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Detection of Human Papillomavirus in Squamous Papilloma of the Esophagus

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Abstract

Introduction: The etiology of esophageal squamous papilloma (ESP) is largely unknown. Previous studies have shown a variable association with human papillomavirus (HPV) with conflicting data. The aim of this study was to further investigate the possible association of HPV in our ESP series using RNA in-situ hybridization (ISH) and compare study groups from the United States of America and China. *Methods:* Demographic and clinical data of patients with ESP were retrieved from the University of California Los Angeles (UCLA) (1/2016-3/2019) and Peking Union Medical College Hospital (PUMCH) (9/2014-3/2019) pathology databases. Hematoxylin and eosin slides were reexamined. Confirmed cases were examined by high- and low-risk HPV RNA ISH. *Results:* For the UCLA cohort, 13 429 upper endoscopies were performed and 78 biopsies from 72 patients were identified as ESP (F:M = 45:27, 66.7% > 45 years). Seventy-four (94.9%) biopsies were designated as polyps or nodules and 46.6% were located in the mid-esophagus. Other abnormal findings included gastroesophageal reflux disease (48.6%), hiatal hernia (38.9%), and esophagitis (36.1%). For the PUMCH cohort, 63 754 upper endoscopies were performed and 73 biopsies from 71 patients were identified as ESP (F:M = 48:23, 71.8% > 45 years). Sixty-four (87.7%) biopsies were designated as polyps or nodules and 57.5% were located in the mid-esophagus. Other abnormal findings included esophagitis (19.7%), and hiatal hernia (8.5%). No features of conventional cytologic dysplasia or viral cytopathic change were found. None of the cases was associated with squamous cell carcinoma, and none showed positive HPV RNA ISH results. *Conclusions:* No association was found between ESP and active HPV infection in our 2 cohorts. Other etiopathogenetic mechanisms, such as aging, might contribute to the development of these innocent lesions.

Keywords

esophagus, squamous papilloma, human papillomavirus, RNA in-situ hybridization

Introduction

Esophageal squamous papilloma (ESP) is a small papillary lesion of the squamous epithelium lining the esophagus. The reported prevalence is between 0.01% and 0.57%.[1–3] Most ESPs are solitary and incidentally found at endoscopy. The etiology of this benign lesion is controversial and largely unknown. Considering the higher prevalence at the lower third of the esophagus in a few studies,[4–6] chronic irritation was suggested as a causative mechanism along with gastroesophageal reflux disease (GERD), chemical injury, and mechanical injury. Infection by human papillomavirus (HPV) is another proposed etiology.[7] HPV-mediated pathogenesis from papilloma to carcinoma is well known in the uterine cervix, anogenital region, and oropharynx. However, the role of HPV in ESP pathogenesis remains debatable. Previous studies have shown a variable association with HPV with conflicting data. In some studies, the presence of HPV in ESPs was not detected,[2,4,8] whereas in others, up to or more than 50% of cases were found to harbor HPV.[1,5,7] The purpose of this study was to further investigate the possible association of

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HPV in our ESP series by using the up-to-date RNA in-situ hybridization (RNA ISH) technology, and by comparing study groups from the United States and China.

Methods

Patients

Demographic and clinical data of patients with a biopsy-proven diagnosis of ESP were retrieved from the UCLA (University of California Los Angeles, USA) pathology database from January 2016 through March 2019, and PUMCH (Peking Union Medical College Hospital, Beijing, China) pathology database from September 2014 through March 2019. Hematoxylin and eosin (H&E)-stained slides were reexamined by 2 authors (YL and HLW) to confirm the diagnosis. Esophageal squamous cell carcinoma, which can grow as papillomatous lesions, as well as papillomatosis, were not included in this study.

Data Collection

Data obtained from electronic medical records included patient demographics, size and location of the lesion in the esophagus (distance from incisor teeth), indications of endoscopy, and endoscopic findings. The location was categorized as proximal (<24 cm from incisors), mid (24-32 cm from incisors), and distal (>32 cm from incisors) portions of the esophagus. The medical records were also searched for any history of oropharyngeal, gastrointestinal, or other malignancies. Any subsequent upper endoscopy for recurrence of the lesions was noted. *Helicobacter pylori* (*H. pylori*) status, if available, was recorded.

