

Human papillomavirus 16 and 18 in squamous cell carcinoma of oral cavity and sexual practices: A pilot study at a Tertiary Care Hospital of North India

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ABSTRACT

Context: Oral squamous cell carcinoma (OSCC) is the most common malignancy in India and tobacco and betel nut chewing are well established risk factors. Despite successful campaigns to help people shun this habit in developing countries the incidence has rather gone up and HPV and sexual practices are now definitely implicated for this. **Aim:** An attempt was made to generate Indian data on role of HPV and sexual practices in relation to OSCC. **Settings and Design:** A prospective observational study was conducted on 50 patients with oral squamous cell carcinoma. **Materials and Methods:** Tissue biopsies from fifty patients of oral squamous cell carcinoma (OSCC) were subjected to PCR analysis to look for presence of HPV 16 and 18. Fifty patients with benign lesions were taken as control. **Statistical Methods Used:** The data was statistically analysed using SPSS version 22 and chi square test. **Results:** 42% of OSCC patients were found to harbour HPV 16 and 18 whereas only 8% of patients with benign lesions had HPV 16 and 18. A significant number of HPV positive patients i.e. 9 out of 21 gave history of multiple sexual partners and oral sex. **Conclusions:** This high percentage of HPV in OSCC in an Indian population from a tertiary care centre in north India and its association with prevailing sexual practices is quite significant.

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INTRODUCTION

Oral squamous cell carcinoma (OSCC) is most common malignancy in India where it constitutes 20–30% of all cancers.^[1] Tobacco in both smoking and oral forms, alcohol consumption, and betel nut chewing are

well-established risk factors for OSCC. There have been successful campaigns to help people shun the habit of smoking and alcohol consumption in various developing countries, but this has not translated into a decrease in the prevalence of OSCC, especially in certain

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subsites such as oral cavity, oropharynx, and tongue.^[2] Moreover, a small proportion (15–20%) of OSCC occurs in nonsmokers and nondrinkers; therefore, other risk factors have been studied, namely viruses such as human papillomavirus (HPV) and Epstein–Barr virus, poor oral hygiene, sharp dentures, and micronutrient deficiencies.^[3]

Recent epidemiology and molecular data suggest that out of various strains of HPV, infection of the upper airway with HPV 16 and 18 may promote head and neck tumorigenesis.^[4,5] Whereas worldwide HPV infection in oral cancer has been increasingly reported, Indian oral cancer has largely been attributed to tobacco and prevalence of HPV has been studied in few Indian studies only. We planned a pilot study to look for the presence of HPV 16 and 18 in OSCC by polymerase chain reaction (PCR) analysis of tissue biopsy.

MATERIALS AND METHODS

The present prospective observational study was conducted on 50 patients with OSCC, who were treated on outdoor or indoor basis in the Department of Surgery, ENT, or Dental Sciences in Post Graduate Institute of Medical Sciences (PGIMS), Rohtak. Thorough history was taken, and clinical examination was done. History of tobacco and alcohol consumption and sexual practices, i.e., oral sex and multiple sexual partners was specifically asked. Apart from this, sharp tooth, poor oral hygiene, and premalignant lesions were analyzed. A punch biopsy was taken from the lesion after taking informed consent. Half of the specimen was sent to the department of pathology to confirm the diagnosis of SCC, and the other half was stored in phosphate buffer solution at -20°C for detection of HPV DNA (16 and 18) by PCR. Fifty samples were collected from patients with benign lesions of the head and neck, and these were taken as controls for comparison of prevalence of HPV. Processing of samples was done in a laminar flow cabinet in a class 2 biosafety facility at centre for biotechnology, Maharshi Dayanand University, Rohtak. For this, samples were homogenized (grinded) in very small pieces and made into pestle. These homogenized samples were used for DNA isolation. Isolation of DNA from tissue samples was performed according to the manufacturer's instructions (DNeasy Blood and Tissue Kit from Qiagen). These isolated DNA were used for HPV subtype 16 and 18 DNA detection by PCR. Figures 1 and 2 depict the PCR assay for HPV 16 in all the 50 cases.

