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Clinicopathological Characteristics of Esophageal Squamous Papillomas in Japanese Patients—With Comparison of Findings from Western Countries

Kuniko Takeshita[1], Shin-ichi Murata[2], Shoji Mitsufuji[1], Naoki Wakabayashi[1], Keisho Kataoka[1], Yasunari Tsuchihashi[3] and Takeshi Okanoue[1]

[1]Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Kyoto, Japan, [2]Department of Pathology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan and [3]Department of Clinical Pathology Research, Louis Pasteur Centre for Medical Research, Kyoto, Japan

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To clarify the characteristics of esophageal squamous papillomas (ESPs) in the Japanese population, we investigated 38 ESPs of 35 Japanese patients from a file with 17,387 upper gastrointestinal endoscopies in our university hospital. ESPs accounted for 0.20% of the total number of endoscopies and comprised 21 females and 14 males with an average age of 59.2 years. More than half of the ESPs (52.6%) were located in the middle esophagus. The ratio of human papilloma virus (HPV) positive ESPs was 10.5% and all were located in the middle esophagus of female patients only. HPV-positive ESP cases were younger (46.8 years) than HPV-negative cases (60.8 years). Based on comparison with the reports from western countries, we attribute the low prevalence in the lower esophagus to the relatively fewer occurrences of severe reflux esophagitis (RE) due to chronic gastritis with low gastric acid secretion among Japanese patients.

Key words: squamous papilloma, esophagus, gastroesophageal reflux diseases, reflux esophagitis, human papilloma virus

I. Introduction

Esophageal squamous papilloma (ESP) is a relatively rare, benign, squamous epithelial tumor [2], which is generally small, single, round and elevated sessile lesions with smooth or rough surfaces. Two etiological factors of ESPs have been posited. One is hyper-regenerative response of the mucosa to chemical and mechanical irritation such as minor trauma, chronic food impaction, alcohol consumption, cigarette smoking, previous gastroesophageal surgery and gastroesophageal reflux diseases (GERDs) [7, 8, 18, 19, 23]. The mucosal irritant theory is clinically supported by the high prevalence of ESPs in the lower esophagus, the site most severely affected by GERDs [7, 8, 18, 23]. The other is human papilloma virus (HPV) infection [4, 5, 20, 22]. Since Syrjanen *et al.* demonstrated the presence of HPV antigens in ESPs, HPV infection has been considered one of the etiological factors of ESPs [28], although the exact pathogenetic importance of the HPV is not yet clear.

The prevalence rate of ESPs is estimated at 0.01 to 0.45% according to the results of autopsy and endoscopic studies [6–8, 15, 17, 23, 26]. ESPs have been thought not to be endemic [13]. Japanese patients with ESPs have interesting features, because the usual Japanese population does not have severe reflux esophagitis (RE), which has been considered an etiological factor of ESPs [7, 8, 16]. So, the geographic distribution and etiological factors of ESPs in Japan are likely to be different compared with western countries. However, no studies have been published in English concerning Japanese patients with ESPs based on a sufficient number of cases. In this report, we studied 38 ESPs for the assessment of various clinical, pathological and virological parameters from the etiological and geographic point of view of ESP in Japan.

Correspondence to: Shin-ichi Murata, Department of Pathology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, 1110 Shimokato, Tamaho-cho, Nakakoma-gun, Yamanashi 409–3898, Japan. E-mail: smurata@yamanashi.ac.jp

II. Methods

Clinical study (patients and samples)

We reviewed a file of 17,387 upper gastrointestinal endoscopies with biopsies performed consecutively over a period of 13 years (1989–2001) at Kyoto Prefectural University Hospital to detect patients with ESPs. The data from the clinical and endoscopic reports that were studied comprised gender, age, endoscopic appearance, location in the esophagus, number of tumors and association with GERD and chronic gastritis. The GERDs included RE, hiatal hernia, post-therapeutic state of endoscopic injection sclerotherapy and post-surgical state of gastrectomy. RE was endoscopically assessed by experienced endoscopists using the Los Angeles (LA) classification of esophagitis [3]. The location of the papillomas was designated as either upper esophagus (less than 24 cm from the incisors), middle esophagus (between 24 and 32 cm from the incisors), or lower esophagus (more than 32 cm from the incisors). Chronic gastritis was diagnosed on the basis of endoscopic findings and/or biopsy specimens.

