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Association of Human Papilloma Virus with Oral Squamous Cell Carcinoma among Bangladeshi Patients

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Abstract: ***Background:*** *Cancer is a major challenge for our society today. Oral squamous cell carcinomas might be related to human papilloma virus infection.*
Objective: *The purpose of the present study was to find the association of Human Papilloma virus (HPV) and oral squamous cell carcinomain Bangladesh.*
Methodology: *This analytical cross sectional study was conducted at the Bangabandhu Sheikh Mujib Medical University (BSMMU), Bangladesh and at the University of Dhaka, for one year. All cases with oral squamous cell carcinomawere examined histologically to detect HPV positivity by identifying HPV altered epithelial cells. The samples were further analyzed with real time PCR after DNA extraction to detect HPV infection as a cause of oral squamous cell carcinoma.*
Result: *A total number of 30 patients were recruited for this study after fulfilling the inclusion and exclusion criteria. Maximum age incidence was found in 40 to 60 years age group which was 17(56.67%) cases.The buccal mucosa found mostly affected which was 16(53.33%) cases followed by lesions involving retromolar region in 16.67% patients (n=5), palatal mucosa in 13.33% patients (n=4), labial mucosa in 10% patients (n=3) and only 6.67% patients having oral squamous cell carcinoma in tongue (n=2). No sample was found positive for human papilloma virus infection. During histopathological evaluation, we are failed to detect any koilocytes in all oral squamous cell carcinoma samples. The result was consistent with the real time PCR detection as well.*
Conclusion: *In conclusionfor Bangladeshi patients, we have found no association between oral squamous cell carcinomaand human papilloma virus.*
Keywords: *Association; Human Papilloma Virus; Oral Squamous Cell Carcinoma; Bangladeshi Patients*

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I. Introduction

The problem of cancer is universal; the only variation occurs in the type, site or other clinic-epidemiological parameters[1]. Oral cancer is a serious public health problem with over 200,000 new cases reported annually worldwide[2-4]. The overall mortality rate for oral cancer remains high at approximately 50 percent even with recent medical treatments is probably due to the diagnosis only at the advanced stage of this disease[2].

Oral squamous cell carcinoma is a subtype of head and neck cancer in which cancerous tissue growth is located in the oral cavity[5]. Oral squamous cell carcinoma may involves the tongue, floor of the mouth, cheek lining, gingiva (gums), retromolar region, lips, or palate (roof of the mouth). Most of the malignant oral

squamous cell carcinoma tend to spread rapidly. However, the tumorigenesis of oral squamous cell carcinoma is complicated. Smoking and other tobacco use are associated with about 75.0% of oral cancer cases[6].

As a member of the papilloma virus family of viruses, HPV can infect humans by attacking the squamous cell of skin and mucous membranes. Since the first evidence of HPV in the etiology of oral squamous cell carcinoma was shown in 1977, numerous studies have been conducted in this regard supporting the role of HPV as a causative agent for oral squamous cell carcinoma[7]. Among 200 genotypes of HPV by DNA sequencing, 85 HPV genotypes have been well characterized[8] of which HPV16 and HPV18 have been found to be associated with oropharyngeal cancer[9-12]. Though the true prevalence of HPV in oral squamous cell carcinoma is still under research, studies have estimated up to 60% positivity in oral squamous cell carcinoma[13-14].Therefore, it was necessary to implement an analysis which was aimed to find the association of Human Papilloma virus (HPV) and oral squamous cell carcinomain Bangladesh.

II. Methodology

This analytical cross-sectional study was conducted in the Department of Oral and Maxillofacial Surgery, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Department of Pathology, BSMMU, Dhaka, Department of Virology, BSMMU, Dhaka, Department of Genetic Engineering & Biotechnology, University of Dhaka, Dhaka, and at the National Forensic DNA Profiling Laboratory, Dhaka Medical College, Dhaka. The protocol was approved by Institutional Review Board of BSMMU, Dhaka, Bangladesh. Out-patients and in-patients visiting the study place was used as the study population. The tissue samples from 30 patients diagnosed with oral squamous cell carcinoma was served as cases. Tumor staging was assessed according to cTNM classification and histological grading was obtained from the report given by the histopathologist. The cases were assigned to sub categories based on their characteristics (age, sex and risk factors). The samples were collected after obtaining institutional review board approval and with their informed consent. Purposive type of non-probability sampling technique was used as per inclusion and exclusion criteria. Subjects were adults aged 18 to 90 years. All subjects were Bangladeshi. Cases were clinically and histopathologically diagnosed as oral squamous cell carcinoma. Generally healthy patients who were fit for surgery. Recurrent cases of oral squamous cell carcinoma, Secondary oral squamous cell carcinoma cases and medically unfit patient for surgery were excluded from this study. All study participants were answered a questionnaire (Appendix-2). General information including patient's name, age, sex, address and details of personal habits was recorded in the preformed standard questionnaire. Clinical examination of primary site and lymph nodes were performed. The findings were noted and data spread sheet was prepared. The oral squamous cell carcinoma samples were stained with hematoxylin and eosin to find koilocytes. The pathologists were not aware of the PCR HPV test results.Again, as a reliable method to detect HPV, real time PCR is thought to be an invaluable tool. So, to further confirm the histological findings we also performed real time PCR to detect HPV in oral squamous cell carcinoma samples. After compilation, all data were presented in the form of tables, figures, and graphs as necessary. Statistical analysis of the results was done by using computer based statistical software, SPSS 20.00 version (SPSS Inc., Chicago, USA). Categorical variables were analyzed by using chi-square test and continuous variables with t test. Significance level was set at 5% (p-value of < 0.05) for all analyses.