RESULTS

The study population comprised 50 patients of OSCC, which included 44 (88%) males and 6 (12%) females.

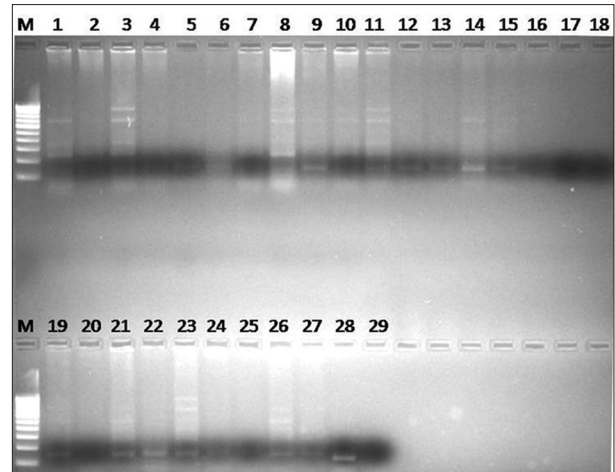


Figure 1: PCR assay for HPV 16 (case 1-27). Lanes 1-27, samples; Lane 28, PCR positive control HPV 16; Lane 29, PCR negative control HPV 16; Lane M, 100 bp ladder

The youngest patient was 35 years old and eldest was 75 years old. 34 (68%) patients were between 51 and 70 years. The mean age at presentation was 55.32 ± 10.20 . Majority of patients, i.e., 44 (88%) were found to be smokers and 34 (68%) were alcoholics, whereas 14 (28%) had a history of sexual practices such as multiple sexual partners and oral sex. All the patients had poor oral hygiene, and none of them had sharp tooth and premalignant lesions.

Base of tongue and tonsil were the most common sites. 16 (32%) specimens were from the base of tongue, and 15 (30%) were from tonsil. Site distribution of various lesions is depicted in Table 1.

Most of the patients in the study were tumor-node-metastasis Stage III and IV. Of the 50 patients, 24 (48%) were Stage IV and 11 (22%) were Stage III. Squamous cell carcinoma was found to be moderately differentiated in 44 (88%), poorly differentiated in 4 (8%), and well-differentiated in 2 (4%) patients.

Detection of human papillomavirus

All 50 patients of OSCC were subjected to PCR for detection of HPV subtype 16 and 18. HPV 16 and 18 DNA was detected in 21 (42%) patients, whereas 29 (58%) patients did not have HPV DNA. HPV 16 was the predominant type which was present in 20 (95.2%) and HPV 18 was detected in 1 (4.7%) patient. These data demonstrate that HPV was present in 42% of patients in this study. 50 control specimens from benign lesions in head and neck were studied for the presence of DNA of HPV subtype 16 and 18. Of these 50 control specimens, 4 were found to be positive for HPV 16 and none for HPV 18; therefore, HPV in control sample was detected in 8%. This difference of HPV detection among oral cancer patients (42%) and control (8%) was found to be statistically highly significant ($P = 0.000$).

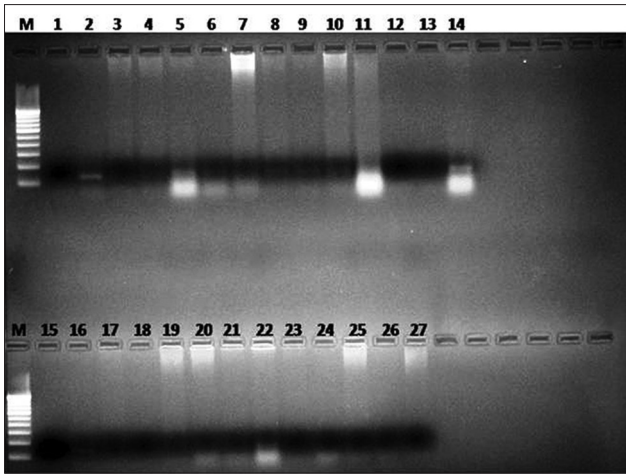


Figure 2: PCR assay for HPV 16 (case 28-50). Lane M: Molecular marker 100 bp; Lane 1 and 15: PCR negative control; Lane 2 and 16: PCR positive control HPV16; Lane 3-14 and 17-27: Samples 28-50

