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HUMAN PAPILLOMA VIRUS PREVALENCE IN SQUAMOUS CELL CARCINOMA OF ORAL TONGUE

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Abstract:

Background and purpose: *Oral squamous cell carcinoma usually affects men between sixth and eighth life decade. The main causative elements are alcohol consumption and tobacco use. An increase in the patient's number with oral cancer deprived of these recognized causative factors proposes that human papillomavirus (HPV), a viral infection may be accountable for this upsurge by substitute as an oncogenic factor. In this study, the appearance of HPV infection and the clinical-pathological significance in oral SCC were examined.*

Place and Duration: *In the Pathology, Oral and Maxillofacial surgery and Otolaryngology Department of Services Hospital Lahore for one year duration from March 2018 to March 2019.*

Methods: *Tissue blocks were selected from 50 cases (SCC patients of the oral tongue) and 50 controls (benign diagnosed palatine tonsil tissues) were included. DNA was mined from cancerous and non-cancerous tissue blocks. Dispersed HPV DNA, conventional PCR polymerase chain reaction (PCR) and detection of high-risk genotypes were obtained using HPV 16 and 18, and the results were correlated with clinical pathological parameters.*

Results: *HPV was positive in 50 patients (26 males and 24 females, mean age 58.36 ± 12.18 years and age range 28-87 years) and 10 (14%) patients were positive for HPV. None of the subjects in the control group had positive results in HPV DNA (p value 0.012). The HPV 16/18 genotype has not been identified in positive patients. There was no statistically substantial relationship between HPV status and age, gender, tumor stage, tumor grade or involvement of lymph nodes.*

Conclusion: *Though the incidence of HPV is much higher in oral tongue, its relationship with carcinogenicity in this area requires further investigation.*

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INTRODUCTION:

Head and Neck Squamous Cell Carcinoma is the 6th most communal malignancy observed globally. The oral tongue squamous cell carcinoma contains about fifty percent of the oral squamous cell carcinoma; this usually affects men from 6th to 8th decades[1-2]. The main traditional possibility reasons for oral squamous cell carcinoma, including the tongue cavity, are consumption of alcohol and tobacco. Though, in spite of the decrease in its prevalence of habits, cancer of the tongue and mouth is increasing, especially in young adults[3].

Human papillomavirus (HPV) is deliberated an oncogenic virus and might be accountable for this growth. For HNSCC; this virus is a significant etiological factor especially oropharyngeal and tonsil cancer, but its relationship with oral squamous cell carcinoma is uncertain[4-5]. In squamous cell carcinoma; incidence of HPV of oral tongue is very adjustable and ranges from zero to hundred percent in various studies. This may be because of sample type, sample size, detection methods, storage conditions, incorrect classification of the oropharyngeal tongue as a subset of the oral cavity and geographical variability[6-7].

Positive tumors for the human papillomavirus are much communal in young subjects and have minor tumors with progressive involvement in the neck. These tumors are poorly distinguished histologically and is SCC (non-keratinized) with basaloid properties[8-9]. Tumors that are positive for human papillomavirus react well to radiation and chemotherapy and show improved survival and prognosis than tumors which are negative for HPV[10].

Limited information is available on the SCC and HPV epidemiology in Pakistan. In this analysis, clinical features and the status of HPV in SCC of oral tongue were assessed.

MATERIALS AND METHODS:

This study was held in the Pathology, Oral and Maxillofacial surgery and Otolaryngology Department of Services Hospital Lahore for one year duration from March 2018 to March 2019. Tissue blocks were selected from 50 cases (SCC patients of the oral tongue) and 50 controls (benign diagnosed palatine tonsil tissues) were included. The two pathologists reviewed hematoxylin and eosin (H&E) slides and previous reports. For comparative analysis; 50 blocks of palatine tonsil tissue (chronic tonsillitis and follicular hypertrophy) of benign patients were taken as a control group. According to earlier analysis of our study, people aged ≤45 years were considered young patients.

Molecular study

6 µm sections from all paraffin-embedded tissue blocks, formalin- fixed for DNA extraction were prepared by means of the mini DNA Extraction Tissue Kit conferring to the instruction's manual.

