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Original Research Article

Correlation of p53 over expression with the clinicopathological prognostic factors in gastric adenocarcinoma

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Abstract

Background: Gastric cancer is one of the leading causes of cancer deaths. The etiology of gastric cancer is complex multistep process characterised by histopathologic precursor lesions and molecular genetic alterations involving APC, K-ras and p53 genes. Overexpression of p53 in gastric carcinoma is associated with bad prognosis.
Aim: To establish correlation between p53 overexpression and clinicopathological features of gastric carcinoma.
Materials and methods: A cross-sectional, descriptive hospital based study of clinical, histopathological and immunohistochemistry (IHC) features of gastric carcinoma was conducted on gastrectomy specimens received to the Department of Pathology from January 2008 to June 2013. The clinical features, tumour morphology and p53 status by immunohistochemistry, were evaluated in each case.
Results: The most common histologic type of gastric carcinoma was of intestinal type and predominantly were grade III tumours (60%). Most (75%) of them presented with lymph node metastasis. Majority were in stage IIIA (27.5%) or stage IV (20.0%). Majority of gastric carcinomas (35%) had 1+ intensity of p53 expression but and it was not statistically significant. Majority of gastric adenocarcinomas showed p53 positivity in <5% tumour cells. Overall, 62.5% gastric adenocarcinomas showed p53 positivity and 10 out of 40 (25%) were intensely positive. There was no significant correlation between p53 status and age of patient, tumor site, tumor size, histological type, grade, lymph node status and TNM stage.
Conclusion: Mutation in p53 plays a vital role in development of gastric carcinoma. However, a significant correlation between gastric carcinoma and clinicopathological parameters could not be established. A prolonged follow up study on a large number of cases can help to find out whether p53 overexpression by standard IHC could be a useful marker for prognosis of gastric carcinoma.
Keywords: Gastric adenocarcinoma, p53, Immunohistochemistry, Grade, TNM stage, Lymph node metastases

1. Introduction

Gastric cancer is one of the leading causes of cancer deaths making it the 5th and 6th cause among males and females respectively in India. The estimated newly diagnosed cases were 23,785 in men and 11,819 in women. [1] The etiology of gastric cancer is a complex multistep process characterised by histopathologic precursor lesions and molecular genetic alterations.

The p53 is a tumor suppressor gene, located on short arm of chromosome 17p 13.1 and plays a pivotal role in regulating cell growth.[1] p53 mutation is commonly encountered in many human cancers. Frequency of p53 overexpression in gastric adenocarcinoma is 26 to 65%.[1] It has also been documented that p53 expression varies with location and histological type of tumor.[3]

Overexpression of p53 protein is directly related to enhance proliferative activity [4] and an increased propensity of lymphovascular invasion, lymph node metastasis and advanced TNM stage [5], representing greater tumour aggressiveness. Laboratory analysis of p53 gene status can be currently done by three methods: 1) polymerase chain reaction (PCR) 2) immunohistochemistry (IHC) and 3) Detection of serum p53 antibody in peripheral blood samples.[2] In comparison to DNA sequencing, immunohistochemical methods are cheaper, easier and more familiar to pathologists as a standard procedure in routine diagnosis. The present study was undertaken to establish correlation between p53 overexpression in gastric cancer by IHC and various prognostic factors including the clinical features, tumour histopathology, tumour grade, lymph node status, and stage of the tumour.

2. Materials and Methods

A cross-sectional, descriptive hospital based study of clinical, histopathological and IHC features of gastric carcinoma was conducted on total/subtotal gastrectomy specimens received at the Department of Pathology from January 2008 to June 2013. The detailed clinical history and results of relevant investigations were collected from the patient's case files. Paraffin blocks were retrieved and sections of 5µm thickness were stained by haematoxylin and eosin (H and E) for histopathological study. In addition, 4µm sections were cut from a paraffin block of tumour tissue and taken on a glass slide coated with adhesive (silane) for immunohistochemistry (IHC) to detect p53 expression.

2.1 Processing for immunohistochemistry:

The technique for IHC included antigen retrieval in citrate buffer in a microwave oven, blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody against p53 protein (Biogenex), linking with rabbit anti mouse secondary antibody (Novocastra, UK), enzyme labelling with streptavidin-horseradish peroxidase (Novacastra, UK), developing chromogen with deaminobenzidine (DAB) and counterstaining with haematoxylin. Positive and negative controls were run with each batch of slides.