III. Results

A total number of 30 patients were recruited for this study after fulfilling the inclusion and exclusion criteria. In this study the age of the patients ranges from 18 to 90 years. Maximum age incidence was found in 40 to 60 years age group which was 17(56.67%) cases followed by Less Than 40 Years and More Than 60 Years which were 7(23.33%) cases and 6(20.0%) cases (Table 1).

Table 1: Age Distribution of the Study Patients

Age Group	**Frequency**	**Percent**
Less Than 40 Years	7	23.33
40 To 60 Years	17	56.67
More Than 60 Years	6	20
Total	**30**	**100**

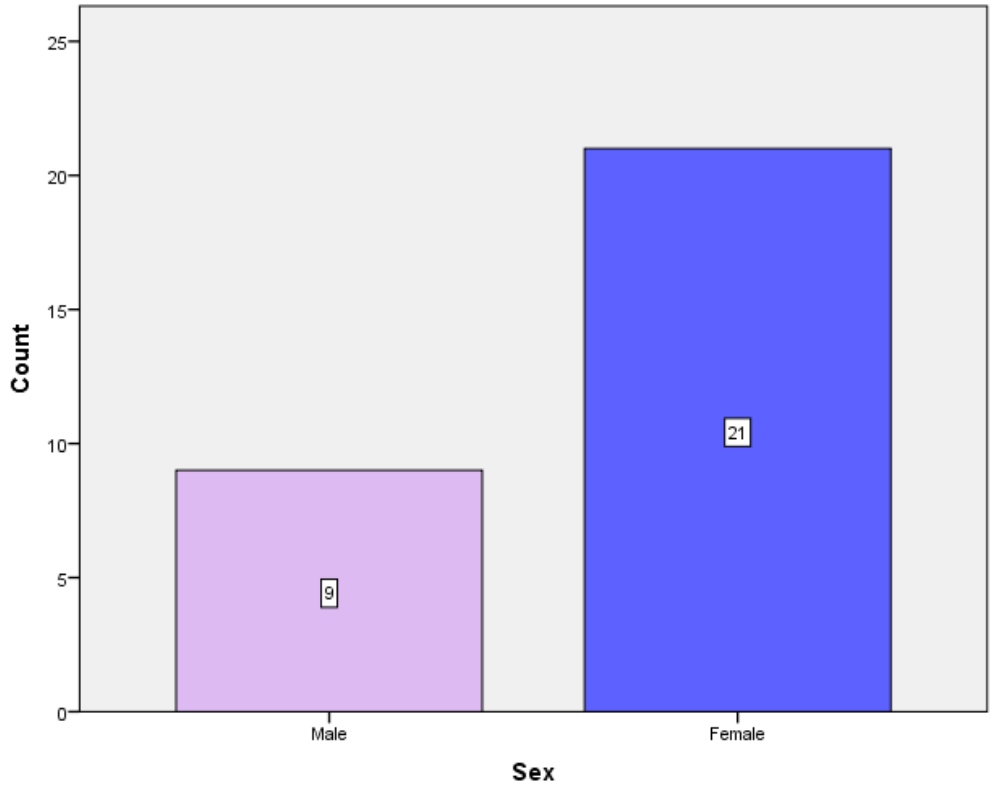

Figure I: Distribution of the study patients by sex

Out of 30 patients, 21 (70%) were female and 9 (30%) were male. In this study, female were more sufferer as a patient of oral squamous cell carcinoma than male (Figure I).