HPV RNA ISH

All confirmed cases with sufficient available tissue samples were tested by HPV RNA ISH using cocktail RNAscope® probes that covered the most common high- and low-risk HPV serotypes provided by Advanced Cell Diagnostics (ACD; Hayward, CA, USA). The cocktail for high-risk HPV (BOND RNA Scope HPV-High Risk 18 PROBE [ACD BIO 312598]) recognizes E6/E7 mRNA of 18 different subtypes including HPV 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82. The cocktail for low-risk HPV (BOND RNA Scope HPV-Low Risk 10 PROBE [ACD BIO 314558]) recognizes E6/E7 mRNA of 10 different subtypes including HPV 6, 11, 40, 43, 44, 54, 69, 70, 71, and 74. Positive controls included cervical low-grade squamous intraepithelial lesion (LSIL) for low-risk HPV probes and cervical adenocarcinoma in situ for high-risk HPV probes. Control probes included BOND RNA Scope HPV RNA positive control probe (PPIB, Leica RS7755) and BOND RNA Scope HPV RNA negative control probe (dapB, Leica RS7756), which were used to determine the integrity of mRNA within the cells. Cases positive by PPIB but negative by dapB were considered suitable for analysis by the testing probes for HPV. RNAscope® was performed using the BOND III advanced staining clinical platform (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's instructions for UCLA cases. The PUMCH cases were performed manually according to the supplier's instructions. Briefly, formalin-fixed, paraffin-embedded tissue sections in 5-μm thickness were deparaffinized and dehydrated, followed by serial treatments with the Pre-Treatment 1 solution and Pre-Treatment 2. Pre-Treatment 3 was performed overnight. The sections were hybridized in a HybEZ^{TM} Oven (ACD, Hayward, USA) with HPV probes through a serial application of Amp 1-6. Diaminobenzidine was used to visualize amplified signals. The sections were counterstained with hematoxylin, dehydrated with graded ethanol and xylene, and coverslipped for microscopic observation.

All slides were initially interpreted by YL and interdependently verified by HLW (for UCLA cases) and WZ (for PUMCH cases). Cases were considered positive if cells of interest demonstrated dark brown, fine granular cytoplasmic, and nuclear positivity easily visible under a 10 × objective lens.

Results

Clinical and Endoscopic Features

For the UCLA study group, 13 429 upper endoscopies were performed from January 2016 through March 2019,

Table 1. Demographic and Clinical Data of UCLA and PUMCH Patients.

		UCLA (n = 72) No. (%)	PUMCH (n = 71)[a] No. (%)
Sex	Male:Female	27:45	23:48
Age (years)	Mean (range)	51.3 (23-81)	49.9 (24-77)
	< 45	24 (33.3)	20 (28.2)
	≥ 45	48 (66.7)	51 (71.8)
ESP location in esophagus	Proximal	22 (30.1)	14 (19.2)
	Mid	34 (46.6)	42 (57.5)
	Distal	17 (23.3)	17 (23.3)
	N/A	5	0
No. of lesion	Single	62 (86.1)	61 (85.9)
	Multiple	10 (13.9)	10 (14.1)
Endoscopic description	Polyp	44 (56.4)	18 (24.7)
	Sessile polyp	5 (6.4)	5 (6.8)
	Nodule	26 (33.3)	41 (56.2)
	Papule	1 (1.3)	
	Wart-like mucosal projection	1 (1.3)	5 (6.8)
	White plaque	1 (1.3)	4 (5.5)
Size (mm)	Mean (range)	4.1 (1-10)	3.7 (1-15)

Abbreviations: ESP, esophageal squamous papilloma; N/A, not available; UCLA, University of California Los Angeles; PUMCH, Peking Union Medical College Hospital.

[a]One patient with 4 biopsy specimens diagnosed as esophageal squamous papillomatosis is not included.

of which 9870 had esophageal biopsies (Table 1). Among them, 78 biopsies (0.58%) from 72 patients were diagnosed with ESP. There were 27 men and 45 women (male:female = 1:1.7). The mean age was 51.3 years (range, 23-81 years), with 48 (66.7%) patients being over 45 years. Sixty-two (86.1%) patients had a single lesion. The other 10 patients had 2 to "a few" lesions. Most ESPs were located in the mid-esophagus (34/73, 46.6%), followed by proximal esophagus (22/73, 30.1%), and distal esophagus (17/73, 23.3%). The location of the other 5 lesions was not specified in endoscopy reports. Seventy-five (96.2%) biopsies were described as polyps, sessile polyps, or nodules endoscopically. The remaining 3 biopsies were described as a papule (n = 1), wart-like mucosal projection (n = 1), and white plaque (n = 1). The size of the lesions varied from 1 to 10 mm (mean, 4.1 mm), and 49/78 (62.8%) lesions were <5 mm in size. Only 2 cases (2.6%) measured 10 mm and none of the lesions were >10 mm. Follow-up endoscopy was done in 8 subjects (11.1%) after 3 to 30 months; none showed recurrence of the esophageal lesions.

