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Detection of human papilloma virus in potentially malignant and malignant lesions of the oral cavity and a study of associated risk factors

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Abstract

Background: Squamous cell carcinoma of the head and neck is the 6th most frequently occurring cancer worldwide, with over 400,000 cases projected annually. Multiple factors such as tobacco, alcohol, irradiation, virus, and chronic irritants are involved in the development of oral squamous cell carcinomas (OSCCs). The most important risk factors are chronic exposure to tobacco and alcohol. Although the evidence that implicates virus is increasing, particularly (human papillomavirus [HPV]), in the carcinogenesis process, the role of virus is not well established. **Aim and Objective:** This study is designed to assess the presence of HPV in potentially malignant and malignant lesions of the oral cavity as well as to correlate the presence of HPV with addictive habits and histopathological grading of the disease. **Materials and Methods:** Biopsy samples of OSCC and potentially malignant lesions were obtained and 3, 5 μm thickness sections were cut using a microtome. The sections were collected using a sterile brush and transferred to an Eppendorf tube. DNA extraction and polymerase chain reaction for the detection of HPV were done. **Results and Conclusion:** The association between histopathological grading and presence of HPV was assessed using Chi-square test and the values thus obtained were found to be statistically significant. HPV was more predominantly seen in well-differentiated carcinomas and moderately differentiated carcinomas as compared to poorly differentiated carcinomas.

Key words: Carcinoma, human papilloma virus, squamous cell carcinoma

Introduction

The most prevalent form of cancer in the oral cavity is squamous cell carcinoma. Squamous cell carcinoma of the head and neck is the 6th most frequently occurring cancer worldwide, with over 400,000 cases projected annually.[1] The most important risk factors are chronic exposure to tobacco and alcohol.[2,3] The important viruses implicated in the process of carcinogenesis are human papillomavirus (HPV), Epstein–Barr Virus, and herpes simplex virus.[4] Although the evidence that implicates virus is increasing, particularly (HPV), in the carcinogenesis process, the role of virus is not well established.

HPVs are small (55 nm), nonenveloped, icosahedral, epitheliotropic DNA tumor viruses that are transmitted in the early life. Previous studies that evaluated oral cavity and oropharyngeal dysplasia have reported widely varying HPV prevalence ranging between 0% and 100%.[3]

In oral squamous cell carcinoma (OSCC), there can be two different subgroups: Those associated with HPV and those not associated with HPV. The latter are associated with long-standing use of tobacco and alcohol. However, investigators showed that tobacco and alcohol use increases the risk not only of developing HPV-independent head and neck squamous cell carcinomas (HNSCCs) but also of developing HPV-associated HNSCCs.[5] Since the treatment modality for OSCC can be modified based on the presence of HPV from surgical removal to chemo-radiotherapy or concurrent chemo-radiotherapy, detection of HPV immediately after diagnosis will help in reducing morbidity of the patient.

This study is designed to assess the presence of HPV in potentially malignant and malignant lesions of the oral cavity as well as to correlate the presence of HPV with addictive habits and histopathological grading of the disease. This hypothesis if proven can lead to the development of vaccines based on virus-like particles, both to reinforce the immune defense of patients with cancers and for prevention.

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Materials and Methods

Study design

This is a case–control study.

Study population

The sample size for the study is 90 samples.

The study group consisted of 67 clinically and histopathologically diagnosed samples of OSCC and potentially malignant lesion and 23 samples (exfoliated oral epithelial cells) from healthy individuals. Out of 67 cases, 3 groups were made. Group I - 47 samples with histopathologically diagnosed OSCC. Group II – 10 samples with potentially malignant lesions of oral cavity with severe dysplasia. Group III – 10 samples with potentially malignant lesions of oral cavity with moderate dysplasia. Group IV (controls) - 23 healthy individuals without any clinically diagnosed carcinoma or potentially malignant lesions of oral cavity. Any intraoral lesions other than malignancies or potentially malignant lesions and biopsy samples with inadequate amount of tissue were excluded from the study.

Procedure

Biopsy samples of OSCC and potentially malignant lesions were obtained. A 3 and 5 μm thickness sections were cut using a microtome. DNA extraction and polymerase chain reaction (PCR) for the detection of HPV were done in the Department of Microbiology, KMC, Mangalore, using standard protocol as described by Jalouli J *et al*.[3]

Exfoliated oral epithelial cells were collected from healthy individuals without any oral mucosal lesions.

Single polymerase chain reaction assay

A single PCR assay was used to detect HPV DNA. Before testing the samples, the specificity of the method was examined using HeLa cell lines as positive control.

