Association of Human Papilloma Virus Type 16 and 18 with Squamous Cell Carcinoma in Oral Cavity and Oropharynx

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Oral squamous cell carcinoma (OSCC) is one of the most common cancers in our subcontinent.There is growing evidence that human papilloma virus (HPV); the predominant type HPV-16 associated with oral and oropharygeal squamous cell carcinoma. The association is strongest in the tonsil followed by cancers of the tongue and of the buccal mucosa.The chief objective of this study was to evaluate the status between HPV type 16 & 18 with oral and oropharyngeal squamous cell carcinoma. The descriptive cross-sectional type study was carried out in the Department of Pathology of Chittagong Medical College,Chittagong , Bangladesh during the period from January 2013 to December 2013. A total of 82 histopathologically diagnosed SCC patients were selected as samples by purposive sampling technique. Histopathological examination with routine Hematoxylin and Eosin (H & E) stain was done of biopsy material. HPV type 16 and 18 DNA were detected in OSCC patients by real time PCR assay from paraffin embedded tissue. Patients habit regarding smoking, tobacco chewing with or without betel leaf, alcohol consumption and history of sexual behaviour were recorded also.To examine the relationship between variables, statistical significant tests were done. Patients' ages ranged from 24 to 84 years with the mean of 57.09 years and SD was 10.57. Out of 82 patients 60 (73.2%) were in age group 50-69 years, 48 (58.5%) patients were male and 34(41.5%) were females with a male-female ratio was 1.41:1. HPV type 16 and 18 was detected in19 (23.2%) cases , out of whom 13 (68.4%) were associated with HPV16, 5 (26.3%) with HPV18 and 1(5.2%) were co infected. Among 19 HPV type16/18 infected patients, 12(63.1%) were male and 7(36.8%) were female with male-female ratio was 1.71:1. In this study tonsil was the tumour site in 36.8 % of the HPV 16 & 18 positive patients followed by buccal mucosa (31.6%) and tongue (21.1%). In HPV positive patients, histologically 11(57.9%) were in grade-1 and 8(42.1%) were in grade-2 squamous cell carcinoma.

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Key words: Oral squamous cell carcinoma, Human papilloma virus

Introduction

Head and neck squamous cell carcinomas (HNSCC) are a biologically heterogeneous group of cancers. 90% are squamous cell carcinoma (SCC) that ranks sixth among all malignancies world wide.[1] In the Indian subcontinent, oral squamous cell carcinoma (OSCC) accounting up to 40-50 % of all malignant cancers. Its incidence in the SAARC countries is among the highest in the world and represents 12% of all cancers in male and 8% in the female population.[2]

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Evidence of the role of human papilloma virus (HPV) in the development of OSCC is increasing . Although the role is less clear in OSCC than in cervical cancer, HPV DNA has been identified in primary tumors of the tonsil, oral cavity, tongue & nasopharynx as well as cellines derived from a variety of head and neck carcinoma.[3]

The participation of HPV in oral and oropharyngeal carcinogenesis[4] on the basis of following evidences: the epitheliotropic nature of HPV, the oncogenic potential of HR genotype in the pathogenesis of cervical squamous cell carcinoma[5] and the morphological similarities between oropharyngeal and genital epithelia.[6]

The most frequently detected genetic alteration in cancers of the oral cavity is the loss of tumor suppressor protein such as p53, antiproliferative proteins, and the product of retinoblastoma gene (pRb) .This is a result of either genetic mutation or interaction with viral oncoproteins like HPV E6/E7.[7]

Of the HPV family, 24 types have been found in oral lesions.[8] Among them HPV-16 and HPV-18 were detected in 80% of cases.[9] As in genital lesions, HPV-16 is the most common type and is associated with a wide range of oral lesions, from benign to pre malignant and malignant.[10]

The prevalence of HPV in OSCC which was initially underestimated to be between 20-30% is now considered to be as high as 50.[11] HPV prevalence has been reported to be twice as high in premalignant lesions as in normal mucosa and is nearly five times higher in OSCC.[12] The association is strongest in the oropharynx, most notably in the tonsil[13] followed by cancers of the tongue and of the buccal mucosa. The purpose of this study was to determine the frequency of HPV type 16 and 18 and to evaluate the status of histopathological diagnosis of squamous cell carcinoma in oral cavity and oropharynx with HPV DNA type 16 and 18.

Methods

The study was the cross-sectional descriptive study. It was carried out in the Department of Pathology, Chittagong Medical College, Chittagong, Bangladesh. Study period was from January 2013 to December 2013. By purposive sampling technique, a total of 82 patients were selected according to the inclusion and exclusion criteria.