For the PUMCH study group, 63 754 upper endoscopies were performed from September 2014 through March 2019, of which 2850 had esophageal biopsies. Among them, 73 biopsies (0.11%) from 71 patients showed histologically confirmed diagnosis of ESP. There were 23 men and 48 women (male:female = 1:2.1). The mean age was 49.9 years (range, 24-77 years), with 51 (71.8%) patients being over 45 years. Sixty-one (85.9%) patients had a single lesion. The other 10 patients had 2 or more lesions. The majority of ESPs were located in the mid-esophagus (42/73, 57.5%), followed by the distal (17/73, 23.3%), and proximal (14/73, 19.2%) esophagus. Sixty-four (87.7%) biopsies were described as polyps, sessile polyps, or nodules endoscopically. The remaining 9 lesions were described as wart-like mucosal projection (n = 5) or plaque (n = 4). The size of the lesions varied from 1 to 15 mm (mean, 3.7 mm), and 59 (80.8%) lesions were <5 mm in size. Four cases (5.5%) were ≥10 mm. Follow-up endoscopy was done in 12 patients (16.9%) after 12 to 30 months. Three patients who had ESPs partially removed during the initial endoscopy showed the existence of residual lesions. The other 9 patients showed no recurrence of the lesions during follow-up.

Indications for Endoscopy and Additional Endoscopic Findings

For the UCLA cohort, the indication for endoscopy was unavailable for one patient. For the remaining 71 patients, 22 (31.0%) had the procedure for GERD, 15 (21.1%) for abdominal pain, 12 (16.9%) for dysphagia, 7 (9.9%) for eosinophilic esophagitis, 4 (5.6%) for Crohn disease, 3 (4.2%) for ulcerative colitis, and 3 (4.2%) for early satiety. Other indications for upper endoscopy included dyspepsia (n = 1), Barrett esophagus (n = 1), esophagitis (n = 1), juvenile polyposis syndrome (n = 1), and small bowel bleeding (n = 1). In addition to ESP, other endoscopic findings in the esophagus included reflux esophagitis (n = 35, 48.6%), hiatal hernia (n = 28, 38.9%), esophagitis (not specified) (n = 26, 36.1%), eosinophilic esophagitis (n = 7, 9.7%), inlet patch (n = 2, 2.8%), and Barrett esophagus (n = 1, 1.4%). *H. pylori* gastritis was found in 1.6% (1/61) of patients who also underwent gastric biopsies.

In terms of the PUMCH study group, the indication for endoscopy was unavailable for 3 patients. For the remaining 68 patients, 18 (26.5%) had the procedure for abdominal pain, 14 (20.6%) for GERD, 12 (17.6%) for dysphagia, and 11 (16.2%) for gastritis. Other reasons included prior history of esophageal mucosal lesion (n = 2), gastric cancer (n = 2), gastric polyp (n = 2), dyspepsia (n = 2), bloating (n = 2), esophagitis (n = 1), GI bleeding (n = 1), and routine check-up (n = 1). In addition to ESP, other endoscopic findings in the esophagus included esophagitis (not specified) (n = 14, 19.7%), and hiatal hernia (n = 6, 8.5%). *H. pylori* gastritis was found in 22.5% (16/71) of patients who also underwent gastric biopsies.

Histopathologic Features

Histologic examination of biopsied esophageal lesions showed acanthotic squamous fronds with fibrovascular lamina propria. Two distinctive growth patterns were recognized. The endophytic pattern was characterized by a dome-like smooth surface with an inverted papillomatous configuration of squamous proliferation (Figure 1A and B). The exophytic pattern featured finger-like projections (Figure 1C and D). No features of conventional cytologic dysplasia nor viral cytopathic change were found (Figure 1E). None of the cases was associated with squamous cell carcinoma.

HPV RNA ISH Results

A total of 148 biopsies with a confirmed diagnosis of ESP (78 from UCLA, 70 from PUMCH) were tested for high- and low-risk HPV by RNAscope®. Positive controls worked well but none of the tested cases showed positive results (Figure 2). The test could not be performed in 3 samples from PUMCH due to the lack of adequate tissue.

