ABSTRACT: BACKGROUND: Oral cancer is amongst the most prevalent cancers worldwide. Tobacco and alcohol are the two most important risk factors for development of oral cancer. Recently there has been mounting evidence that human papilloma virus plays an important role in development of a subset of head and neck squamous cell carcinoma. There is considerable variation in the frequencies of HPV positive tumors in published studies in the world and also from India. AIM: This work was carried out to investigate the prevalence of human papilloma virus (HPV) infection in oral and oropharyngeal squamous cell carcinomas in a tertiary care hospital in Bareilly city of Uttar Pradesh state of India. METHODS: Conventional Polymerase Chain Reaction technique using consensus HPV primers was used to determine HPV positivity in biopsy specimens from 100 patients of oral and oropharyngeal squamous cell carcinomas. HPV positive and negative tumors were compared with respect to clinicopathological characteristics like age, gender, site, exposure to tobacco and alcohol, grade and stage of tumor. RESULTS: HPV was detected in 18% cases. HPV positivity was significantly associated with poorly differentiated cancers. HPV positive and negative tumors did not differ significantly with regard to mean age, gender, site, tobacco use, alcohol intake and stage at presentation. CONCLUSION: HPV infection plays a role in a proportion of oral and oropharyngeal squamous cell carcinomas.

KEYWORDS: Oral squamous cell carcinoma, Oropharyngeal squamous cell carcinoma, Human papilloma virus.

INTRODUCTION: Oral and pharyngeal cancer, grouped together, is the sixth most common cancer in the world.1 There is a wide geographical variation in the incidence of oral cancer, with approximately two-thirds of patients in the developing countries of Southeast Asia, Eastern Europe and Latin America.2 This variation reflects the differences in prevalence of specific risk factors. A high incidence of lip cancer is found among white races exposed to solar radiation. High rates of incidence of cancers of intra oral sites are reported from communities with high consumption of tobacco, particularly among users of smokeless tobacco, often in association with areca nut in the form of betel quid.3 Tobacco, alcohol, poor oral hygiene, and genetics are important risk factors for head and neck tumors overall.4 Approximately 20% of oral cancers occur in people lacking these established risk factors and there is strong epidemiologic and experimental evidence indicating that Human Papillomavirus (HPV) may account for the majority of these cancers.5 HPVs are small double-stranded DNA tumor viruses infecting oral mucosa and genital tracts. HPVs responsible for benign epithelial hyperproliferation are named “low-risk” types (HPV-LR), while HPVs associated with premalignant lesions and invasive squamous cell carcinoma are named “high-risk” types (HPV-HR).6 HPV 6 and 11 are the two most common low-risk types; they account for the majority of genital warts, which rarely progress to malignancy. While two high-risk HPV types 16 and 18 are closely associated with cervical cancer, HPV-16 is predominant in head and neck cancers.7
Since the 1980’s, there is growing evidence of an etiological link between HPV infection and a subset of head and neck squamous cell carcinomas (HNSCC), especially those involving the oropharynx. It was first proposed in 1983 by Syrjanen K et al and then supported by several other authors.

The frequencies of HPV-positive tumors in HNSCC show considerable variation in published studies. Some studies report frequencies of 0% in oral and laryngeal carcinomas, whereas others report up to 93% in oropharyngeal carcinomas. In a meta-analysis of 4852 HNSCC the pooled prevalence of HPV DNA in the overall samples was 34.5%, in Oral SCC it was 38.1%. HPV -16 is the most common subtype observed in HNSCC.

HPV-positive head and neck SCCs are histologically distinct from HPV-negative tumors. In contrast to the non-HPV-related HNSCC, which are usually moderately differentiated and keratinizing, HPV-associated HNSCC are consistently poorly differentiated and non-keratinizing, and have a distinct basaloid appearance. This malignant disease has a better prognosis than HPV negative tumors, due in part to increased sensitivity of cancers to chemotherapy and radiotherapy.

The objective of this study was to determine the prevalence of HPV infection in oral and oropharyngeal malignancies and to determine if HPV positive and negative tumors were different in various clinicopathological characteristics.