The nested PCR was used for HPV Co-DNA detection and high-risk genotypes, HPV 16 and 18; traditional PCR was used. β actin primers (317 bp) were cast-off as internal control. From human papilloma virus positive cervical tissue; DNA extract was identified as the positive control, was used for each PCR reaction. In a 25 µL volume containing 200 ng of DNA template; each amplification reaction was performed, 1.5 mM mgCl2, 200 µM dNTP, 1X PCR buffer, 0.2 µM each primer and 1 unit of taq DNA polymerase.

PCR conditions were as follows; The first denaturation was annealed for five minutes at 94 ° C, then for 45 seconds at 94 ° C for 40 denaturation cycles, 45 seconds at 55 ° C, extension for 45 seconds at 72℃ with a last allowance for 5 minutes at 72℃. On 2.5% agarose gel; amplicons were run, imaging with transilluminator and surveyed by Gel Red staining.

Human papillomavirus genotype

Conventional PCR was performed separately on positive HPV samples to identify HPV 16 and HPV 18. The nested PCR was used for HPV Co-DNA detection and high risk genotypes, HPV 16 and 18; traditional PCR was used. As an internal control; β actin primers (317 bp) were used. From human papilloma virus positive cervical tissue; DNA extract was identified as the positive control, was used for each PCR reaction. In a 25 µL volume containing 200 ng of DNA template; each amplification reaction was performed, 1.5 mM mgCl2, 1X PCR buffer, 200 µM dNTP, 0.2 µM each primer and 1 unit of taq DNA polymerase. With initial denaturation for 1 minute at 94 ° C; The PCR reaction was analyzed, followed by thirty five denaturation cycles for 1 minute at 94 ° C, for two minutes annealing at fifty eight degree, extension for three minutes at 72 ° C and final for 10 minutes elongation at 72 degree. On 2.5% agarose gel; amplicons were run, imaging with transilluminator and surveyed by Gel Red staining.

Statistical analysis

The association between status of human papilloma virus and clinical-pathological parameters in SCC of oral tongue was analyzed using SPSS software using S-square tests and Fisher's full tests (version 14). P values below 0.05 were measured significant statistically.

RESULTS:

27 to 86 years was the patients age range (average age 57.36 ± 12.18 years), and 36% were men and

64% were women. 46% of the control group were male, 54% were female, and 29 to 68 years was the age range (average age 49.73 ± 9.10 years) (p> 0.05). 14 cases (28%) were ≤45 years old and therefore were measured as young patients (Table 1).

Table 1. Correlation of Human Papillomavirus Status with the Characteristics of Oral Tongue Squamous Cell Carcinoma Cases

Characteristics	HPV		P-Value
	Positive (n=7) (%)	Negative (n=43) (%)	
Age			
≤45 years	2 (14.3%)	12 (85.7%)	1.000
>45 years	5 (13.9%)	31 (86.1%)	
Gender			
Male	5 (27.8%)	13 (72.2%)	0.083
Female	2 (6.2%)	30 (93.8%)	
Tumor Differentiation			
Well (n= 26)	6 (23.1%)	20 (76.9%)	
Moderately (n=18)	0 (0%)	18 (100%)	0.092
Poorly (n=6)	1 (16.7%)	5 (83.3%)	
Tumor stage			
I	1 (12.5%)	7 (87.5%)	
II	2 (28.6%)	5 (71.4%)	
III	2 (11.1%)	16 (88.9%)	0.755
IV	2 (11.8%)	15 (88.2%)	
Lymph node status			
Free	3 (14.3%)	18 (85.7%)	1.000
Involved	4 (14.8%)	23 (85.2%)	

From 50 SCC cases from the oral cavity; 7 (14%) of cases had Human papillomavirus in DNA.
The HPV genome was not detected in all tonsil tissues; this alteration was important statistically (p value = 0.012).
The patients mean age with and without HPV was 56.80 ± 10.70 and 57.50 ± 11.20, correspondingly (p-value 0.91).

In males; HPV infection was more communal than women, but the variance was not significant statistically (28.08 vs. 6.3%, p value 0.084). None of the samples (0%) showed positive results in the HPV 16 and 18 genotype. 2 of the 15 (15.01%) young subjects (≤45 years old) were HPV positive. In HPV positive patients, although the difference was not statistically significant (P1 value), the majority of patients were older than 45 years (71.4%) compared to younger patients (28.6%).