Histopathological examination with routine Hematoxylin and Eosin (H & E) stain was done on biopsy material. Patients habit regarding smoking,tobacco chewing, alcohol consumption and history of sexual behaviour were recorded also. HPV DNA extracted from paraffin embedded tissue using QIAamp FFPE tissue kit (**Qiagen**). HPV type 16 and 18 DNA were amplified and detected by means of Real Time PCR machine named Rotor-Gene 3000/6000/Q (Corbett research, Qiagen) using type specific primer **(Sacace biotechnologies, Italy).** Results obtained through the presence of crossing of fluorescence curve with the threshold line on the green and orange channel.

Table I: Primer used for PCR amplification

Primer	Sequences	Target Gene	Amplimer Length
TS 16	5′ ATATATGTTAGATTTGCAACCAGAGACAAC 3′	E7	196 bp
TS 16	5′ GTCTACGTGTGTGCTTTGTACGCAC 3′		
TS 18	5′ CCGAGCACGACAGGAGAGGCT 3′	E7	172 bp
TS 18	5′ TCGTTTTCTTCCTCTGAGTCGCTT 3′		
b globin	5′ CCACACTGTGCCCATCTACG 3′		99 bp
	5′ AGGATCTTCATGAGGTAGTCAGTCAG 3′		

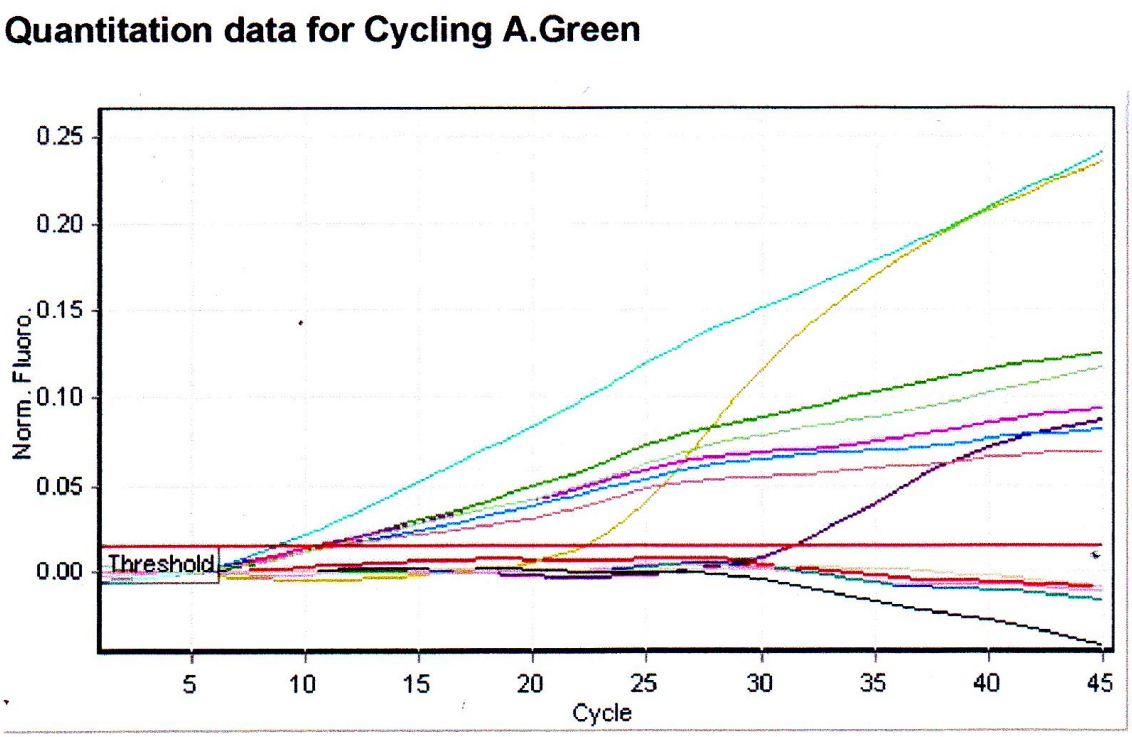

Figure 1. PCR amplification of HPV 16 by Real Time

Results

It was revealed from the study that majority of the patients 31 (37.8%) were in age group 50-59 years and 29 (35.4 %) were in age group 60-69 years. The mean age was 57.09 years. Male were 48 (58.5%) Female were 34 (41.5%). Male to female ratio was 1.41:1.[Table II]