Histopathological study

All biopsies were formalin-fixed and paraffin-embedded, and 4 μm thick sections were stained with hematoxylin and eosin (HE) for histopathological studies. The HE-stained sections of ESPs were investigated for the following assessments.

1) Histological diagnosis was performed according to the criteria of the Armed Forces Institute of Pathology (AFIP) (Fig. 1a) [13].

2) The extent of neutrophil infiltration in the epithelium of ESPs was determined by using three categories: mild (infiltration only of the surface epithelium), moderate (random infiltration of the epithelium), and marked (random infiltration of the epithelium and subepithelial stromal tissue) (Fig. 1b).

3) The presence of koilocytosis, which was defined as squamous cells with irregular nuclei surrounded by a perinuclear cytoplasmic clearing, was also studied (Fig. 1c).

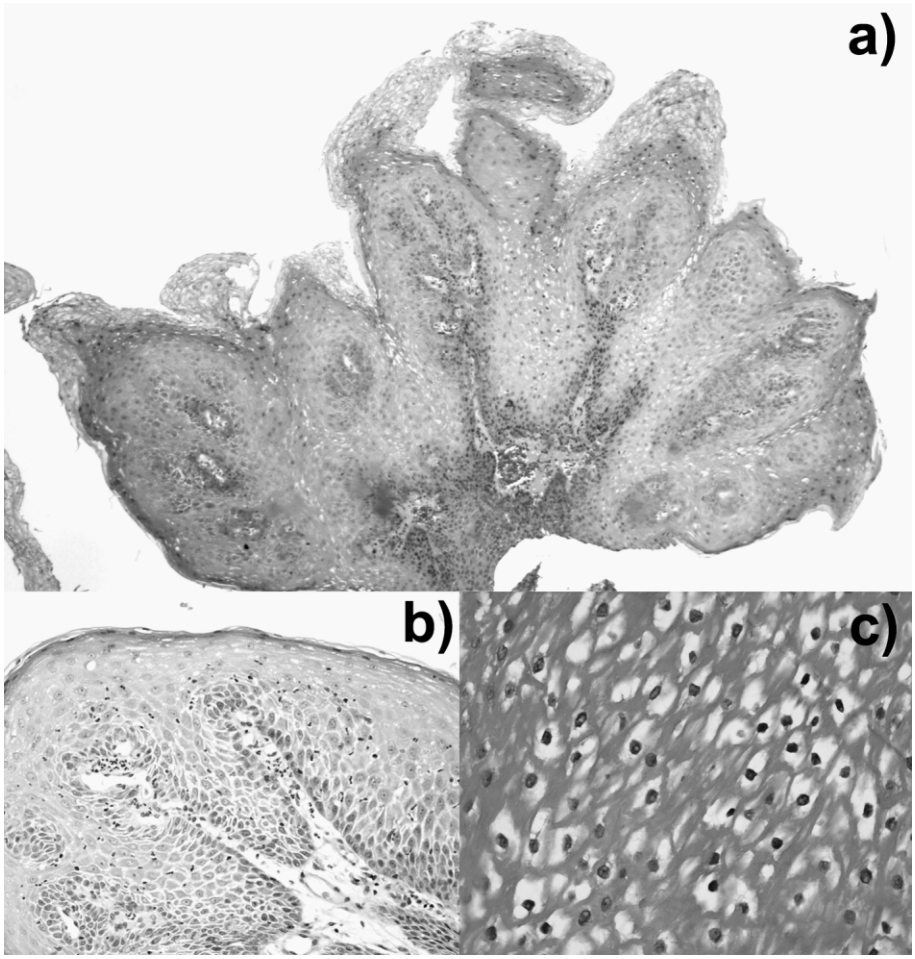

Fig. 1. Histological findings of esophageal squamous papilloma. **a)** Representative histological features of ESP. The ESP shows a papillary projection lined with acanthotic squamous epithelium (original magnification: ×10). **b)** Marked neutrophil infiltration in the whole tumor epithelium (original magnification: ×25). **c)** Koilocytosis in ESP is vague compared with dysplasias of uterine cervix (original magnification: ×25).