HPV infection was more common in men than women, but the difference was not statistically significant (27.8 vs. 6.2%, p value 0.083). None of the samples (0%) showed positive results in the HPV 16/18 genotype. Two of the 14 (14.3%) young patients (≤45 years old) were positive for HPV. In positive HPV cases, although the difference was not statistically significant (P1 value), the majority of patients were older than 45 years (71.4%) compared to younger patients (28.6%).

The HPV infection incidence was greater in well distinguished tumors than tumors which are poorly distinguished, but the transformation was not substantial statistically (22.2 vs. 17.07%, p value 0.092). The HPV was not positive among moderately differentiated tumors.

In positive HPV patients, maximum tumors were differentiated well, but the variance was not significant statistically (86.07% vs. 13.93%, 0.092 p value).

Although more common for HPV positive stage II tumors (29.16%), no statistically noteworthy difference was noted between tumor stage and HPV status (0.775 p-value).

In addition, no significant statistically difference was noted between positive HPV and status of lymph nodes (P1 value).

There was no statistically important relationship among HPV status and age, gender, tumor stage, tumor grade or involvement of lymph nodes. The association of status of HPV with case characteristics are given in Table 1.

DISCUSSION:

Many researches have revealed the causal character of infection with HPV at the beginning of palatine tonsils and SCC of tongue at its base, but their part as in SCC risk factor for oral tongue is debatable. In this study, DNA of HPV was identified in 15% of SCC in the oral tongue and high-risk HPV 16/18 genotypes were not detected.
Paz et al. Frozen tissue samples from 168 Indian patients were analyzed. In 13% of patients with tongue SCCs; DNA of HPV is found[11-12]. This analysis exhibited that patients with HPV positive had an advanced level than patients with negative HPV, but there was no noteworthy alteration in the

survival rate for 3-year. Kantola et al examined 105 cases of SCC in oral tongue and in all cases, HPV was noted.

Tsimplaki et al. In Greece, 53 SCC of oral tongue patients were evaluated. While 11.5% was the general positive HPV DNA rate, 7.5% of the patients were noticed to have high-risk HPV. They proposed that HPV is more communal in women than in men, and the infection is not concomitant with young age[13].

In India, men have more usual HPV than women, with no important alteration in the HPV infections frequency in young (≤46 years) and older (> 46 years) patients. In Liang et al study; 51 patients with oral SCC to analyze the presence of HPV DNA analyzed new frozen tumor by PCR, and only 1 tumor was positive for HPV (HPV-16) (1.96%); This patient was young.

The incidence of high-risk HPV varies significantly across several studies and in altered geographical areas. In our study, HPV infection was more common in men than in women[14-15]. There was no significant difference between the average age of patients with and without HPV positive. In addition, no significant differences were found in the incidence of positive HPV between young and old patients.

CONCLUSION:

Although no high-risk HPV was detected in our study, the incidence of HPV in SCC of Oral tongue was significantly higher than in the normal control group. No statistically noteworthy relationship was found between HPV DNA positivity and clinical-pathological traits.

This study results did not confirm the HPV infection role in carcinogenesis in SCC of oral tongue. Possible genetic and environmental factors of the host should be considered.

REFERENCES:

