Original Article

Human Papilloma Virus Related Oral Carcinomas

# The Frequency of Human Papilloma Virus Related Oral Squamous Cell Carcinomas by P16 Immuno Histochemical Stain

**Asmah Afzal[1], Rajia Liaqat[2], Faiza Shafqat[3], Farah Kalsoom[4], Asif Loya[1]**

## ABSTRACT

**Objective:** To determine the frequency of HPV positive oral squamous cell carcinomas by applying p16IHC stain in Pakistani population.

**Study Design:** Descriptive, Cross-sectional study

**Place and Duration of Study:** This study was conducted at the Shaukat Khanum Memorial Hospital and Research Centre for six (06) months from August 2018 to the February 2019.

**Materials and Methods:** In this research 140 patients with oral squamous cell carcinomas, histologically diagnosed, of all ages and either gender. Patients with treatedcases (surgery, chemotherapy, radiotherapy) were excluded. The biopsy was performed and fixed in 10% neutral bufferedformalin. Hematoxylin and eosin were used in the staining of the tissues. The Immunohistochemical (IHC) stainp16 was performed in the same batch according to the specification given by the manufacturer.

**Results:** Mean age in our study was 48:86 ± 9:37 years. Out of the 140 patients, 114(81:43%) were maleand 26(18:57%) were females resulting in male to female ratio of 4:4:1. HPV associated oral squamous cellcarcinomas by p16 immunocytochemistry were found in57(40:71%) patients. It was also found that therewas a significant difference (p <0:05) of HPV associated oral squamous cell carcinomas between differentage groups while no significant difference (p >0:05) of HPV associated oral squamous cell carcinomas werefound between gender and stage of tumor.

**Conclusion:** This study concluded that there is a high frequency of HPV associated oral squamous cellcarcinomas by p16 immunocytochemistry in our population with a positive association with younger age andmale gender.

**Key Words:** Squamous Cell Carcinoma, Oral, Human Papilloma Virus.

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## INTRODUCTION

Oral cavity cancers are amongst the most common malignancies in Pakistan and many other countries of the world.[1,2] Among the subgroups, about 95% of cancers are squamous cell carcinomas (SCC). The non-SCC include adenocarcinoma of minor salivary glands, malignant melanomas, clear cell, and adenoid cystic carcinomas.[3]Bhurgi Y et al[4] reported an annual SSC incidence rate of 4.1 and 4.0 per 100,000/year in males and females respectively.

SCC is of epithelial origin, squamous cell, which isa major component of oral cavity. SCC originatesfrom the uncontrolled proliferation of mutated cells. Clinically, the carcinoma may present in the form of white plaques, ulcers and fungating masses. A major regional influenceof chewing tobacco, betel nut, pan, cigarette smoking and huqqa in Pakistan suggest common etiological factors in the pathogenesis of oral cancers. Other factors include alcohol use, nutritional deficiencies, syphilis, immune deficiency disorders, radiations, poor oro-dental hygiene, chronic irritation, trauma, and viruses.[5]

Among viruses, human papilloma virus (in particular type 16) has been identified in 10% to 15% of oral carcinomas and appears to be among the strongest possible risk factor.[6]Immuocytochemistry, in situ hybridization and polymerase chain reaction are commonly used techniques for virus detection in tumors. P16 IHC stain is considered as a reliable

[1] Department of Pathology, Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH& RC), Lahore.
[2] Department of Pathology, Al Aleem Medical College, Lahore.
[3] Department of Pathology, Bakhtawar Amin Medical & Dental College/ Hospital. Multan.
[4] Department of Pathology, Fatima Jinnah Medical University (FJMU), Lahore.

Correspondence: Asmah Afzal, Resident Doctor, Department of Histopathology, Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH& RC)
Contact No: 03457276924
Email: asmahafzal@gmail.com

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surrogate marker with a sensitivity of 100% and specificity of 74%[7] while in another study the sensitivity was 76% and specificity was 71%.[8]

In this research, the frequency of human papilloma virus (HPV) related oral squamous cell carcinomas (OSCC) by p16 IHCis studied for the Pakistani population.

