

Original Research Article


Study of expression of P53 in Gastric Carcinoma – As a prognostic indicator

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Abstract

Cancer of stomach remains the major cause of mortality worldwide despite a marked decline in the incidence of gastric carcinoma in the industrialized nations. Gastric cancer is the end result of multifactorial, multigenetic, multistep process. The knowledge of the factors that influence gastric carcinogenesis is determinant for the development of strategies effective for prevention and treatment. Our study emphasizes upon the role of p53 as a prognostic indicator of gastric carcinoma. 50 cases of gastric biopsies were taken at random over a period of 4 years, and p53 expression of these lesions was studied. Gastric carcinoma cases were followed and the TNM staging was assessed. In our study, p53 expression was associated with younger age group, increased depth of invasion and lymph node metastasis, thereby indicating that p53 is a prognostic indicator for gastric carcinoma.

Key words

Gastric carcinoma, p53, Prognosis.

Introduction

Cancer of stomach remains the major cause of mortality worldwide despite a marked decline in the incidence of gastric carcinoma in the industrialized nations. Highest incidence is seen in china, japan and Italy. In India, overall cancer incidence is 100-121/ 100000 male population and 59-70/100000 in females. As such, they account for 3.1% to 12.9% of all cancer deaths in

different parts of India, highest of this being in southern India (ICMR bulletin 2015). Gastric cancer is the end result of multifactorial, multi genetic and multistep process. Environmental exposures and genetic factors have been reported to cause gastric cancer, but the debate about whether environment or heritability plays the principle role- continues. It is widely acknowledged, that most of the carcinomas of stomach are accompanied and often preceded by

a phase of dysplasia either low grade or high grade, of which, the later is considered as carcinoma in situ. Over a hundred cancer related genes have been discovered which control cell proliferation and issue homeostasis. Tumor suppressor genes are vulnerable sites for critical DNA damage, because they normally function as physiological barriers against clonal expansion or genomic mutability and are able to hinder the growth and metastasis of cells driven to uncontrolled proliferation by oncogenes. The p53 tumor suppressor gene is the most striking example as it is mutated in about half of all the types of cancers arising from a wide spectrum of tissues. There has recently been an increase in the number of studies on p53 mutations and abnormal expression of p53 in gastric carcinomas, with variable expression rates of 22-61%. Although TNM staging remains the most important prognostic factor for gastric cancer, there is a need for new prognostic and predictive factors, as prognosis varies among the patients of the same stage. P53 is a DNA binding protein localized to the nucleus, which when called into action, functions primarily by controlling the transcription of several other genes. The major functional activities of p53 protein are cell cycle arrest and initiation of apoptosis in response to DNA damage, by transcription of several genes that mediate cell cycle arrest and apoptosis. It is a tetrameric structure with a large beta- sandwich that acts as a scaffold for 3 loop-based elements. The prognostic value of p53 nuclear expression in gastric carcinoma is still unclear as shown by discordant results reported in literature. The objective of the study is to determine the value of p53 accumulation as a marker of tumor progression and clinic pathological variables in gastric carcinoma.

Aim and objectives

- To determine the percentage of expression of p53 nuclear protein in gastric carcinoma.
- Distribution of p53 positivity according to histopathological parameters.

- To determine the relationship between p53 over expression and clinico pathological variables in gastric carcinoma.
- To analyze the prognostic significance of p53 in gastric carcinoma.
- To compare the present study with other studies in literature.

Materials and methods

The study was conducted at Gandhi Hospital, a tertiary care Centre; Hyderabad, in the department of pathology and gastroenterology. A total of 50 cases of gastric biopsies were picked out at random from the year 2012-2016 for a period of 4 years. The tissues were fixed in 10% formalin, processed and embedded in paraffin. The individual case was typed histologically according to WHO classification. Out of 50 cases, 32 cases were of intestinal type and 11 were of diffuse. 3 cases were dysplasia, 3 of atrophic gastritis, and 1 was small cell carcinoma. These cases were followed up for gastrectomy in carcinoma patients. The lymph node status and depth of tumor invasion were assessed according to TNM staging. The biopsy blocks of respective cases were taken up for immunohistochemical analysis of p53 tumor suppressor protein. Sections from colonic carcinoma were taken as positive control and those from normal gastric biopsies were taken as negative control. The correlation between p53 positivity and histological variants, lymph node status, depth of invasion were determined. Immune localization of p53 protein was performed using Streptavidin- Biotin- Immuno peroxidase method protocol described by DAKO. Grade of staining was assessed by 2 competent pathologists, each unaware of patient's data. The intensity of staining was graded as per **Table – 1**.

