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A STUDY OF P53 AND PANCYTOKERATIN EXPRESSION IN SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA OF OESOPHAGUS IN OESOPHAGEAL BIOPSIES

Pathology

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ABSTRACT

BACKGROUND Oesophageal cancer is one of the deadliest malignancies worldwide. India, especially the North-eastern states, have a very high prevalence of this cancer. Squamous cell carcinoma and adenocarcinoma are the two most common histologic types of oesophageal carcinoma. Due to varied etiopathogenetic factors and management options of these two types, it is crucial to make an accurate diagnosis; however, it is not always easy to do so in poorly differentiated cancers, particularly in small biopsy specimens. Molecular pathogenesis of both Squamous cell carcinoma and Adenocarcinoma of oesophagus involves alterations of the tumour suppressor gene, P53. Oesophageal cancers show immunohistochemical positivity for a number of cytokeratins. **AIM AND OBJECTIVE** The objective of our study was to evaluate P53 and pancytokeratin expression by immunohistochemical techniques in oesophageal cancer biopsy specimens. **MATERIALS AND METHODS** The study was conducted in the department of Pathology, Gauhati Medical College and Hospital [in collaboration with Department of Gastroenterology, Gauhati Medical College and Hospital] for a period of one year (March, 2019 to February, 2020). Eighty-one cases of oesophageal carcinoma were included in the study. Haematoxylin & Eosin staining, P53 and pancytokeratin immunohistochemical staining were performed on the oesophageal biopsy tissue sections. **RESULTS** Out of 81 oesophageal carcinoma cases, 72 cases (88.9%) were squamous cell carcinoma, 8 cases (9.9%) were adenocarcinoma and 1 case (1.2%) was adenosquamous carcinoma. Most commonly affected age-group in oesophageal carcinoma was 61-70 years. A male:female ratio of 1.3:1 was observed. 34 cases (47.22%) of oesophageal squamous cell carcinoma and 7 cases (87.5%) of oesophageal adenocarcinoma were positive for P53 on immunohistochemistry. 69 cases (95.8%) of oesophageal squamous cell carcinoma and 5 cases (62.5%) of oesophageal adenocarcinoma were positive for pancytokeratin on immunohistochemistry. **CONCLUSION** A higher proportion of oesophageal adenocarcinoma cases showed positive staining for P53 than oesophageal squamous cell carcinoma. In contrast, a higher proportion of cases of oesophageal squamous cell carcinoma showed positive staining for pancytokeratin than oesophageal adenocarcinoma. However, further extensive research studies are required for validation of the same.

KEYWORDS

P53 expression; Pancytokeratin, oesophageal adenocarcinoma, oesophageal squamous cell carcinoma, immunohistochemistry

INTRODUCTION:

Oesophageal cancer is one of the commonest and deadliest cancers worldwide. Although oesophageal cancer is common in both the sexes, there is a male preponderance. In India, a high prevalence has been observed in the north-eastern states, that can be partly attributed to life-style habits like betel nut intake, consumption of alcohol and tobacco amongst both males and females. Eighty-percent of oesophageal cancers in India are squamous cell carcinomas; adenocarcinomas are the second most prevalent histologic type. Prevalence of oesophageal adenocarcinoma, which mostly affects the lower-third of the oesophagus and gastro-oesophageal junction, is showing a rising trend due to changing lifestyles[1,2]. Differentiating these two histologic types is important because they have diverse etiopathogenesis and management options. However, differentiating these based on morphology alone may be challenging in small biopsy specimens, especially the poorly-differentiated subtypes. IHC can be of tremendous help when attempting to establish differentiation in a poorly differentiated esophageal carcinoma of uncertain differentiation.

Molecular pathogenesis of oesophageal squamous cell carcinoma involves loss of expression of tumour suppressor genes, including p53. Majority of oesophageal adenocarcinomas have mutated p53 leading to p53 overexpression[3].

Oesophageal cancers, like other epithelial cancers, show immunohistochemical positivity for a number of cytokeratins. Immunoreactivity for cytokeratin 5/6 in >50% of tumour cells is 100% specific for squamous cell carcinoma. If IHC for cytokeratin 5/6 demonstrates no immunoreactivity or immunoreactivity in a minority of the tumour cells, it is recommended to perform additional studies with AGR2, MUC5AC and/or PASd to confirm the diagnosis of esophageal adenocarcinoma. Diffuse AGR2 expression and MUC5AC positivity is highly sensitive for esophageal adenocarcinoma., particularly in diminished cytokeratin 5/6 positivity[3].

