60% of periungual SCC specimens.[32] A systematic review of ungual and periungual SCC lesions deduced the critical role of HPV16 in tumor progression in the nails. The 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.[37] In our specimens, the presence of koilocytes was significantly higher in these regions than in others; 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 six-fold more than other head and neck malignancies. HPV-associated SCCs were mostly diagnosed in younger patients (about 80% of patients were under 60 years). Furthermore, HPV infection in the oral SCC is associated with a better survival and treatment response rate.[41-43]

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

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)

IQR, interquartile range.

being the predominant subtypes. The highest frequency of HPV was seen in SCC lesions with warty and basaloid histological features.[34] Previously, HPV DNA was found in about 60% of genital malignancies in women.[36]

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.[44-46] For instance, the 5-year survival rate for patients with HPV16 E6 seropositive oropharyngeal tumor was about 30% higher than those who were seronegative.[47] A study conducted by Ang *et al.*[48] among patients with oropharyngeal SCC revealed a favorable response to the treatments and a higher sensitivity to radiotherapy in HPV-positive lesions. The better prognosis could probably be due to less genomic instability and comparatively less aneuploidy and chromosomal aberrations.[49]

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.[50] A prospective investigation with biological assessments besides histopathological examination could provide more precise results with more validity for evaluating the HPV role in SCC progression.

References

1. Tavakolpour S, Daneshpazhooh M, Mahmoudi H. Skin Cancer: Genetics, Immunology, Treatments, and Psychological Care. In: Mehdipour P. (ed.). Cancer Genetics and Psychotherapy. Cham: Springer International Publishing; 2017.

2. Prieto-Granada C, Rodriguez-Waitkus P. Cutaneous squamous cell carcinoma and related entities: Epidemiology, clinical and histological features, and basic science overview. Curr Probl Cancer 2015;39:206-15.

3. Gloster HM Jr., Neal K. Skin cancer in skin of color. J Am Acad Dermatol 2006;55:741-60; quiz 761-4.

4. Aldabagh B, Angeles JG, Cardones AR, Arron ST. Cutaneous squamous cell carcinoma and human papillomavirus: is there an association? Dermatol Surg 2013;39:1-23.

5. Jensen P, Hansen S, Møller B, et al. Skin cancer in kidney and heart transplant recipients and different long-term immunosuppressive therapy regimens. J Am Acad Dermatol 1999;40:177-86.

6. Lindelöf B, Sigurgeirsson B, Gäbel H, Stern RS. Incidence of skin cancer in 5356 patients following organ transplantation. Br J Dermatol 2000;143:513-9.

7. Arron ST, Jennings L, Nindl I, et al. Viral oncogenesis and its role in nonmelanoma skin cancer. Br J Dermatol 2011;164:1201-13.

8. Vajdic CM, Mcdonald SP, Mccredie MR, et al. Cancer incidence before and after kidney transplantation. JAMA 2006;296:2823-31.

9. Koss LG. The 57th birthday of koilocytes. Cancer Cytopathol 2012;120:421.

10. Gheit T. Mucosal and Cutaneous Human Papillomavirus Infections and Cancer Biology. Front Oncol 2019;9.

11. Duensing S, Münger K. Mechanisms of genomic instability in human cancer: Insights from studies with human papillomavirus oncoproteins. Int J Cancer 2004;109:157-62.

12. Krawczyk E, Suprynowicz FA, Liu X, et al. Koilocytosis: a cooperative interaction between the human papillomavirus E5 and E6 oncoproteins. Am J Pathol 2008;173:682-8.

13. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nat Rev Cancer 2002;2:342-50.

14. Cardoso JC, Calonje E. Cutaneous manifestations of human papillomaviruses: a review. Acta Dermatovenerol Alp Pannonica Adriat 2011;20:145-54.

15. Syrjänen S. The role of human papillomavirus infection in head and neck cancers. Ann Oncol 2010;21:vii243-5.

16. Harwood CA, Surentheran T, Sasieni P, et al. Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. Br J Dermatol 2004;150:949-57.

17. Cho NH, Kang S, Hong S, et al.. Multinucleation of koilocytes is in fact multilobation and is related to aberration of the G2 checkpoint. J Clin Pathol 2005;58:576-82.

18. Lawson JS, Glenn WK, Heng B, et al. Koilocytes indicate a role for human papilloma virus in breast cancer. Br J Cancer 2009;101:1351-6.

19. Okodo M, Okayama K, Teruya K, et al. Koilocytic changes are not elicited by human papillomavirus genotypes with higher oncogenic potential. J Med Virol 2020;92:3766-73.

20. Reid R, Stanhope CR, Herschman BR, et al. Genital warts and cervical cancer. I. Evidence of an association between subclinical papillomavirus infection and cervical malignancy. Cancer 1982;50:377-87.

21. Roteli-Martins CM, Derchain SF, Martinez EZ, et al. Morphological diagnosis of HPV lesions and cervical intraepithelial neoplasia (CIN) is highly reproducible. Clin Exp Obstet Gynecol 2001;28:78-80.

22. Boon ME, Kok LP. Koilocytotic lesions of the cervix: the interrelation of morphometric features, the presence of papilloma-virus antigens, and the degree of koilocytosis. Histopathology 1985;9:751-63.