Table II: Distribution of the OSCC Patients by age and sex

Age groups	Frequency	
	N	%
< 40	3	3.7
40-49	9	11.0
50-59	31	37.8
60-69	29	35.4
70 and above	10	12.2
Sex		
Male	48	58.5
Female	34	41.5
Total	82	100.0

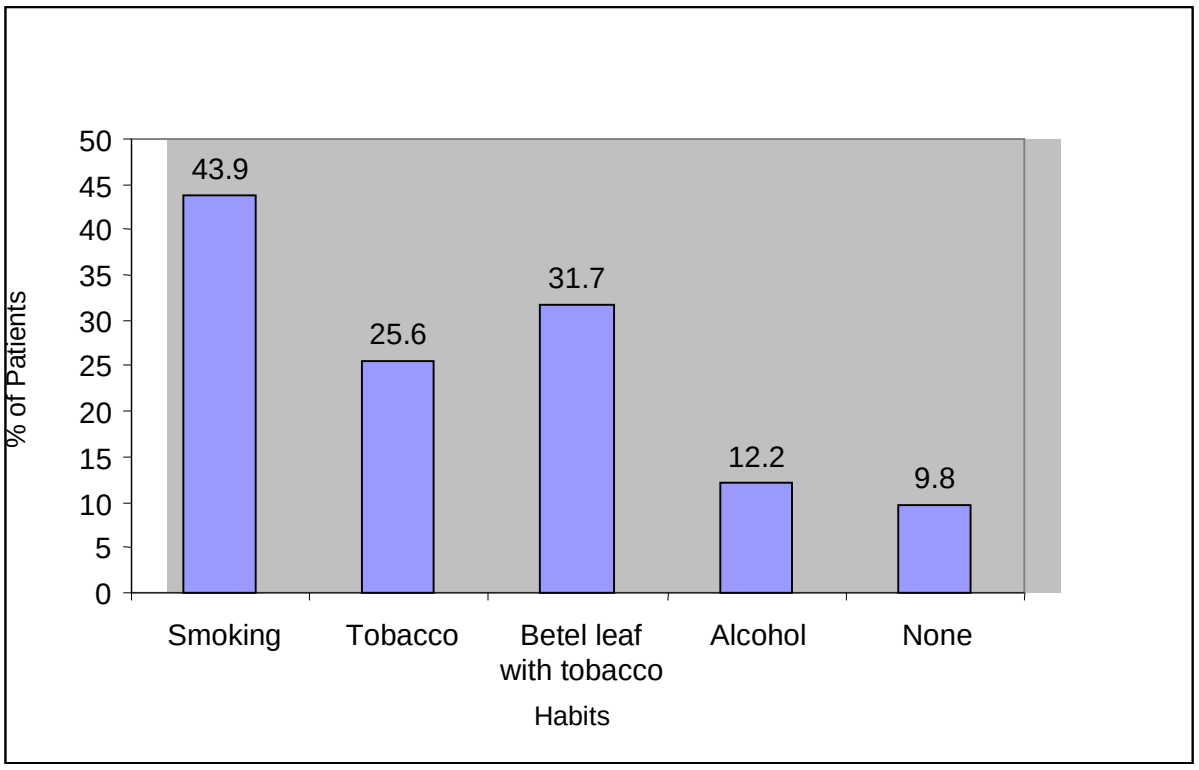

Figure 2. Distribution of patients by personal habit

Figure 2 shows the distribution of personal habit of patients with OSCC. Out of 82 patients, Smoker were 43.9% , Tobacco chewer in the form of jarda, gul, sadapata were 25.6%. Tobacco chewer with betel leaf were 31.7%.and . It also showed that 9.8% patients had no habit.

Table III: Distribution of OSCC patients by HPV type 16 and 18

HPV 16/18 result	Frequency	
	N	%
Cases Positive	19	23.2
Cases Negative	63	76.8
Total	82	100.0

Regarding detection of HPV type 16 and 18 among the OSCC patients, Table III shows positive patients were 19(23.2%) .

Table IV: Distribution of OSCC patients by HPV type 16/18 and sex

Sex	HPV 16/18			
	Positive		Negative	
	N	%	N	%
Male	12	25.0	36	75.0
Female	7	20.6	27	79.4
Total	19	23.2	63	76.8

HPV 16 and18 infected patients, 12(63.15%) were male and 7 (36.84%) were female with male to female ratio was 1.71:1. HPV type 16/18 and sex of the OSCC patients, it was found that out of 48 males; 12(25%) were positive and 7(20.6%) out of 34 females were positive respectively.

