The Professional Medical Journal
www.theprofesional.com

https://doi.org/10.29309/TPMJ/2021.28.08.3814

ORIGINAL PROF-0-3814

Human papilloma virus prevalence and its correlation with clinical presentation and histological grading in oral squamous cell carcinoma.

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Article received on:
13/06/2019
Accepted for publication:
28/08/2020

ABSTRACT… Objectives: To determine the prevalence of Human Papilloma virus 16 in patients with oral squamous cell carcinoma to correlate the existence of Human Papilloma virus 16 in squamous cell carcinoma with clinical presentation, habits and histological grading. **Study Design:** Interventional Study. **Setting:** Department of Oral Surgery, Isra Dental College Hospital. **Period:** July 2017 to December 2017. **Material & Methods:** A sample of 60 patients presenting were selected by non-probability purposive sampling. Patients reporting to Isra Dental College, Oral Surgery OPD Isra University during the study period with clinical suspicion of Oral Squamous Cell Carcinoma were included. **Results:** Age category was from 20 till 60+ years of age whereas age group of 40-49 (36.7%) was the most frequent age in the study population followed by 30-39 (30%) years and 50-59 (23.3%) years of age respectively. The mean and standard deviation was 43.35 ± 10.156 respectively. Maximum numbers of people were seen to have more than one habit i.e. they were using guthka with smoking or areca nut with guthka and smoking etc. areca nut, cigarette smoking and guthka were among the others most used. The mean was 6.32 with standard deviation of 2.954. Most of the patients had submucous fibrosis on the right side of buccal vestibule 24 (40%) and 22 (36.7%) had submucous fibrosis on the left side of buccal vestibule. 56 (93.3%) of the patients had well differentiated squamous cell carcinoma whereas 4 (6.7%) has moderately differentiated squamous cell carcinoma. No patients were seen to have poorly differentiated squamous cell carcinoma. **Conclusion:** In conclusion, the Prevalence of HPV 16 in our study was found to be 11.7%. There was no association between habits and histological grading with Human Papillomavirus 16 in patient with oral squamous cell carcinoma. But there was association between HPV16 and clinical presentation of OSCC because of p-value (.015) which was significant.

Key words: HPV, Histological Grading, OSCC.

Article Citation: Ahmed F, Shahid H, Shafique S, Ahmed A, Shaikh AA, Ali SS. Human papilloma virus prevalence and its correlation with clinical presentation and histological grading in oral squamous cell carcinoma. Professional Med J 2021; 28(8):1067-1075. https://doi.org/10.29309/TPMJ/2021.28.08.3814

INTRODUCTION

Oral cancer is a serious worldwide public health problem, with high incidence and mortality rates. According to data from the International Agency for Research on Cancer (IARC), approximately 263,900 new cases and 128,000 deaths by cancer of the oral cavity are estimated to have occurred in the world in 2008.[1,2] Among the malignant tumors of this anatomic site, more than 90% are oral squamous cell carcinomas (OSCC). Several risk factors are related to oral cancer, with the main being: tobacco use, alcohol consumption, and infection by high-risk genotypes of human papillomavirus (HPV).[2,3]

Squamous cell carcinoma (SCC) is the most common malignant neoplasm of the oral mucosa, representing more than 90% of theses malignant tumors.[4] A study of Elango et al, states that probably due to the habits reduction in high-risk countries, there is an overall reduction in the incidence of head and neck cancers especially as laryngeal carcinoma and buccal-gum carcinoma that associate with abuse of tobacco in both urban and rural communities.[5] Despite general decrease in the incidence of head and neck cancers, but its increased incidence in certain regions such as oropharynx, tongue and oral cancers in the younger adults has been observed.[4,5]

In the last decade, oral cancer institute of America (OCIOA) has announced that OSCC rate has decreased in men than women because women increasingly are smoking and using alcohol.[6] Over the past three decades, information support that the human papilloma virus (HPV) is as a causative factor in the development and progression of head and neck cancers, particularly those that originate from the oropharynx. It seems that constant infection by HPV might increase the risk of SCC.[6,7]