This study aims to evaluate the expression of p53 and pancytokeratin in squamous cell carcinoma and adenocarcinoma of oesophagus in oesophageal biopsies by application of immunohistochemical techniques.

MATERIALS AND METHODS:

A cross-sectional study was carried out in department of Pathology, Gauhati Medical College and Hospital for a period of 1 year (March, 2019 to February, 2020). The study was approved by Institutional Ethical Committee of Gauhati Medical College and Hospital. A written informed consent, detailed history and investigations were obtained from the patients aged 30-80 years who underwent endoscopic biopsy for clinicoradiological suspicion of esophageal carcinoma. Patients already diagnosed with non-malignant or pre-malignant conditions of the oesophagus were excluded from the study. After routine processing of the tissues, prepared slides were stained with haematoxylin and eosin stain. Tissue sections diagnosed as squamous cell carcinoma or adenocarcinoma were further treated with p53 and pancytokeratin IHC staining. The slides were microscopically examined for positivity of p53 (diffuse nuclear expression due to accumulation of stable mutant p53) and pancytokeratin (diffuse cytoplasmic expression). Validity of the study was assessed by determining the P-value which was calculated by Chi-square test. P-value of less than 0.05 was considered significant.

RESULTS:

The distribution of oesophageal carcinoma in the different age-groups among males and females in this study showed that the maximum peak of cases of oesophageal carcinoma was observed in 61-70 years age-group.

The male:female ratio of oesophageal carcinoma cases in our study was 1.3:1.

Most of the cases of oesophageal squamous cell carcinoma affected individuals aged 41-50 years (Table No. 1).

Table1: The Distribution Of The Different Histologic Types Of Esophageal Carcinoma {squamous Cell Carcinoma (scc), Adenocarcinoma (adc) And Adenosquamous Carcinoma (adsc)} In Different Age Groups In This Study.

AGE-GROUP (YEARS)	SCC	ADC	ADSC
0-10	0	0	0
11-20	0	0	0
21-30	1	0	0
31-40	6	1	0
41-50	23	0	0
51-60	16	2	0

61-70	21	3	1
71-80	5	2	0
81-90	0	0	0
91-100	0	0	0
TOTAL	72	8	1

Therapeutic procedure was done in 52% (n=16) of the patients which includes appendicectomy 55 %, adhesiolysis 33 %, hernioplasty 11%. 17% (n=5) of the patients had enlarged mesenteric nodes in the terminal ileum which was taken up for biopsy and reports showed the features of non specific adenitis. No abnormality is noted in 7% (n=2) of the patient that means negative laparoscopy present in our study.

Squamous cell carcinoma (SCC) of oesophagus was found to be the most predominant histologic type in both males and females (Table No. 2). This observation was found to be statistically significant (p-value: 0.049).

TABLE 2: The distribution of the different types of oesophageal carcinoma in males and females in this study.

GENDER	SCC	ADENOCARCINOMA	ADENOSQUAMOUS CARCINOMA
MALE	38	7	1
FEMALE	34	1	0

Most of the cases of squamous cell carcinoma of oesophagus in both males and females were moderately-differentiated. Most adenocarcinoma cases were seen in males and were WD ADC and PD ADC (Table No. 3). This observation was statistically significant (P-value: <0.0001). The most frequently affected site in esophageal squamous cell carcinoma and adenocarcinoma was the middle-third and the lower-third of the oesophagus, respectively. This observation was also statistically significant (P-value: <0.0001).

Table 3: The Distribution Of The Three Grades [well-differentiated (wd), Moderately Differentiated (md) And Poorly-differentiated (pd)] Of Oesophageal Squamous Cell Carcinoma (scc) And Adenocarcinoma (adc) In Upper-third, Middle-third And Lower-third Of Oesophagus In This Study.

	SCC			ADC			TOTAL
	WD	MD	PD	WD	MD	PD	
UPPER-THIRD	5	12	1	0	1	0	19
MIDDLE-THIRD	4	42	4	0	0	0	50
LOWER-THIRD	0	3	1	3	1	3	11
TOTAL	9	57	6	3	2	3	80

Table 4: Distribution Of Cases With Immunohistochemical Staining For P53 In Cases Of Squamous Cell Carcinoma (scc) And Adenocarcinoma (adc) Of Oesophagus In Oesophageal Biopsies In This Study.

