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ORIGINAL ARTICLE

Relationship between α-genus human papillomavirus and non-genital seborrheic keratosis: Report of new cases and updated review

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Abstract

Background: Seborrheic keratoses (SK) are the most common acquired benign tumor that affects middle-aged or older adults with great cosmetic concern. Clinical and histopathological similarities of SK and common warts have been addressed by investigating the possible presence of human papillomavirus (HPV) DNA in SK. Previous studies suggested the association between α-genus HPV and SK located on genital skin, whereas the causal relationship between α-HPV and non-genital SK remains controversial.

Aim: This study aimed to clarify the pathogenic involvement of α-HPV in the development of non-genital SK.

Methods: We analyzed α-HPV DNA prevalence and HPV genotypes using a PCR-based microarray on 51 skin samples presenting with histologically confirmed SK without any malignant changes. Correlation between the histological subtype of SK and their HPV DNA-positive reactivity was also evaluated.

Results: Of 51 non-genital SK, two (3.9%) skin samples were positive for α-HPV DNA; high-risk HPV 31 and low-risk HPV 42 were found. Evaluation of HPV prevalence in different histological types of SK showed that both HPV-positive cases were acanthotic type; 14.3% of acanthotic SK lesions were positive, while all of the other types were negative for α-HPV.

Conclusions: This study demonstrates that α-HPV positivity is very rare in common non-genital SK. The rare α-HPV-positive SK lesions histologically belonged to the acanthotic type, implying a potential impact of HPV infection on epidermal hyperproliferation. Although a possible association cannot be excluded, our findings suggest that α-HPV is not a major causative factor for non-genital SK.

KEYWORDS
human papillomavirus, non-genital, seborrheic keratosis, α-HPV

Tobias Nellessen and Rui Aoki should be considered as joint first author.

1 | INTRODUCTION

Seborrheic keratoses (SK) are the most common acquired benign epithelial tumor that can occur anywhere on the skin of middle-aged or older adults.[1] Despite their high incidence, the etiology of SK is not entirely clear. SK are considered to result from clonal expansion of mutated epidermal keratinocytes.[2] Also, they exhibit histologic evidence of proliferation with hyperkeratosis, papillomatosis, and acanthosis as seen also in verrucae vulgares (VV). SK become progressively verrucous, and multiple SKs can occur patterned linearly[1]; this configuration can also be seen in VV and is known as Koebner phenomenon.[3] Over the past 30 years, these clinical and histopathological similarities between SK and VV have been addressed many times by investigation of the possible presence of human papillomavirus (HPV) DNA in SK.[3-14] Multiple studies suggested the association between α-genus HPV and SK located on genital skin; the detection rates of HPV DNA ranged from 42% to 72%.[4-7] Reports analyzing non-genital SKs showed HPV positivity rates ranging from 0% to 91%.[3,5-11] Hence, this topic remains controversial. To clarify the causal relationship between α-HPV and non-genital SK, we examined the presence of α-HPV DNA from non-genital SK lesions by polymerase chain reaction (PCR). Furthermore, we evaluated which histologic types of SK could be related to HPV positivity.

ETHICAL APPROVAL

This study was approved by the local ethics commission of the LMU-Munich (Project-ID: KB 20/002).

3 | MATERIALS AND METHODS

Fifty-one skin samples of patients presenting with histologically confirmed, non-genital SK without any malignant changes were collected from the pathology database of our institution. All histological samples were evaluated by experienced dermatohistopathologists. The study was approved by the local ethics committee. Clinical and histological characteristics of patients are depicted in Table 1. DNA was extracted from formalin-fixed paraffin-embedded tissue in the form of 10 μm-thick unstained slides. Deparaffinized tissue samples were digested as described previously.[15,16] The amplification of HPV DNA was performed via PCR using the HPV primer mix obtained from the VisionArray HPV Chip 1.0 kit (ZytoVision GmbH, Bremerhaven, Germany). The assay contained an internal positive control (by amplification of *HLA-DQA1*). The amplified HPV DNA was visualized by 1.5% agarose gel electrophoresis and then used for hybridization detecting 41 α-HPV subtypes (6, 11, 16, 18, 26, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 61, 62, 66, 67, 68a, 68b, 69, 70, 72, 73, 81, 82 IS39, 82 MM4, 83, 84, 90, and 91) according to the manufacturer's instructions, evaluated using VisionArray Analyzer software (ZytoVision GmbH, Bremerhaven, Germany).