Discussion

ESP is a benign wart-like exophytic or endophytic squamous lesion that rarely exceeds 1 cm in size. Patients with ESP are typically asymptomatic and the diagnosis is almost always incidentally made during upper endoscopy performed for a different indication. The prevalence of

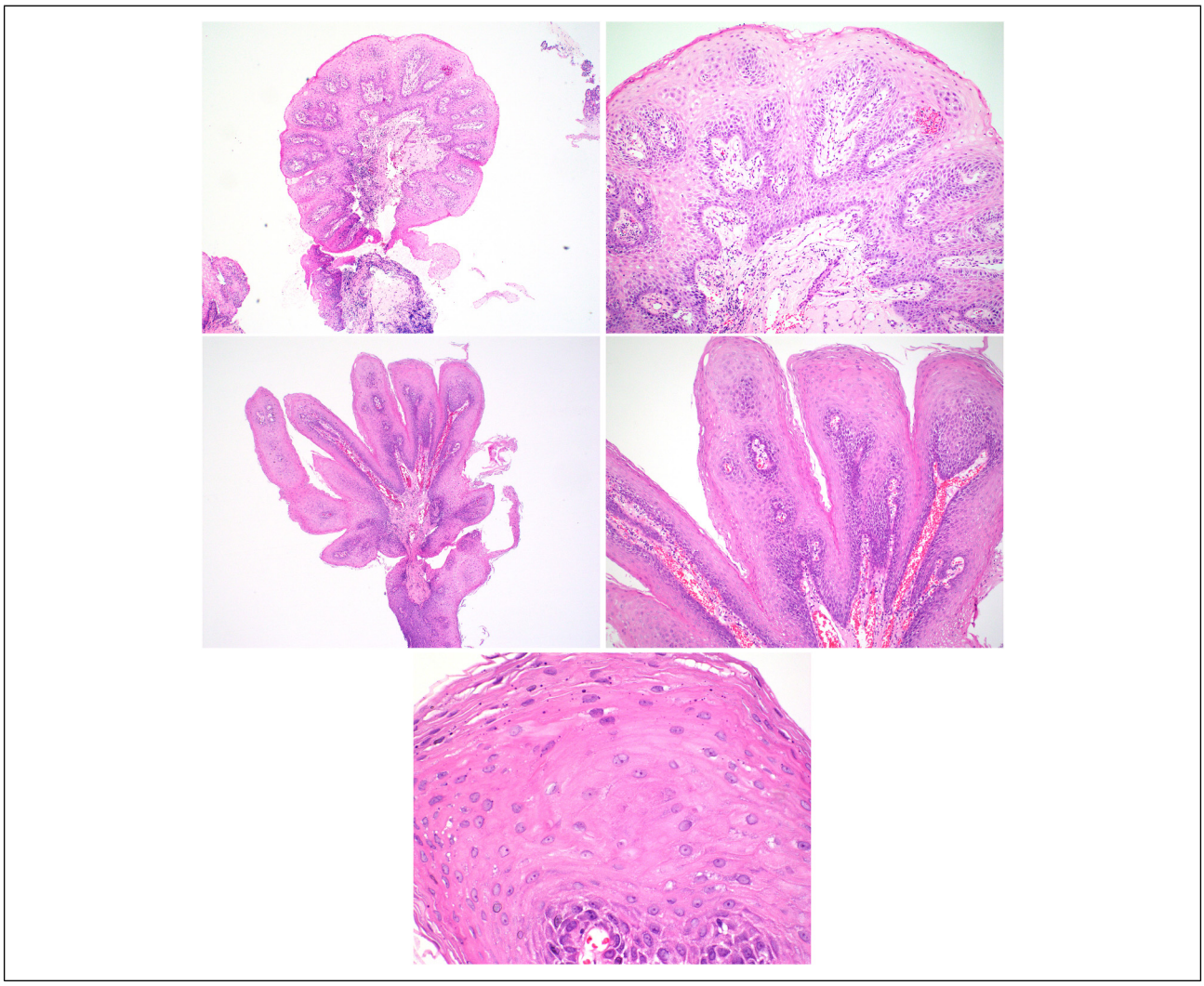

Figure 1. Histologic features of esophageal squamous papilloma. The endophytic type has a dome-like smooth surface, (A) Hematoxylin and eosin (H&E), original magnification 40×, and exhibits an inverted papillomatous configuration of squamous proliferation, (B) H&E, original magnification 100×. The exophytic type is characterized by finger-like papillary projections, (C) H&E, original magnification 40× with delicate fibrovascular cores lined by squamous epithelium, (D) H&E, original magnification 100×. No cytologic dysplasia or viral cytopathic change is demonstrated, (E) H&E, original magnification 400×.

