



## Association Of Human Papilloma Virus In Squamous Cell Carcinoma Of Oral Cavity: A Study In A Tertiary Health Care Centre

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### Abstract

**Aim-** To study the association of Human Papilloma Virus in squamous cell carcinoma of oral cavity. **Methodology-** The patients who were detected with histopathological diagnosis of oral squamous cell carcinoma via punch biopsy were subjected to PCR for detection of HPV DNA. **Results-** This scientific study suggests association of HPV in OSCC mainly HPV 16 subtype. The sociodemographic pattern and ethnicity suggests HPV positivity amongst males of low socioeconomic status of mainly rural areas. **Conclusions-** HPV positive were found in 11 out of 100 cases of OSCC studied. Also history of cervical carcinoma and multiple partners were found amongst the positive cases, which suggests a direct link. This study has a direct implications in prognosis of OSCCs, as HPV positive patients were found to have better prognosis than HPV negative OSCCs and hence early cure can help in longevity and survival.

**Keywords:** Oral squamous cell carcinoma, Human Papilloma Virus

### Introduction

Oral cancer is a major neoplasm worldwide and accounts for most head and neck cancers. Approximately 90% of oral cancers are squamous cell carcinoma (OSCC).<sup>[1]</sup> OSCC represents 12% of all cancers in men & 8% of all cancers in the female population. It is particularly common in developing world. Its incidence in India is rated as one of the highest in the world. The etiology of OSCC appears to be multifactorial and strongly related to lifestyle, mostly habits and diet. The widespread use of tobacco was thought to play a major role in the etiology of OSCC. Now the constant detection of the Human Papilloma Viruses (HPVs) in OSCC patients who do not habitually use tobacco or alcohol, points a close association.<sup>[1,3,5,10,12]</sup>

The HPV involvement in oral & oropharyngeal cancers was first proposed in 1983 by Syrjanen et al.

Studies have estimated up to 60% positivity in OSCC, while HPV 16 is the most predominant type. Generally the mean age for diagnosis of OSCC has been approximately 60years, but now more young adults are involved. Also classical site of occurrence changed from buccal mucosa to tongue & its surrounding regions, amounting to around two-third of HPV positive OSCC cases, with tonsils being the closest second next in queue.<sup>[3,5,6]</sup>

Whatever may be the TNM staging, HPV positive OSCCs seems to be largely preventable and curable with a good prognostic value.<sup>[11-15, 52, 86, 93]</sup>

Therefore, screening for HPV in suspected individuals, though not commonly done in case of oral lesions unlike cervical ones, can provide a great insight to future for early detection, as it happens to be mostly curable or preventable. HPV is now one of the most commonly associated entity with oral

cancer. HPV positivity varies from around 60% (Rakesh S et al, Miranda M et al) & 80% in South Indian region (Ajila V et al) to almost 3% HPV positivity in Bangladesh (Akhter M et al) indicating a wide range of detection rate variation owing to difference in ethnicity & geographical location & also influenced by dietary habits & genetic predisposition (Rakesh S et al, Gupta K et al, Patel KR et al [3,4,5,6,7,8])

Kiocyte is a major cytological feature of detection (Rakesh S et al) whereas PCR is a gold standard to detect HPV-DNA was used by many researchers like Sreejyoti HK et al, Ajila V et al.<sup>[3,5,10]</sup> Recently p16 has also gained importance in HPV detection as Ajila V et al as most HPV positive tumours show p16 over-expression (Gupta S et al).<sup>[5,9]</sup>

HPV-16 was the most predominant type in most of the studies made till date. It is estimated up to as low as 2-3% to as high as 60% HPV positivity in OSCC.<sup>[2,3,5]</sup> HPV 18 is the second most common type. HPV 18 has a special tropism for glandular tissue and is most frequently detected type in adenocarcinomas of cervix.