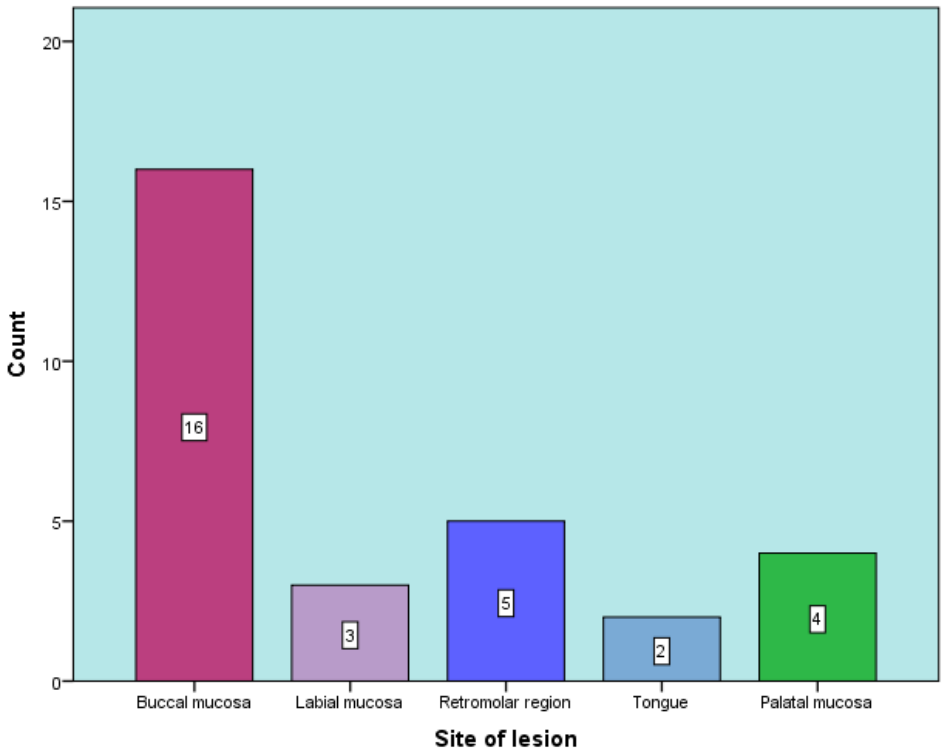

Figure II: Distribution of the oral squamous cell carcinoma patients by site of the lesion

The distribution of site of the lesion among the oral squamous cell carcinoma patients were recorded. Among the study subjects, buccal mucosa (n=16) found mostly affected (53.33%), followed by lesions involving retromolar region in 16.67% patients (n=5), palatal mucosa in 13.33% patients (n=4), labial mucosa in 10% patients (n=3) and only 6.67% patients having oral squamous cell carcinoma in tongue (n=2) (Figure II).

Table 2: HPV detection in the study patients

HPV Detection	**Oral Squamous Cell CarcinomaPatients (n=30)**
Koilocyte Present	0(0%)
HPV DNA detection by Real Time PCR	0(0%)

The findings of HPV detection by koilocyte identification using histopathology and real time PCR method for detecting HPV DNA in the oral squamous cell carcinomasamples were recorded. No sample was found positive for human papilloma virus infection. That is during histopathological evaluation, we are failed to detect any koilocytes in all oral squamous cell carcinoma samples. The result was consistent with the real time PCR detection as well (Table 2).

IV. Discussion

The search for an infectious cause of human cancers remains a matter of great interest in research arena. The discovery of a viral agent in a specific tumour may have huge implications not only for diagnosis and better understanding of the tumour's pathophysiology, but also on its prevention and/or treatment. Cabibiet al[15] have reported that the sensitivity of detection of HPV infection by identification of koilocytes was 74% and the specificity was 72%. This has led many scientists to believe that presence of koilocytes in histopathologic sections from secondary oral squamous cell carcinoma cases makes it simple and reliable criteria to detect the presence of HPV in these lesions. So, as an inexpensive method of detecting HPV infection in secondary oral squamous cell carcinoma cases, 5-µm thick sections obtained from the paraffin embedded secondary oral squamous cell carcinoma samples were stained with hematoxylin and eosin to find koilocytes. This has been well documented in the link between cervical carcinoma and high risk types of HPV, and for liver cancer and hepatitis B or C infection[16-17].

Although at least 15 HPV types are thought to have oncogenic potential, the most prevalent type caused HPV-associated oral squamous cell cancers is HPV16, the same type that leads to HPV-associated anogenital cancers[18]. Because of the morphological similarities[19]and epitheliotrophic nature of HPV, a link between oral squamous cell carcinoma and HPV seemed logical and has been the focus of numerous studies.

