Human Papillomaviruses (HPV) in Tissue Specimens of Oral Squamous Cell Papillomas and Normal Oral Mucosa

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Abstract. Background: The etiology of oral squamous cell papillomas (OSCP) is still unresolved. Materials and Methods: The presence of human papillomavirus (HPV) was examined, using PCR and three different consensus primers, in tissue specimens obtained from 49 patients with OSCP and 49 tissue specimens of histologically-normal oral mucosa obtained from the same number of individuals, who matched the patients with OSCP in age, gender and localization of the obtained tissue specimens. Results: Amplifiable DNA was recovered from 44 out of 49 and 45 out of 49 tissue specimens of OSCP and normal oral mucosa, respectively. HPV-6 was detected in three and HPV-16 in one out of 44 OSCP specimens tested. Three tissue specimens of normal oral mucosa were HPV DNA-positive, harboring HPV-6, HPV-11 and HPV-31. Conclusion: Since no significant difference in the prevalence of HPV DNA between the patients with OSCP and the control subjects (9.1% vs. 6.7%; p=0.694) was observed, HPV is deemed to play a limited role in the etiology of OSCP, at least in Europe.

Oral squamous cell papilloma (OSCP) is a benign hyperplastic wart-like localized proliferation of the oral epithelium and is considered the most frequent benign tumor of the oral epithelium (1). Histopathologically, it appears as an exophytic lesion comprising folds of hyperplastic stratified epithelium, that are usually thickly para- or orthokeratinized or may also be non-keratinized. OSCP is most common in children and in adults in the third and fifth decades, although it may be found at any age. The gender incidence is almost equal, with a slight male predominance (1). Despite extensive research, the etiology of OSCP still remains unresolved. Some studies, performed in the last two decades, indicated a possible etiological connection between OSCP and human papillomavirus (HPV) infection (summarized in Table I). However, understanding of the etiological role of HPV is hampered by the fact that there is a marked variation in the HPV DNA prevalence rate in OSCP specimens in published studies (2-27), varying from 0% to 100% (Table I). To further elucidate the putative role of HPV in the etiology of OSCP, a comparative study of the presence of HPV DNA was carried out on formalin-fixed, paraffin-embedded tissue specimens obtained from 49 patients with OSCP and on 49 formalin-fixed, paraffin-embedded tissue specimens of histologically-normal oral mucosa, obtained from the same number of individuals who matched the patients with OSCP in age (±5 years), gender and localization of the obtained tissue specimens. The presence of HPV DNA was ascertained in all tissue specimens using polymerase chain reaction (PCR), and the three most commonly used consensus primers targeting the HPV L1 and E6 genes. To the best of our knowledge, the present study represents the first comparative investigation of the HPV DNA prevalence in patients with OSCP with age- and gender-matched control subjects, and the first study in which more than one consensus PCR primer was used for the determination of the presence of HPV in either OSCP or normal oral mucosa.

Materials and Methods

OSCP specimens. The first study group comprised 49 patients with OSCP (22 men and 27 women), treated at the Department of Maxillofacial and Oral Surgery, University Medical Center, Ljubljana, Slovenia. Localization of OSCP was as follows: buccal mucosa (six cases), tongue (nine cases), floor of the mouth (ten cases), retromolar trigonum (eleven cases), and soft palate (13 cases). The patient age range was 44–78 years (mean age 50.5).
Alcohol consumption was reported by 14 out of 49 (28.5%) patients and smoking by 21 out of 49 (42.8%) patients. Immediately after resection, the tissue was fixed in 10% neutral-buffered formalin, embedded in paraffin or paraplast, processed to 5-μm-thick sections and stained with hematoxylin-eosin. Histologically normal oral mucosa specimens.

The second study group (controls) consisted of 49 histologically normal oral mucosa formalin-fixed, paraffin-embedded tissue specimens obtained by autopsy from the same number of individuals, who matched the case subjects in age (±5 years), gender and localization of the obtained tissue specimens. The autopsy tissue specimens were retrieved from the histological files of the Institute of Pathology, Medical Faculty, University of Ljubljana, Slovenia.

Isolation of DNA and internal control amplification. DNA was isolated from formalin-fixed paraffin-embedded tissue sections as described previously (28, 29). The quality of each DNA sample was verified by the amplification of a 536-bp fragment of the ubiquitous human beta-globin gene using the LightCycler-FastStart DNA Master SYBR Green I Kit (Roche Applied Science, Mannheim, Germany) and the KM29/RS42 primers, as described earlier (30-32). Successful amplification of the beta-globin gene fragment indicated that the DNA sample was adequate for the HPV DNA analysis and that no PCR inhibitors were present.

Detection of HPV DNA. The PCR amplification was performed on all samples using the PCR Core Kit plus (Roche Applied Science, Mannheim, Germany) and three different consensus HPV primer sets: PGMY09 and PGMY11 targeting an approximately 450-bp fragment of the L1 HPV gene (33), GP5+ and GP6+ targeting an approximately 140 to 150-bp fragment of the HPV L1 gene (34) and WD72, WD76, WD66, WD67 and WD154 targeting an approximately 240-bp fragment of the HPV E6 gene (35), as described earlier (31, 36-38). All known precautions, to avoid a PCR product carry-over and sample-to-sample contamination, were rigorously taken, as described previously (32). To determine the HPV genotypes, the PGMY09/11 PCR products were digested using seven restriction endonucleases (BamHI, DdeI, HaeIII, HinfI, PstI, RsaI and Sau3AI) and analyzed by agarose gel electrophoresis, as described earlier (28, 37, 39).

Statistical analysis. The χ²-test was employed.