The H and E stained slides were studied for the tumour histology, grade, lymph node metastasis and other features. The immunostained slides were examined for nuclear staining with anti-p53 antibody. In each case, the proportion of positive staining tumor cells (expressed as percentage) and the average intensity of staining (expressed as 1+, 2+, 3+ and 4+) were evaluated.

Table 1: The format for reporting p53

Percentage of cells showing positive staining	Intensity	Result
0%	0	Negative
<5%	1+	Negative
5-25%	1+/2+	Weak positive
26-75%	2+/3+	Moderately positive
>75%	3+/4+	Intense positive

The relationship between various parameters such as age, sex, duration of disease, anatomic site of tumour, size and extent of tumour, histologic type and grade, lymph node status, and staging of the tumour with overexpression of p53 were studied.

2.2 Statistical analysis

Statistical analysis was done using Chi-square test of significance and "p" value of less than 0.05 was accepted as indicating statistical significance. Data analysis was carried out using Statistical Package for Social Science (SPSS, V 10.5) package.

3. Results

During a period of 66 months, 40 gastrectomy specimens were received with age ranging from 25 to 75 years. The female preponderance was noted. The frequent presenting complaints were vomiting (37.5%) and dyspepsia (37.5%) followed by abdominal pain (35%).

The common site for gastric carcinoma was pyloric antrum (60%) and majority (20/40, 70%) of tumors were > 4cm in size. Intestinal type of adenocarcionma (55%) were predominant according to Lauren's classification (Table 2) and according to WHO classification diffuse type was frequently seen (11/40, 27.5%) (Table 3). Majority of patients presented with grade III gastric carcinomas (24/40, 60%) (Table 4).

Gastric carcinomas more commonly (30/40, 75%) presented in advanced stage with lymph node metastasis. Majority of patients presented in stage IIIA (27.5%) or stage IV (20.0%) (Table 5). There was no statistical significance between TNM stage and histological type of gastric carcinoma (Table 6).

Majority of gastric carcinomas (14/40, 35%) had 1+ intensity of p53 positivity, followed by 2+ intensity (12/40, 30%), 4+ intensity (7/40, 17.5%); however this difference was not statistically significant. Majority of gastric adenocarcinomas showed p53 positivity in <5% tumour cells (14/40, 35%). Overall, 62.5% (25/40) gastric adenocarcinomas showed p53 positivity and 10 out of 40 (25%) were intensely positive. There was no significant correlation between p53 status and age of patient, tumor site, tumor size, histological type, grade, lymph node status and TNM stage.

Table 2: Lauren classification of gastric adenocarcinoma

Lauren Classification	Frequency	Percent
Intestinal	22	55.0
Diffuse	18	45.0
Total	40	100.0

Table 3: WHO classification of gastric adenocarcinoma

Histological Type – WHO	Frequency	Percent
Intestinal	7	17.5
Diffuse	11	27.5
Papillary	2	5.0
Tubular	9	22.5
Mucinous	3	7.5
Signet Ring Cell	7	17.5
Oncocytic	1	2.5
Total	**40**	**100.0**

Table 4: Grade of gastric adenocarcinoma in the cases studied

Grade	Frequency	Percent
Grade I	2	5.0
Grade II	14	35.0
Grade III	24	60.0
Total	40	100.0

Table 5: TNM staging in the cases of gastric carcinoma

TNM Stage	Frequency	Percent
Stage IA	1	2.5
Stage IB	5	12.5
Stage II	5	12.5
Stage III	2	5.0
Stage IIIA	11	27.5
Stage IIIB	6	15.0
Stage IIIC	2	5.0
Stage IV	8	20.0
Total	40	100.0