Proliferative activity study

Proliferative activity of 28 ESPs from 28 patients was assessed by using immunohistochemical staining of anti–Ki-67 antibody (MIB-1). For immunohistochemical staining, additional sections pretreated by steamer heat (10 mM sodium citrate buffer, 15 min) were stained using a 1:50 dilution of monoclonal mouse antibody MIB-1 (DAKO, Glostrup, Denmark) and an EnVision kit (DAKO, Carpinteria, CA, USA). To evaluate the proliferative activity of ESPs in each case, we counted about 500 nuclei in the entire layer of the squamous epithelium. The MIB-1 index in each case was calculated as the number of positive cells divided by the total number of cells examined.

Virological study

Thirty-eight formalin–fixed ESPs were examined for human papilloma virus (HPV) by means of the polymerase chain reaction (PCR) method using the following procedures [9, 16, 30].

1) DNA extraction

Three serial 5-μm-thick sections from the formalin-fixed and paraffin-embedded materials of 38 ESPs were deparaffinized and digested in 50 μl of Proteinase K buffer (50 mM Tris-hydrochloric acid pH 8.3, 1 mM EDTA, 0.5% Tween 20 with 0.5 mg/ml of Proteinase K) overnight at 5°C. After heat-inactivation (90°C, 10 min) of Proteinase K, part of the supernatant was used for PCR. Twenty-eight non-neoplastic esophageal epitheliums were also examined as negative control.

2) PCR analysis

Extracted DNA samples were amplified with a consensus primer pair for HPV gene (TaKaRa Biomedicals, Osaka, Japan). The consensus primer (forward primer, 5'-TGCTAATTCGGTGCTACCTG-3'; reverse primer, 5'-GAGCTGTCGCTTAATTGCTC-3') was designed within the E6 and E7 open reading frame (ORF) for the amplification of HPV genotypes 6 and 11 [9]. Amplification reactions were performed with a heat-stable Thermus aquaticus (Taq) polymerase (TaKaRa Biomedicals). The template DNA (1 μg) was subjected to PCR. The reaction mixture of 50 μl contained 100 mM KCl, 20 mM Tris-HCl pH 8.0, 25 mM $MgCl_2$, 2.5 mM of each dNTP, 2.5 units of Taq polymerase and 100 pmol of each consensus primer. The reaction mixture was subjected to 35 cycles of amplification using the DNA Thermal Cycler (MJ Research, Waltham, MA, USA). Each cycle consisted of a denaturation step at 94°C for 0.5 min, an annealing step at 55°C for 1 min, and a chain elongation step at 72°C for 1 min.

3) Restriction enzyme analysis of PCR products

To identify the HPV subtypes 6 and 11, the PCR products (2 μl) were digested with three units of digestion enzyme Afa I (TaKaRa Biomedicals) in 20 μl reaction mixture for 1 hour at 37°C. The PCR products cut into 132 and 96 bp were HPV subtype 6, and those cut into 166 and 62 bps were HPV subtype 11. The entire PCR and all digested products (10 μl each) were electrophoresed on a composite gel and photographed with ethidium bromide under UV light.

Statistical analysis

All the differences between grades were assessed with one or two sample t-tests and Kruskal-Wallis test analyses. Statistical significance was set at a p-value of less than 0.05.

III. Results

Clinical study

From our hospital file of 17,387 upper gastrointestinal endoscopies with biopsies, 38 ESPs from 35 patients were found (prevalence, 0.20%). The patients with ESPs comprised 21 females and 14 males with an average age of 59.2 years (25–83 years). All ESPs were endoscopically identified as small (less than 5 mm), sessile or semi-pedunculated and whitish polypoid lesions. The location of the ESPs is shown in Table 1. More than half of the ESPs (52.6%) were located in the middle esophagus. Most patients had a single ESP except for three cases with double tumors. Associated GERDs and chronic gastritis are shown in Table 2. Most of the patients with ESPs located in the lower esophagus had GERDs (Table 2). Five (13.2%) of the ESPs were associated with RE and six (15.8%) with hiatal hernia. All the cases with RE were classified as grade A, which is the least severe grade according to the LA classification of esophagitis [3]. Almost all the patients except for younger persons (73.3%) suffered from chronic gastritis, which has a very high prevalence in Japan.