Intensity of p53 IHC stain	SCC			TOTAL	ADC			TOTAL
	WD	MD	PD		WD	MD	PD	
STRONGLY POSITIVE	2	12	6	20	2	2	2	6
MODERATELY POSITIVE	0	6	0	6	1	0	0	1
WEAKLY POSITIVE	1	6	1	8	0	0	0	0
NEGATIVE	6	32	0	38	0	0	1	1

TABLE 5: Distribution of cases with immunohistochemical staining for pancytokeratin in cases of squamous cell carcinoma (SCC) and adenocarcinoma (ADC) of oesophagus in oesophageal biopsies in our study.

Intensity of pancytokeratin IHC stain	SCC			TOTAL	ADC			TOTAL
	WD	MD	PD		WD	MD	PD	
STRONGLY POSITIVE	9	36	0	45	2	1	0	3
MODERATELY POSITIVE	0	20	4	24	1	0	1	2
WEAKLY POSITIVE	0	0	0	0	0	0	0	0
NEGATIVE	0	1	2	3	0	1	2	3

There was one case of oesophageal adenosquamous carcinoma in our study which showed moderate positivity on immunohistochemical staining for both p53 and pancytokeratin.

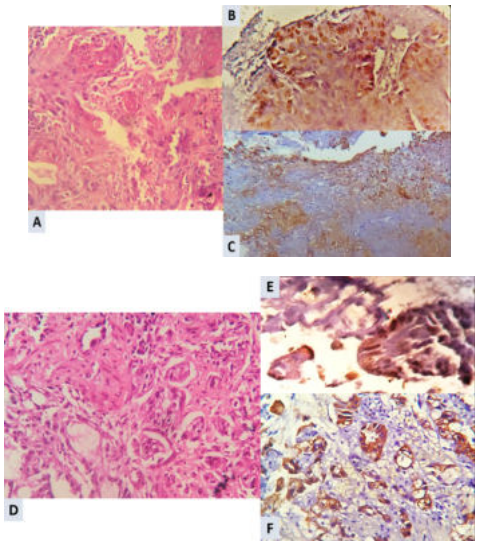

Fig. 1: Photomicrographs of oesophageal carcinoma seen in this study.

A: Haematoxylin and Eosin-stained sections of poorly-differentiated squamous cell carcinoma of oesophagus seen on low-power microscopy/100x magnification

B: Oesophageal squamous cell carcinoma showing nuclear positivity for the immunohistochemical marker, p53 (high-power/400x magnification)

C: Oesophageal squamous cell carcinoma showing cytoplasmic and membranous positivity for the immunohistochemical marker, pancytokeratin (low-power/100x magnification)

D: Haematoxylin and Eosin-stained sections of adenocarcinoma showing an area of gland formation by malignant signet ring cells seen on low-power microscopy/100x

E: Oesophageal adenocarcinoma showing nuclear positivity for the immunohistochemical marker, p53 (high-power/400x magnification)

F: Oesophageal adenocarcinoma showing cytoplasmic and membranous positivity for the immunohistochemical marker, pancytokeratin (low- power microscopy/ 100X magnification).

DISCUSSION

In the present study, the maximum peak of oesophageal carcinoma cases was observed in the age-group of 61-70 years amongst both males and females (30.43% and 34.3% in males and females, respectively). Oesophageal squamous cell carcinoma mostly affected people in the age-group of 41-70 years, with the highest peak in 41-50 years age-group (31.94%). Oesophageal adenocarcinoma mostly affected people in age-group of 51-80 years with the maximum peak in 61-70 years age-group (37.5%). Only 1 case of adenosquamous carcinoma of oesophagus was observed. It was seen in a 62 years old male. According to a study conducted by Choksi D in 2019, the mean age of esophageal carcinoma was 54.83 years (range 25–89 years)[4].In a study conducted by Samarasam I in Christian Medical College Hospital (CMC), Vellore, it was observed that mean age of esophageal cancer was 52 years[1]. A comparatively lower age of onset of oesophageal squamous cell carcinoma seen in the present study can be attributed to an early exposure to risk-factors of oesophageal squamous cell carcinoma like betel-nut consumption, smoking, alcohol intake and smoked food consumption in the north-eastern states of India. Poor nourishment and consumption of hot beverages, hot spicy food may be responsible for esophageal cancer in non-smokers[1].

Even though oesophageal carcinoma continues to be a male-predominant disease, the ratio of the disease in males to that in females has shown a change in trend with an increase in proportion of cases in the females. A probable cause for this could be nearly equal exposure to the risk factors of oesophageal carcinoma like betel-nut intake, consumption of smoked food, alcohol intake in both males and females in north-east India. According to the study conducted by Choksi D in

2019, the male: female ratio was 1.67 whereas the study conducted by Samarasam I in Christian Medical College Hospital showed a male: female ratio of 3:1[1,4]. However, in the present study, this ratio has decreased to 1.3:1.