HPV is an epitheliotropic virus implicated in the development of skin warts and papillomatous lesions in mucosae.[8] Nowadays, more than 100 HPV genotypes are known, and based on their potential for induction of malignant transformation, the several genotypes are classified as "low risk" and "high risk" for the development of genital malignancy.[9] Structurally, this virus is characterized by a non-enveloped icosahedral capsid, with circular double-stranded deoxyribonucleic acid (DNA) genome, approximately 8,000 base pairs (pb) long.[10] Its genome may encode six early and two late genes. Among the early genes, E6 and E7 deserve attention, because their homonymous products, among other effects, promote the degradation of tumor suppressors p53 and pRb, respectively, an event considered crucial for the neoplastic transformation of infected cells.[11,12] The mechanism that makes a high-risk HPV induce the malignant progression of previously benign lesions is, primarily, this genotype capacity to integrate its genome to that of the host (viral integration). This provokes the break of a viral DNA segment that contains E2 gene, which, among other functions, inhibits the expression of E6 and E7 genes, culminating in the over expression of E6 and E7. This cascade of events leads to excessive and unregulated cell proliferation, with involvement of repair mechanisms, which favors the accumulation of mutations and the occurrence of chromosomal aberrations, as well as apoptosis inhibition.[13]

The relationship between HPV and OSCC was first suggested in 1983 by Syrjänenet al, when they discovered koilocytoticatypias in malignant oral lesions by optical microscopy.[14] But the presence of viral DNA was only confirmed two years later, by means of in situ hybridization (ISH).[14,15] HPV infection in the oral cavity is associated with risky sexual behaviors, mainly to orogenital sex. However, mouth-to-mouth contact, vertical birth transmission and autoinoculation resulting from chewing warts are also transmission modes of this virus to the oral mucosa.[16] The viruses isolated in OSCC are low-risk genotypes, including HPV-6, 11, 16, 18, 22, 31, 33, 35, 38, 58, 68, and 70.[17] Nevertheless, in around 80% of the cases of infected oral squamous carcinomas, the identified genotypes were HPV-16. Co-infections with two HPV genotypes have been reported; the findings reveal that in these cases, neoplasms tend to occur, on average, a decade earlier than in individuals infected by a single genotype, or not infected.[18] In a study done by SMA ALI (2008)[19] in Karachi, it was seen that HPV positive result was 68%, they used conventional PCR technique for their study. Another study done by Iqbal, A et al in Lahore in 2014, concluded that 52% HPV were positive by the use of immunohistochemistry (IHC). But we used real time PCR i.e. it was totally computerized, which is also the first time it is been used in Pakistan for the detection of HPV in Sindh, Pakistan.[20]

Hence, the intend of this study is to strengthen the global trend of pronouncing HPV 16 as a risk factor for OSCC, as HPV positive OSCC are believed to be more chemo sensitive.The objectives of the study are to determine the prevalence of Human Papilloma virus 16 in patients with oral squamous cell carcinoma presenting at Isra University Hospital.To correlate the existence of Human Papillomavirus 16in squamous cell carcinoma with clinical presentation, habits and histological grading.

MATERIAL & METHODS

This study is an interventional study of the general population N= 60. Patients visiting the Oral Surgery department of Isra dental college, Isra University from July 2017 to December 2017were recruited. Patients coming to the OPD of Isra Dental College are generally both from rural and urban areas as Hyderabad is a small city and adjoining cities don't have tertiary based hospitals

in their localities. Patients with clinical suspicion of Oral Squamous Cell Carcinoma were included into this non-probability purposive sampling study. Patients above the age of 20 years and of both genders were included. Patients having poorly controlled systemic disorder which was contraindicating biopsy, any other lesion except OSCC and all patients below 20 years of agewere excluded from this study. The purpose was to strengthen the global trend of pronouncing HPV 16 as a risk factor for OSCC, as HPV positive OSCC are believed to be more chemo sensitive. SPSS version 22 was used to analyze the data. Prior to the study, the ethical approval for this study was obtained from the ethical review board of the institute.

Verbal and written Consent was taken for history, clinical examination & for incisional biopsy procedure. All participants of the study underwent careful clinical evaluation including a full medical history and clinical examination to confirm the diagnosis of oral squamous cell carcinoma. Determination of whether the patient fulfills the inclusion / exclusion criteria. Written, witnessed informed consent was obtained and a copy given to the patient. The patient was then assigned a study number. After taking careful history, relevant clinical features were recorded on Performa. Incisional biopsy of the lesion was performed under local / general anesthesia and specimen was sent to histopathology laboratory.

RESULTS

Age distribution of study subjects are shown in Table-I. Age category was from 20 to 60+ years of age whereas age group of 40-49 (36.7%) was the most frequent age in the study population followed by 30-39 (30%) years and 50-59 (23.3%) years of age respectively. The mean and standard deviation was 43.35 ± 10.156 respectively. Table-II shows the gender distribution of male to females. Frequency of male to female subjects was 35 (58.3%) males and 25 (41.7%) females respectively. Table-III shows the distribution of study population in relation to personal habits. Maximum numbers of people were seen to have more than one habit i.e. they were using guthka with smoking or areca nut with guthka and smoking etc. areca nut, cigarette smoking and guthka were among the others most used. The mean was 6.32 with standard deviation of 2.954.