Table 6: TNM stage in relation to the histological type in gastric carcinoma

Histological Type-WHO	TNM Stage								Total	χ^2 value	p' value
	Stage IA	Stage IB	Stage II	Stage III	Stage III A	Stage III B	Stage III C	Stage IV			
Intestinal	0	1	3	1	1	0	0	1	7	44.0	0.67
	.0%	14.3%	42.9%	14.3%	14.3%	.0%	.0%	14%	100.%		
Diffuse	0	4	1	0	2	2	0	2	11		
	.0%	36.4%	9.1%	.0%	18.2%	18 %	.0%	18.%	100.%		
Papillary	0	0	0	0	2	0	0	0	2		
	.0%	.0%	.0%	.0%	100.0%	.0%	.0%	.0%	100.%		
Tubular	1	0	1	1	2	2	1	1	9		
	11.1%	.0%	11.1%	11.%	22.2%	22.2%	11.1%	11.1%	100.0%		
Mucinous	0	0	0	0	2	0	0	1	3		
	.0%	.0%	.0%	.0%	66.7%	.0%	.0%	33.3%	100.0%		
Signet Ring Cell	1	0	1	1	2	0	1	1	7		
	14.%	.0%	14.3%	14.%	28.6%	0%	14.%	14.%	100.%		
Oncocytic	0	0	0	0	0	0	0	1	1		
	.0%	.0%	.0%	.0%	.0%	.0%	.0%	100%	100.%		
Total	2	5	6	3	11	4	2	7	40		
	5.0%	12.5%	15.0%	7.5%	27.5%	10.%	5.0%	17.%	100.%		

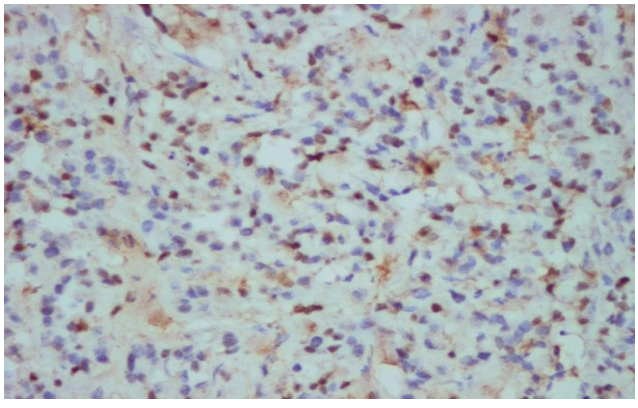

Figure 1: P53 positivity 2+, 26-75% (moderate) in a stomach adenocarcinoma (anti-p53-polyhorse radish peroxidase-DAB chromogen, x100)

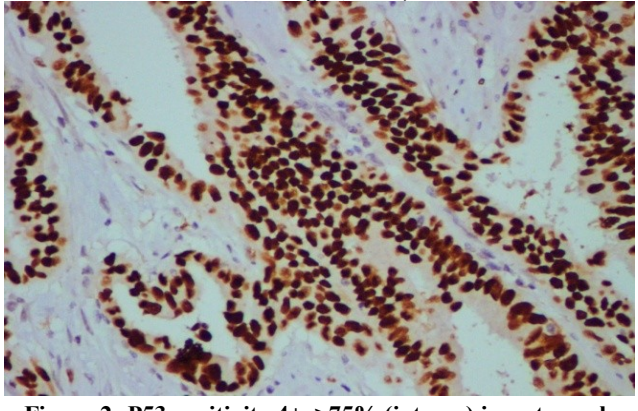

Figure 2: P53 positivity 4+, >75% (intense) in a stomach adenocarcinoma, intestinal type (anti-p53-polyhorse radish peroxidase-DAB chromogen, x400)

4. Discussion

The p53 gene codes a protein that is important in cell cycle and thus functions as a tumor suppressor. It has been attributed as the "guardian of genome" as it plays a pivotal role by preventing genome mutation and conserving stability. It is involved in cell cycle regulation, DNA repair and in apoptosis. Since it is involved in many biological processes it plays a pivotal role in carcinogenesis. Thus, loss of p53 function plays a crucial role in tumor development. [6]

In nonmalignant stomach, p53 immunopositive cells are found in neck, gastric proliferative zone and in Helicobacter pylori infection. In H. pylori gastritis, free radicals produced by activated leucocytes cause mucosal DNA damage and thus nuclear p53 expression can be expected to be seen in proliferative zone.[7] p53 overexpression has been reported in 17-90.7% of invasive tumors. The nuclear staining of p53 can be seen in both intestinal and diffuse type of gastric tumors even though common in intestinal type. The degree of p53 expression correlates with proliferative rate of tumors which can be explained by higher incidence of p53 positivity in intestinal type of tumors.[8] p53 expression is common in poorly differentiated tumors than well differentiated carcinomas.[9] Its expression is more common in tumors from proximal stomach compared to distal tumors.[10] Evaluation of p53 as a prongostic marker has yielded conflicting results. Thus, this study was conducted to establish correlation between p53 overexpression in gastric cancer by IHC and various prognostic factors including the clinical features, tumour histopathology, tumour grade, lymph node status, and stage of the tumour.