Both males and females showed a higher proportion of squamous cell carcinoma of oesophagus than the other histologic subtypes (82.6% in males and 97.1% in females). A p-value of 0.05 was observed for this finding.

The results of the overall analysis in the present study suggested that till date squamous cell carcinoma remains the predominant histologic subtype (88.9%) of oesophageal malignancies, followed by the less commoner, adenocarcinoma of oesophagus (9.9%). Adenosquamous carcinoma constituted only 1.2% of cases of oesophageal carcinoma. This is in concordance with the studies conducted separately by Samarasam I in CMC, Vellore and Choksi D in 2019 in Department of Gatsroeneterology, Lokmanya Tilak Hospital, Mumbai where SCC was found to be the most common type of esophageal cancer in the Indian subcontinent[1,4].

It has been reported that in countries with higher human development index (HDI), there is a higher incidence of adenocarcinoma (AC) of the oesophagus. For example, in the US, the incidence of AC of the oesophagus has increased by over 400% over the past 25 years. In contrast, in countries with low HDI, like India, there is a higher incidence of esophageal squamous cell carcinoma (SCC)[4,5].

Majority of cases of oesophageal squamous cell carcinoma in both males and females were moderately differentiated (71.79% in males and 85.29% in females). Poorly-differentiated squamous cell carcinoma constituted only 12.82% of oesophageal squamous cell carcinoma in males, and 2.94% in females. Majority of the oesophageal squamous cell carcinoma cases reported in the present study were found to be moderately-differentiated whereas most oesophageal adenocarcinomas were found to be well-differentiated or poorly-differentiated. This observation was found to be statistically significant with a p-value <0.0001.

The overall majority of oesophageal carcinomas in the present study were found to arise in the middle-third of the oesophagus (62% cases), whereas the upper-third of the oesophagus was the least affected site (14% cases). The middle-third of the oesophagus was the most frequently affected site in oesophageal squamous cell carcinomas (69.44%), while the lower-third was the least affected site (5.55%). In contrast, oesophageal adenocarcinomas affected mostly the lower-third (87.5%); no case of oesophageal adenocarcinoma was seen in the middle-third. This observation also was found to be statistically significant with a p-value <0.0001.

Majority of well-differentiated squamous cell carcinoma of oesophagus affected the upper-third of oesophagus (55.55%), whereas the moderately- and poorly-differentiated ones mostly affected the middle-third (73% and 66.67%, respectively).

100% cases of well-differentiated and poorly-differentiated adenocarcinoma of oesophagus affected the lower-third of the oesophagus, whereas the moderately-differentiated ones affected the upper-third and lower-third in equal proportions (that is, 50% each).

According to the study conducted by Samarasam I in CMC, Vellore, the most common location of oesophageal carcinoma was found to be the distal third of the esophagus[1]. Choksi D observed in his study that the most common location of esophageal carcinoma was mid-esophagus with 229 patients (41.48%) followed by 208 patients (37.68%) in the lower esophagus[4].

IHC makes it possible to visualize the distribution and spatial localization of specific cellular components (cytoplasmic/ nuclear/ cell membrane/ lipid/ protein antigens) within a cell or tissue. Oesophageal cancers, like other epithelial cancers, show immunohistochemical positivity for a number of cytokeratins. Pancytokeratin AE1/AE3 is a mixture of both AE1 and AE3, where AE1 reacts with type I cytokeratins and AE3 with type II cytokeratins. AE1/AE3 is widely used as a pan-cytokeratin marker but lacks the reactivity with cytokeratin 18[6-13].

In this study, 50% (36 cases) moderately-differentiated squamous cell carcinoma and 12.5% (9 cases) of well-differentiated squamous cell carcinoma showed strong positivity for the immunohistochemical marker pancytokeratin; 27.7% (20 cases) moderately-differentiated squamous cell carcinoma and 5.6% (4 cases) poorly-differentiated squamous cell carcinoma was moderately positive for pancytokeratin (see Figure 1). Negative immunostaining for pancytokeratin was seen in 1.4% (1 cases) moderately-differentiated squamous cell carcinoma and 2.8% (2 cases) poorly-differentiated squamous cell carcinoma. None of the squamous cell carcinoma cases showed weak positivity for pancytokeratin.