Histopathological study

1) Histological diagnosis

Histological findings of all ESPs showed they had exophytic, endophytic or spiked features and were lined with acanthotic stratified squamous epithelium with conserved normal cellular orientation but without signs of cytological atypia (Fig. 1a).

2) Epithelial infiltration of neutrophils

The relationship between the degree of neutrophil infiltration in the epithelium of ESPs and the tumor location is noted in Figure 2. ESPs in the lower esophagus were more markedly inflamed than at any other site of the esophagus ($p<0.003$, Kruskal-Wallis test). In particular, the five ESPs associated with RE were all markedly inflamed (Fig. 1b).

Table 1. *Distribution of esophageal squamous papillomas*

Location of ESPs in the esophagus	No. of ESPs	No. of patients
Upper	9 (23.7%)	8 (22.9%)
Middle	20 (52.6%)	18 (51.4%)
Lower	9 (23.7%)	9 (25.7%)
Total	38	35

Table 2. *Association of gastroesophageal reflux disease with esophageal squamous papillomas*

Location of ESPs in the esophagus	Gastroesophageal reflux diseases*				Chronic gastritis
	RE	HH	Post-EIS	Post-GR	
Upper	0	0	0	0	4
Middle	1	0	1	0	14
Lower	4	6	0	1	4
Total (%)**	5 (13.2%)	6 (15.8%)	1 (2.6%)	1 (2.6%)	22 (73.3%)***

* RE, reflux esophagitis; HH, hiatal hernia; post-EIS, post therapeutic state of endoscopic injection sclerotherapy; post-GR; post-surgical state of gastrectomy.
** Five of six hiatal hernia were complicated with reflux esophagitis.
*** Thirty cases could be used for the study of chronic gastritis.

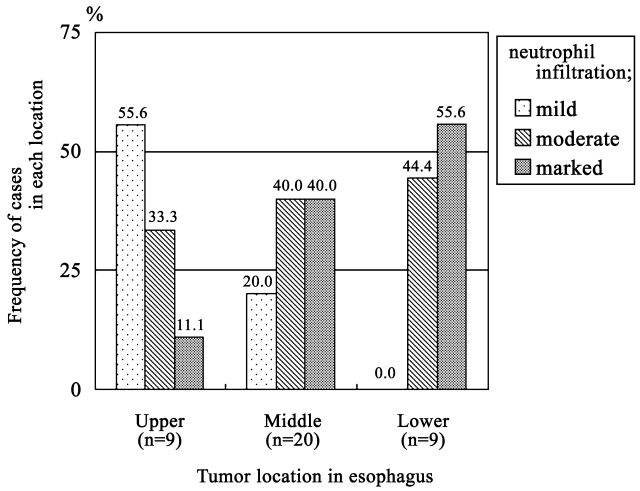

Fig. 2. Correlation of tumor location with neutrophil infiltration in the tumor epithelium ($p<0.003$).

3) Presence of koilocytosis

The epithelium of 23 of the ESPs (61%) showed the presence of koilocytosis, which was focal compared with that in the dysplasia of the uterine cervix (Fig. 1c).

Proliferative activity study

The nuclei of the basal, parabasal and sporadically superficial epithelial cells in the tumors were positive for MIB-1 (Fig. 3). The ESPs with a high MIB-1 index included positive cells diffusely distributed in the lower half of the tumor epithelium. The average number of MIB-1 index in 28 ESPs was 29.2±1.5 (mean±SE). Table 3 shows correlations between the MIB-1 index and clinicopathological parameters. A higher MIB-1 index was observed in the ESPs with more severe epithelial infiltration of neutrophils ($p=0.01$), and the tumor MIB-1 index was significantly higher in RE-affected than in non-affected ESPs ($p=0.007$).

Virological study

Four tumors from four patients (10.5%) were positive for HPV subtype 6, and no HPV subtype 11 could be identified (Fig. 4). All non-neoplastic esophageal epitheliums were negative for HPV. Table 4 shows the correlation of HPV status with clinicopathological features. All the ESPs with HPV were single and located in the middle esophagus and were found more often in relatively young female patients. While no HPV-positive ESPs were associated with RE, they showed mild to moderate, but not marked infiltration of neutrophils. No relation could be established between MIB-1 and HPV status.