HPV has special affinity for epithelial cells. High risk HPV types 16 and 18 are linked to OSCCs. It enters the host cells by binding to cell surface receptors. The HPV genome is then integrated into the host genome. The integration of HPV-DNA results in formation of 'E6 protein' and 'E7 protein' that helps in oncogenesis.<sup>[3]</sup>

It is still debatable whether habitual correlation has an impact on HPV prevalence. History of sexual practices suggest a lot about the newer generation lifestyle. Many patients gave a history of multiple sexual partners & oral sex. Women of HPV cervix had more frequent oral infections, due to poor hygiene maintenance (Ajila V et al).<sup>[5]</sup>

Rakesh S et al found that even though mean age for OSCC was 60 years classically, now less than 40 years adults too have OSCC.<sup>[3]</sup> Most of the studies made like Sherin N et al & Marur S et al considered tongue as most common site for young adults & buccal mucosa for older age but others suggest primary tonsillar cancers (Akhter M et al, Chaturvedi AK et al) & mandibular region (Sreejyothi HK et al).<sup>[6,10,11,12,13]</sup> Prasad S et al could not suggest any definite anatomical site predilection.<sup>4</sup>

Whatever may be the presentation, HPV with OSCC has a good prognosis than non-HPV associated OSCC, & it is a widely known fact from most of the studies.<sup>[3,4,7,9,10,11,12,13,15,16]</sup> Also it is possible to prevent HPV by vaccination & antioxidants & by modifying life style habit. So, when researches repeatedly pose a threat about HPV associated OSCCs taking up our world, & side ways also suggesting it as a preventable and curable, studies are required more to make this disease to be diagnosed more early & make people get treated much before.

## Objective

This study was taken up to estimate proportion of Human Papilloma Virus infection in oral squamous cell carcinoma of patients and their molecular characterization and subtyping. This study also is taken up to evaluate the association of Human Papilloma Virus positivity according to socio-demographic status and ethnicity of the studied subjects.

## Materials and methods

A cross sectional observational study was taken up in the Department of Otorhinolaryngology. and head and neck surgery, Department of Pathology and Department of Microbiology, AGMC for 1.5 years (Sept 2019- Mar 2021). In my study, total 100 number of patients with oral lesions confirmed as squamous cell carcinoma were registered. Census sampling was done for the present study to select eligible cases.

**Inclusion Criteria:** (a) All patients with suspected oral lesions attending ENT OPD & IPD at AGMC & GBP Hospital, giving consent to participate in the study, (b) Patients with a histopathological diagnosis of oral squamous cell carcinoma, who haven't undergone any treatment, (c) Patients of all age group and sex group.

**Exclusion Criteria:** (a) Patients who came negative for biopsy, (b) Patients who had undergone surgical treatment or radiotherapy or chemotherapy, (c) Patients who are terminally ill.

**Study Procedure:** 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 OSCC, and the other half was stored in phosphate buffer solution at -20°C for

detection of HPV DNA (16 and 18) by PCR. For HPE, the tissue sample was kept in a formalin (10% neutral buffered formaldehyde in water) containing glass container before sending it to the pathologist. There the sample was processed and histological sections were made out, which were then studied under microscope. To see the tissue under a microscope, the sections were stained with eosin, which revealed the cellular components.

After the studied subject were confirmed as squamous cell carcinoma, the other half of specimen stored in phosphate buffer solution, were sent to VRDL. The 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 were performed according to the manufacturer's instructions (DNA extraction kit from Qiagen). All samples were subjected to PCR, using primers specific for consensus sequence spanning the E6 open reading frame of high-risk HPV type 16 & 18. The PCR conditions were as follows: initial incubation at 94 °C for 5 min followed by 35 cycles of reaction with step of denaturation at 95 °C for 1

minute, annealing at 45 or 46 °C for 1 minute and elongation at 72 °C for 2 minutes, and the 35th cycle was followed by a step of final elongation at 72 °C for 10 min and then at 4°C. PCR product was checked for amplification in a 3% agarose gel stained with ethidium bromide. The optimum size of the product was ascertained comparing it with 100 bp DNA ladder. Positive samples were subjected to PCR, using HPV 16 and 18 type-specific primers.

After collecting data, it was recorded, entered and analyzed with computer using SPSS version 15.0.

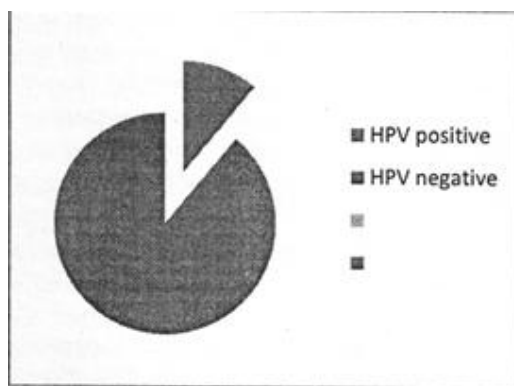
The study were conducted after approval of the Institutional Ethics Committee of Agartala Government Medical College.

