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The Immunohistochemistry for HPV infection in High Grade Squamous cell lesions of Esophagus Diagnosed on Endoscopic Biopsies

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Abstract

Background: The recent report in the literatures refers to the role of HPV in pathogenesis of esophageal cancer with geographical biases. There were not many reports from India where in relationship between HPV and esophageal squamous cell high grade lesions were analysed. Such studies are required to explore the role of HPV in esophageal Squamous cell carcinoma for its implication of prevention and treatment.

Aim: The aim of study was to observe the pathogenetic relationship between high grade squamous cell lesion and HPV detected on the tissue biopsies by immunohistochemistry .The another objective of the study was to study relationship of HPV with grades of SCC.

Methods: Fifty-six paraffin embedded blocks that were diagnosed as high grade squamous cell lesions were selected and the immunostaining for HPV was performed using mouse monoclonal Anti-Human Papillomavirus (HPV) antibodies (DAKO code M3528) against HPV.

Results: The study group 56 cases endoscopic biopsies of high grade SCC showed HPV positive IHC staining in total of 12 cases (21.42%) of which 1 case (1.79%) was of carcinoma in-situ and 11(20%) was of invasive SCC. All the 11 cases of invasive SCC were of well differentiated SCC which showed positive HPV IHC staining. The p-value for the results of HPV for high grade squamous cell lesions of esophagus was 0.0022 which was significant.

Conclusions: There exist the association between HPV and esophagus high grade squamous cell lesions. These aspects of pathogenicity are required to be propped further at large sample studies to be carried out in divergent populations.

Keywords: Squamous cell carcinoma • Human papilloma virus • Immunohistochemistry

Introduction

Globally cancer of esophagus is one of the leading causes of cancer mortality. The commonest subtype of esophageal cancer is Squamous cell carcinoma and the other common one is Adenocarcinoma which is known sequelae to barretts' esophagus. The esophageal SCC in past two decades has been the topic of interest for researchers for its evaluation for etiopathogenesis. To the contexts of Indian population esophageal SCC by the figures of ICMR cancer registry occupies 8th rank with Age Standardized incidence Rate (ASR) of 6.5 in 100000 population for males and 4.2 per lakh population for females of the total body cancer. Since the introduction of endoscopy at diagnostic evaluation of lesions of gastrointestinal tract, the endoscopic biopsies of esophageal lesions became an integral part and have enhanced the cancer detection rate of the esophageal lesions. The one subtype that has found to be the commonest chunk of malignant neoplastic lesions diagnosed on esophageal biopsies is SCC. There are several risk factors listed for esophageal SCC including viral oncogenesis which implicates Epstein- Barr virus, Herpes Simplex virus and Human papilloma virus. Of these risk factors, HPV have been cited in many studies to have the causal role in etiopathogenesis of esophageal cancer. The literature on these aspects of HPV is mostly drawn from China [1].

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Where the evidences have been generated for detection of HPV on the tissue of esophageal SCC by adapting to by various methods of detection such as PCR, IHC, ISH. However, there are scarce studies in India at evaluation of role of HPV in genesis of esophageal cancer. There exists a gap of understanding as Indian medical literature do not have many publications which has evaluated the role of HPV and its association with high grade squamous cell lesions of esophagus. The aim of study was to observe the pathogenetic relationship between high grade squamous cell lesion and HPV detected on the tissue biopsies by IHC. The another objective of the study was to study relationship of HPV with grades of SCC [2].