IV. Discussion

The prevalence of ESPs is generally estimated at less than 0.1% [6–8, 17, 23], except for three Italian studies (prevalence: 0.12%, 0.45% and 0.35%) [15, 26, 29]. In our study, the prevalence of ESPs was 0.20%, and the endoscopic analysis from four different hospitals in Japan showed similar prevalence of ESPs (0.15 to 0.3%, average 0.23%; unpublished data). These figures are higher than those previously reported from Europe (Table 5). The majority of the patients in our study was middle-aged (average 59.2 years), similar to the age of the populations in previously published studies. On the other hand, most of reports from western countries indicated male predominance and high prevalence in the lower third esophagus [5, 8, 16, 17, 22, 23], whereas, the male/female ratio was 1/1.57 and more than half of the ESPs (52.6%) occurred in the middle esophagus in our study. Mosca *et al.* also reported the geographic difference of ESPs from a comparative analysis between Italy and other western countries based on the literature [15]. Mosca *et al.* and our results suggest that there might be considerable differences in the geographic distribution of ESPs due to environmental agents functioning as etiological factors.

Chronic esophageal inflammation secondary to GERDs including RE and hiatal hernia has been suggested as an etiological factor of ESPs [7, 8, 16]. The prevalence of Japanese patients with RE has recently been increasing (about 15%), but severe RE is still not common in Japan [10]. The patients with chronic gastritis, which has a quite higher prevalence in Japan than in western countries, showed reduced gastric acid secretion [1]. We consider the low prevalence of ESPs in the lower esophagus to be one of the characteristic features of Japanese ESP patients and to be due to low prev-

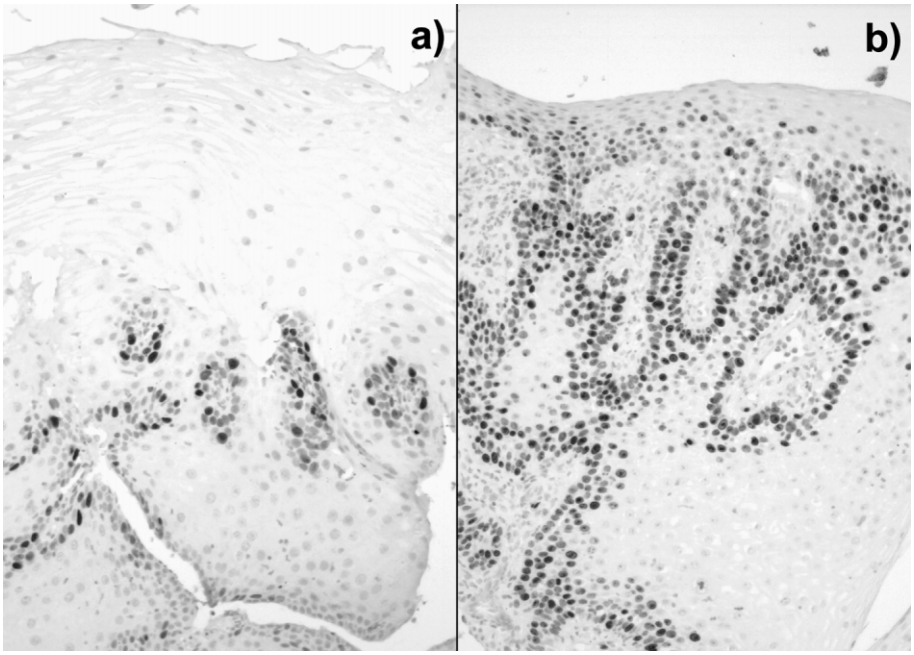

Fig. 3. Representative findings of MIB-1 immunohistochemical stains of esophageal squamous papillomas (original magnification: ×10). **a)** A papilloma case with low MIB-1 index. **b)** A papilloma with high MIB-1 index.