# MATERIALS AND METHODS

It is a descriptive, cross-sectional study which is conducted in the Pathology department of SKMCH & RC, Lahore, for a period of six (06) months(the 6th of August 2018 to the 5th of February 2019). The sample size of 140 cases was calculated with 8% margin of error and 95% confidence level with an expected frequency of HPV related oral squamous cell carcinoma35%. Non-probability, purposive sampling technique was used in the sampling process. For the sample selection, the following are the inclusion and exclusion criteria.

Inclusion Criteria

i. All patients with histological diagnosis of OSCC.
ii. Patients of all ages.

**Exclusion Criteria**

i. Unfixed and poorly preserved specimen.
ii. Treated cases (surgery, chemotherapy, radiotherapy).
iii. Site other than the oral cavity.

**Data Collection Procedure**

Cases of OSCC fulfilling inclusion criteria were included instudy after approval of the ethical committee of SKMCH & RC and informed consent fromthe respective patient. The biopsy was performed and fixed in 10% neutral buffered formalin. Tissuewas processed and stained with hematoxylin and eosin. The personal bias was controlledby showing all cases to one consultant with a minimum of 5 years experience. TheIHC stain p16 was performed in the same batch according to the specificationgiven by the manufacturer. Data was collected through a prescribed and approved form which contained two parts. First Part included the patient's bio-data while second part contained the study variables.

**Data Analysis Procedure**

The collected data is analysis through state of the art tool for data computation i.e. "SPSS version 19". The quantitative variables of the study were calculated as mean and deviation from the mean i.e. standard deviation. The qualitative variables like gender and HPV positive SCC were presented in the form of frequencies and/ or percentages. Stratification was done on three variables which are age, gender and stage to explore the impact of these variables on HPV positive SCC. Post stratification chi-square test was applied with a significant P-value at $\leq 0.05$.

# RESULTS

The age range in this study was from 18 to 80 years with a mean age of 48.86 ± 9.37 years. Majority of the patients 51 (36.43%) were between 36 to 50 years of age as shown in Table I. Out of the 140 patients, 114 (81.43%) were male and 26 (18.57%) were females with male to female ratio of 4.4:1. HPV associated OSCC by p16 immunocytochemistry was found in 57 (40.71%) patients, whereas there were no HPV associated oral squamous cellcarcinomas in 83 (59.29%) patients as shown in Figure I. The percentage of patients according to stage of disease have shown in Figure II which showed that majority of patients were with stage II i.e. 58 (41.43%).

When Stratification of HPV associated oral SSC with respect to age groups was done, it was found that there was significant difference ($p<0.05$) of HPV associated OSCC between different age groups as shown in Table III while the stratification of HPV associated OSCC with respect to gender and stage of carcinoma has shown in Table IV & V respectively which showed no significant difference ($p>0.05$).

**Table No.1: Age distribution according to gender (n=140)**

| Age (years) | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | No. of patients | %age | No. of patients | %age | No. of patients | %age |
| 18-35 | 17 | 14.91 | 01 | 3.85 | 18 | 12.86 |
| 36-50 | 44 | 38.60 | 07 | 26.92 | 51 | 36.43 |
| 51-65 | 29 | 25.44 | 11 | 42.31 | 40 | 28.57 |
| 66-80 | 24 | 21.05 | 07 | 26.92 | 31 | 22.14 |
| Total | 114 | 81.43 | 26 | 18.57 | 140 | 100.0 |