| Anatomical sites | Number of patients (%) |
|---------------------------|------------------------|
| Base of tongue | 16 (32) |
| Tonsil | 15 (30) |
| Soft palate | 4 (8) |
| TI sulcus | 4 (8) |
| Vallecula | 3 (6) |
| Buccal mucosa | 2 (4) |
| Posterior pharyngeal wall | 2 (4) |
| Tongue | 2 (4) |
| Floor of mouth | 1 (2) |
| Hard palate | 1 (2) |
| Total | 50 (100) |

Human papillomavirus and uncommon sexual practices

All 50 oral cancer patients were questioned regarding sexual practices such as multiple sexual partners or oral sexual practices. Total of 14 (14/50, i.e., 28%) patients admitted to have such sexual practices and 9 of them tested positive for HPV, i.e., 42% HPV-positive oral cancer patients (9 out of 21) had a history of multiple sexual partners or oral sexual practices compared to 17.2% (5 out of 29) HPV-negative oral cancer patients. This association was found to be statistically significant ($P = 0.003$).

DISCUSSION

OSCC is the most common cancer in Indian males, comprising 19% of the total malignancies and it is the third most common cancer in Indian females. Tobacco and alcoholism have been thought to be the main etiological factors in India, but interestingly few studies have shown that HPV infection is also a significant factor. The role of HPV as an etiologic factor for oral SCC has been repeatedly proven in various studies worldwide.

We could detect HPV 16 and 18 in 42% of patients of OSCC. HPV 16 was the major subtype as it was present in 20 of 21 (95.2%) and HPV 18 was detected in 1 of 21 (4.8%) patients. HPV 16 and 18 in benign lesions of head and neck could be detected in only 8%. There is a wide variation in the prevalence of HPV shown in many studies worldwide.^[6-11] It ranges from 15% in a study done by Benzamin *et al.* to 49% in another study done by Hammarstedt *et al.* Recent figures estimate that 25.6% of all oropharyngeal cancers are attributable to HPV infection with HPV 16 being the most frequent type.^[12] It is well-accepted that the prevalence of HPV is maximum in oropharynx; hence, researchers who studied on patients of oropharyngeal SCC or tonsillar carcinoma exclusively had a higher prevalence of HPV. Hammarstedt *et al.* and Hong *et al.* studied on tonsillar SCC only and they detected HPV in 49% and 47% of their patients, respectively.^[6,10] Similarly Chaturvedi *et al.* also studied exclusively in oropharyngeal squamous cell carcinoma and the prevalence of HPV in their study was 44.1%.^[9]

Various Indian studies show wide geographical variation in the prevalence of HPV ranging from 0% to 74% [Table 2]. Patel *et al.* reported that HPV was not found in the population of Western India.^[13] South Indian studies have shown that OSCC patients there have maximum HPV prevalence, 74% in the study by Balaram *et al.* and 48.3% by Elango *et al.*^[14,15] The reason for such a high prevalence of HPV in the study done by Balaram *et al.* is because he detected HPV 6 and HPV 11 also apart from oncogenic subtypes HPV 16 and HPV 18. The prevalence of HPV 16 was 42%, and HPV 18 was 47% in his study which is similar to this study.^[14] Koppikar *et al.* showed the prevalence of HPV was 31% but the prevalence of oncogenic HPV, i.e., HPV 16 and 18 was less.^[16] They also studied 102 patients with benign lesions and the HPV prevalence was only 5% in them.