In the present study, 40 gastrectomy specimens were studied and correlation of p53 status with clinical features and tumor histopathology was analysed. The peak age group who presented with gastric carcinoma was between 60 and 69 years and thus elderly age is a risk factor for development of gastric carcinoma. Lymph node metastasis at time of diagnosis was seen in 75% of patients whereas in studies by Ghaffarzadegan *et al*[11] and Aurello *et al*[12] it was 57.6% and 64% respectively. Intestinal type of gastric adenocarcinoma was the common type of gastric adenocarcinoma observed in our study which was similar to that noted in studies by Nabi *et al*[13] and Omran *et al*[14]. In our study most of the tumors were of grade III (60%) whereas it was grade II in studies by Chiaravalli *et al*[15] and Choi sang-wook *et al*[16].

In the present study p53 positivity was seen in 25 out of 40 cases (62.5%) of gastric adenocarcinoma. 59.3% of tumours in the pyloric antrum (14/24 cases) and 100% of gastroesophageal junction tumours (3/3 cases) showed p53

positivity. Studies by Fenoglio-Preiser *et al*[17] and Brito *et al*[18] showed p53 immunoreactivity in 17-19% and 35% of gastric carcinomas respectively. Ghaffarzadegan *et al*[11] noted p53 positivity in 75% of gastric carcinomas and found that p53 alterations occur much more commonly in proximal lesions than in distal ones, suggesting that the molecular events leading to the development of gastric carcinoma may be very different in proximal versus distal tumors. Overall, however, it appears that p53 alterations occur early in the development of gastric carcinoma, being present even in the non neoplastic mucosa and they increase in frequency as one progress along the pathway of gastric carcinoma development.

In our study, p53 positivity was seen in 4 out of 7 cases (57.1%) of signet ring cell adenocarcinomas and 5 out of 9 (55.6%) of tubular adenocarcinomas. P53 positivity was seen in 18 out of 30 (60%) cases with lymph node metastasis and 7 out of 10 cases (70%) without lymph node metastases, but this was not statistically significant (p=0.646). A study by Brito *et al*[18] showed that the frequency of p53 positivity in tumours of tubular histological type (46%) was significantly higher than that in signet ring tumours (10%) (p = 0.006), and neoplasms that invaded deeply into the submucosa were more frequently positive (45%) than others (30%). In their study, five of eight (62%) T1 tumours with lymph node metastases were p53 immunoreactive. Their findings showed that immunocytochemically demonstrable overexpression of p53 correlates with other morphological markers of aggressiveness in T1 gastric adenocarcinoma.

Azarhoush *et al*[3] showed that p53 alterations correlate well with gastric location, and are frequent in adenocarcinomas of cardia than that of antrum. There was no difference in clinicopathologic characteristics between p53 positive and p53 negative gastric carcinomas. In our present study, p53 alterations did not correlate with clinicopathological parameters. Filiz *et al*[19] found no relation between p53 staining and parameters such as nuclear grade, invasion depth, lymph node involvement.

The present study showed p53 positivity to be equal in intestinal and diffuse type of gastric adenocarcinoma. Filiz *et al*[19] found p53 positivity in 17 out of 20 (85%) intestinal type and 13 out of 22 (59%) diffuse carcinoma. Ghaffarzadegan *et al* found p53 positivity in 59 out of 100 cases and they noted a significant correlation between rate of p53 overexpression and histologic type of tumor.[11] There was no significant association between protein accumulation and lymph node status in our study which was supported by study by Lazar *et al*[20].

5. Conclusion

Mutation in p53 plays a vital role in development of gastric carcinoma. However, a significant correlation between gastric carcinoma and clinicopathological parameters could not be established. A prolonged follow up study on a large number of cases can help to find out whether p53 overexpression by standard IHC could be a useful marker for identification of gastric carcinoma.

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