TABLE 1 Clinical and histological characteristics of patients' samples with seborrheic keratoses (SK)

Characteristic	Cases (%) $n = 51$
Age (years)	
mean ± SD	67.2 ± 13.2
range	21–91
Gender	
Female	21 (41.2)
Male	30 (58.8)
Location	
Scalp	4 (7.8)
Face	20 (39.2)
Neck	1 (2.0)
Trunk	23 (45.1)
Arm	1 (2.0)
Leg	2 (3.9)
Histological type	
Acanthotic	14 (27.5)
Hyperkeratotic	14 (27.5)
Irritated	12 (23.5)
Mixed	5 (9.8)
Reticulated	4 (7.8)
Clonal	2 (3.9)

Abbreviation: SD, standard deviation.

4 | RESULTS

The mean age of patients with SK was 67.2 ± 13.2 years, ranging from 21 to 91 years (Table 1). All SK samples were derived from non-genital localizations. Sun exposed skin of face (39.2%) and trunk (45.1%) were common sites for SK. Of all 51 samples, 2 (3.9%) were positive for α-HPV DNA; high-risk HPV type 31 was found in a face sample from a male patient at age 77, and low-risk HPV type 42 was found in a leg sample also from a male patient at age 57.

SK show a variety of histological subtypes, and more than one type is often found in the same lesion. We divided our SK samples into the following six categories: acanthotic, hyperkeratotic, irritated, reticulated, clonal, and mixed type (Table 1). Evaluation of HPV prevalence in different SK types revealed that both HPV-positive cases belonged to the acanthotic type; two of 14 acanthotic SK samples (14.3%) were positive, while all of the other types were negative for HPV (Table 2). No viral cytopathic effect (koilocytosis) was found in the HPV-positive skin samples.

5 | DISCUSSION

The pathogenic contribution of HPV to the development of SK has been discussed in previous studies (Table 3). Regarding genital SK,

TABLE 2 Prevalence of high-risk and low-risk α-human papillomavirus (HPV) genotypes in different histological subtypes of seborrheic keratoses (SK)

Histological type	High-risk α-HPV		Low-risk α-HPV	
	Positive case (%)	Genotype	Positive case (%)	Genotype
Acanthotic ($n = 14$)	1 (7.1)	31	1 (7.1)	42
Hyperkeratotic ($n = 14$)	0	–	0	–
Irritated ($n = 12$)	0	–	0	–
Mixed ($n = 5$)	0	–	0	–
Reticulated ($n = 4$)	0	–	0	–
Clonal ($n = 2$)	0	–	0	–
Total ($n = 51$)	1 (2.0)		1 (2.0)	

TABLE 3 Previous reports on human papillomavirus (HPV) prevalence in genital and non-genital seborrheic keratoses (SK) and the clinical and histological features of HPV-positive cases (from 1990 to 2021)

Report	HPV prevalence	HPV genotype	Features of HPV-positive cases			
			Age (Mean)	Gender (M/F)	Location	Histological type
Leonardi (1991)	24/57 (42%) of genital SK	6, 11, 16	31.9	18/6	Genitalia, crus	ND
Soler (1992)	1/1 of non-genital SK[a]	5	ND	ND	Non-genitalia	ND
Zhu (1992)	23/43 (53%) of genital SK	6, 11, 33	32.6	12/11	Genitalia, thigh	NSD[e]
	1/29 (3.4%) of non-genital SK	6				
Tsambaos (1995)	34/173 (20%) of non-genital SK[a]	6/11, 31/33/35	ND	ND	ND	Acanthotic and hyperkeratotic type
Lee (2001)	0/40 (0%) of non-genital SK[b]	–	–	–	–	–
Bai (2003)	18/25 (72%) of vulvar SK	6, others	ND	ND	Vulva, non-genitalia	ND
	3/20 (15%) of non-genital SK	6, 16/18				
Gushi (2003)	30/104 (29%) of non-genital SK[a]	18, 6, 2, 1	ND	ND	Head, trunk, leg	ND
	95/104 (91%) of non-genital SK[c]					
Li (2004)	42/55 (76%) of non-genital SK[d]	20, 23, 5, X7, 17, 37, 17b, X4, X4b, SK3	ND	28/14	Face, neck, trunk, extremities	ND
Tardio (2012)	28/40 (70%) of genital SK	6, 18, 35, 55	32	23/5	Genitalia	NSD
	2/20 (10%) of non-genital SK	6	33	1/1	Groin, suprapubic	
Reutter (2014)	3/21 (14%) of vulvar SK	6, unknown	60.7	0/3	Vulva	NSD
	0/10 (0%) of non-genital SK	–	–	–	–	–
Part (2014)	1/1 of genital SK	6	33	1/0	Penis	Acanthotic type
Wu (2015)	5/17 (29%) of SK with bowenoid transformation	ND	66	ND	Face, abdomen, thigh	Hyperkeratotic type with bowenoid transformation
Current report (2021)	2/51 (3.9%) of non-genital SK	31, 42	67	2/0	Face, leg	Acanthotic type

Abbreviations: F, female; M, male; NSD, no significant difference between HPV-positive and HPV-negative cases; ND, not described; SK, seborrheic keratosis.