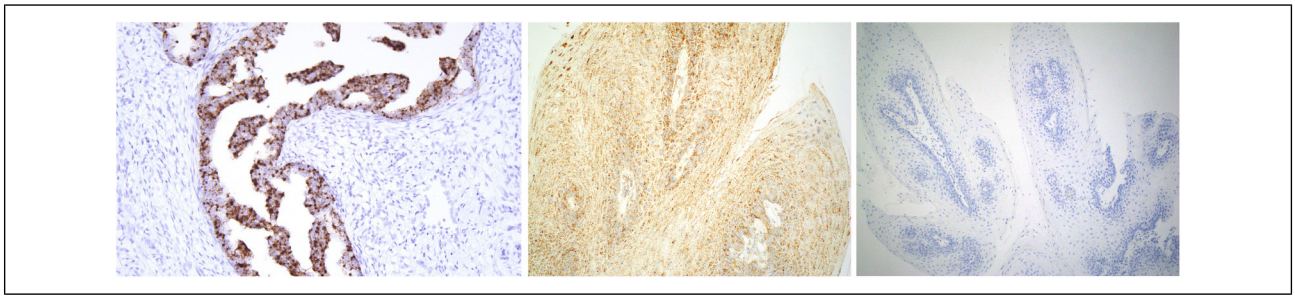

Figure 2. Positive high-risk HPV RNA ISH in cervical adenocarcinoma in situ case, (A) RNA Scope, original magnification 200×. RNA Scope HPV RNA positive control probe (PPIB) confirms the integrity of mRNA within the tissue, (B) RNA Scope, original magnification 200×. An example of esophageal squamous papilloma shows negative staining for high- and low-risk HPV probes, (C) RNA Scope, original magnification 100×.
Abbreviations: ISH, in situ hybridization; HPV, human papillomavirus.

ESP is reported to increase from 0.13% in 2000 to 0.57% in 2013,[1] but the difference likely results from varied endoscopic detection and/or biopsy rates, rather than a true increase in prevalence. Our data show a higher ESP detection rate in UCLA patients (0.58%) than in PUMCH patients (0.11%), which appears explainable by a much higher biopsy rate of the esophagus during upper endoscopies in UCLA patients. It is likely that some of the ESPs are not biopsied or removed during upper endoscopies in PUMCH patients because of their small size and benign appearance. A biopsy or removal may be thought unnecessary by endoscopists.

The etiopathogenesis of ESP is not fully understood. Two main hypotheses have been proposed. One is prolonged and localized chemical and/or mechanical irritation of the esophageal mucosa by factors such as reflux,[8] minor trauma,[6,9,10] and tobacco and/or alcohol abuse,[9] which may elicit a cellular damage-repair response followed by hyperregeneration. In our cohorts, the endoscopic indications for UCLA patients appear mainly esophagus-related, such as GERD, dysphagia, etc, but the indications for PUMCH patients appear less esophagus-centered. In fact, the frequency of *H. pylori* gastritis is higher in PUMCH patients than that in UCLA patients. Our data show a predilection of ESP to occur in the mid-esophagus in both patient populations. Specifically, 52% of ESP cases in our patient cohorts are seen in the mid-esophagus. Only 23% of cases are seen in the distal esophagus, similar to the frequency occurring in the proximal esophagus (25%). Similarly, some other studies also showed the mid-esophagus to be the most common site for the detection of ESP (Table 2).[2,9,11–13] Also in concordance with our findings, the frequency of ESP detection in the distal esophagus is similar to that in the proximal esophagus in a couple of earlier studies.[9,11] Together, these observations argue against reflux disease to be a common etiology for

Table 2. HPV Detection in Esophageal Squamous Papilloma—Literature Review.