Table-IV shows the frequency of clinical presentation of oral squamous cell carcinoma present in study population. It is seen that most of the patients presented with Exophytic lesion i.e. 38 (65%), while endophytic was 8 (13.3%) and ulcerative lesions were 7 (11.7). The mean was 2.10 and SD was 1.838. Table-V shows the distribution of study population in association with site of biopsy. Most of the patients had submucous fibrosis on the right side of buccal vestibule 24 (40%) and 22 (36.7%) had submucous fibrosis on the left side of buccal vestibule. Table-VI shows the histopathology report of the patients in relation with well differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma and poorly differentiated squamous cell carcinoma. 56 (93.3%) of the patients had well differentiated squamous cell carcinoma whereas 4 (6.7%) has moderately differentiated squamous cell carcinoma. No patients were seen to have poorly differentiated squamous cell carcinoma.

Table-VII shows the presence of HPV in patients. It is seen that 7 (11.7%) had positive HPV while 53 (88.3%) had no presence of HPV. Table-VIII shows the association of HPV with site of biopsy. Most of the patients with positive HPV are seen having squamous cell carcinoma on the right side of buccal vestibule 3 out of 7. 1 each was seen on left side of buccal vestibule, mandibular alveolus, maxillary alveolus and dorsum of the tongue. Lower anterior lip had no presence of HPV. The was 5.628, df=5, p-value = .344.

Table-IX shows the association of HPV in relation with personal habits. Most of the patients with positive HPV are patients who had a habit of one or more eating items 3 of the 7. One each presence of HPV was seen respectively in patients with eating habits of Manipuri, guthka, areca nut and cigarette smoking. The was 1.094, df=8, p-value = .998. Table-X shows the histopathology report of the patients i.e. well differentiated squamous cell carcinoma, moderately differentiated squamous cell carcinoma and poorly differentiated

squamous cell carcinoma in relation with HPV. Well differentiated squamous cell carcinoma has the most number of patients positive with HPV i.e. 6 (n=7) whereas 1 (n=7) is positive in moderately differentiated squamous cell carcinoma. None are seen in poorly differentiated squamous cell carcinoma. The was .739, df=1, p-value = .390. Table-XI shows the frequency of clinical presentation of oral squamous cell carcinoma in association with HPV 16 present in study population. It is seen that patients with Endophytic, erythroleukoplakic and ulcerative lesion had two patients each with positive HPV 16. One patient was positive with exophytic lesion. The was 10.484, df=4, p-value = .033.

Age	Frequency	%	Mean	SD
20 - 29	3	5	43.35	10.156
30 - 39	15	30		
40 - 49	29	36.7		
50 - 59	31	23.3		
60 +	43	5		
Total	60	100		

Table-I. Shows Age Distribution of Study Population (n=60).

Gender	Frequency	%
Male	35	58.3
Female	25	41.7
Total	60	100

Table-II. Gender Distribution of Study Population.

	Fre-quency	Percent	Mean	SD
Birhi	1	1.7	6.32	2.954
Naswar	2	3.3		
MainPuri	6	10.0		
Guthka	6	10.0		
Areca Nut	9	15.0		
Cigarette Smoking	8	13.3		
Pan	1	1.7		
More than One Habit	25	41.7		
Smokeless Tobacco	2	3.3		
Total	60	100.0		

Table-III. Frequency of personal habits of study population of study sample (n=60).

	Frequency	%	Mean	SD
Exophytic	39	65	2.10	1.838
Endophytic	8	13.3		
Erythroplakic	1	1.7		
Erythroleukoplakic	5	8.3		
Ulcerative Lesions	7	11.7		
Total	60	100		

Table-IV. Clinical presentation of oral squamous cell carcinoma.

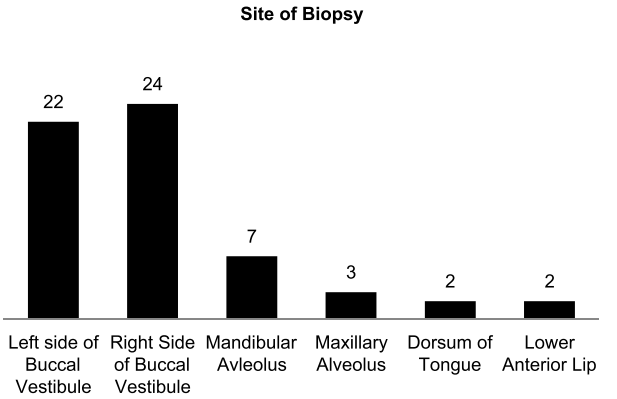

Table-V. Site of Biopsy of study population.