Table 3. *Correlation of the MIB-1 index with clinicopathological parameters in esophageal squamous papillomas*

Findings	No. of cases	MIB-1 index mean±SE	p
Location in esophagus			
Upper	4	27.5±3.0	
Middle	15	28.5±2.2	NS
Lower	9	31.1±3.0	
Neutrophil infiltration			
Mild	3	25.9±1.7	
Moderate	15	27.4±2.8	0.01
Marked	10	34.6±2.7	
Associated reflux esophagitis			
Affected	5	37.2±2.7	0.007
Non-affected	23	27.2±1.5	

NS: no significance

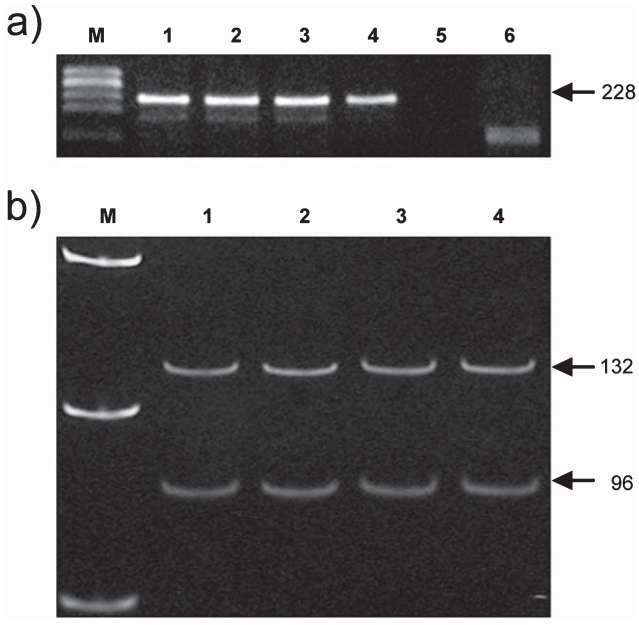

Fig. 4. Results of PCR analysis to detect human papilloma virus (HPV) infection in ESPs. **a)** PCR products using consensus primers-PCR. Lanes 1-4: PCR products from four cases are HPV-positive. Lane 5, negative control (no DNA); Lanes 6, positive control (synthetic DNA). **b)** PCR products digested by restriction enzyme Afa I. Lanes 1–4 indicate HPV subtype 6.

alence of severe RE, which is caused by chronic gastritis with reduced gastric acid secretion. In support of this consideration, the prevalence of severe RE was also low in Italy [14, 27], and all three studies which showed findings similar to those of our study, that is, a high prevalence of ESPs, female dominant sex distribution and high prevalence in the middle esophagus, were from Italy (Table 5) [15, 26, 29].

Although no pathological studies of the proliferative activity of ESPs have been reported, the proliferative activity of ESPs was found to be higher than that of the non-neoplastic squamous epithelium (less than 10%) [11]. The finding that the majority of MIB-1 positive nuclei were located in the basal and parabasal epithelial cells in the tumors suggests that the ESPs continued to preserve the proliferative orientation of non-neoplastic squamous epithelium. Moreover, the tumor MIB-1 index was significantly associated

Table 4. *Correlation of HPV infection in esophageal squamous papillomas with clinicopathological parameters*

Parameters (%)	HPV infection	
	Positive (n=4)	Negative (n=34)
Age (average)	46.8	60.8
M : F	0 : 4.0	1 : 1.2
Tumor location		
upper	0 (0%)	9 (25.8%)
middle	4 (100%)	16 (45.2%)
lower	0 (0%)	9 (29.0%)
Associated reflux esophagitis	0 (0%)	5 (16.1%)
Neutrophil infiltration		
mild	1 (25%)	8 (23%)
moderate	3 (75%)	12 (34%)
marked	0 (0%)	14 (40%)
MIB-1 index (average)	29.4	29.2
Koilocytosis	4 (100%)	19 (55.9%)

Statistical analysis was not done, because the number of HPV-positive cases was too few.

with inflammation and affected patients' proportion of RE. These results indicate that inflammation due to GERDs has an important influence on tumor cell proliferation and may act as an etiological factor of ESPs. As for severely inflamed ESPs located in the upper to middle esophagus, other environmental irritation factors such as smoking, alcohol consumption and GERD-related chronic ear-nose-throat disorders, may play a role in tumorigenesis.