Number of patients/cases	Location in esophagus	Testing methods	Number of HPV-positive cases % (HPV+/tested)	Countries of authors	Year of publication
143/151	Mid (52%), proximal (25%), distal (23%)	RNAscope®	0 (0/148)	USA, China	2023, the present study
10/12	Proximal (60%), distal (40%)	FISH	0 (0/12)	Lebanon	2021[14]
51/55	Mid (51%), proximal (27%), distal (22%)	N/A	N/A	Turkey	2021[11]
38/38	Mid (68%), distal (21%), proximal (11%)	PCR	18.4% (7/38)	Turkey	2017[12]
60/60	Distal (58.3%), mid (28.3%), proximal (13.3%)	PCR	47.4% (9/19)	USA	2017[1]
78/78	Distal	IHC (1), ISH (5)	0 (0/6)	France	2015[8]
45/45	N/A	PCR	4.4% (2/45)	USA	2010[15]
18/19	Proximal (58%), mid (16%), unknown (26%)	ACISH, PCR	87.5% (14/16), 85.7% (12/14)	Mexico	2008[7]
35/38	Mid (53%), distal (23.7%), proximal (23.7%)	PCR	10.5% (4/38)	Japan	2006[9]
155/172	Mostly mid and proximal	PCR	46.2% (12/26)	Hungary	2005[16]
9/9	Mid (78%)	DNA ISH	0 (0/9)	Italy	2001[2]
42/42	Mid (55%), distal (29%), proximal (17%)	PCR	4.8% (2/42)	Italy	2000[13]
11/11	N/A	PCR	63.6% (7/11)	Germany	1999[17]
9/9	Distal (77.8%), mid (11.1%), proximal (11.1%)	N/A	N/A	China	1996[18]
26/26	N/A	DNA ISH	10% (1/10)	Korea	1996[19]
10/10	Distal (80%), mid (20%)	HPV IHC	0 (0/10)	Saudi Arabia	1995[20]
28/29	Distal (61.1%), mid (19.4%), proximal (19.4%)	DNA ISH, PCR	ISH negative; PCR 3.4% (1/29)	Slovenia, Poland	1995[21]
17/23	Distal (65%), mid (17%), proximal (17%)	DNA ISH, PCR	4.3% (1/23)	USA	1994[10]
33/38	Distal (71%), mid (24%), proximal (5%)	PCR	50% (13/26)	Canada	1993[5]
14/14	Distal (92%)	DNA ISH, PCR	0 (0/14)	Finland	1991[4]
35/35	Mid (46%)	N/A	N/A	Italy	1988[3]
6/6	Distal (83%)	N/A	N/A	Spain	1986[6]
15/15	Distal (67%), proximal (20%), mid (13.3%)	N/A	N/A	Italy	1983[22]

Abbreviations: HPV, human papillomavirus; FISH, fluorescence in-situ hybridization; PCR, polymerase chain reaction; ACISH, amplified chromogenic in-situ hybridization; ISH, in-situ hybridization; IHC, immunohistochemistry; N/A, not available.

ESP, as it would predispose the distal esophagus to be the most vulnerable place for ESP occurrence.

The second proposed etiopathogenetic factor is HPV infection. Previous studies have described the prevalence of HPV infection in ESPs with variable results (Table 2). Bohn et al[7] reported that as high as 87.5% of ESPs in their study were associated with HPV detected by DNA ISH and 85.7% by polymerase chain reaction (PCR). Lavergne and de Villiers[17] reported 63.6% and Odze et al[5] reported 50% of their cases that were positive for HPV, also by PCR. On the other hand, others failed to detect HPV in their cases[2,4,8,14,20] or reported a very low positivity rate,[10,13,15,21] also using DNA ISH and/or PCR methodologies. Poljak et al[21] reported one ESP case that was positive for HPV by PCR but negative by DNA ISH. Our previous study also showed positive HPV PCR in 2 of 45 ESP samples.[15]

The present study has employed the most up-to-date RNA ISH technology and has examined the largest tissue samples. Our data show that none of the 148 ESP cases from the United States and China is positive for HPV RNA. The apparent discrepancy from previous studies is clearly not resulted from geographic or patient population variations. Instead, the differences in detection methodologies and the functional status of the virus may be the best explanations. All previous studies showing a high rate of HPV detection have employed DNA-based tests, which might have detected the presence of HPV DNA in lesional tissue that is uncoupled to its functional activity. Surprisingly, HPV can be detected in up to 35% of normal skin of immunocompetent hosts.[23] Therefore, the mere presence of HPV DNA in lesional tissue does not necessarily mean that the virus is transcriptionally or functionally active. Taking oropharyngeal squamous cell carcinoma as an example, the detection of transcriptionally active oncogenic HPV in tumor tissue has been recommended to be the standard to help clinical decision-making.[24] Previous studies have shown that up to 50% of HPV DNA-positive oropharyngeal squamous cell carcinoma cases are negative for E6/E7 mRNA expression.[25] Among HPV-positive oropharyngeal squamous cell carcinoma, only HPV transcriptionally active tumors showed significantly better survival compared to tobacco/alcohol-related oropharyngeal squamous cell carcinoma.[26] In addition, the HPV E6/E7 oncoproteins are central to dysplastic progression in the uterine cervix. Detection of E6/E7 expression could help differentiate benign/reactive squamous epithelium from low-grade squamous intraepithelial lesions in cervical samples.[27] Furthermore, RNAscope® has been shown to have a higher specificity and an equivalent sensitivity in comparison to other methods for HPV detection.[28,29] Therefore, the detection of transcriptionally active HPV by RNAscope® would be more clinically relevant. Our study has employed RNA ISH technology designed to detect the E6/E7 viral oncogene mRNA of both low- and high-risk HPV types in the largest number of ESP cases. Our negative results support the notion that HPV, if present, is dormant and transcriptionally inactive in ESP tissue, and likely to be just a bystander. These data help explain why ESP rarely (if any) progress to malignancy[8] and argue against an etiologic role of HPV in the pathogenesis of ESP.