Table V: Distribution of HPV type 16 and 18 positive patients

HPV	Frequency	
	N	%
HPV type 16	13	68.4
HPV type 18	05	26.3
HPV 16 & 18	01	5.2
Total	19	100.0

Table V shows out of 19 HPV detected patients 13 (68.4%) were HPV type 16 positive, 5 (26.3%) were type 18 positive and 1(5.2%) were both 16 & 18 positive.

Table VI: Distribution of site of tumour and HPV type 16/18

Site of tumour	HPV type 16/18			
	Positive		Negative	
	N	%	N	%
Buccal mucosa	6	31.6	25	39.7
Tongue	4	21.1	12	19.0
Tonsil	7	36.8	9	14.3
Palate	0	.0	3	4.8
Retro molar region	1	5.3	8	12.7
Oropharynx	1	5.3	6	9.5
Total	19	23.2	63	76.8

About the distribution of tumour site and HPV type 16/18 positive patients, tonsil was involved in majority of the (36.8%) patients. It was found 31.6% patients had buccal mucosa involvement, 21.1% patients showed tongue and 5.3% patients had oropharynx & retromolar region involvement each.[Table VI]

Table VII: Distribution of OSCC patients by histomorphology and HPV type 16/18

SCC	HPV type 16/18			
	Positive		Negative	
	N	%	N	%
Verrucous carcinoma	0	0.0	1	1.6
SCC Grade-1	11	57.9	43	68.3
SCC Grade-2	8	42.1	18	28.6
SCC Grade-3	0	0.0	1	1.6
Total	19	23.2	63	76.8

This study revealed that, HPV type 16 and 18 were detected in 19 patients. Among them majority (57.9%) were in invasive SCC grade-1 followed by 42.1% patients were in grade-2 histologically diagnosed SCC ($p > 0.05$).

Discussion

A complex, multistep process is likely in carcinogenesis of the aero digestive tract epithelium.[14] The age range of 82 patients was 24 to 84 years. Mean age was 57.09 years (SD $\pm$10.57). It was almost similar to two studies, where mean age of the patients were 63 yeas and 50 years respectively.[15,16]

In this study male to female ratio was 1.41:1. In a study, the male-female ratio was 1.4:1 which consistent with this study.[16] Among HPV 16 and 18 positive patients our study showed the male to female ratio was 1.71:1 .

In this study, 25% male sex found HPV 16&18 positive which was more than female (20.6%). Whereas in HPV 16 & 18 negative

patients female sex was more (79.4%) than male sex (75%). Previous reports showing a tendency for higher HPV prevalence in male patients.[17]

In this study, HPV type 16 & 18 was detected in 23.2% of oral and oropharnyngeal squamous cell carcinoma, which is within the prevalence of HPV in OSCC that ranges between 20-50%.[11]

Among HPV positive patients 68.4% were HPV type 16 positive and 26.3% were HPV type 18 positive. Only one patient was coinfected with both type 16 and 18. In one study HPV 16 was detected 70.8% infected cases either alone or in combination with other viral types.[18] Another study by Nagpal in India[19] has also found HPV 16 as the most frequent type involved. Studies have reported detection rate among head and neck SCC tumors with 90% of the HPV types identified as HPV 16.[12] Ali , 2008[16] found HPV 16 in 90% positive patients.
HPV 16 and 18 DNA causes upregulation of E6 and E7 oncoproteins, inactivate p53 and pRb tumour suppressor gene that control both the cell cycle and apoptosis.The high-risk HPV E6 oncogenic activity is degradation of the p53 tumour-suppressor gene. The functions of p53 in the cell cycle include controlling the G1 transition to the S phase of the cell cycle at the G1 checkpoint by inducing expression of cyclin inhibitors p16, p21 and p27 that block the activities of cyclin-CDKs complexes, thus mediating arrest of the cell cycle. E7 oncoprotein functionally inactivates the Rb family of proteins resulting in overexpression of E2F transcription factor with upregulation of cell cycle genes resulting in DNA replication, in the transition of the cell from the G1 to the S phase, and in increased cell proliferation.[20]

Among HPV positive patients present study also showed tonsillar cancer was highest (36.8%) followed by buccal mucosa 31.6% and tongue 21.1%. One study was near similar to present study which showed approximately 25-75% of oropharyngeal cancers have been tested HPV positive, with rates in tonsillar cancer being highest, followed by cancers of the tongue and of the buccal mucosa.[21]

In histological grading, HPV was detected more (57.9%) in SCC grade-1 than in SCC grade- 2 (42.1%). This finding is similar with an study that showed HPV positive patients (71.4%) were in grade-1 followed by 14.3% in grade-2 SCC.[22] Ali, 2008 showed more HPV was detected in grade- 2 SCC in another study.

