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IMMUNOHISTOCHEMICAL EVALUATION OF P53 IN GASTRIC CARCINOMA AND CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS

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ABSTRACT **BACKGROUND:** Gastric cancer is one of the most aggressive type of cancer with a very high mortality rate worldwide. The immunohistochemical protein expression of mutant p53 has been proposed as a potential tool to study the biological behavior of gastric cancer. The role of p53 as a prognostic indicator in case of gastric carcinoma is debatable; hence this study was conducted to know the immunoexpression of p53 in gastric cancer and to evaluate the correlation with clinicopathological parameters. **METHODS:** It is a hospital based cross sectional study which included a total 50 cases of gastric carcinoma. Surgically resected gastric specimen were first analyzed by routine H&E and then subjected to immunohistochemistry for p53. The findings were then compared with clinicopathological parameters like gender, age, tumor location, Lauren's classification, WHO subtype, histological grade and TNM staging. **RESULTS:** GCs had a peak incidence in the age group of 51 to 60 years. The youngest age of presentation of gastric cancer was at 32 years in this study. 33 (66%) cases were reported in males and 17 (34%) cases were reported in females. 31(62%) cases involved the Pyloro-antrum, 14 (28%) involved body, 2 (4%) involved the cardia, 3 (6%) cases involved fundus. Ulcero-proliferative type was the most common (64%) gross type followed by infiltrative type (28%). 60% cases were poorly differentiated cancer. 38% and 24% cases belong to TNM stage III and IV respectively. P53 positivity was observed in 74% cases of GC. Level of p53 positivity was found significantly associated with tumor grade and tumor stage with p value of 0.000851 and 0.0078 respectively. **CONCLUSION:** Immunohistochemical evaluation of p53 is simple & effective modality to determine the prognosis of gastric cancer and could be helpful in identifying a group of patients having a higher risk of poor survival.

KEYWORDS : gastric carcinoma, p53, immunohistochemistry

INTRODUCTION

According to Global Cancer Statistics 2020 (GLOBOCAN 2020), gastric carcinoma (GC) is one of the most prevalent cancers in the world and is responsible for over one million new cases in 2020 and an estimated 769,000 deaths (equating to one in every 13 deaths globally), ranking fifth for incidence and fourth for mortality globally [1]. The incidence of gastric carcinoma in India is overall less compared to the worldwide incidence. It ranks third among males and eighth among females for incidence in India according to GLOBOCAN INDIA 2020 statistics [2]. Incidence of GC varies widely among the various regions within India due to diverse culture and related food habits. North Eastern region of India has the highest age adjusted incidence rate for occurrence of GC according to the report of National Cancer Registry Programme 2020 published by Indian Council of Medical Research [3]. Aizawl district of Mizoram has the highest incidence of stomach cancer in India [3]. Arunachal Pradesh has registered the second-highest stomach cancer cases in the world after China [4]. This strikingly high incidence of GC in the region of NE India is mainly due to some of the ethnic food habits like consuming smoked meat or fish and high rate of consumption of alcohol and smoking habits [5].

Adenocarcinoma is the most common malignancy of the stomach, comprising more than 90% of all GCs. The major risk factors for gastric carcinoma are Helicobacter pylori, dietary factors and gastroesophageal reflux disease [6]. The prognosis of gastric carcinoma is mainly dependent on the stage of the disease. Because of the variability of prognosis within a clinical or pathological stage of GC, there has been a constant search for specific biological markers in order to identify subgroups of patients with more aggressive course of disease [7]. The immunohistochemical expression of p53 has been proposed as a potential tool for the evaluation of the biological behavior of GC [8].

P53, also known as TP53 or tumor protein is a gene that codes for a protein that regulates the cell cycle and hence functions as a tumor suppressor. The human p53 gene is involved in development of many malignancies including gastric carcinoma. Expression rate of p53 detected by immunohistochemistry is reported as 13-54% in GC in various studies [9]. Because of this, prognostic significance of the abnormal increase of p53 expression detected by immunohistochemistry in GC is evaluated in this study.

MATERIALS AND METHODS

The current study was undertaken to study the immunoexpression of p53 in gastric carcinoma and to correlate their expression with clinicopathological parameters. So, on the basis of that, we have a definite inclusion and exclusion criteria to consider. All the gastric carcinoma cases reported in surgically resected gastric specimens irrespective of the age and sex were included in this study. Whereas patients already diagnosed with non-malignant or premalignant conditions of stomach and patients already diagnosed with Gastric tumours other than Gastric adenocarcinoma were excluded from this study. The present study is a hospital based cross sectional study conducted in the Department of Oncopathology, State Cancer Institute and Department of Pathology, Gauhati Medical College and Hospital, Guwahati, Assam for a period of one year from July 2021 to June 2022. 50 cases of gastric adenocarcinoma diagnosed on surgically resected Gastrectomy specimen by routine histopathological examination were included in this study.