In evaluation of the cases of esophageal adenocarcinoma for the IHC marker pancytokeratin, it was observed that 25% (2 cases) of well-differentiated adenocarcinoma and 12.5% (1 case) of moderately-differentiated adenocarcinoma showed strong positivity for pancytokeratin; 12.5% (1 case) of well-differentiated adenocarcinoma and 12.5% (1 case) of poorly-differentiated adenocarcinoma showed moderate positivity for pancytokeratin; 12.5% (1 case) of moderately-differentiated adenocarcinoma and 12.5% (1 case) of poorly-differentiated adenocarcinoma were negative for pancytokeratin. None of the adenocarcinoma cases showed weak positivity for pancytokeratin (see Table no. 5).

Table no. 6 summarizes the findings of a similar immunohistochemical study with cytokeratin5/6, cytokeratin 7 and other markers in oesophageal squamous cell carcinoma and adenocarcinoma cases conducted by DiMaio MA et al in 2012 where they observed that whereas CK5/6 is a good marker for squamous cell carcinoma of oesophagus, CK7 was more specific for oesophageal adenocarcinomas[3]. In the present study it has been observed that pancytokeratin is a good marker for SCC in comparison to ADC.

TABLE 6 Comparison of the IHC marker analysed in the present study to that of other studies

STUDY		Cytokeratin			
		SCC		ADC	
YEAR	AUTHOR	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE
2012	Dimaio et al[3]	CK5/6 97.56%	CK5/6 2.44%	CK5/6 13%	CK5/6 87%
2012	Dimaio et al[3]	CK7 73%	CK7 27%	CK7 94%	CK7 6%
2020	Present study	PanCK 95%	PanCK 5%	PanCK 62.5%	PanCK 37.5%

P53 gene mutation and protein accumulation may occur at very early stages of oesophageal carcinogenesis like precancerous lesions. However, in carcinomas, there is a higher frequency of p53 gene mutations, which accounts for most of the cases with IHC-detectable p53 protein accumulations[14].

In this study, less than half of oesophageal squamous cell carcinoma cases (47.2%) stained positive for p53 on immunohistochemistry with only 27.8% of them showing strong positivity; whereas majority of oesophageal adenocarcinoma cases (87.5%) stained positive for p53, with approximately 75% of the positive cases showing strong positivity. 54.2% cases of oesophageal squamous cell carcinoma and 12.5% cases of oesophageal adenocarcinoma were negative for p53 on IHC staining. Among the few adenocarcinomas that stained negative for p53, most were poorly-differentiated. A p-value of 0.01 for this finding indicated that it was statistically significant.

P53 overexpression is often associated with inactivation of p53[15]. But the sensitivity of IHC to assess p53 mutation is generally poor (the expression rate of p53 detected by IHC may range from 33-70% in oesophageal cancers) as truncated mutants can lead to complete loss of p53 staining and be missed by IHC. Also, in some cases, nonsense mutations or a quickly degraded mutant p53 protein can cause lack of expression of p53[16-18].

In 2001, Okuda E et al observed in his study that a vast majority of esophageal squamous cell carcinomas were p53 positive[19]. However, the study conducted by Shimada H in 2018 showed that most esophageal carcinomas were negative for p53[20]. Two studies, one in 2000 and another in 2019, conducted separately by Ireland AP et al and Melling N et al, respectively, showed an almost equal proportions of cases of oesophageal adenocarcinoma staining positive and negative for p53 on immunohistochemistry[21,22].

In the present study, there was only 1 case of adenosquamous cell

carcinoma, and it showed moderate positive staining for both p53 and pancytokeratin.

Hence, this study indicates that the immunohistochemical marker pancytokeratin is a good marker for oesophageal squamous cell carcinoma but not for adenocarcinoma of oesophagus. In contrast, the immunohistochemical marker p53 is a good marker for oesophageal adenocarcinoma but not very reliable for squamous cell carcinoma of oesophagus. Most of the cases of oesophageal adenocarcinoma that were negative for both pancytokeratin and p53 were found to be poorly differentiated. Therefore, it also suggests that loss of differentiation may affect the expression of these markers.

However, the level of significance of the results may have been affected by the limited sample size of this study. Also, as this is a hospital-based study, the results obtained with this sample may not truly reflect that at the community level.

CONCLUSION

In our study, the immunohistochemical marker pancytokeratin was found to be a good marker for oesophageal squamous cell carcinoma but not very reliable for adenocarcinoma of oesophagus. However, P53 was found to be a good marker for oesophageal adenocarcinoma but not very reliable for squamous cell carcinoma of oesophagus. The results and observations of this study may be applied to the general population at the community level only after proper validation of the study by conducting a similar study with a sample size large enough to adequately represent the entire population.

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