[a]Using *in situ* hybridization.

[b]Using *in situ* PCR or PCR with specific primers for HPV 6, 11, 31, and 33.

[c]Using PCR coupled with southern blot hybridization and sequencing.

[d]Using PCR with specific primers for epidermodysplasia verruciformis (EV)-associated HPVs.

[e]Perinuclear vacuolization of epithelial cells, mitosis, and dyskeratotic cells were more marked.

four previous reports showed a strong association with α-HPV.[4-7] α-HPV DNA was detected in 42%–72% of the genital SK samples from these groups,[4-7] while only the latest study reported a much lower positivity in 14% of vulvar SK by PCR.[11] Reutter et al. suggested that a possible reason for the low rate of HPV positivity could be the older age of the cohort with vulvar SK (67.5 years) compared to that of patients in the previous studies (eg, 41.4 years).[11] Although SK in genital lesions is frequently found in a younger age group with higher sexual activity compared to SK in non-genital lesions, they had no patient younger than 50 years old in their report.

In contrast to the genital lesions, α-HPV was infrequently found in non-genital SK, ranging from 0% to 20%[5-9,11] in most reports, including ours (Table 3), whereas two studies reported a high frequency of HPV, which accounted for 76% and 91% of non-genital SK.[3,10] The variation was probably due to molecular techniques including DNA extraction and PCR primers used for HPV detection as well as the criteria of sample selection. As the authors documented, these higher incidences can be attributed to the use of highly sensitive methods; Gushi et al. used PCR coupled with southern blot hybridization[3]; Li et al. used nested PCR with specific primers for epidermodysplasia verruciformis (EV)-associated HPVs.[10] Li et al. detected EV-associated HPV DNA in 15% of non-genital SK specimens in the first-step PCR, while after the second-step PCR, the detection rate reached 76%, suggesting a low copy number for EV-associated HPV DNA.[10] In addition, the prevalence of EV-associated HPV DNA increased with an increase in numbers of non-genital SK lesions: 91% in patients with more than five lesions and 43% in patients with only one lesion.[10] These results indicated that EV-associated HPV might be involved in the pathogenesis of non-genital SK; however, its role has remained poorly understood. In the present study, α-HPV DNA was only detected in 3.9% of non-genital SK cases despite using a reproducible and sensitive microarray-based assay. A limitation of our study is the fact that EV-associated HPVs were not addressed; thus, the possibility of EV-associated HPV infection in SK cannot be excluded.

The principal HPV DNA detected in SK lesions was low-risk α-HPV 6, which is usually present in benign warts, comprising 67% to 96% of HPV-positive cases investigated in previous studies.[3-7,11] Other HPV types were found only rarely, such as HPV 11, 16, and 33. We did not detect type 6, but instead found high-risk type 31 and low-risk type 42 in non-genital SK lesions. Both of the positive cases were described histologically as acanthotic type. Tsambaos et al. found that a large percentage of the acanthotic type were associated with HPV type 31/33/35.[8] This finding supports our data. It is possible that HPV infection might promote epithelial cell proliferation or epidermal hyperplasia might prolong the residence and replication of HPV.

Furthermore, Wu et al. reported HPV DNA detection in 29% of 17 rare cases of SK with bowenoid transformation, suggesting a possible association of HPV with malignant transformation in SK.[12] Accumulating evidence has suggested the etiopathogenetic role of HPV in epithelial carcinogenesis. As seen in our previous report, high-risk α-HPV DNA was detected in 58% of 76 squamous cell carcinoma (SCC) and 38% of 34 Bowen's disease lesions, whereas no high-risk α-HPV was detected in 48 actinic keratosis (AK) lesions.[16] Our current data of HPV prevalence in non-genital SK were similar to the control data showing that 1 of 10 healthy control skins were positive for high-risk HPV type 16.[16]