To detect the presence of HPV infection, various methods had been applied. Histopathological analysis under light microscopy is the most commonly used method for oral pathology diagnosis and it is a useful method for the observation of viral particles when molecular biology methods are not available. The histological diagnosis of HPV infection is made on the basis of some cytopathic effects, such as koilocytosis, dyskeratosis, papillomatosis, hyperkeratosis, acanthosis and parakeratosis. Among these, koilocytosis is the most common cytopathic effect and is considered by pathologists to be a pathognomonic sign of HPV-associated lesions[20]. Although koilocytosis denotes an important morphological marker for HPV infection, it is not a precise basis for HPV diagnosis because it results in approximately 30% false-positives, which should be considered[21]. In a meta-analysis[22] including 62 studies suggests that PCR is the technique of choice, as it is the most sensitive assay. They found the accuracy of HPV detection was greater when evaluated by means of a high sensitive assay (oral squamous cell carcinoma, PCR based: 39.9%),compared with oral squamous cell carcinoma, ISH based (29.8%). These findings support the assumption that use of more sensitive HPV DNA detection methods, should be considered as essential prerogatives in designing studies on the viral prevalence of these tumors. Therefore, we used histology to find koilocytes as a simple method to detect HPV related changes and to further confirm it we performed real time PCR to detect HPV DNA.

As the best of our knowledge, so far, it is the first time to explore the prevalence of HPV infection in oral squamous cell carcinoma patients in a Bangladeshi population using histology to detect koilocytes and real time PCR to detect HPV infection in human purified genomic DNA obtained from oral squamous cell carcinoma samples. The major aim of this study was to see the possibility of having any evidence for HPV association as a risk factor of oral squamous cell carcinoma in Bangladeshi population. In the current study, no specimen showed to have HPV infection histologically. No koilocytes were observed among 30 oral squamous cell carcinoma cases. Also real time PCR failed to detect any HPV DNA in the DNA extracts obtained from the paraffin embedded oral squamous cell carcinoma samples. The first study from the Bangladesh in this context was done by Akhter et al. in 2009 when they studied 34 oral squamous cell carcinoma cases, and concluded that oral squamous cell carcinoma with HPV is almost absent in Bangladeshi population[23]. They found only one positive case using PCR method to detect HPV. Al-Qahtani et al. conducted a retrospective study of 199 patients with oral squamous cell carcinoma in the year 2007 and the authors found positive PCR-based HPV detection in

only one sample, establishing HPV was negative in approximately 99.3% of the samples[24]. The first study[25] in Sudan which has been done with paraffin embedded oral squamous cell carcinoma tissues, all were also found negative for HPV DNA with the PCR. These findings are supporting our results.

There are some other controversial definitions need to be clarified. In many documents, scientists tend to classified all oral anatomic sites into the oral cancer which included oral cavity, oropharyngeal region and the palatine tonsils also, leading to misinterpretation of the results[26-28]. Studies that analyze exclusively for oral cancer rather than oropharyngeal sites showed low prevalence and non-significant association between HPV and oral cancer[29]. The implication of HPV in the development of oral squamous cell carcinoma, seems poor and is probably overestimated because of the anatomical confusion described above[18]. Among our study patients, all the cases selected were exclusively oral cancer patients, though we had two patients with tongue cancer, none of the lesion were located in the base of the tongue. Also the most common cancer site in our study is buccal mucosa which is also not a part of the oropharynx. These anatomic locations of the lesion may also contribute to our negative result.

The use of fresh tissue will increase the detection rate due to better quality of DNA material. But in the current study, we used formalin fixed paraffin embedded oral squamous cell carcinoma tissues. Therefore, it may not accurately indicate viral infection. Formalin cross linking may cause DNA damage. Again, unlike in the cervix, HPV integration into host DNA is not common in oral cancer[30].

However, the methods like histopathology and real time PCR and the relatively small sample size used in this study may not be adequate enough to provide accurate information regarding the association of the virus with the disease. Therefore, to further confirm the relationship between the HPV and oral squamous cell carcinoma we should perform immunohistochemistry (IHC) and PCR with larger sample size.

V. Conclusion

This study has been carried out to evaluate the relationship between oral squamous cell carcinomaand HPV in Bangladeshi population. We have found no positive association between oral squamous cell carcinomaand HPV. The association between oral squamous cell carcinomaand HPV is still a matter of debate and the aetiological factors are still unknown. As this study has been conducted on relatively small sample size and within a short period of one year only, we have recommend further study using larger sample size and HPV detection by immunohistochemistry and PCR. Moreover, for more sensitive detection, we should keep in mind that HPV infection in oral squamous cell carcinomaalso have anatomical site preferences.

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