ORIGINAL RESEARCH PAPER
Volume - 10 | Issue - 03 | March - 2021 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

EXPRESSION OF P53 IN SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA OF OESOPHAGUS BY IMMUNOHISTOCHEMISTRY IN OESOPHAGEAL BIOPSIES

Pathology

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ABSTRACT

BACKGROUND: Oesophageal carcinoma is one of the commonest cancers in India, particularly in the north-eastern states. Non-specific symptoms and low treatment remission rates make it one of the deadliest cancers. Molecular pathogenesis of both Squamous cell carcinoma and Adenocarcinoma of oesophagus involves alterations of the tumour suppressor gene, P53. Differentiating these two histologic types of oesophageal carcinoma is important because they have diverse etiopathogenesis and management options. **AIM / OBJECTIVE:** The objective of our study was to evaluate P53 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 and P53 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.**CONCLUSION:** A comparatively higher proportion of oesophageal adenocarcinoma cases showed positive staining for P53 than oesophageal squamous cell carcinoma. However, further extensive research studies are required for validation of the same.

KEYWORDS

P53 Expression; Oesophageal Adenocarcinoma; Oesophageal Squamous Cell Carcinoma; Immunohistochemistry; Oesophageal Biopsy

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[1]. 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[2,3]. Eighty-percent of oesophageal cancers in India are squamous cell carcinomas; adenocarcinomas are the second most common 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[2,4]. Differentiating these two histologic types is important because they have diverse etiopathogenesis and management options. Treatment modalities vary considerably from surgical treatment (oesophagogastrectomy, preferred for adenocarcinoma of lower oesophagus) to chemoradiation (preferred for squamous cell carcinoma of upper oesophagus) [5]. However, differentiating these two histologic types based on morphology alone may be challenging in small biopsy specimens, especially the poorly-differentiated subtypes.

P53, a tumour-suppressor gene located on Chromosome 17p13.1, encodes the wild-type nuclear protein p53 that plays a key-role in cell-cycle regulation, inhibition of DNA synthesis, repair of damaged DNA, differentiation, autophagy and apoptosis[6-9]. Hence, p53 plays a fundamental role in maintenance of genomic stability. Under normal conditions, p53 levels are low to undetectable. In unstressed cells, p53 is held at bay through its association with MDM2, leading to degradation by proteasome, and thus, p53 is virtually undetectable immunohistochemically in normal cells[4,10]. But when mutated p53 becomes stabilized, it resists degradation, thereby, accumulating in the cell nucleus[11]. In esophageal cancers, p53 shows nuclear staining due to accumulation of the stable mutant p53, thereby, being detectable immunohistochemically using monoclonal antibodies[11,12]. 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[13]. This study aims to evaluate the expression of p53 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. The oesophageal biopsy samples were collected from department of Gastroenterology in Gauhati Medical College and Hospital. The biopsy materials were fixed in 10% formalin, processed in automated tissue processor. Sections were trimmed up to 4 – 5 micron thickness using rotary microtome and stained with Haematoxylin and Eosin stains. Tissue sections diagnosed as squamous cell carcinoma or adenocarcinoma were retrieved and incubated for 30 – 60 minutes after adding primary antibody (anti-p53). After washing, secondary antibody was added followed by addition of chromogen substrate, counterstaining and mounting. The slides were microscopically examined for positivity of p53: diffuse nuclear expression (due to accumulation of stable mutant p53) was considered positive. A cell without p53 mutation is negative for immunohistochemical staining of p53 because n dye accumulation occurs in the cell. Immunohistochemical scoring system was based on the intensity and extent of staining[14]. 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. Master-charts, prepared in Microsoft excel sheet, were used for data recording and statistical analysis. Vancouver style of referencing was used for bibliography.

RESULTS:

The distribution of oesophageal carcinoma in the different age-groups among males and females in this study has been summarised in table no. 1. The male:female ratio of oesophageal carcinoma cases in our study was 1.3:1. The maximum peak of cases of oesophageal carcinoma was observed in 61-70 years age-group.

Table No. 1: The distribution of esophageal carcinoma in the different age-groups among males and females in this study

AGE-GROUP (YEARS)	MALES	FEMALES
0-10	0	0
11-20	0	0
21-30	1	0
31-40	2	5
41-50	12	9
51-60	13	6

61-70	14	12
71-80	5	2
81-90	0	0
91-100	0	0
TOTAL	47	34

The distribution of the various histologic types of oesophageal carcinoma in different age-groups in this study has been summarized in Table no. 2. Most of the cases of oesophageal squamous cell carcinoma affected individuals aged 41-50 years.