To what extent HPV infection contributes as an etiological factor of ESPs is still controversial. While the prevalence of HPV-positive ESPs was 10.5% in our study, differences in detection methods have resulted in variations in published results ranging from 0% to 64% (Table 5) [4, 6, 12, 15, 16, 20–22, 29]. The PCR method which we used in this study is more sensitive than *in situ* hybridization and immunohistochemical methods. Moreover, the PCR assay using consensus primers for a portion of the E6–7 open reading frame is more reliable than the ones using the E1 and L1 region primers, because it is less likely to produce false-negative results [9, 30]. Although HPV genotypes 6 and 11 are major subtypes in ESPs [4, 12, 20–22], only one study with a sufficient number of cases and a few case reports have reported the detection of cancer-associated HPV subtypes [16, 31]. Koilocytosis, which is a histological finding indicating virus infection, was also found in cases in which the PCR method could not detect HPV infection. This seems to indicate that either koilocytosis in some cases is not related to virus infection [12, 16], or that we still cannot exclude the infection of other HPV subtypes [12, 24, 25, 29]. The latter point is supported by the fact that so far the full genomic sequences of 85 HPV genotypes have been identified, and that more than 120 putative novel types have been partially characterized [32].

All the HPV-positive cases were female and their tumor was located in the middle esophagus, and none of the female patients with HPV-positive ESPs were associated with dysplasia or squamous cell carcinoma of the genital tract. The mean age of the HPV-positive cases was young-

Table 5. *Summary of published studies including more than five esophageal squamous papilloma cases*

No. of patients	Prevalence %	Mean age & gender (M : F)	Dominant location	Esophagitis	HPV	Country	Author (year)
35	0.20	59.2 14 : 21	Middle (53%)	13.2%	10.5%	Japan	Our study
42	0.35	45 y.o. 1 : 1	Middle (55%)	17%	4.8%	Italy	Talamini[29] (2000)
35	0.45	45 y.o. 18 : 17	Middle (46%)	11%	NR	Italy	Sablich[26] (1988)
33	NR	50 y.o. 24 : 9	Lower (71%)	61%	50%	Canada	Odze[16] (1993)
28	NR	50 y.o. 1 : 1	Lower (55%)	NR	3.4%	Slovenia Porland	Poljak[21] (1995)
25	NR	57 y.o. 17 : 5	Lower (64%)	48%	NR (4.3%)[6]	USA	Carr[4,5] (1994)
15	0.08	48 y.o. 11 : 4	Lower (67%)	33%	NR	Italy	Franzin[8] (1983)
12	0.07	49 y.o. 6 : 6	Lower (92%)	NR	0%	Finland	Chang[6] (1991)
11	NR	57 y.o. (NR)	NR	NR	64%	Germany	Lavergne[12] (1999)
9	0.12*	43 y.o. 4 : 5	Middle (78%)	0%	NR	Italy	Mosca[15] (2001)
6	0.04	63 y.o. 2 : 4	Lower (83%)	100%	NR	Spain	Fernandez[7] (1986)

* Nine ESPs from 7,618 upper gastrointestinal endoscopies.
NR; not recorded in detail or not studied.

er than that of HPV-negative cases. The past literature also reported similar results in that the middle esophagus was the most common location of the tumor of HPV-positive ESP cases, and that such patients were relatively younger than HPV-negative ESP cases [16]. Our results and the literature might indicate that the middle esophagus represents a favorable environment for HPV infection.

In conclusion, we investigated three aspects of 38 ESPs in 35 Japanese patients, clinical, histopathological and virological. From the geographic point of view, there is no English report studying Japanese ESP patients with comparison of findings from western countries. We posit that the characteristics of ESPs in Japanese patients are relatively high prevalence (0.20%), female dominance and high frequency of location in the middle esophagus. We attribute the dominant middle esophagus distribution (the equivalent of low prevalence in the lower esophagus) to high prevalence of chronic gastritis in Japan, which brings in low prevalence of severe RE. Also similar with previous studies, HPV-positive ESPs cases showed female predominance, relatively young age and high prevalence of location in the middle esophagus. The high prevalence of ESPs seems to indicate that other etiological factors than RE may contribute more to ESP tumorigenesis in Japan. This is the first report in English of a study of Japanese ESP patients based on a sufficient number of cases to provide meaningful results.

V. References

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