Differences in inclusion/exclusion criteria in various studies may also contribute to the discrepant results. In our study, we did not find any ESP case that exhibited features of dysplasia or viral cytopathic change during our diagnosis confirmation. However, in the study by Bohn et al who detected low-risk HPV (6/11) in 10 and high-risk HPV (16) in 2 of the 14 ESP cases they investigated, koilocytosis was described to be present in all cases.[7] In the study by Odze et al who detected high-risk HPV (16 or 16/18) in 12 and low-risk HPV (6/11) in 1 of 26 ESPs, koilocytosis was described to be present in 50% of cases but there was no significant association between koilocytosis and HPV status.[5]

Interestingly, our data show that nearly 70% of patients with ESP are over 45 years old. The average age of ESP patients reported in the literature is also over 45 years old.[1,4,6,8–10,13,30] These findings suggest that the occurrence of ESP may be an aging-related process, although ESP can also occur in children.[14] On the other hand, ESPs are almost always found incidentally during upper endoscopy for an unrelated indication. An alternative explanation would be that older patients have more frequent endoscopic procedures for various reasons.

ESP is considered benign in nature. There have been only a couple of case reports on its malignant transformation.[8,31] None of our cases is associated with malignancy simultaneously or asynchronously or shows progression to cytologic dysplasia or squamous cell carcinoma. Since ESP is typically a small lesion, endoscopic removal with biopsy forceps, snare polypectomy, and cautery is currently considered adequate.[32] There have been no guidelines or recommendations for the surveillance of these patients.

ESP is single in most cases but can be multiple. Esophageal squamous papillomatosis, characterized by numerous ESP-like lesions diffusely involving the entire esophagus, is extremely rare. Different from ESP, esophageal papillomatosis carries a significant risk of malignant transformation, as squamous cell carcinomas were identified in almost half of reported cases or during surveillance (Table 3). Therefore, it has been recommended that esophageal papillomatosis be considered a premalignant condition,[30] despite its histologic similarity to ESP. At PUMCH, we have seen one patient, a 9-year-old boy, not included in this study, who presented with heartburn and dysphagia. Upper endoscopy showed carpet-like sessile, verrucous lesions up to 1.5 cm in size involving the entire esophagus in a circumferential fashion. Several larger polypoid lesions were biopsied. Histologic

Table 3. Data Summary of Esophageal Papillomatosis Reported in the Literature and its Association With Malignancy and HPV Infection.