Table No. 2: The distribution of the different histologic types of esophageal carcinoma (squamous cell carcinoma, adenocarcinoma and adenosquamous carcinoma) in different age groups in this study

AGE-GROUP (YEARS)	SQUAMOUS CELL CARCINOMA	ADENOCARCINOMA	ADENOSQUAMOUS CARCINOMA
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

The distribution of the different types of oesophageal carcinoma in males and females in this study has been summarised in Table no. 3. Squamous cell carcinoma (SCC) of oesophagus was found to be the most predominant histologic type in both males and females. This observation was found to be statistically significant (p-value: 0.049).

Table. No. 3: 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

The distribution of the three grades [well-differentiated (WD), moderately differentiated (MD) and poorly-differentiated] of oesophageal squamous cell carcinoma (SCC) and adenocarcinoma (ADC) in upper-third, middle-third and lower-third of oesophagus has been summarised in table no. 4. 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. 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 No. 4: The distribution of the three grades [well-differentiated (WD), moderately differentiated (MD) and poorly-differentiated] of oesophageal squamous cell carcinoma (SCC) and adenocarcinoma (ADC) in upper-third, middle-third and lower-third of oesophagus in our 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	

The results for immunohistochemical staining for p53 in cases of squamous cell carcinoma (SCC) and adenocarcinoma (ADC) of oesophagus in oesophageal biopsies has been summarised in Table no. 5. There was one case of oesophageal adenosquamous carcinoma in our study which showed moderate positivity on immunohistochemical staining for p53.

Table No. 5: The results for immunohistochemical staining for p53 in cases of squamous cell carcinoma (SCC) and adenocarcinoma (ADC) of oesophagus in oesophageal biopsies in our study.

	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

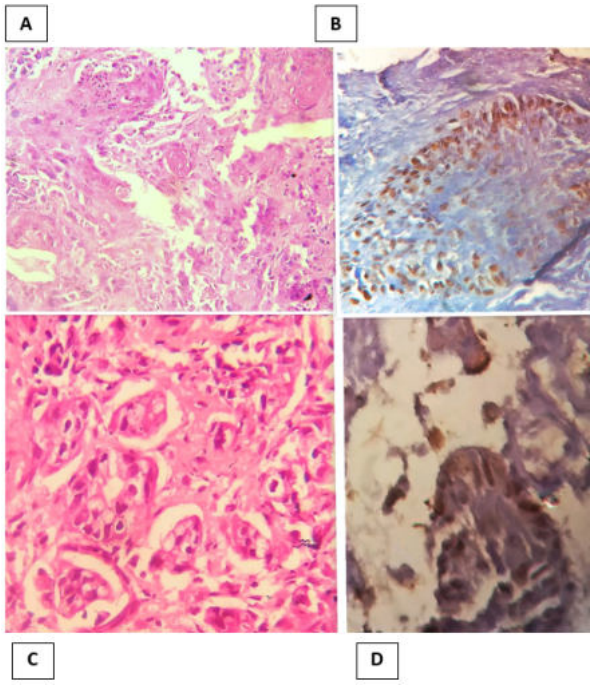

Fig. 1: Photomicrographs of oesophageal carcinoma seen in this study.

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 (low-power/100x magnification)

C: Haematoxylin and Eosin-stained sections of of adenocarcinoma showing an area of gland formation by malignant signet ring cells seen on high-power microscopy/400x

D: Oesophageal adenocarcinoma showing nuclear positivity for the immunohistochemical marker, p53 (low-power/100x magnification)

DISCUSSION

Cancer of the esophagus is the leading site in the registries in the states of Assam, Meghalaya, Mizoram, and Nagaland[15].

In the present study, the different histologic subtypes of oesophageal cancer in various age-groups and different parts of the oesophagus, amongst both male and female population, has been studied. Also, the expression pattern of p53 in oesophageal squamous cell carcinoma and adenocarcinoma has been studied by using immunohistochemical detection methods.

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)[16]. 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. 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[2].

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 [2,16]. 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[2,16].

It has been reported that in countries with higher human development index (HDI), there is a higher incidence of adenocarcinoma (AC) of the esophagus. For example, in the US, the incidence of AC of the esophagus 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)[16,17].

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 where the most common location of oesophageal carcinoma was found to be the distal third of the esophagus[2]. 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[16].

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[18].

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[19]. 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[12,20,21].

In 2001, Okuda E et al observed in his study that a vast majority of esophageal squamous cell carcinomas were p53 positive[22]. However, the study conducted by Shimada H in 2018 showed that most esophageal carcinomas were negative for p53[23]. 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[24,25].

In the present study, there was only 1 case of adenosquamous cell carcinoma, and it showed moderate positive staining for p53..

Hence, this study indicates that 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 p53 were found to be poorly differentiated. Therefore, it also suggests that loss of differentiation may affect the expression of this marker.

However, the limited sample size of this study may have affected the level of significance of the results. 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, P53 was found to be a good marker for oesophageal adenocarcinoma but not very reliable for squamous cell carcinoma of oesophagus. However, to apply the observations of this study to general population at the community level, proper validation of the study is required by conducting a similar study with a sample size large enough to adequately represent the entire population.

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