Statistical analysis was done and correlation between p53 positivity and clinic pathological variables was calculated. A P value of <0.05 was considered as statistically significant. The following variables were included in the study-

Tumor Grade (G1, G2, G3), Depth of invasion (T1, T2, T3), Lymph node status (N0, N1, N2), and Histological type of tumor according to WHO classification (**Photo – 1 to 6**).

Table – 1: Intensity of staining.

	Intensity of stain	Interpretation
1	<5% of tumor cells showing nuclear stain	Negative
2	5-20% of tumor cells showing nuclear stain	Weakly positive
3	20-50% of tumor cells showing nuclear stain	Moderately positive
4	>50% of tumor cells showing nuclear stain	Strongly positive

Results

45 cases of gastric cancer, out of which intestinal types were 32, and diffuse types were 12 were studied. 3 cases of dysplasia, 2 of atrophic gastritis, 1 of small cell carcinoma were studied. Out 50 cases, 42 were male and 8 were female. Those aged less than 50 years were 15 in number. 35 patients were more than 50 years of age and 2 patients were aged less than 40 years and showed strong positivity for p53. Over expression of p53 was found to be in 18 out of 32 cases (60%) of intestinal type and 8 out of 12 cases of diffuse type (69.3%). 1 case of small cell carcinoma showed strong positivity(100%). There was significant correlation between the expression of p53 and tumor grade G (P value 0.0497) as per **Table - 2**.

Statistical analysis also revealed a significant correlation between the depth of the tumor invasion and P53 staining (P value 0.0001). In addition significant correlation was found between expression of p53 and lymph node status (P value 0.0038).

There was no statistically significant correlation between P53 expression and histologic tumor type. Cases of dysplasia and atrophic gastritis did not express P53 at all, in contrary to some studies which have stated that P53 was expressed in precancerous conditions.

Discussion

The P53 gene encoded on chromosome 17p is believed to be an important negative regulator of cellular proliferation and malignant progression

[1, 4]. Although molecular mechanism of its action is not clearly understood however, it seems that the mutational inactivation of this gene facilitates carcinogenesis. Most of the mutations alter the conformation of nuclear protein product, which can inactivate any wild type P53 protein present. The half-life of wild type P53 gene product is short, whereas the half-life of some mutant forms is prolonged. Therefore most of the protein detected by IHC is a mutated form is a mutated gene product. Elevated expression of P53 protein or mutational inactivation of P53 gene has been shown in various human malignant tumors including carcinomas of colon, rectum, breast, prostate, esophagus and stomach. Purpose of the study was to clarify the relationship between clinicopathological variables and P53 expression in gastric carcinoma. The expression of P53 was studied in 44 gastric carcinoma using paraffin embedded surgical biopsies results showed that 65% of gastric cancers expressed elevated levels of P53 protein. Starzynska, et al. [2] have found significant correlation between increase in tumor size and P53 expression. Seo, et al. [3] studied the prognostic significance of P21 and P53 expression in gastric cancer by Immunohistochemistry. In this study they correlated expression of P53 and P21 with clinicopathological variables. They conclude that the P21 and P53 expression might help in predicting the aggressive behavior of gastric cancer. Johanna Mrena, et al. [4] studied expression of cyclooxygenase 2 and nuclear p53 accumulation in gastric carcinoma. They noticed that COX2 and P53 expression are independent poor

prognostic factors causing shorter survival in patients. In our study, p53 expression was associated with increased depth of invasion, lymph node metastasis, and the tumor grade; which was in concurrence with this study. Aoyagi, et al. [5], investigated the expression of p53, p21 and TGFβ in gastric carcinoma. They

found that cases with p53 +ve and p21-ve expression were in advanced stage with increased depth of invasion, lymph node and distant metastasis. This finding was in concurrence with our study. They concluded that combination of p53 and p21 expression is a useful prognostic marker of gastric carcinoma.

Table – 2: Clinicopathological variables.