The specimen were received in 10% neutral buffered formalin and 3-4 µm thick tissue sections were prepared. After processing in automated tissue processor, paraffin blocks were made. Further, formalin-fixed paraffin-embedded tissue samples were subjected to H&E stain and histopathological diagnosis was done on the basis of WHO classification of Gastric tumors, 2019 and CAP cancer protocol stomach. For IHC, additional sections were prepared on Ply-l-lysine coated slides and were subjected to IHC for P53. The findings were then compared with clinicopathological parameters like gender, age, tumor location, Lauren's classification, WHO subtype, histological grade and TNM staging.

Interpretation for P53: Tumor cells were scored positive when there was golden-brown nuclear staining in the neoplastic cells.

P53-negative (-): immunostaining in <10% of the tumor nuclei.
P53-positive (+): immunostaining in >10% of the tumor nuclei

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS for windows 21.0 software. The clinical properties of the patients were calculated using mean and percentage values. Parametric parameters were investigated using Chi square test and P value of <0.05 is considered as statistically significant.

RESULTS

The age of the patients ranges from 32-79 years with an average age of 56.38 years and a peak incidence in the age group of 51-60 years

(36%). The youngest age of presentation was 32 years in this study. Out of the 50 cases 33 cases (66%) were male and 17 cases (34%) were female with male to female ratio of 1.94:1. Among the 50 cases of gastric carcinoma, 31 (62%) of cases involved the Pyloro-antrum, 14 (28%) involved the body, 3 (6%) cases involved the fundus and only 2 (4%) cases involved the cardia region of the stomach. Based on gross morphology, gastric carcinoma cases were divided into 4 groups. Ulcero-proliferative type (64%) was the most common gross appearance followed by infiltrative type (28%). The 50 cases of gastric carcinoma were grouped according to Lauren's classification, out of which 56% (28 cases) belonged to Diffuse type and 44% (22 cases) belonged to Intestinal type. In our study, it was observed that maximum gastric adenocarcinoma were of Poorly Cohesive type (60%) followed in decreasing frequency by signet ring type (16%), tubular type (12%), mucinous (8%). The least common type was found to be papillary (4%) in our study. The 50 cases of GCs were graded histologically and divided into three groups, out of which 10 cases (20%) were well differentiated (G1), 10 cases (20%) cases were moderately differentiated (G2) and 30 cases (60%) were poorly differentiated (G3). In this study 6 cases showed invasion upto the submucosa (T1), 18 cases showed infiltration into the muscularis propria or subserosa (T2), 18 cases showed infiltration into the serosa and 13 cases showed infiltration beyond serosa (T4). Out of the 50 cases, maximum number of cases presented with 7 or more lymph nodes positive for carcinoma. Out of the 50 cases, 30 cases (60%) showed Lymphovascular invasion while 20 cases (40%) showed no invasion. In the present study, 6 cases (12%) belonged to stage I, 13 cases (26%) belonged to stage II, 19 cases (38%) belonged to stage III and 12 cases (24%) belonged to stage IV.

In this study, 37 (74%) cases showed positive expression for p53 while 13 (26%) cases were negative.

Correlation of p53 expression with various clinicopathological parameters are as follows:

The age ranged between 32-79 years with a mean of 56.38 years. There were 34 (68%) cases above the age of 50 years and 16 (32%) below 50 years. The age distribution of p53 expression was compared between these two groups. p53 positivity was observed in 72.73% of male and 76.47% of female cases. In our study, p53 positivity was observed in 80.64% of tumors of the Pyloro – antrum, 64.28% of tumors of the body, 50 % of tumors of the cardia and 66.67% of tumors of the fundus. Among the various gross types, p53 positivity was found in 1 case (50%) of Borrmann type I, 1 case (50%) of Borrmann type II, 27 cases (84.37%) of Borrmann type III and 8 cases (57.14%) of Borrmann type IV. Among WHO histological subtypes, 20% of tubular adenocarcinomas, 50% of papillary carcinomas, 75% of mucinous, 62.5% of signet ring cell and 90% of poorly cohesive carcinomas showed p53 positive expression. When Lauren's type was compared, a greater percentage of p53 positive expression with diffuse type cancers (82.14%) in comparison with intestinal type carcinomas (63.63%) was observed. An increasing percentage of p53 positivity was observed with increasing tumor grade. 30% of well differentiated tumors (G1), 70% of moderately differentiated tumors (G2) and 90% of poorly differentiated tumors (G3) showing positivity for p53 was observed in our study which is found to be statistically significant with a P value of 0.000851. According to the T stage, a progressive increase in the number of p53 positive cases were noticed from T2 to T3 which is statistically significant (p=0.0077).