**MATERIAL AND METHODS:** The present study was carried out at Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly, after obtaining approval from the ethical committee of the institute.

This was a prospective study of 100 biopsy proven cases of oral cavity and oropharyngeal malignancies received in the pathology department, SRMS IMS, Bareilly from January 2008 to December 2013. Punch biopsy from the lesion was obtained and half of the sample was formalin fixed for light microscopy with haematoxylin and eosin stain to confirm the diagnosis, while the remaining half was subjected to PCR. The variables analyzed were age, sex, site, history of use of tobacco and alcohol, histopathological diagnosis, grade and stage of tumor. The pertinent clinical details were obtained from the patients' clinical records.

The WHO grading system was used for the histopathological grading of oral cavity and oropharyngeal squamous cell carcinomas. This system is largely based on the Broders classification and uses three grades Grade I (Well differentiated), Grade II (Moderately differentiated) and Grade III (Poorly differentiated). The staging of oral cavity and oropharyngeal cancers was based on the TNM system of the American Joint Committee on Cancer (AJCC).

On the basis of stage at presentation the patients were divided into two groups: those with early stage disease (Stage I/II) and those with late stage disease (Stage III/IV).

Prevalence of HPV infection in oral and oropharyngeal malignancies was evaluated by Polymerase Chain Reaction (PCR) method.

Chi-square test was employed to test the association of different variables with HPV status and a p value of less than 0.05 was taken as significant.

**PCR TECHNIQUE:** Genomic DNA was isolated from the biopsy tissue by phenol–chloroform extraction method. PCR was carried out with the HPV consensus primers MY09/11. Negative samples were tested using GP5+/6+ primers to exclude false negatives. The details of primers are as follows:
The PCR reaction conditions were as follows: MY09/11: initial denaturing step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 10 minutes.

GP5+/GP6+: Initial denaturing step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 40°C for 2 minute and extension at 72°C for 10 minutes. PCR was performed using 300ng DNA as template. Results were analysed on 1.5% agarose gel containing 0.5 µg /ml ethidium bromide. A band at 450 base pair specific for HPV was seen.

RESULTS: Of the 100 cases of oral cavity and oropharyngeal malignancies, 88(88%) were males and 12(12%) were females. Mean age at diagnosis was 51.11years in males and 54.24 years in females. Table 1 shows the clinicopathological profile of HPV positive and HPV negative oral cancer patients.

It was observed that in a total 100 patients of oral cavity and oropharyngeal carcinomas 18 (18%) were found to be HPV positive and 82(82%) were found to be HPV negative (Figure 1).

In the HPV positive group 15(83.33%) were males and 3(16.37%) were females and in the HPV negative group 73(89.02%) were males and 9(10.98%) were females. This difference in gender was found to be statistically insignificant with a p value of 0.25.

The mean age of HPV positive patients were found to be 51.33 years and for the HPV negative patients to be 53.24 years. This was found to be statistically insignificant with a p value of 0.85.

It was found that 61 patients were tobacco users and 39 were not. In the HPV positive patients 8(44.45%) were tobacco users and 10(55.55%) were not. In the HPV negative patients 53(64.63%) were tobacco users and 29(35.37%) were not. This was found to be statistically insignificant with a p value of 0.11.

It was observed that 41 patients had history of alcohol intake and 59 patients had no history of alcohol intake. In the HPV positive patients 6(33.33%) had history of alcohol intake and 12(66.67%) did not have history of alcohol intake. However in the patients with HPV negative 35(42.68%) had history of alcohol intake and 47(57.32%) did not. This was found to be statistically insignificant with a p value of 0.46.

It was observed that 49 patients had malignancy of oral cavity and 51 had malignancy of oropharynx. In the HPV positive group, 6(33.33%) patients had malignancy of oral cavity and 12(66.67%) had malignancy of oropharynx. Whereas among HPV negative patients 43(52.44%) had malignancy of oral cavity and 39(47.56%) had malignancy of oropharynx. This was found to be statistically insignificant with a p value of 0.14.