	Frequency	Percent
Well Differentiated Squamous Cell Carcinoma	56	93.3
Moderately Differentiated Squamous Cell Carcinoma	4	6.7
Poorly Differentiated Squamous Cell Carcinoma	0	0
Total	60	100.0

Table-VI. Histopathological Report.

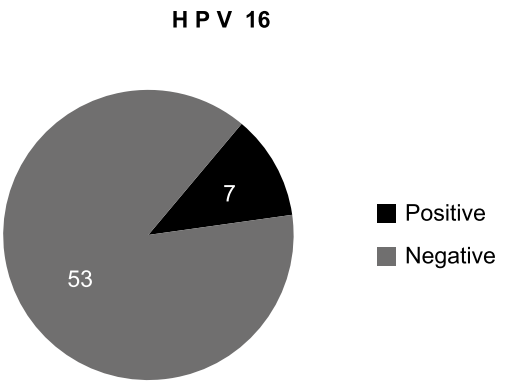

Table-VII. Presence of HPV in study population.

	H P V 16				
Site of Biopsy	**Positive**	**Negative**	**x^2**	**df**	**P-Value**
Left Side of Buccal Vestibule	1	21	5.628	5	.344
Right Side of Buccal Vestibule	3	21			
Mandibular Alveolus	1	6			
Maxillary Alveolus	1	2			
Dorsum of Tongue	1	1			
Lower Anterior Lip	0	2			
Total	7	53			

Table-VIII. Site of Biopsy in Association with HPV.

Habits	HPV 16					
	Positive	**Negative**	**Total**	**x^2**	**df**	**P-Value**
Birhi	0	1	1	1.094	8	.998
Naswar	0	2	2			
Mainpuri	1	5	6			
Guthka	1	5	6			
Areca Nut	1	8	9			
Cigarratte Smoking	1	7	8			
Pan	0	1	1			
Smokeless Tobacco	0	2	2			
More than One Habit	3	22	25			
Total	7	53	60			

Table-IX. HPV in association with Personal Habits.

	H P V 16				
	Positive	**Negative**	**x^2**	**df**	**P-Value**
Well Differentiated Squamous Cell Carcinoma	6	50	.739	1	.390
Moderately Differentiated Squamous Cell Carcinoma	1	3			
Poorly Differentiated Squamous Cell Carcinoma	0	0			
Total	7	53			

Table-X. HPV in association with Histopathological grading.

Clinical Presentation of OSCC	HPV 16					
	Positive	**Negative**	**Total**	**x^2**	**df**	**P-Value**
Exophytic	1	38	39	10.484	4	.033
Endophytic	2	6	8			
Erythroplakic	0	1	1			
Erythroleukoplakic	2	3	5			
Ulcerative Lesions	2	5	7			
Total	7	53	60			

Table-XI. Clinical Presentation of OSCC in Relation with HPV.

DISCUSSION

The present prospective study is an original research conducted by the department of oral and maxillofacial surgery, Isra University, Hyderabad. All patients coming into the OPD of oral and maxillofacial surgery with suspicion of oral squamous cell carcinoma were recruited for study.

Cancer of the oral cavity is the 10th most frequent cancer for men and 14th for both genders worldwide[21] and is increasing. This is partly ascribed infection with human papillomavirus (HPV), particularly type 16 which is increasing in those diagnosed with malignancy and they do not present the traditional risk factors. This new and rapidly growing younger group where males slightly outnumber females is infected

with the same type of virus responsible for most cervical cancers. However, survival rates for oropharyngeal cancer (OPC) is poor although it varies widely depending on the original location, and the extent of the disease. The use of tobacco is common and the use of non-smoked tobacco is growing popularity due to intensive marketing word wide. This together with the fact that more people are infected with the sexually transmitted virus means that the health care providers face a new and difficult situation in preventive medicine. Therefore more studies are needed in order to clarify the different aspects of virus involvement. The health of the oral mucosa is affected by the general health of the individual and the nutritional status plus factors such as exposure to microorganisms, chemical, thermal, and mechanical agents. This is summarized by Bundgaard et al.[22] and Rosen Quist et al.[23-25] who underlined that oral cancer is a lifestyle-related disease to a great extent and demonstrated a clear relationship between marital status, inadequate dental situation not acceptable oral hygiene and oral cancer.