**Results**

The study included 100 of oral squamous cell carcinoma patients.

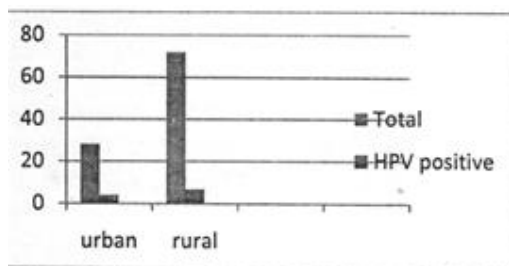
11 of the OSCCs were found to be HPV positives (11%). Only 1 of 11 (9.1%) was found to be HPV 18 & rest all (90.9%) were HPV 16 positives, as per subtyping HPV genomes in PCR.

**Fig 1 : HPV association among oral squamous cell carcinoma patients**



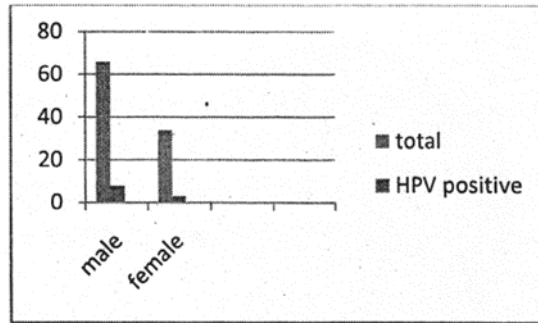
Among 100 OSCCs, 72 belonged to rural & 28 belonged to urban. Whereas only 4 out of 28 people from urban (14.3%) & 7 out of 72 from rural (9.7%) were HPV positive

**Fig 2: Geographical distribution of the studied patients**



Also 66 were male & 34 were female. And only 8 of 66 males (12.1%) and 3 of 34 females (8.8%) were HPV positives.

Fig 3: Gender distribution among patients



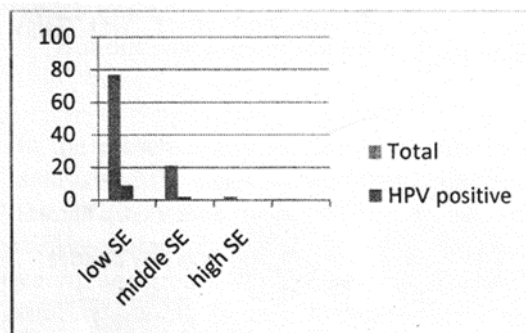
For analysis, we divided the age group in the following manner and the table 1 shows the corresponding number of cases and males and females in each age distribution group. This suggests highest number of HPV positives in age group 41-60yrs (14.3%).

Table No. 1: Age distribution of cases who participated in the study

Age group in years	No of cases, (HPV positive among them)	Male, Female
<20yrs	2 ,(1)	M-1 F-1
21-40	28 ,(0)	M-13, F-15
41-60	42, (6)	M-32, F-10
Above 61	28 ,(4)	M-21, F-7

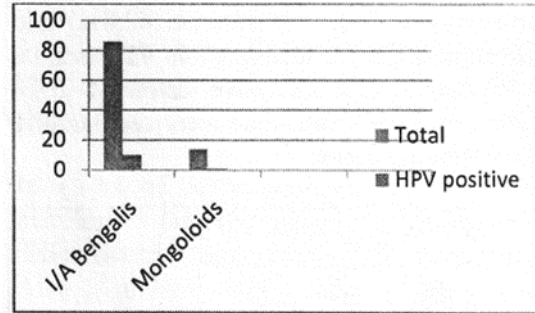
Among 100 OSCC cases, low SE (earning < Rs5000/-per month) were more HPV affected (9 in 77 i.e 11.7%) compared to middle SE status (earning Rs 5000-500007-per month) (2 in 21 i.e 9.5%). None were affected amongst high SE status (earning >Rs 50000/- per month).

Fig 4: Socioeconomic status of patients studied



10 of 86 (11.6%) were Indo-Aryan Bengalis, but 1 in 14(7%) was mongoloid amongst the HPV positives.