Among the 100 patients it was observed that 43 patients had well differentiated malignancy, 47 had moderately differentiated malignancy and 9 had poorly differentiated malignancy (Figures 2, 3 and 4). In HPV positive cases, 6(33.33%) were well differentiated, 7(38.88%) were moderately differentiated and 5(27.79%) were poorly differentiated. However in HPV negative cases, 38(46.34%) were well differentiated, 40(48.78%) were moderately differentiated and 4(4.88%) were poorly differentiated. This difference was found to be statistically significant with a p value of 0.009.
Among the 100 patients, 22 patients had stage I malignancy, 25 had stage II, 26 had stage III and 27 had stage IV. In HPV positive cases 3(16.66%) were stage I, 4(22.22%) were stage II, 6(33.33%) were stage III and 5(27.79%) were stage IV. Whereas in HPV negative cases 19(23.17%) were stage I, 21(25.61%) were stage II, 20(24.40%) were stage III and 22(26.82%) were stage IV. This difference was not found to be statistically significant with a p value of 0.84.

**DISCUSSION:** India is a high-risk region for oral and oropharyngeal cancers due to a high prevalence of tobacco use, particularly chewing (in both sexes), bidi smoking and alcohol drinking in male population. Tobacco and alcohol are the two most important known risk factors for the development of oral cancer. Cofactors in oral squamous cell carcinoma include dietary factors, immunodeficiency and viral infections like HPV 16/18.18

PCR study for HPV was done in 100 patients out of which 18(18%) were HPV positive and 82 (82%) were HPV negative. This is in accordance with Smith et al who found prevalence of high risk HPV types to be 20% in cancer patients.19 Other studies done in India show 69% of head and neck squamous cell carcinoma patients to be HPV positive in Eastern India20 as compared to 67% in South India,21 15% of oral cancers in Western India22 and 64.5% of oral cancers in north India.23 The reported rates of detection of HPV DNA in head and neck SCC in various studies range from 0% to 100%. The extreme variation in reported prevalence may be owing to lumping together of essentially different lesions, to different sample numbers ranging from 2 to 100 samples and to difference in the sampling techniques, in the ethno-geographic origins of the subjects examined and in the HPV detection methods applied.24

In our study out of 18 HPV positive patients, we found 15(83.33%) males and 3(16.37%) females and 73(89.02%) males and 9(9.98%) females to be HPV negative. This difference was not found to be statistically significant. This finding was similar to the study by Smith et al.19 However the male patients were significantly more HPV positive in a study done by Mitra et al.20

In this study mean age was found to 51.33 in HPV positive patients and 53.24 in HPV negative patients. HPV 16 DNA was more common in case subjects of oral SCC less than 50 years of age at diagnosis than case subjects greater than or equal to 50 years of age at diagnosis.25 Some studies have documented that high-risk HPV types are three times more likely to be detected in OSCC in young patients than in patients above 60 years of age.5

The history of tobacco and alcohol use was found to be less common in HPV positive patients than HPV negative patients. However this difference was not found to be significantly significant. Gillison et al26 found that as compared with HPV-negative oropharyngeal cancers, HPV-positive oropharyngeal cancers were less likely to occur among moderate to heavy drinkers and smokers.

In our study among HPV positive patients greater number of cases i.e. 12(66.67%) were present in the oropharynx while in HPV negative patients greater number of cases i.e. 43(52.44%) were present in the oral cavity. Klussmann et al27 found that among HNSCC the frequency of HPV positive lesions was maximum (45%) for oropharynx. In a meta-analysis of an association between HPV 16 and HNSCC, Hobbs et al28 found that the association between HPV16 and cancer was the strongest for the tonsils, intermediate for the oropharynx, and weakest for the oral cavity and larynx.

