

Detection of Human Papilloma Virus Type 16 in Oral and Oropharyngeal Squamous Cell Carcinoma

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The objective of this study was to evaluate the status of HPV type 16 with oral and oropharyngeal squamous cell carcinoma. The descriptive cross-sectional type study was carried out 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. HPV type 16 DNA was detected in OSCC patients by real time PCR assay from paraffin embedded tissue. Patients habit regarding smoking, tobacco chewing, 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 10.569. 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 was detected in 14 (17%) cases, out of whom 9 (64.2%) were male and 5 (35.7%) were female with male-female ratio was 1.8:1. In this study tonsil and buccal mucosa was the tumour site in 35.7% of the HPV16 positive patients followed by tongue (21.4%) and oropharynx (7.1%). In HPV positive patients, histologically 9 (64.2%) were in grade-1 and 5 (35.7%) were in grade-2 squamous cell carcinoma.

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Key words: Oral squamous cell carcinoma, Human papilloma virus

Introduction

Head and neck cancer is the sixth most common cancer worldwide.¹ In the Indian subcontinent, oral squamous cell carcinoma (OSCC) accounting up to 40-50% of all malignant cancers.² 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.³ Evidence of the role of human papilloma virus (HPV) in the development of OSCC is increasing.⁴ The prevalence of HPV in OSCC which was initially underestimated to be between 20-30% is now considered to be as

high as 50% if not more; in other words, it is likely that every alternate OSCC patient is HPV positive.² 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.⁵ The association is strongest in the oropharynx, most notably in the tonsil⁶ 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 to evaluate the status of histopathological diagnosis of squamous cell carcinoma in oral cavity and oropharynx with HPV DNA type 16.

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Methods

The cross-sectional descriptive study 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 was 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 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/		
b globin	5/CCACACTGTGCCCATCTACG 3/ 5/AGGATCTTCATGAGGTAGTCAGTCAG 3/		99 bp

Quantitation data for Cycling A.Green

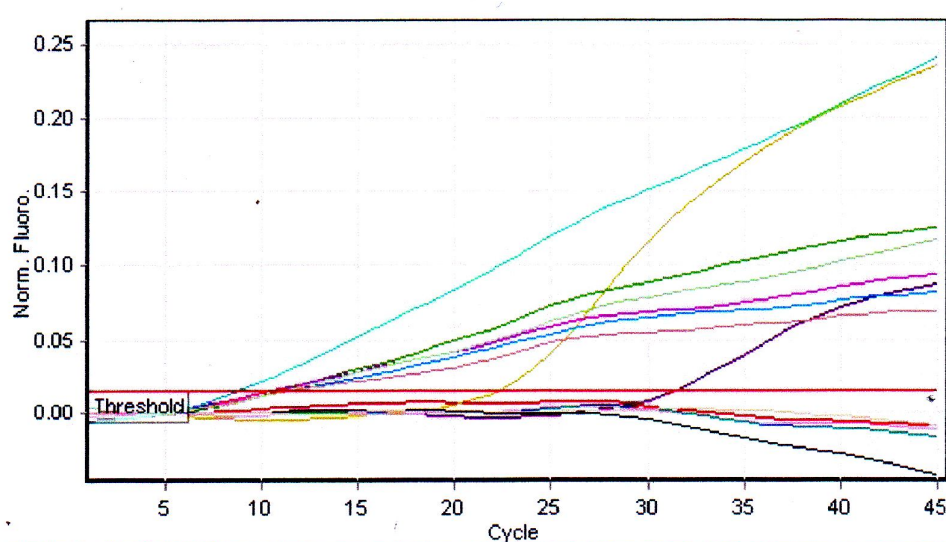


Figure 1. PCR amplification of HPV 16 by Real Time

Results

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

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 to female ratio was 1.41:1.[Table II]

This study also showed the distribution of personal habit of patients with OSCC. Out of 82 patients, smokers were 43.9%. Tobacco chewer in the form of Jarda, Gul, Sadapata were 25.6%. Tobacco chewer with betel leaf were 31.7%. It also showed that 9.8% patients had no habit.

Table III: Distribution of OSCC patients by HPV type 16

HPV type 16 result	Frequency	
	N	%
Cases Positive	14	17.0
Cases Negative	68	83.0
Total	82	100.0

Regarding detection of HPV type 16 among the OSCC patients, Table 3 showed positive patients were 14(17%).

Table IV: Distribution of site of tumour and HPV type 16

Site of tumour	HPV type 16			
	Positive		Negative	
	N	%	N	%
Buccal mucosa	5	35.7	26	38.2
Tongue	3	21.4	13	19.1
Tonsil	5	35.7	11	16.1
Palate	0	.0	3	4.4
Retro molar region	0	.0	9	13.2
Oropharynx	1	7.1	6	8.8
Total	14	17.0	68	83.0

About the distribution of tumour site and HPV type 16 positive patients, tonsil and buccal mucosa was involved in majority of the (71.4%) patients. It was found 21.4% patients had tongue involvement and oropharynx involvement was only 7.1% [Table IV].

Table V: Distribution of OSCC patients by histomorphology and HPV type 16

SCC	HPV type 16			
	Positive		Negative	
	N	%	N	%
SCC Grade-1	9	64.2	46	67.6
SCC Grade-2	5	35.7	21	30.8
SCC Grade-3	0	0.0	1	1.4
Total	14	17.0	68	83.0

This study revealed that, HPV type 16 was detected in 14 patients. Among them majority (64.2%) were in invasive SCC grade-1 followed by 35.7 patients were in grade-2 histologically diagnosed SCC. Chi-square test was done and the result was not significant. ($\chi^2 = 1.984$, $df = 3$, $p > .05$)

Discussion

The age range of 82 patients was 24 to 84 years. Mean age was 57.09 years (SD ± 10.57). It was almost similar to two studies, where mean age of the patients were 63 years and 50 years respectively.^{7,8} 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.⁸ Among HPV 16 positive patients our study showed the male to female ratio was 1.8:1.

In this study, due to smaller sample size HPV type 16 was detected in 17% of oral and oropharyngeal squamous cell carcinoma, which is near the prevalence of HPV in OSCC that ranges between 20-50%.²

Another study by Nagpal in India⁹ 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 16.⁵ Ali, 2008⁸ 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.¹⁰

Among HPV positive patients present study also showed tonsillar and buccal mucosal cancer was highest (35.7%) followed by tongue 21.4%. 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.¹¹

In histological grading, HPV was detected more (64.2%) in SCC grade-1 than in SCC grade- 2 (35.7%). 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.¹² Ali, 2008 showed more HPV was detected in grade- 2 SCC in another study.

Conclusion

In conclusion we detected HPV DNA type 16 in 17% OSCC patients. In the PCR based present study tonsil and buccal mucosa were the commonest site of tumour and histologically grade-1 patients were more frequent in HPV positive patients.

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