Age	Sex	Symptom	Site	Clinical management	Associated malignancy	HPV detection	HPV test methods	Year of publication
9	M	Dysphagia	Entire esophagus	Biopsy and surveillance	NEG	NEG	RNA ISH	2023, present case
67	M	Dysphagia, hematemesis	30-39 cm from incisors	Esophagectomy	SCC in situ	N/A	N/A	2020[33]
40	F	Acanthosis nigricans and tripe palms, no digestive system abnormality	Entire esophagus	Biopsy	NEG	N/A	N/A	2020[42]
61	M	Heartburn	34-38.5 cm from incisors	Cryospray ablation	NEG	N/A	N/A	2019[32]
23	F	Heartburn, dysphagia	Entire esophagus	Polypectomy	NEG	NEG	N/A	2018[43]
53	F	Dysphagia	Piriform sinus to esophagogastric junction	Total esophagectomy	Diffuse LGD, focal HGD	POS (subtype 16)	DNA ISH	2016[36]
67	F	Dysphagia	N/A	Liquid nitrogen cryotherapy	NEG	N/A	N/A	2016[41]
45	M	Acanthosis nigricans	Entire esophagus, middle and lower pharynx	Biopsy	NEG	NEG	PCR	2016[44]
51	M	Dysphagia	$4 \times 1.6 \times 0.7$ cm hemicircumferential lesion	Esophagectomy	NEG	N/A	N/A	2014[45]
37	M	Incidental	Entire esophagus	Biopsy	NEG	NEG	PCR	2014[46]
62	F	Heartburn	Gastroesophageal junction	EMR	NEG	NEG	PCR	2012[37]
74	M	Heartburn, regurgitation	32-37 cm from incisors	Radiofrequency ablation, esophagectomy	HGD, SSC in situ	NEG	DNA ISH	2009[39]
70	M	Dysphagia	22-39 cm from incisors	Total esophagectomy	SCC	NEG	DNA ISH	2009[30]
70	F	Dysphagia	Pharyngoesophageal junction to the distal esophageal sphincter	Esophageal dilatation	SCC	NEG	DNA ISH	2005[47]
84	M	Dysphagia	Middle and distal esophagus	Photodynamic therapy	SCC	NEG	DNA ISH	2004[38]
74	F	Dysphagia	20-30 cm from incisors	Esophagectomy	SCC	NEG	DNA ISH	2004[35]
83	M	Dysphagia, nausea	Entire esophagus	Complete regression	NEG	POS (subtypes 16, 33)	PCR	2003[48]
56	M	Dysphagia	23-33 cm from incisors	Progressive dilation	NEG	NEG	PCR	2002[49]
28	M	Dysphagia, hoarseness	Upper and middle esophagus	Esophagectomy	SCC	N/A	N/A	2000[34]
53	F	Chronic diarrhea	Entire esophagus	Biopsy	NEG	POS (subtypes 51, 52, 56)	PCR, DNA ISH	1998[50]
28	F	Dysphagia, retrosternal pain	Upper two-thirds of esophagus	Biopsy	NEG	NEG	PCR, DNA ISH	1996[51]
75	F	GERD, history of Billroth II procedure	Gastroesophageal junction	Snare polypectomy	Adenocarcinoma	N/A	N/A	1995[52]
27	M	Epigastric pain, dysphagia	Distal esophagus, cardia, main and intermediate bronchus	Laser evaporation, interferon treatment	LGD	POS (subtype 11)	DNA ISH	1989[40]

Abbreviations: HPV, human papillomavirus, POS, positive; NEG, negative; N/A, not available; SCC, squamous cell carcinoma; PCR, polymerase chain reaction; GERD, gastroesophageal reflux disease; ISH, in situ hybridization; LGD, low-grade dysplasia; HGD, high-grade dysplasia.

examination showed features of squamous papillomas with no cytologic dysplasia or viral cytopathic change. The case was diagnosed as esophageal squamous papillomatosis. The case was tested for HPV by RNA ISH, which showed negative results. Next-generation sequencing showed *KRAS* exon 2 somatic missense mutation (NM_033360.3:C.35G > A (p.G12D)). The patient has been surveilled endoscopically for 5 years now. No squamous dysplasia or squamous cell carcinoma was found thus far. The treatment of esophageal papillomatosis can be challenging, as the optimal management and surveillance guidelines remain unclear because of its rarity. Some patients with extensive lesions may require esophagectomy because of associated dysplasia and progression to carcinoma.[30,33–36] Other treatment options include endoscopic resection,[37] photodynamic therapy,[38] radiofrequency ablation,[39] laser evaporation,[40] and cryospray ablation.[32,41] As shown in Table 3, our case is unique because it is the first report of esophageal papillomatosis occurring in a child. Although no malignancy has developed, long-term follow-up is warranted. It is interesting to mention that none of the reported cases was associated with respiratory papillomatosis except for one case that originated in the distal esophagus but spread into the main and intermediate bronchus.[40]

In conclusion, this is the largest cohort study to investigate the association between ESP and HPV, and the first time using the up-to-date RNAscope® technology to detect E6/E7 viral oncogene mRNA of both low- and high-risk HPV types. The results show no association between ESP and active HPV infection in both North American and Chinese populations. Therefore, there is no clinical significance for HPV detection in ESP lesions. Other etiopathogenetic mechanisms, such as chronic localized inflammatory reactions and aging, may contribute to the development of these innocent lesions.

Declaration of Conflicting Interests

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Ethical Approval

This project is reviewed and approved by the University of California Los Angeles Institutional Review Board (Protocol No. 19-000444).

Informed Consent

Not applicable, because this article does not contain any clinical trials.

Trial Registration

Not applicable, because this article does not contain any clinical trials.

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