1. Tealab, S.H., Sedhom, N.F., Hassouna, A., Gouda, I. and Ismail, H., 2019. Prevalence of human papilloma virus in oropharyngeal, tongue and lip squamous cell carcinoma: an experience from the Egyptian National Cancer Institute. *Journal of Investigative Medicine*, *67*(7), pp.1061-1066.
2. Huang, K., Ni, G., Ma, B., Chen, S., Luan, Y., Wang, T., Walton, S., Frazer, I., Liu, X. and Pan, X., 2020. Importance of human papillomavirus infection in squamous cell carcinomas of the tongue in Guangdong Province, China. *Journal of International Medical Research*, *48*(1), p.0300060519897187.
3. Vidal Loustau, A.C., Dulguerov, N., Curvoisier, D., McKee, T. and Lombardi, T., 2019. Low prevalence of HPV-induced oral squamous cell carcinoma in Geneva, Switzerland. *Oral diseases*, *25*(5), pp.1283-1290.
4. Hernandez, B.Y., Lynch, C.F., Chan, O.T., Goodman, M.T., Unger, E.R., Steinau, M., Thompson, T.D., Gillison, M., Lyu, C. and Saraiya, M., 2019. Human papillomavirus DNA detection, p16INK4a, and oral cavity cancer in a US population. *Oral oncology*, *91*, pp.92-96.
5. Boon, S.S., Chen, Z., Li, J., Lee, K.Y., Cai, L., Zhong, R. and Chan, P.K., 2019. Human papillomavirus type 18 oncoproteins exert their oncogenicity in esophageal and tongue squamous cell carcinoma cell lines distinctly. *BMC cancer*, *19*(1), pp.1-12.
6. Last, A.S., Pipkorn, P., Chen, S., Kallogjeri, D., Zenga, J., Rich, J.T., Paniello, R., Zevallos, J., Chernock, R., Adkins, D. and Oppelt, P., 2020. Risk and Rate of Occult Contralateral Nodal Disease in Surgically Treated Patients With Human Papillomavirus− Related Squamous Cell Carcinoma of the Base of the Tongue. *JAMA Otolaryngology–Head & Neck Surgery*, *146*(1), pp.50-56.
7. Bygani, S.K., Sabarinath, B., Sivapathasundharam, B. and Karthik, L., 2020. Role of Human Papilloma Virus in Oral Squamous Cell Carcinoma of Patients with Smoking and Non-Smoking Habits Based on Immunoexpression of p16: An Immunohistochemical Study. *Archives of Advances in Biosciences*, *10*(4), pp.64-74.
8. Martin-Gomez, L., Fulp, W.J., Schell, M.J., Sirak, B., Abrahamsen, M., Isaacs-Soriano, K.A., Lorincz, A., Wenig, B., Chung, C.H., Caudell, J.J. and Giuliano, A.R., 2019. Oral gargle-tumor biopsy human papillomavirus (HPV) agreement and associated factors among oropharyngeal squamous cell carcinoma (OPSCC) cases. *Oral oncology*, *92*, pp.85-91.
9. Wittekindt, C., Wagner, S., Bushnak, A., Prigge, E.S., von Knebel Doeberitz, M., Würdemann, N., Bernhardt, K., Pons-Kühnemann, J., Maulbecker-Armstrong, C. and Klussmann, J.P., 2019. Increasing incidence rates of oropharyngeal squamous cell carcinoma in Germany and significance of disease burden attributed to human papillomavirus. *Cancer Prevention Research*, *12*(6), pp.375-382.
10. Bukhari, N., Joseph, J.P., Hussain, S.S., Khan, M.A., Wakim, M.J.Y., Yahya, E.B., Arif, A., Saleem, A. and Sharif, N., 2019. Prevalence of Human Papilloma Virus Sub Genotypes following Head and Neck Squamous Cell Carcinomas in Asian Continent, A Systematic

Review Article. *Asian Pacific Journal of Cancer Prevention*, *20*(11), pp.3269-3277.

11. Haeggblom, L., Attoff, T., Yu, J., Holzhauser, S., Vlastos, A., Mirzae, L., Ährlund-Richter, A., Munck-Wikland, E., Marklund, L., Hammarstedt-Nordenvall, L. and Ye, W., 2019. Changes in incidence and prevalence of human papillomavirus in tonsillar and base of tongue cancer during 2000-2016 in the Stockholm region and Sweden. *Head & neck*, *41*(6), pp.1583-1590.
12. Dalakoti, P., Ramaswamy, B., Bhandarkar, A.M., Nayak, D.R., Sabeena, S. and Arunkumar, G., 2019. Prevalence of HPV in Oral Squamous Cell Carcinoma in South West India. *Indian Journal of Otolaryngology and Head & Neck Surgery*, *71*(1), pp.657-664.
13. Rettig, E.M., Gooi, Z., Bardin, R., Bogale, M., Rooper, L., Acha, E. and Koch, W.M., 2019. Oral Human Papillomavirus Infection and Head and Neck Squamous Cell Carcinoma in Rural Northwest Cameroon. *OTO open*, *3*(1), p.2473974X18818415.
14. Yaaqob, M.A., Hairon, S.M., Kannan, T.P. and Rahman, N.A., 2019. Factors associated with mortality of oral squamous cell carcinoma (OSCC) patients managed at Hospital USM. *Archives of Orofacial Science*, *14*(1).
15. Ramesh, P.S., Devegowda, D., Singh, A. and Thimmulappa, R.K., 2020. NRF2, p53, and p16: Predictive Biomarkers to Stratify Human Papillomavirus Associated Head and Neck Cancer Patients for De-Escalation of Cancer Therapy. *Critical Reviews in Oncology/Hematology*, p.102885.