Materials and Methods

Fiftysix paraffin embedded blocks that were diagnosed as high grade squamous cell lesions on histopathology were selected from Jawahar lal Nehru Medical College Sawangi during the period from August 2017 to October 2019. Patient identification data were retrieved from patients file including age, tumor grade, endoscopic findings, and history of habitution and the immunostaining for HPV that includes subtype of HPV-6,11,16,18,31,33,42,51,52,56 and 58 was perfomed using mouse monoclonal Anti-Human Papillomavirus (HPV) antibodies against HPV. The immunohistochemical staining was carried out on 4um thickness section from formalin fixed, paraffin embedded tumor were cut and mounted onto poly-lysine coated slides followed by deparafinnization in xylene, Rehydration through descending grades of alcohol and was placed in distill water for 1 min. samples were steamed for antigen retrieval in pressure cooker done for 15 minutes, Washed with distill water by giving a single dip. Transfer the slides into buffer for at least 5 minutes at room temperature. Peroxidase activity was blocked for 30 minutes using 3, 5 Hydrogen Peroxide + methanol. Wash with buffer 3 times for 5 minutes each. Apply mouse monoclonal anti HPV antibody for HPV at room temperature and kept for 1 hour. Wash with buffer thrice for 5 minutes each at room temperature. Envision to be undertaken by labeled polymer for 30 minutes at room temperature [3]. Apply chromogen 3, 3'-diaminobenzydene (DAB) for 15-20 minutes. (Working DAB solution: 1 ml DAB buffer + 25 µL DAB concentrate). Wash with buffer 3 times each for 5 minutes. Wash with buffer for 10 minutes and slides were counter stain with harris hematoxylin , wash with running tap water , mount with DPX.

Interpretation of IHC

The negative and positive IHC results were interpreted and were scored with the help of references available in literature. Distinct granular brown discoloration was generally considered as positive staining for IHC. The IHC was interpreted for its positive or negative results on following 3 parameters using light microscopy with minimum of 1000 cells and screened under high power objective nuclear/cytoplasmic staining, Granular staining/intensity of signal, No. of cells showing granular signals in cytoplasm and nucleus. The following scoring system for the Interpretation of IHC results for high risk HPV were used. For area scoring : 0 indicated no positive staining; 1, 10% of tissue stained; 2, 11%-50% of tissue stained; 3, 51%-75% of tissue stained; and 4, .75% of tissue stained. Cytoplasmic signal staining in absence of nuclear staining too is considered positive for interpretation of IHC [4].

Statistics Analysis

The data obtained was analyzed using the statistical programs software Statistical package for the social sciences version (22.0), Fischer Exact test and Chi square test were used to calculate the association between detection of HPV by IHC with the diagnosis of high grade squamous cell lesions of esophagus i.e. SCC in-situ, invasive SCC along with grades involved in the study. P-value less than 0.05 (<0.05) was considered statistically significant.

Results

Patient characteristics

56 subjects (26) out of them were males (46.2%) and 30 (53.58%) were females, with (0.8:1) male to female ratio , the patient age ranged between 31-90 years , With the mean age about 59 ± 1.48 years. The majority of high grade squamous cell lesions patients were observed in the age range of 51-60 years as shown in (Table 1).

Age(years)	Male	Female	Total	Percentage %
31-40	2	2	4	7.1
41-50	1	10	11	19.6
51-60	10	8	18	32.14
61-70	10	6	16	28.57
71-80	2	4	6	10.71
81-90	1	0	1	1.79

Table 1. Age and gender distribution of subjects (n-56).

Histomorphological diagnosis

Of total 56 subjects, out of which one case of carcinoma in situ and 55 were of invasive SCC. The frequency of invasive SCC were 30(53.57%) well differentiated SCC, 9(16.07%) moderately differentiated SCC, 16(28.57%) poorly differentiated SCC. The study group of 56 cases endoscopic biopsies of high grade SCC showed HPV positive IHC staining in total of 12 cases (21.42%)of which 1 case (1.79%) was of carcinoma in-situ and 11(20%) was of invasive SCC. There were scores of IHC staining for HPV and in 12 cases interpretated as positive revealed that all thehad strong positive signals with (p-0.029) and was significant as shown in Table 2.

Diagnosis		Total	HPV (IHC) status				Positive %
			Negative		Positive		
			Male	Female	Male	Female	
Study group	CIS	1	0	0	0	1	100%
(SCC)N=56	Invasive SCC	55	22	22	4	7	20%
Control group	Normal mucosa	20	9	11	0	0	0%
N=20							
P value	Fischer's Exact test p-value=0.029						

Table 2. IHC results for HPV in SCC cases (n-56) and control group (n-20).

Grading of invasive SCC

Of the total 55 cases of invasive SCC, the maximum biopsies 30(53.57%) showed grade I well differentiated , 09(16.07%) grade II moderately differentiated and 16(28.57%) grade III poorly differentiated, grade of invasive SCC.