Gel electrophoresis

Aliquots of 15 ml of the PCR product were analyzed on 2% agarose gel (agarose from HiMedia) containing ethidium bromide (Merck KGaA, Darmstadt, Germany). Products were visualized

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under ultraviolet light. The size of the amplified product was determined by comparison with a base-pair ladder size marker (Gene Ruler, 100 bp, 50 bp DNA Ladder Plus, Fermentas, India).

Statistical analysis

In this study, the Chi-square test was used to investigate the difference in HPV prevalence between controls and squamous cell carcinoma patients. In all analyses, the *P* values were considered significantly different if $P < 0.05$. For description of the data, mean values and 95% confidence intervals were calculated.

Results

The prevalence of HPV in the samples enrolled in the study is listed in Table 1. Among 47 samples to OSCCs tested, 40.4% (19/47) of OSCC cases studied were positive for HPV whereas 59.6% (28/47) were negative for HPV [Figure 1]. Fifteen percent of the female patients and 22.9% of male patients included in the study showed positivity for HPV. The Pearson Chi-square test was done to correlate the presence of HPV with gender. This value, however, was not found to be statistically significant (0.548).

All the potentially malignant lesions were negative for HPV [Figure 2]. The association between histopathological grading and presence of HPV was assessed, and the values thus obtained were found to be statistically significant.

Discussion

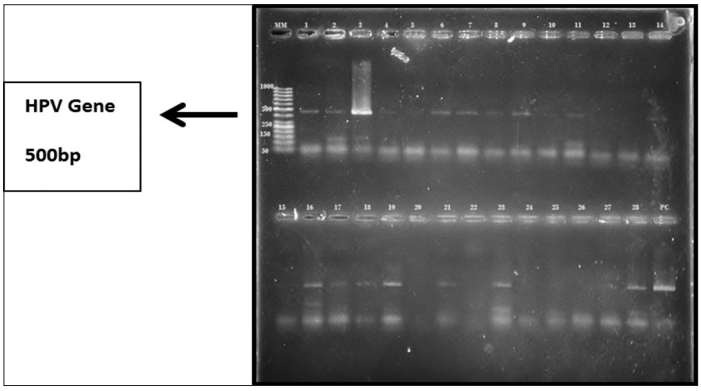
The role of HPV in oral cancer (OSCC) has been under debate since the first report suggested this association in 1983. Keeping this in mind, we conducted a study to detect the presence of HPV in potentially malignant and malignant lesions of the oral cavity.

In the present study, we included 90 samples (70 males and 20 females) which consisted of 4 groups namely Group 1 (47 OSCC biopsies), Group 2 (10 severe dysplasia biopsies), Group 3 (10 moderate dysplasia biopsies), and Group 4 (exfoliated superficial epithelial cells from 23 controls). The age range of subjects was from 20 to 86 years with a mean age of 55 ± 14.69 years.

Table 1: Prevalence of human papillomavirus in all the samples tested (*n*=90)

Clinical diagnosis	Total samples tested	HPV positive (%)	HPV negative (%)
Controls	23	0/23	23/23 (100)
Potentially malignant lesions	20	0/20	20/20 (100)
Oral squamous cell carcinomas	47	19/48 (40.4)	28/47 (59.6)

HPV=Human papillomavirus



HPV	Lane number
Positive	1,2,3,4,6,7,8,911,14,16,17,18,19,21,23,28
Negative	5,10,12,13,15,20,22,24,25,26

Figure 1: Gel picture of the ethidium bromide-staining in 2% agarose gel and showing presence of human papillomavirus infection in 17 out of 27 oral squamous cell carcinoma cases (MM - Molecular marker, PC - Positive control, Lane 1-28-oral squamous cell carcinoma samples [Lane 11-Verrucous proliferation inside odontogenic keratocyst])

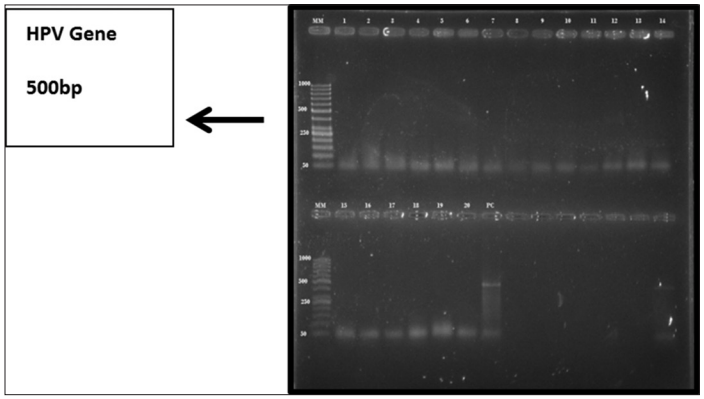
The prevalence of HPV in OSCC in the present study was 40.4%. A study conducted by Bouda *et al*. using nested PCR to detect the presence of HPV in OSCC and normal oral mucosa revealed the presence of HPV DNA in 48 of the 53 (91%) OSSC samples analyzed.[6] Chaudhary *et al*. (2009) conducted a study using PCR and hybrid capture assay II (HCA II) to detect HPV in OSCC showed that 32.4% were positive by PCR and 31.4% were positive by HCA II.[7]