This study demonstrates that α-HPV positivity is very rare in common non-genital SK. The rare α-HPV-positive cases histologically belonged to the acanthotic type; this result implies a potential impact of HPV infection on epidermal hyperproliferation. Otherwise, the acanthotic epidermis in SK might just play as a reservoir for HPV. Based on the previous reports, α-HPV may be related to SK at least partially in the genital area; however, the direct evidence has not been provided to date. Overall, our findings show a low positivity rate of α-HPV in non-genital SK, suggesting that α-HPV is probably not a major causative factor for non-genital SK.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Markus Reinholz (MR) designed this study. Tobias Nellessen (TN), Rui Aoki (RA), and Claudia Kammerbauer (CK) carried out the experiment. CK also provided enhanced technical support. TN and RA analyzed and interpreted the data, discussing with MR. TN drafted the manuscript, and RA critically revised it with support from MR. Benjamin M. Clanner-Engelshofen commented on the manuscript. Lars E. French supervised the project. All authors approved and contributed to the final version of the manuscript.

DATA AVAILABILITY STATEMENT

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

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REFERENCES

1. Kang S, Amagai M, Bruckner A, et al. *Fitzpatrick's Dermatology*. 9th ed. New York City: Mc Graw Hill Education; 2019.
2. Nakamura H, Hirota S, Adachi S, Ozaki K, Asada H, Kitamura Y. Clonal nature of seborrheic keratosis demonstrated by using the polymorphism of the human androgen receptor locus as a marker. *J Invest Dermatol*. 2001;116(4):506-510.
3. Gushi A, Kanekura T, Kanzaki T, Eizuru Y. Detection and sequences of human papillomavirus DNA in nongenital seborrhoeic keratosis of immunopotent individuals. *J Dermatol Sci*. 2003;31(2):143-149.
4. Leonardi CL, Zhu WY, Kinsey WH, Penneys NS. Seborrheic keratoses from the genital region may contain human papillomavirus DNA. *Arch Dermatol*. 1991;127(8):1203-1206.

5. Zhu WY, Leonardi C, Penneys NS. Detection of human papillomavirus DNA in seborrheic keratosis by polymerase chain reaction. *J Dermatol Sci*. 1992;4(3):166-171.
6. Bai H, Cviko A, Granter S, Yuan L, Betensky RA, Crum CP. Immunophenotypic and viral (human papillomavirus) correlates of vulvar seborrheic keratosis. *Hum Pathol*. 2003;34(6):559-564.
7. Tardio JC, Bancalari E, Moreno A, Martin-Fragueiro LM. Genital seborrheic keratoses are human papillomavirus-related lesions. A linear array genotyping test study. *APMIS*. 2012;120(6):477-483.
8. Tsambaos D, Monastirli A, Kapranos N, et al. Detection of human papillomavirus DNA in nongenital seborrhoeic keratoses. *Arch Dermatol Res*. 1995;287(6):612-615.
9. Lee ES, Whang MR, Kang WH. Absence of human papillomavirus DNA in nongenital seborrheic keratosis. *J Korean Med Sci*. 2001;16(5):619-622.
10. Li YH, Chen G, Dong XP, Chen HD. Detection of epidermodysplasia verruciformis-associated human papillomavirus DNA in nongenital seborrhoeic keratosis. *Br J Dermatol*. 2004;151(5):1060-1065.
11. Reutter JC, Geisinger KR, Laudadio J. Vulvar seborrheic keratosis: is there a relationship to human papillomavirus? *J Low Genit Tract Dis*. 2014;18(2):190-194.
12. Wu YH, Hsiao PF, Chen CK. Seborrheic keratosis with bowenoid transformation: the immunohistochemical features and its association with human papillomavirus infection. *Am J Dermatopathol*. 2015;37(6):462-468.
13. Soler C, Chardonnet Y, Euvrard S, Chignol MC, Thivolet J. Evaluation of human papillomavirus type 5 on frozen sections of multiple lesions from transplant recipients with in situ hybridization and non-isotopic probes. *Dermatology*. 1992;184(4):248-253.
14. Part M, Svecova D, Brezova D, Breza J. Giant seborrheic keratoses on penis. *J Sex Med*. 2014;11(12):3119-3122.
15. Marsela E, Fischbeck AJ, Hildebrand JA, et al. Coexistence of oncogenic human papillomavirus genotypes in condylomata acuminata among children and adolescents. *Acta Derm Venereol*. 2020;100(4):adv00061.
16. Aoki R, Clanner-Engelshofen BM, Charnowski S, Ruzicka T, Reinholz M. Distribution of high-risk alpha-genus human papillomavirus genotypes impacts cutaneous neoplasms. *J Eur Acad Dermatol Venereol*. 2019;33(7):1304-1311.

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