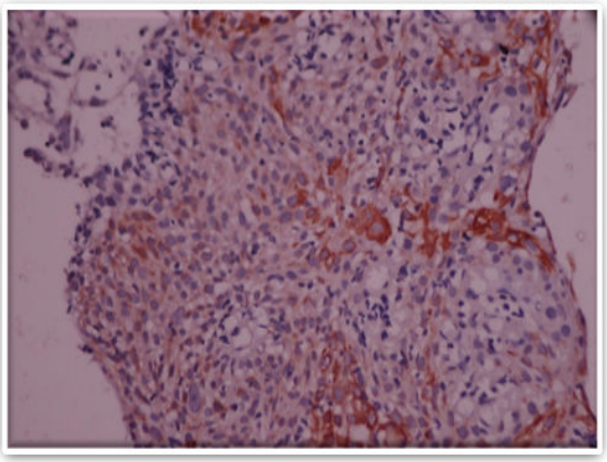

Figure 1. HPV positive (carcinoma in situ) shows brown discrete staining of cytoplasmic and occasional nuclei (anti HPV monoclonal antibody stain) intensity score 3+, 40X.

The 11 cases of well differentiated SCC showed positive HPV IHC staining; no case from the category of moderate or poorly differentiated SCC showed positive results for IHC for HPV. A single case of SCC Carcinoma in situ showed positive immunostaining for HPV IHC (Figure 1), p-value was 0.0022 for this observation and was significant.

Discussion

The distribution of patients of age ranges in the present study revealed that the age range of 41-50 years showed the maximum number of cases of SCC followed by 51-60 years. The similar observation for age for esophageal SCC has been observed in the studies. Youngest patient in the study was 35 years and the incidence of SCC in esophagus before 4th decade of life was observed to be 7.2% in the present study. The SCC occurring in the lower age of 4th decade of life have also been reported. Old age in itself is a risk factor for development of SCC has been quoted in the text. The present study observed 23 patients were older, that is past of 6th decade of life. Similar observations for occurrence of SCC in old age has made in the studies. The gender bias has been recorded in most of the studies reviewed for the present work which observed that male suffered more of SCC than females and attributed the results for risk factor like tobacco user, alcohol and others. There is an observation unusual for one of the female who was positive for HPV on IHC for esophageal SCC on biopsy and had simultaneous investigation of cervical cytology which shows HPV associated koilocytic atypia [5].

Most of the studies which have originated in China have found high incidence and correlation of high risk HPV as pathogenetic factor for ESCC. The present study is in no agreement with the results of South American and European studies, where low evidence for HPV immunoreactivity by IHC has been observed with esophageal SCC. The present study have concordant finding (21.43%) with the studies. The present study did not observe the very high positive % for HPV as has been documented in the few studies originating in China for the reason that the prevalence of HPV or viral antigenicity in the subjects within the present study from the central India probably has low endemicity of prevalence of HPV.

Conclusion

The observation in the present study for association between HPV and squamous cell lesion including SCC implicates there causal relationship this aspect of pathogenicity is required to be further studied as a large sample at multiple centre engaging the workup of esophageal SCC.

References

1. Hardefeldt, H A, Cox M R, and Eslick G D. "Association between Human Papillomavirus (HPV) and Oesophageal Squamous Cell Carcinoma: A Meta-Analysis." Epidemiol Infect 142 (2014): 1119-1137.
2. Liyanage, Surabhi S, Segelov Eva, Garland Suzanne M, and Tabrizi Sepehr N, et al. "Role of Human Papillomaviruses in Esophageal Squamous Cell Carcinoma." Asia-Pac J Clin Oncol 9 (2013): 12-28. Syrjanen,
3. K J "HPV Infections and Oesophageal Cancer." J Clin Pathol 55 (2002): 721-728.
4. Mir, M Muzaffar, and Dar Nazir Ahmad. "Esophageal Cancer in Kashmir (India): An Enigma for Researchers." *Int J Health Sci* 3 (2009): 71.
5. Shuyama, K, Castillo A, Aguayo F, and Sun Q. "Human Papillomavirus in High-and Low-Risk Areas of Oesophageal Squamous Cell Carcinoma in China." Br J Cancer 96 (2007): 1554-1559.

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