HPV 16 was found positive in approximately 12% of study population and 86% negative. This finding is in contrast to a study done by Feng, Y. et al in USA in 2016, as well as a study done by Pina, A.R. in Brazil in 2016, where 75 % of the study population had positive presence of HPV 16 and 25% had negative.[25,26] This finding is also in contrast to a study done by Zafareo, M.E. in 2016 in USA where 30% of the total study population has positive HPV 16.[27] This finding is in contrast to a study done by Amini, A. et al in USA in 2016, where 62 % of the study population had positive presence of HPV 16 and 38% had negative HPV 16.[28] This study are also in contrast to a study done by Iqbal, A et al in Lahore in 2014 where 52% were positive and 48% were negative. This can be due to the fact that the studies used immunohistochemistry (IHC) for HPV detection. We used real time PCR.[20] These findings are also in contrast to a study done by S.M.A. ALI in 2008 who's HPV positive result is 68%. They in there research concluded that HPV16 was 90% positive and they used conventional PCR technique for their study.[19]

However our finding is in consistent to study done by Singh V et al in 2015 in India where 9% of the study population had HPV positive result.[29]

In the present study HPV 16 was seen more in males as compared to females, this finding is consistent to a study done by Satgunaseelan, L in Australia in 2016, Amini, A. et al in USA in 2016 and Iqbal, A et al in Lahore in 2014 where males showed more presence of HPV 16 as compared to females.[20,28,30]

Although only 7 cases was found positive among them HPV 16 was seen in majority of the patients between the ages of 40 – 49 (57.1%).This finding is in contrast to a study done by Saito, T. in Japan in 1995, where 60 -69 years old patients had HPV 16 (60%).[12,31] However, it is consistent to a study done by Iqbal, A et al in Lahore in 2014 where the majority of patients were between the age of 41-51, but without any statistical significance.[20]

In the present study 6 out of 7 patients seen with HPV 16, were with well differentiated SCC. This finding is consistent to a study done by Saito, T. in Japan in 1995, where 90% patients had HPV with well differentiated SCC and also is consistent to a study done by Iqbal, A et al in Lahore in 2014 where majority of the HPV positive patients had also well differentiated SCC.[12,20]

In the present study, association of habit profile with HPV 16 positivity of study indicated that more patients were found indulged in more than one habits (42%). Areca nut users were (15%). This is in contrast to a study done by Znoar, et al in India in 2003, where majority of the patients were involved in smokeless tobacco.[32]

However this finding is in consistent to study done by Singh V et al in 2015 in India where majority of the patients had more than one habits.[29] Buccal vestibule was the most common site for SCC in our study population. This is consistent to studies done by Iqbal A et al in Lahore in 2014. They also reported that buccal mucosa as the most frequent site of lesion.[20] It is not obvious that tobacco and viruses interact in the development of oral cancer. It might be two different pathways involved;

development of malignancies induced by tobacco specific carcinogens in a classical multistep process and a virus induced transformation in the lymphatic tissues intra orally.

One key issue to further study is the effect of tobacco mutagens on the virus propagation, and the effects on the regulating proteins controlling the process of morphologic transformation of the target tissue in the oral cavity.

Small sample size with unequal distribution of gender were some of the limitations encountered in the study. Site of biopsy was also limited to buccal mucosa.

CONCLUSION

Prevalence of HPV 16 in our study was found to be 11.7%.There was no association between habits and histological grading with Human Papillomavirus 16 in patient with oral squamous cell carcinoma. But there is association between HPV16 and clinical presentation of OSCC because p-value (.015) which was significant.

RECOMMENDATIONS

The predominance of the HPV high-risk types, HPV16, in HPV-positive tumors of the OSCC, provides further evidence for its etiologic importance. Although prevalence of HPV16 was found low in our study but these results might not be representative of total population. However, additional research is required in a number of areas. The current literature on HPV and survival is rather sparse. Progress in the understanding of the significance of HPV in head and neck cancer can be made only with multidisciplinary efforts, as is evident from the research history of cervical cancer.

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1	Fahad Ahmed	Idea, Abstract, Methodology.	
2	Hassan Shahid	Data Interpretaion, References, Formatting.	
3	Salman Shafique	Introduction, Literature Search/ Reveiw.	
4	Aswad Ahmed	Data analysis.	
5	Alvina Ali Shaikh	Data collection, Data analysis.	
6	Sheikh Sajjad Ali	Discussion, Conclusion.	