**Mean ± SD = 48.86 ± 9.37 years**

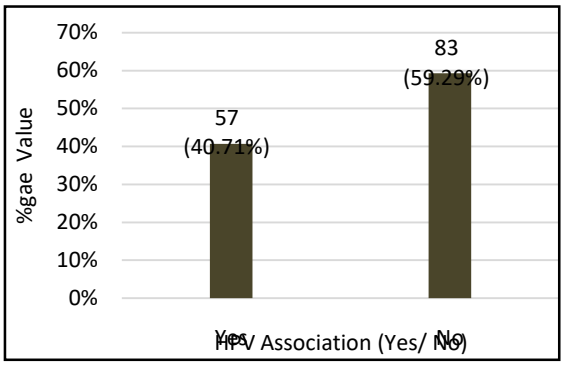


**Figure No.1: %age of the Patients with HPV associated oral SSC**

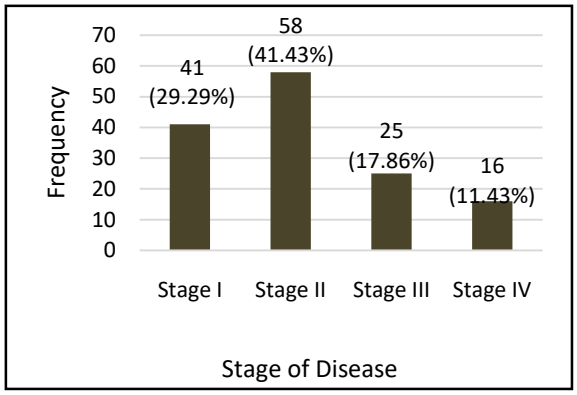


**Figure No.2: %age of patients with HPV associated oral SSCAccording to the Stage**

**Table No.2: Stratification of HPV Associated Oral SSC with Respect to Age Groups**

| Age (years) | HPV associated oral SSC | | p-value |
|---|---|---|---|
| | Yes | No | |
| 18-35 | 10 (55.56%) | 08 (44.44%) | 0.009 |
| 36-50 | 28 (54.90%) | 23 (45.10%) | |
| 51-65 | 11 (27.50%) | 29 (72.50%) | |
| 66-80 | 08 (25.81%) | 23 (74.19%) | |

**Table No.3: Stratification of HPV Associated Oral SSC with Respect to Gender**

| Gender | HPV associated oral SSC | | p-value |
|---|---|---|---|
| | Yes | No | |
| Male | 49 (42.98%) | 65 (57.02%) | 0.253 |
| Female | 08 (30.77%) | 18 (69.23%) | |

**Table No.4: Stratification of HPV Associated Oral SSC with Respect to Stage of Disease**

| Stage of disease | HPV associated oral SSC | | p-value |
|---|---|---|---|
| | Yes | No | |
| Stage I | 17 (41.46%) | 24 (58.54%) | 0.743 |
| Stage II | 26 (44.83%) | 32 (55.17%) | |
| Stage III | 09 (36.0%) | 16 (64.0%) | |
| Stage IV | 05 (31.25%) | 11 (68.75%) | |

# DISCUSSION

According to the literature, there is a strong tie between the oral HPV infection and OSSC in the cases where the subjects are addicted to the tobacco and alcohols.[9] In this context, it is possible that HPV transmission occured through the oral—genital or direct mouth-to-mouth or other means of contact.

Due to the heterogeneous nature of the OSCC, finding a relation between the HPV and OSCC is no easy task. Furthermore, it becomes more difficult due to a very small number of cases which are HPV positive. Syrjänenet al. for the first time reported in a study that some OSCC have morphological and IHC features which reveal HPV as etiological factor.[9]In this study involvement of the HPV in the pathogenesis of a subset of HNSCC was indicated.[9]According to the literature, the identification of the HPV-associated OSCC is a very difficult task which is often basaloid in histology.