The histopathological grading of OSCC cases in the present study showed that among HPV positive patients maximum were moderately differentiated 7(38.88%) followed by well 6(33.33%) and poorly differentiated 5(27.79%) respectively. Similar findings were observed in HPV negative patients with 40(48.78%) of moderately differentiated cancer followed by 38(46.34%) well and
4(4.88%) poorly differentiated cancers. However the percentage of HPV positive patients with poorly differentiated tumour morphology was significantly higher than HPV negative patients (p=0.009). Several studies have reported that detection of HPV DNA is highly correlated with poor tumor differentiation grade.26,29,30

In the present study more number of cases was found to be in stage III/IV (61.12%) compared to stage I/II (38.88%). Among HPV negative patients also more number of patients was in stage III/IV (51.22%) as compared to stage I/II (48.78%). These findings were in accordance with the study of Mitra S et al.20 In general HPV-associated oropharyngeal cancers at presentation are stage III or IV.4

CONCLUSION: Thus in the present study 18% patients were positive for HPV infection. There was a statistically significant difference between the histological grade of HPV positive and HPV negative SCC with significantly higher number of HPV positive patients presenting with poorly differentiated SCC. HPV positive tumors did not differ significantly from HPV negative tumors with regard to patient age, gender, tobacco and alcohol use, tumor site, and stage at presentation.

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| Characteristics | Total No. of cases | HPV + (18)(%) | HPV- (82)(%) | P value |
|-----------------|--------------------|---------------|--------------|---------|
| Gender          |                    |               |              |         |
| Male            | 88                 | 15(83.33)     | 73(89.02)    | 0.25    |
| Female          | 12                 | 3(16.37)      | 9(10.98)     |         |
| Mean age        | 100                | 51.33         | 53.24        | 0.85    |
| Tobacco         |                    |               |              |         |
| Yes             | 61                 | 8(44.45)      | 53(64.63)    | 0.11    |
| No              | 39                 | 10(55.55)     | 29(35.37)    |         |
| Alcohol         |                    |               |              |         |
| Yes             | 41                 | 6(33.33)      | 35(42.68)    | 0.46    |
| No              | 59                 | 12(66.67)     | 47(57.32)    |         |
| Site            |                    |               |              |         |
| Oral cavity     | 49                 | 6(33.33)      | 43(52.44)    | 0.14    |
| Oropharynx      | 51                 | 12(66.67)     | 39(47.56)    |         |
| Grade           |                    |               |              |         |
| WD              | 44                 | 6(33.33)      | 38(46.34)    | 0.009   |
| MD              | 47                 | 7(38.88)      | 40(48.78)    |         |
| PD              | 9                  | 5(27.79)      | 4(4.88)      |         |
| Stage           |                    |               |              |         |
| I               | 22                 | 3(16.66)      | 19(23.17)    | 0.84    |
| II              | 25                 | 4(22.22)      | 21(25.61)    |         |
| III             | 26                 | 6(33.33)      | 20(24.40)    |         |
| IV              | 27                 | 5(27.79)      | 22(26.82)    |         |

Table 1: Clinicopathological parameters in HPV positive and HPV negative oral and oropharyngeal SCC

WD: Well differentiated
MD: Moderately differentiated
PD: Poorly differentiated
Fig. 1: Prevalence of HPV infection among 100 cases

Fig. 2: Well differentiated squamous Cell carcinoma (H & E, 10X)

Fig. 3: Moderately differentiated squamous cell carcinoma (H & E, 10X)
AUTHORS:
1. Tanu Agrawal
2. Tripti Garg
3. Jaspreet Kaur
4. Rohit Sharma
5. Ratna Saxena

PARTICULARS OF CONTRIBUTORS:
1. Associate Professor, Department of Pathology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly.
2. Junior Resident, Department of Pathology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly.
3. Research Associate, Department of Biochemistry, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly.
4. Professor, Department of ENT & Head & Neck Surgery, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly.
5. Professor & HOD, Department of Pathology, Shri Ram Murti Smarak Institute of Medical Sciences, Bareilly.

NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Tanu Agrawal,
Associate Professor,
Department of Pathology,
Shri Ram Murti Smarak Institute of Medical Sciences, Nainital Road, Bhojipura,
Bareilly-243202, Uttar Pradesh, India.
E-mail: tanuagrawal510@yahoo.co.in

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Fig. 4: Poorly differentiated squamous cell carcinoma (H & E, 10X)