Results

The internal control amplification with human beta-globin primers KM29/RS42 revealed that amplifiable DNA was recovered from 44 out of 49 and 45 out of 49 tissue specimens of OSCP and normal oral mucosa, respectively.
Thus, nine tissue specimens, which failed in the internal control amplification (five OSCP specimens and four tissue specimens of normal mucosa), were excluded from further analysis.

The PCR amplification, with two different HPV L1 primers and one HPV E6 consensus primer, showed the presence of HPV DNA in four out of the 44 (9.1%) OSCP specimens tested. In total, two cases of OSCP from the soft palate, one case from the buccal mucosa and one case of oral OSCP from the tongue were HPV DNA-positive. All three consensus primer sets recognized HPV DNA in three out of four HPV DNA-positive OSCP specimens. The OSCP specimen from the tongue was recognized as HPV DNA-positive by the PGMY and WD consensus primer sets only. For determining HPV genotypes, PGMY09/11 PCR products were digested with seven restriction endonucleases and were analyzed by agarose gel electrophoresis. The restriction patterns were found to be unique for HPV-6 in three case of OSCP obtained from the tongue, buccal mucosa and soft palate, respectively, and for HPV-16 in OSCP obtained from the soft palate.

In addition, HPV DNA was detected in three out of 44 (6.7%) of the tissue specimens of normal oral mucosa tested. In all three HPV DNA-positive tissue specimens of normal oral mucosa, the HPV DNA was detected by all the HPV consensus primer sets used. The HPV DNA-positive normal oral mucosa specimens, obtained from the floor of the mouth, tongue and buccal mucosa, harbored HPV-6, HPV-11 and HPV-31, respectively.

Statistical analysis showed no significant difference in the prevalence of HPV DNA between the patients with OSCP and the age- and gender-matched control subjects (9.1% vs. 6.7%; \( p = 0.694 \)).

Discussion

The etiology of OSCP, the most common oral benign neoplasm, is still unresolved. Since the histology of oral mucosa resembles that of the uterine cervix, the lower genital tract or the skin, where HPV-related neoplastic lesions are frequent, OSCP has been frequently studied for the presence of HPV infection (Table 1). However, despite extensive research, the exact role of HPV in the etiology of OSCP has remained inconclusive, since the HPV DNA prevalence rates in OSCP tissue specimens reported in different studies varied considerably, from 0% to 100%. As shown in Table 1, the HPV DNA prevalence rates reported in different studies of OSCP were, in contrast to the studies of other HPV-related neoplasms (e.g., cervical carcinoma), not predominantly dependent on the sensitivity of the method used for the detection of HPV, but were rather dispersed regardless of the HPV detection method used. Thus, the HPV prevalence rates obtained in the studies which used relatively insensitive HPV detection methods, i.e., immunohistochemistry and in situ hybridization, ranged from 0% to 100% and 13.3% to 100%, respectively, and surprisingly, did not differ significantly from those obtained with PCR (0%-86.7%), which is regarded as the most sensitive method for the detection of HPV. Thus, it is more likely that the obtained differences in the HPV prevalence rates reflected protocol variations (e.g., different anti-HPV antibodies, different probes and primers, stringency conditions, use of anticontamination PCR procedures, monitoring of PCR inhibition with internal control amplification, etc.), or possible geographically-related differences in the prevalence of HPV infection, rather than a choice of method per se. Additionally, the design of the majority of previous studies was not ideal, since many of them included only a few patients (results most probably driven by selection bias) and almost all the studies investigated the prevalence of HPV exclusively in OSCP patients without including a control group. Since the HPV colonization rate of normal oral mucosa is high and may reach even 52% (40), we believe that it is always necessary to detect a "background" prevalence of HPV in normal oral mucosa in age- and gender-matched control subjects to elucidate the clinical significance of the established prevalence of HPV among any type of neoplastic lesion, including OSCP (32). Unfortunately, in contrast to the HPV prevalence studies performed on cervical carcinoma and other anogenital HPV-related neoplastic lesions, where case-control study design has been a standard approach for many years (41), to the best of our knowledge, only one study has comparatively investigated the HPV prevalence in patients with OSCP and in a control group of the subjects with normal oral mucosa (23). In this study from Venezuela, performed on 27 patients with OSCP and 20 subjects with clinically normal oral mucosa, HPV DNA was detected in 40.7% of the OSCP and in 10% of the control samples. However, this group did not use age- and gender-matched controls, which is quite important since it was recently shown that the presence of HPV in the oral cavity is a transient event, which is age-dependent (42).

To overcome the described deficiencies of previous studies, we designed our study in such a way that: (i) the HPV prevalence was determined on a relatively high number of OSCP tissue specimens; (ii) HPV prevalence was studied comparatively in the specimens of histologically-normal oral mucosa obtained from an equally extensive group of age- and gender-matched control subjects; and (iii) highly-sensitive and reliable HPV detection and genotyping methods were used for the determination of HPV. Employing such a study design, we were unable to determine any significant difference in the prevalence of HPV between patients with OSCP and age- and gender-matched control subjects \( (p=0.694) \).
In conclusion, in the study in which the presence of HPV was comparatively examined on tissue specimens from patients with OSCP and age- and gender-matched control subjects, no significant differences were found in the prevalence of HPV between the groups under comparison. Our results support the concept of latent or subclinical HPV infection of the oral mucosa, and suggest that occasional findings of HPV DNA in OSCP tissue specimens may result not from viral infection, but rather represent an incidental HPV colonization of the oral mucosa. According to our results, it seems that, at least in Europe, HPV play a limited role in the etiopathogenesis of the majority of OSCP.

References


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