Clinicopathological variables	Total number	Negative	Positive	Moderately positive	Strongly positive	P value
Histological type	50					n/a
Intestinal	32	12	02	12	06	
Diffuse	12	04	01	02	05	
Small cell type	01	-	-	-	01	
Dysplasia	03	03	-	-	-	
Atrophic Gastritis	02	02	-	-	-	
Depth Of Invasion						0.0001
T1	10	07	02	01	-	
T2	15	06	02	02	05	
T3	20	02	01	02	15	
Lymph node Status						0.0038
N0	09	06	02	01	-	
N1	16	02	04	03	07	
N2	19	02	01	06	10	
Tumor Grade						0.0497
G1	05	02	02	01	-	
G2	23	04	-	09	10	
G3	17	01	02	04	10	

Xiang ming, et al. [6] stated that, over expression of p53 is a predictor of lymph node metastasis, which was in concurrence with our study. Machara, et al. [7] studied the prognostic value of p53 protein expression for patients with gastric cancer. They concluded that the expression of p53 was closely related to the potential of tumor advancement and also a poorer post-operative prognosis for the patients with gastric cancer, which was in concurrence with our study. Kim, et al. [8] and Roviello, et al. [9] have reported that p53 accumulation and positivity correlated with the depth of invasion and lymph node status, as was found in our study. Hurlimann, et al. [10] have found no significant association between p53

accumulation and tumor size, grade, lymph node metastasis, age or sex of patient; in contrast to findings in our study. Monig, et al. [11] stated that the nuclear p53 immuno reaction was closely associated with tumor location, lymph node metastasis and curability. Tumors with positive lymph node reaction frequently metastasize to lymph nodes with metastatic rate of 91.4%, in contrast with tumors with negative p53 stain reactivity having metastatic rate of 71.4% (P value-0.021). They conclude that p53 expression in combination with common prognostic parameters may help to detect the prognostically unfavorable sub groups of gastric carcinoma patients. Kyun Eun Lee, Hyuk- Joon Lee, et al. [12] in their study noted that p53 expression was

positive in 43.2% of all gastric cancer tissues. Older age, male gender, larger tumor size and well differentiated histology were positively correlated with p53 expression. In our study, p53 expression was more positive in younger age group. According to Lauren classification, intestinal type of gastric cancer has more positive p53 expression than diffuse type. We observed more frequent p53 expression in diffuse type. J. Pinto D Souza, F Silva, et al. [13], in their study of 163 cases of gastrectomy, noticed that p53 expression was identified in 41 cases, and was significantly associated with venous invasion, lymph node metastasis and C-erb B2 expression. The p53 expression correlated with overall survival and survival of sub group of patients with intestinal type of carcinoma. They concluded that p53 expression was associated with aggressive biological behavior and was related to the cumulative survival of patients. In our study, p53 expression was more common in diffuse type accounting to 69.3%, in contrast to the studies conducted by Tolbert, et al. [14] and Lin X P, et al. [15], where p53 was expressed more in intestinal type (42% and 50%) respectively, when compared to diffuse type (21% and 34.6%) respectively. Nalan A Babacan, Hatice Reyhan, et al. [16] stated that UHRF-1 p53 were not prognostic factors for gastric carcinoma, but they have diagnostic value in differentiating between gastritis and gastric carcinoma. They noticed no correlation between p53 expression and lymph node involvement. But in our study, p53 expression was found to correlate with lymph node metastasis. Fondevila, et al. [17] demonstrated that p53 is an independent prognostic factor for disease free survival and overall survival in patients with curatively resected gastric cancer and that p53 may also influence response to chemotherapy. In our study, we found that combined with other prognostic factors, p53 is a prognostic indicator in gastric carcinoma. Deveci, et al. [18] demonstrated that p53 positivity was a poor prognostic factor for more than 5 metastatic lymph nodes involved in gastric carcinoma cases. On the other hand, some studies support the prognostic value of p53 in gastric cancer.

Fukunaga, et al. [19] and Gabbert, et al. [20] have found no relationship of p53 expression with liver metastasis and lymph node metastasis, in contrast to findings in our study.

Photo – 1: Total gastrectomy with ulcero proliferative growth.



Photo – 2: Moderately differentiated intestinal adeno carcinoma.