DISCUSSION

Gastric carcinoma is a life threatening disease and represents a significant health problem worldwide. Many biological markers have been studied as possible tools for the evaluation of the biological behavior of gastric cancer in order to predict the clinical outcome. Among these, immunohistochemical detection of cell cycle regulator p53 has been proposed to be of prognostic significance.

Most of the patients (66%) in our study were male. However, there was no statistically significant association with gender. This finding is similar to the findings of Daniela Lazar et al. [10]. Risk of carcinogenesis increases with advancing age, a study done by Honda T et al. [11] confirmed that age group of > 60 years has significantly higher risk for gastric cancer. Similar results were seen in our study i.e. p53 expression is significantly higher in age group of > 60 years. However, no statistically significant association was found.

Histologic grade is considered to be one of the most important prognostic indicators in case of Gastric carcinoma. As grade increases prognosis becomes poorer. We found a statistically significant correlation between p53 expression and histologic grade. Similar results were found by Tushar Hiralal Sankalecha et al. [12], Daniela Lazar et al. [10], and C. M. Servarayan et al. [13]. So overexpression of p53 can be linked with histological aggressiveness of the tumor which is a bad prognostic indicator.

The Lauren's classification divides adenocarcinoma of the stomach into 2 main types: intestinal and Diffuse. Generally diffuse type shows more aggressive behavior which was the most commonly observed Lauren's type in our study. However, this finding is not consistent with the findings of other studies conducted by Saha et al. [14], Rajagopal et al. [15] and Casasola et al. [16] .When p53 expression was compared with Lauren's classification, we found no significant correlation between these two parameters.

Location of the gastric tumor is an important parameter to assess prognosis. The most common site of gastric cancer in this study is found to be the pyloro – antrum (62%) followed by body (28%) which is in accordance with studies conducted by Daniela Lazar et al. [10], Rajagopal et al. [15], Saba El-Gendi et al. [17] and Tzanakis et al. [18]. Stomach cancer has been classified into various histological types according to WHO classification and when compared with p53 expression we found no statistically significant correlation. Similar results were seen in studies done by Akshatha C et al. [19], Daniela lazar et al. [10].

Depth of invasion (T stage), lymph node involvement (N stage) and the clinical stage (TNM stage) are important prognostic parameters in case of GC. As the stage increases prognosis becomes poorer and survival decreases. When compared with these parameters, we found that p53 expression was significantly increased with increasing grades of T, N & clinical stages and statistical significance was observed. Hence p53 expression can also be linked with invasiveness of tumor & clinical aggressiveness of the tumor.

CONCLUSION

The number of gastric cancer patients showing increased expression of p53 is significantly higher. A high percentage of cases with p53 overexpression was noticed with increasing grade, depth of invasion, nodal status and TNM stage which are bad prognostic indicators. To conclude, evaluation of p53 by immunohistochemistry is simple & effective modality to determine the prognosis and survival in various grades & stages of gastric cancer and could be helpful in identifying a group of patients having a higher risk of poor survival.

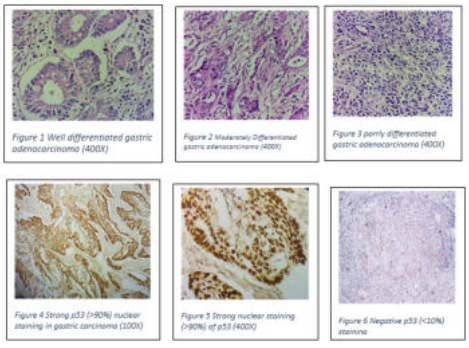
Table 1: comparison of clinicopathological parameters with p53 expression

Characteristics	Total	P53		P value
		Positive	Negative	
gender				
Male	33	24	9	0.775
Female	17	13	4	
age				
<50	16	11	5	0.562
>50	34	26	8	
Tumor location				
Cardia	2	1	1	0.556
Fundus	3	2	1	
Body	14	9	5	
Pyloro-antrum	31	25	6	
Gross morphology				
Ulceroproliferative	32	27	5	0.167
Polypoidal	2	1	1	
Fungating	2	1	1	
Infiltrative	14	8	6	

Table 2: comparison of histological grade and stage with p53 expression

Characteristics	Total	P53		P value
		Positive	Negative	
Histological grade				
Well differentiated	10	3	7	0.000851
Moderately differentiated	10	7	3	
Poorly differentiated	30	27	3	
T stage				
T1	6	1	5	0.0077
T2	18	15	3	
T3	13	11	2	
T4	13	10	3	
N stage				
N0	6	2	4	0.0075
N1	7	3	4	
N2	17	14	3	
N3	20	18	2	
TNM stage				
I	6	1	5	0.0078
II	13	10	3	
III	19	16	3	
IV	12	10	2	

IMAGES:



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Conflicts of interest
There are no conflicts of interest.

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