Conclusion

In conclusion we detected HPV DNA type 16 & 18 in 23.2% OSCC patients and type 16 was predominant (68.4%). Tonsil was the commonest site of tumour and histologically grade-1 patients were more frequent in HPV positive patients in the PCR based present study.

References

1. Parkin DM , et al., (2001). Estimating the world cancer burden : Globocan 2000. Int J Cancer; 94: 153-6.
2. Ferlay J et al GLOBOCAN 2008; Cancer incidence and mortality worldwide.IARC.
3. Woods KV, Shillitoe EJ, Spitz MR, (1993) Analysis of human papillomavirus DNA in oral squamous cell carcinomas. (Review.) J Oral Pathol Med; 22:101–108.
4. Gillison ML, Koch WM, Capone RB, (2000**)** Evidence for a causal association between HPV and a subset of head and neck cancers. J Natl Cancer Inst.; 92(9):709-20.
5. Munoz N, Bosch FX, de Sanjose S, (2003) Epidemiologic classification of human papillomavirus types associated with

cervical cancer. N Engl J Med ; 348: 518-527.
6. Thompson IO, et al.,,(2001) A comparative light-microscopic, electron-microscopic and chemical study of human vaginal and buccal epithelium. Arch Oral Biol; 46: 1091-1098.
7. Das BC, et al.,.(2000) Cancer of the uterine cervix and human papillomavirus infection. Curr Sci ; 78: 52-63.
8. Terai M, Takagi M, Matsukura T (1999 B) Oral wart associated with human papillomavirus type 2. J Oral Pathol Med.; 28(3):137-40.
9. Chocolatewala N M, Chaturvedi P (2009) . Role of HPV in the oral carcinogenesis: An Indian perspective. Review article (2009);vol-5;2;71-77.Tata Memorial Hospital, Mumbai,India.
10. Syrjanen SM, Syrjanen KJ, Happonen RP (1988b). HPV DNA sequences in oral precancerous lesions and SCC demonstrated by in situ hybridization. J Oral Pathol 17:273-278.
11. Sugerman PB, Shillitoe EJ (1997). The high risk human papillomaviruses and oral cancer: evidence for and against a causal relationship. Oral Dis 3:130-147.
12. Miller CS, and Johnstone BM. (2001) Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 91(6):622-35.
13. Dahlstrand H, et al.,(2004). Presence of HPV in tonsillar cancer is a favourable prognostic factor for clinical outcome. Anticancer Res ;24:1829-35.
14. Vokes EE, Weichselbaum RR, Lippman SM,(1993) Head and neck cancer . N Engl J Med; 21; 328(3):184-94.
15. Jan L and Hana V (2011) The role of high risk HPV infection in oral and oropharyngeal SCC in nonsmoking and non-drinkingpatients; a clinicopathological and molecular study of 46 cases. Virchows Arch 458: 179-187.
16. Ali SM , (2008) HPV association and p53 mutation in oral cavity(SCC) cancers of Pakistani patients: Its correlation with histologic variables & disease outcome. Dept. of Pathology and Microbiology. The Aga Khan University, Karachi. P 181-183.
17. Miller CS and White DK.(1996) HPV expression in oral mucosa,premalignant conditions, and SCC: a retrospective review of the literature. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 82(1):57-68.
18. Martha Bouda et al,(2000) High risk HPV types are frequently detected in potentially malignant and malignant oral lesions, but not in normal mucosa. Mod Pathol;13(6) 644-653.
19. Nagpal JK, Patnaik KS, Das BR. (2002) Prevalence of highrisk HPV types and its association with p53 codon 72 polymorphism in tobacco addicted squamous cell carcinoma (OSCC) patients of Eastern India. Int J Cancer 97: 649–653.
20. Feller, L., et al. (2010). Human papillomavirus-mediated carcinogenesis and HPV-associated OSCC. Head Face Med. 2010; 6: 14.
21. Syrjanen S (2005) HPV in head and neck cancer. J Clin Virol. 32: S 59-66.
22. Agrawal G P et al (2013) Role of HPV-16 in Pathogenesis of Oral Epithelial Dysplasia and OSCC and Correlation of p16INK4A Expression in HPV-16 Positive Cases: An Immunohistochemical Study. ISRN Pathology; p 1-7