23. Martins RH, Dias NH, Gregório EA, et al. Laryngeal papillomatosis: morphological study by light and electron microscopy of the HPV-6. Braz J Otorhinolaryngol 2008;74:539-43.

24. Miller BA, Davidson M, Myerson D, et al. Human papillomavirus type 16 DNA in esophageal carcinomas from Alaska Natives. Int J Cancer 1997;71:218-22.

25. Aggarwal S, Arora VK, Gupta S, et al. Koilocytosis: correlations with high-risk HPV and its comparison on tissue sections and cytology, urothelial carcinoma. Diagn Cytopathol 2009;37:174-7.

26. Sias C, Salichos L, Lapa D, et al. Alpha, Beta, gamma human papillomaviruses (HPV) detection with a different sets of primers in oropharyngeal swabs, anal and cervical samples. Virol J 2019;16:27.

27. De Villiers EM, Fauquet C, Broker TR, et al. Classification of papillomaviruses. Virology 2004;324:17-27.

28. Bouvard V, Baan R, Straif K, et al. A review of human carcinogens--Part B: biological agents. Lancet Oncol 2009;10:321-2.

29. Halec G, Schmitt M, Dondog B, et al. Biological activity of probable/possible high-risk human papillomavirus types in cervical cancer. Int J Cancer 2013;132:63-71.

30. Mahmoudi H, Ghodsi SZ, Tavakolpour S, Daneshpazhooh M. Cryotherapy plus oral zinc sulfate versus cryotherapy plus placebo to treat common warts: A double blind, randomized,

placebo-controlled trial. Int J Womens Dermatol 2018;4:87-90.

31. Plummer M, De Martel C, Vignat J, et al. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health 2016;4:e609-16.

32. Ashinoff R, Li JJ, Jacobson M, et al. Detection of Human Papillomavirus DNA in Squamous Cell Carcinoma of the Nail Bed and Finger Determined by Polymerase Chain Reaction. Arch Dermatol 1991;127:1813-8.

33. Dika E, Venturoli S, Patrizi A, et al. The detection of human papillomavirus-16 in squamous cell carcinoma of the nail unit: A case series. J Am Acad Dermatol 2017;76:354-6.

34. Iorga L, Dragos Marcu R, Cristina Diaconu C, et al. Penile carcinoma and HPV infection (Review). Exp Therap Med 2020;20:91-6.

35. Miyahara GI, Simonato LE, Mattar NJ, et al. Correlation between koilocytes and human papillomavirus detection by PCR in oral and oropharynx squamous cell carcinoma biopsies. Memórias do Instituto Oswaldo Cruz 2011;106:166-9.

36. Muñoz N, Bosch FX, De Sanjosé S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 2003;348:518-27

37. Riddel C, Rashid R, Thomas V. Ungual and periungual human papillomavirus-associated squamous cell carcinoma: a review. J Am Acad Dermatol 2011;64:1147-53.

38. Sanderson RJ, Ironside JA. Squamous cell carcinomas of the head and neck. BMJ 2002;325:822-7.

39. Wang J, Aldabagh B, Yu J, Arron ST. Role of human papillomavirus in cutaneous squamous cell carcinoma: a meta-analysis. J Am Acad Dermatol 2014;70:621-9.

40. Forslund O, Iftner T, Andersson K, et al. Cutaneous human papillomaviruses found in sun-exposed skin: Beta-papillomavirus species 2 predominates in squamous cell carcinoma. J Infect Dis 2007;196:876-83.

41. Gillison ML, Koch WM, Capone RB, et al. Evidence for a causal association between human papillomavirus and a sub-

set of head and neck cancers. J Natl Cancer Inst 2000;92:709-20.

42. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14:467-75.

43. Weinberger PM, Yu Z, Haffty BG, et al. Molecular classification identifies a subset of human papillomavirus--associated oropharyngeal cancers with favorable prognosis. J Clin Oncol 2006;24:736-47.

44. Lassen P, Eriksen JG, Hamilton-Dutoit S, et al. Effect of HPV-associated p16INK4A expression on response to radiotherapy and survival in squamous cell carcinoma of the head and neck. J Clin Oncol 2009;27:1992-8.

45. Licitra L, Perrone F, Bossi P, et al. High-risk human papillomavirus affects prognosis in patients with surgically treated oropharyngeal squamous cell carcinoma. J Clin Oncol 2006;24:5630-6.

46. Lindquist D, Romanitan M, Hammarstedt L, et al. Human papillomavirus is a favourable prognostic factor in tonsillar cancer and its oncogenic role is supported by the expression of E6 and E7. Mol Oncol 2007;1:350-5.

47. Kreimer AR, Johansson M, Waterboer T, et al. Evaluation of human papillomavirus antibodies and risk of subsequent head and neck cancer. J Clin Oncol 2013;31:2708-15.

48. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363:24-35.

49. Dahlstrand H, Dahlgren L, Lindquist D, et al. Presence of human papillomavirus in tonsillar cancer is a favourable prognostic factor for clinical outcome. Anticancer Res 2004;24:1829-35.

50. Abadi MA, Ho GYF, Burk RD, et al. Stringent criteria for histological diagnosis of koilocytosis fail to eliminate overdiagnosis of human papillomavirus infection and cervical intraepithelial neoplasia grade 1. Human Pathol 1998;29:54-9.