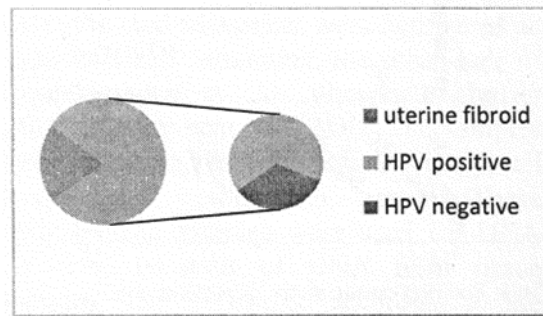
Fig 5: Ethno-Racial distribution among patients



HPV positives were 8 in 83 hindus (9.6%), 3 in 13 muslims (23.1%), but nil in the Buddhists & Christians. Our study shows almost same association in illiterates (5 of 45= 11.1%) & literates (i.e 6 of 55 = 10.9%) for HPV positivity. Among the 11 HPV positives, 7 (63.6%) were having one partner but 3 of them (27.3%) had multiple partners. Only 1(9.1%), was a male of 16yrs who was unmarried and has no sexual exposure. There was no history of oral sex among all the 100 studied OSCC cases.

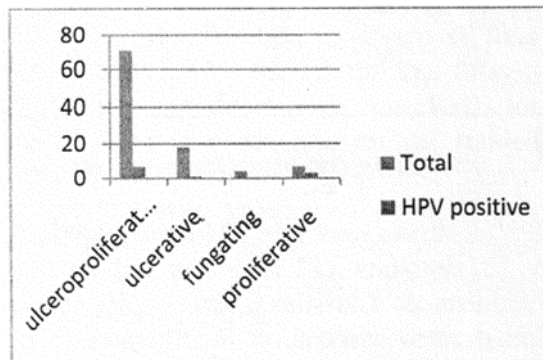
There was history of 4 post op cases of hysterectomy, which on detailed history assessment revealed that 3 were cases of cervical cancer & 1 was uterine fibroid. Among these 3 cervical cancer cases, 2 were HPV positives (66.7%) & 1 was HPV negative.

Fig 6: Association of HPV with Cervical Carcinoma among the studied female cases



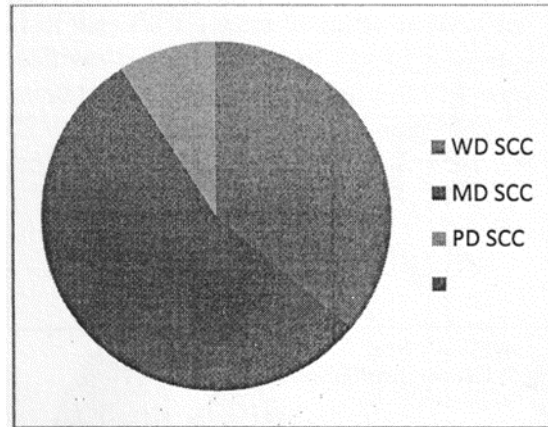
HPV positives were mainly seen in proliferative (3 in 7 cases, i.e. 42.8%) and ulceroproliferative lesions (7 in 71 cases i.e 9.9%) rather than ulcerative lesions (1 in 18 cases, i.e 5.6%). But none were seen among the fungating cases.

Fig 7: Different presentations of OSCC in all HPV positive cases



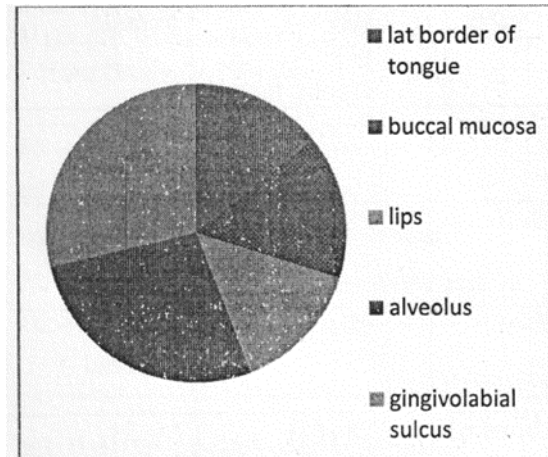
HPV positives were seen mainly in moderately differentiated squamous carcinoma (6 in 31 i.e, 19.4%) and rest well differentiated squamous cell carcinoma (4 in 57 i.e, 7%) & poorly differentiated (1 in 12 i.e 8.3%). Among the HPV positives, moderately differentiated squamous cell carcinoma holds the biggest part (54.5%).