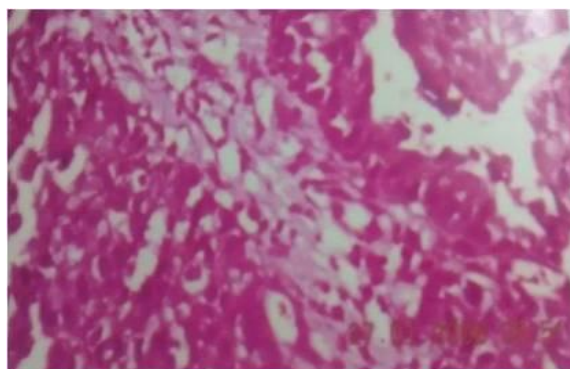
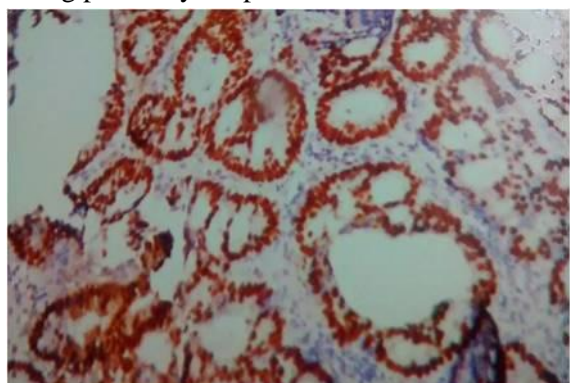


Photo – 3: Intestinal type adeno carcinoma with strong positivity for p53.



Conclusion

In our study, we demonstrated a significant correlation between p53 expression and lymph node metastasis, depth of invasion, and tumor

grade and younger age group in accordance with previous reports. No significant correlation was found between the level of p53 expression and histological type of carcinoma. There was no association between sex, race, and p53 expression. In conclusion, we have deduced that p53 expression occurs in considerable number of cases of gastric carcinoma. A pre-operative assessment of p53 expression could be helpful in identifying patients with high risk of higher grade and more advanced tumors. Immuno histochemical analysis of p53 expression appears to be an accurate and simple method of screening gastric carcinoma. As research indicates, gene therapy for p53 which primarily targets wild type of p53, and modulation of MDM2 protein for induction of apoptosis, is a promising field in near future in the prevention of gastric carcinoma.

Photo – 4: Intestinal type adeno carcinoma p 53 positivity 40X.

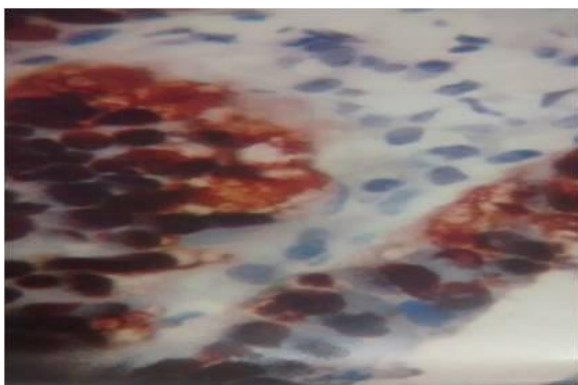


Photo – 5: Diffuse type adeno carcinoma H & E stain 40X.

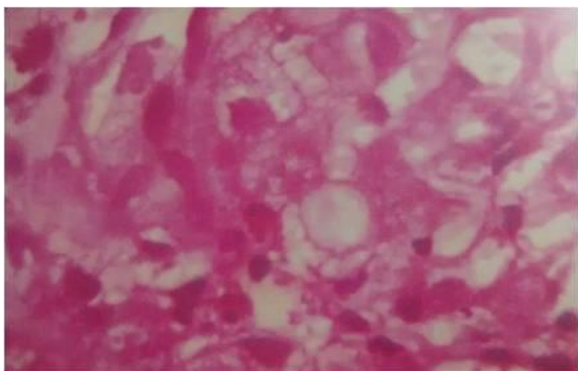
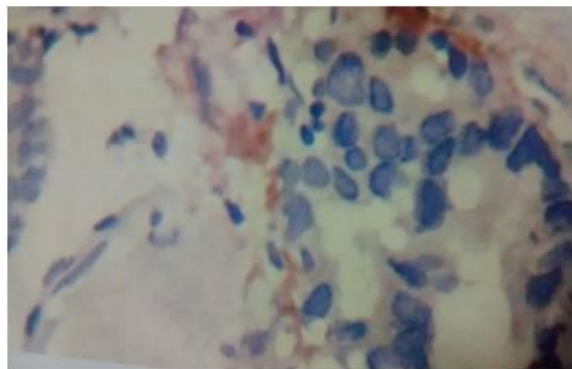


Photo – 6: Intestinal type adeno carcinoma with p53 negativity.



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