We analyzed the correlation between viral presence and gender, but the results were insignificant. Such associations have been reported by some groups but could not be confirmed by others. This could be because of ethnic population and geographic differences in our study populations compared to other study groups.[8]

Paraffin-embedded biopsies from 10 moderate dysplasia cases and 10 severe dysplasia cases were subjected to DNA extraction followed by qualitative PCR using universal primer for HPV. We observed that HPV was absent in all the moderate and severe dysplasia cases studied. This was similar to a study conducted by Ha *et al*., who demonstrated low prevalence of HPV in premalignant lesions.[9]

Exfoliated epithelial cells were collected from the oral mucosa of normal individuals and subjected to DNA extraction and PCR. None of the examined normal samples in the present study were found to be positive. A study conducted by Bouda *et al*. showed the absence of HPV in the normal oral mucosa.[6]

In our study, we included OSCC affecting different anatomical regions of the oral cavity including labial mucosa, buccal mucosa, tongue, alveolus, and palate. We observed that there is a high prevalence of HPV infections in SCC of the alveolus (75% of the cases studied). This was followed by floor of the mouth and tongue. Hormia *et al*. evaluated the presence of HPV in gingival sulcus and found that 26% of the gingival biopsies were positive for HPV. The results suggest that the periodontal pocket might serve as a reservoir of HPVs in oral mucosa.[10] Although HPVs are associated with a number



HPV	Lane number
Positive	None
Negative	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20

Figure 2: Gel picture of the ethidium bromide-staining in 2% agarose gel and showing absence of human papillomavirus infection in all the 20 dysplasia cases (MM - Molecular marker, PC - Positive control, Lane 1–10 - Moderate dysplasia samples Lane 11–20 - Severe dysplasia samples)

Table 2: Prevalence of human papillomavirus based on the anatomical sites

Anatomical site	Total tested (%)	HPV positive (%)	HPV negative (%)
Alveolus	12 (100)	9/12 (75)	3/12 (25)
Buccal/labial mucosa	35 (100)	7/35 (20)	28/35 (80)
Floor of the mouth	3 (100)	1/3 (33.3)	2/3 (66.7)
Tongue	17 (100)	2/17 (11.8)	15/17 (88.2)

HPV=Human papillomavirus

of proliferative epithelial lesions including squamous cell malignancies, the point of entry and the site of replication of HPV in the oral cavity are not known. Since the gingival pocket is the only site in the oral mucosa where basal cells, known to be targets of HPV at other mucosal sites, are normally exposed to the environment, it can act as a point of entry as well as reservoir for HPV.[10] Thus, carcinoma of the alveolus can be the most common site affected by HPV-associated carcinoma.

We compared the prevalence of HPV in different histopathological grades of OSCC. HPV was more predominantly seen in well-differentiated carcinomas and moderately differentiated carcinomas as compared to poorly differentiated carcinomas. This could be the reason for increased response to chemoradiotherapy, targeted therapy, and immunotherapy for HPV-associated carcinomas compared to HPV-negative ones. HPV-related OSCC has increased survival rate due to expression of different molecular markers [Table 2].

Since the study conducted was retrospective and included archival of biopsy samples, habit history was available only for 20 patients studied. Out of this, 10 patients had the history of smoking tobacco, 13 had pan chewing habit, and 5 had the habit of drinking alcohol. It is worth mentioning here that out of the 10 patients with history of smoking tobacco and 5 patients with history of alcohol consumption, all were diagnosed with OSCC. Apart from this, out of 13 patients with history of tobacco chewing, 10 had OSCC. Since the available data are not sufficient, statistical analysis could not be performed.

Conclusion

This study supports the hypothesis that HPV-infection may be a risk factor not only for oral cancers, but also in potentially malignant disorders. Understanding the process of HPV-related carcinogenesis is critical for the development of efficient HPV-targeted prevention strategies.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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