Many researchers have studied the prevalence of HPV in benign lesions of head and neck or normal oral samples as control. They compared the prevalence of HPV in control to prevalence of HPV in head and neck squamous cell carcinoma (HNSCC). They found that as compared to control, the cases are more significantly associated with HPV infection. This further strengthens the idea that HPV as an etiological factor for HNSCC. Our study also shows that presence of HPV in HNSCC is significantly more than the presence of HPV in control. This association is in concordance with the literature.^[11,15-18]

The route of transmission of HPV is sexual, more specifically oral sex and having multiple sexual partners. Gillison *et al.* proved that HPV 16 positive OSCC was independently associated with several measures of

sexual behavior. Association increased with increasing number of oral sex partners.^[19] Smith *et al.* said that HPV-positive oral cancer is associated with oral-genital sexual practice.^[17] Interesting fact is that Indian studies have not mentioned much about the sexual behaviors associated with HPV-positive oral SCC in India. India is traditionally considered a conservative society and even a talk of uncommon sexual practices is considered as taboo. 42% of HPV-positive patients in our study admitted to having such sexual practices as oral sex and multiple sexual partners after probing. This highlights the role of these sexual practices in HPV-positive oral SCC and proves that these sexual practices are prevalent under cover of taboo in Indian society also. This has been observed in a recent review article that inadequate world literature currently is of a low level of evidence to conclude an association between oral sex and HPV. Oral sex is supposedly the most widely practiced unnatural sex worldwide, and any association with HPV transmission needs to be investigated.^[20]

Many western researchers have said that HPV induced OSCC is more common in Oropharynx specially tonsillar fossa.^[7,8,17,21,22] Indian studies had an entirely different picture. Koppikar *et al.* found that maximum prevalence of HPV was in oral cavity.^[16] Nagpal *et al.* showed max infectivity of HPV in the mandible region followed by buccal mucosa.^[23] Barwad *et al.* in his FNAC-based study and Elango *et al.* proved that maximum prevalence of HPV in oral tongue.^[15,24] We did not find any definite anatomical site predilection as depicted in Table 3. The reason for no definite anatomical site predilection

in our study can be attributed to the small size of the study population. We need to compile the data on a much bigger population to come to a definite conclusion regarding anatomical site.

CONCLUSION

It is observed that HPV 16 and 18 could be detected in 42% of patients of OSCC at PGIMS, Rohtak. This high percentage of HPV in OSCC patients at a Tertiary Care Centre of North India is comparable to the available literature from rest of the country. Benign lesions of oral cavity had a very low presence of HPV, i.e., 8%. Sexual practices such as multiple sexual partners and oral sex are not uncommon in our so-called conservative society and were found in 42% of HPV-positive cases. This pilot study clearly points to the need of a larger study of HPV prevalence on patients with OSCC and its association with prevailing sexual practices to clarify specific clinical features, any predilection of HPV infection for a particular anatomical site, pathological features, and outcome of the HPV-positive OSCC patients. These findings if confirmed by such a study may form the basis for starting a vaccination program for primary prevention of OSCC.

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Conflicts of interest

There are no conflicts of interest.

Table 2: Indian studies and prevalence of HPV

| Indian studies | Total number of patients | HPV positive patients | Prevalence of HPV (%) |
|--|--------------------------|-----------------------|-----------------------|
| Balaram <i>et al.</i> ^[14] | 91 | 67 | 74 |
| Elango <i>et al.</i> ^[15] | 60 | 29 | 48.3 |
| Nagpal <i>et al.</i> ^[22] | 110 | 37 | 33.6 |
| Barwad <i>et al.</i> ^[23] | 111 | 36 | 32.4 |
| Koppikar <i>et al.</i> ^[16] | 102 | 32 | 31 |
| Patel <i>et al.</i> ^[13] | 97 | 0 | 0 |

HPV: Human papillomavirus

Table 3: Distribution of anatomical sites according to HPV status

| Anatomical site | HPV status | | Total |
|---------------------------|------------|---------|-------|
| | Absent | Present | |
| Base of tongue | 8 | 8 | 16 |
| Tonsil | 10 | 5 | 15 |
| Soft palate | 2 | 2 | 4 |
| TL sulcus | 2 | 2 | 4 |
| Vallecula | 3 | 0 | 3 |
| Buccal mucosa | 1 | 1 | 2 |
| Posterior pharyngeal wall | 2 | 0 | 2 |
| Tongue | 1 | 1 | 2 |
| Floor of mouth | 0 | 1 | 1 |
| Hard palate | 0 | 1 | 1 |
| Total | 29 | 21 | 50 |

HPV: Human papillomavirus

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