In our study, HPV associated OSCC by p16 immunocytochemistry was found in 40.71% patients, whereas there was no HPV associated OSCCin rest of the 59.29% patients. As compared to our study, Agrawal GP et al[10] reported22.5% positive cases for HPV 16. However, the association between the "high risk human papillomavirus (HR HPV)" and risk of oral cancer development is reported since1983 after the detection of HPV16 OSCC. Since then HPV DNA has repeatedly been observed in head and neck cancers.[11,12]

In another study, OSCC p16 positivity of 86.66% was reported. Of 26/30 positive cases, p16 staining was positive in 70%, 90% and 100% of well-differentiated, moderately differentiated, poorly differentiated OSCC respectively.[13] Kojima A et al[14] in his study has found HPV associated OSCC by p16 immunocytochemistryin 66.66% patients.

A study conducted for Indian population shows that HPV infection is more frequent in OSCC cases which is 33.6% as compared to the 23%, 8-20% and 19% of the Japanese, American and Dutch patients.[15] This variability may be attributable to ethnicity and geography,a small number of samples analyzed, possible contamination and detection technique used.[16]

In another study, Duncan LD et al[17]utilized the p16 IHC as a substitute marker for high-risk HPV and as an alternate test to PCR. Authors of the study observed 55.6% with 0 staining, 27.2% with 1+ staining, and 8.6% with 2+ staining. **de Abreu** PM et al[18]in his study has shown a very low frequency of HPV associated oral squamous cell carcinoma (4.04%). However, in contradiction to the previous studies and our study, Young SK et al[19], and Tsuchiya H et al[20] were unable to find such association between HPV 16 and OSCC.

According to the literature, 25-75% of oropharyngeal cancers results in the HPV positive, where tonsils has the highest ratio followed by tongue and buccal mucosa.[76] The probable reason could be that HPV being an inhabitant of normal crypt epithelia and Waldeyer's ring, an antigen presenting site, may act as the reservoir for HPV.

Similar to the underlying study, Gillison ML et al[21] conducted a cross-sectional study and showedthat the frequency of oral HPV infection only in 6.9% population of the age range 14 - 69 years. The study revealed a higher frequency in male as compared to the female.

In the underlying study, distribution of HPV 16 positive/ negative cases according to the age group had shown a strong correlation with the results reported by Cruz IBF et al[22]. However, the results of this study are not in line with the results reported by the Kurose K et

al.[23]According to the gender-based distribution of the results of positive cases, close similarities are observed with the results reported by the Werness BA et al[24] where the males having predominance. Male predominance is also reported by the Cruz IBF et al[22] and Koppikar P et al.[25]

Statistical analysis of the results shows that there is no correlation between the HPV16 and histopathologic grades of differentiation which is in line with the results reported by the Schlecht NF et al[26] and Badaracco G et al[27] however, Abdelsayed RA[28] reported a strong correlation.The results indicate that the HPV association withOSCC by p16 immunocytochemistry is significant and there is positive association with young age and male gender.

# CONCLUSION

This study concluded that there is a high frequency of HPV associated oral squamous cellcarcinomas by p16 immunocytochemistry in our population revealing positive association with young age and male gender. As we know from recent studies that HPV associated OSCCare more sensitive to the chemo-and-radiotherapy and accordingly require limited resection even after lymph node metastases, it is highly recommended to detect HPV positive association by p16 IHC stain as a mandatory pre-treatment assessment to segregate those who would benefit maximum from the therapy and who would not. Also, it would help in risk stratification to avoid intensification of treatment, to reduce severe acute and late treatment associated side effects, to improve the therapeutic ratio, to minimize unnecessary hospital stayand above all to aid oncologists to develop more effective and advanced treatment modalities to improve patient survival and quality of life.

**Author's Contribution:**

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|---|---|
| Concept & Design of Study: | Asmah Afzal, RajiaLiaqat |
| Drafting: | FaizaShafqat, FarahKalsoom |
| Data Analysis: | FaizaShafqat, FarahKalsoom |
| Revisiting Critically: | Asmah Afzal, RajiaLiaqat |
| Final Approval of version: | Asmah Afzal, Asif Loya |

**Conflict of Interest:** The study has no conflict of interest to declare by any author.

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