**Fig 8: Histopathological grades of HPV positive OSCC cases**



Among the OSCCs, lateral border of tongue (24%) and buccal mucosa (22%) were more prevalent whereas among HPV positives alveolus (27%) , buccal mucosa(18%) and lateral border of tongue (18%) were mainly involved.

**Fig 9: Site of presentation of HPV positive OSCC cases**



## Discussion

In our study the commonest age group of OSCC ranges from 41-60yrs with a mean age of 51.37yrs. This is in accordance with findings observed by Abhinandan et al.<sup>[32]</sup>

Males outnumbered female patients in OSCC, with a male female ratio of 2:1. These findings are in accordance with a study done by Khanna et al. and Varshney et al.<sup>[33,34]</sup>

Most of our patients belonged from lower socioeconomic strata and mainly from the rural areas, almost like 70% for both categories. Similar observations were made by Mehrotra et al, Rakesh et al and others.<sup>[1,3-6,15]</sup>

As stated by Rakesh et al and Khanna et al. OSCCs occur usually in tongue and lips.<sup>[3,33]</sup> In contrast, in our study, the most common site of lesion is lateral border tongue and buccal mucosa, that is 24% and 22% of the cases respectively. These findings were similar with observations made by Akter et al.<sup>[6]</sup>

The reported incidence of oral cancer among young adults in various studies varies from 0.4-5.5% .<sup>[23,27]</sup> Our study showed an average incidence rate of 8 out of 10 patients with oral lesions during the last 1.Syrs. This suggests a very high rate of oral cancer in this region, which should be a cause for concern.

Demographic comparisons show that while oral cancer in younger persons showed almost equal sex distribution, in older adults there tended to be a

marked male predilection. Previous reports by Iype et al, Schanz et al etc reported similar male predominance.<sup>13,11,33,23\_15,27!</sup>

Similarly in our study HPV 16 was found predominant (10 out of 11 ) among the HPV positive cases of OSCCs. I<sup>16,35,48,53!</sup>

A study by Akhter et al in Bangladesh, which is very close to our region suggests association of HPV in OSCC is 3-4 %, where one of 34 OSCC cases was positive .<sup>16!</sup> But our study result is a bit higher, which is 11%.

In the present study finding histopathologic analysis of the cases showed that majority of the OSCC cases had moderately or well differentiated phenotype in both older and young age group. This suggest histologically similar grading is seen in both elderly and young adults.<sup>13,10,86!</sup>

As with OSCCs , HPV positives were also found mainly in the rural and low socioeconomic strata like the studies conducted by Rakesh et al, Ajila et al. and Akhter et al.<sup>13,5,6!</sup>

Indo-Aryan bengalis were found mostly HPV positives and also they had a large share of OSCCs.

In cases of HPV positive OSCCs , the histology pattern seen is moderately differentiated squamous cell carcinoma. Also most of them were of ulceroproliferative variety. This study findings are similar with studies done by Rakesh et al. Marur et al and Sreejyothi et al [3,11,13]

The most common sites of lesion in such cases were in alveolus (3 in 11) followed by lateral border of tongue and buccal mucosa (both 2 in 11).

Most of the studies made like Sherin N et al & Marur S et al considered tongue as most common site for young adults & buccal mucosa for older age.<sup>10,H!</sup>

### Conclusion

It can be concluded that there is a definite association of HPV and oral squamous cell carcinoma (OSCC). Out of which HPV 16

were most frequently isolated. Incidence of OSCC and ' HPV infections were more predominantly found in males of age group 41-60yrs. People from low socioeconomic background with poor literacy were commonly affected. Lateral border of tongue, buccal mucosa, alveolus were commonly involved in HPV

positive OSCCs. It was also felt that HPV positive OSCCs can probably be prevented by undertaking adequate measures in maintaining proper personal hygiene and sexual behavior. However more such studies are required involving large sample size with longer duration of studies for making a definitive conclusion.

The local data is very limited regarding this topic. Screening patients by our study can benefit the patient in getting treated early and curing much better.

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