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Research Paper

Clinicopathological Correlation of P53 Protein Expression in different Histological Subtypes of Gastric Carcinoma

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Abstract

Introduction: *Gastric cancer is one of the common cancers after lung cancer worldwide, especially in the developing countries. p53 immunohistochemical expression has been proposed as a potential tool to evaluate the biological behavior of gastric cancer. However, its practical implication in gastric cancer prognosis or treatment by restoration of mutated p53 function is yet to be fully exploited. Hence, this study with the aim of assessing the yield of p53 expression in gastric carcinoma and its relationship with various clinicopathological parameters was formulated.*

Objectives: *To assess immunohistochemical expression of p53 protein in different histological subtypes of gastric carcinoma cases and to study correlation between p53 protein expression and different clinicopathological parameters like age, gender, tumour location, gross pattern, histological types, degree of tumor differentiation and pathological staging in the above said cases.*

Materials and Methods: *This was a hospital based cross-sectional study done over a period of 18 months on 60 cases of gastrectomies with proven histological diagnosis of gastric carcinoma.*

Observation and Results: *Strong p53 expression was frequent among elderly male patients (>60 years) who had ulceroproliferative tumors, located at pyloric antrum belonging to pTNM stage IIIA. In 87% cases, it was expressed, predominantly in moderately differentiated intestinal type with serosal invasion. Also, cases even with no lymph node metastasis showed p53 expression.*

Conclusion: *Few findings were contradictory to other similar studies e.g. lymph node negative cases showing P53 expression does not support the hypothesis of predicting lymph node status by p53 expression. No significant correlation between p53 expression and different clinicopathological parameters in gastric carcinoma cases was seen.*

Keywords: *P53, immunohistochemical, clinicopathological, histological subtypes, metastasis.*

Introduction

Gastric carcinoma is a malignant epithelial tumour of the stomach mucosa. It remains the second most common cause of cancer related deaths worldwide. Costa Rica and Japan have the first and second highest rate in the world, with a death rate of 77.7 and 50.5 per 100,000 respectively[1] . In India, incidence of gastric carcinoma is higher in southern and north-eastern states [2,3].

p53 gene is a tumor suppressor gene located on the short arm of chromosome 17, producing a 53 kDa nuclear protein. p53 gene by the production of p53 protein controls the cell cycle and prevents genetic mutations for carcinogenesis. p53 gene mutations are genetic events in the pathogenesis of gastric adenocarcinomas[4] .

Overexpression of p53 protein is directly related to enhanced proliferative activity and an increased propensity of lymph node metastasis and advanced TNM stage, representing greater tumor aggressiveness[5]. Immunohistochemical studies show that antibodies raised against mutant p53 protein may be used as screening method for the presence of mutations [7].

Prognosis of gastric cancer is poor as most of patients generally consult health care services in advanced stage of disease. Furthermore, surgery and chemotherapy have limited value in advanced disease. Prognosis and survival depend on early diagnosis and treatment. So, there is need for specific histological and biological markers to identify the subgroups of patients with more aggressive course of illness in the same stage of disease.

Many such potential markers were studied viz. Ki67, HIF, E-cadherin, MMP-1, TGF-B, STAT3, TIMP1, HER2 in gastric cancer but amongst all, p53 was studied considerably[6,7] .

Majority of studies suggest prognostic significance of p53 expression in gastric cancer, however some studies fail to show its role in gastric cancer[8,9] . This conflict in opinion led us to formulate this study.

Objectives

To assess the immunohistochemical expression of p53 protein in different histological subtypes of gastric carcinoma cases and to study correlation between p53 protein expression and different clinicopathological parameters like age, gender, tumour location, gross pattern, histological types, degree of tumour differentiation and pathological staging in the above said cases.

Procedure

This was a hospital based cross-sectional study conducted at Department of Pathology, Govt. Medical College, Thrissur from 01.11.2015 to 30.04.2017 on a sample size of 60. Only those gastrectomy cases with histopathologic confirmation of gastric cancer having proper documentation including the availability of representative paraffin-embedded tissue blocks were included for the study.

All the gastrectomy specimens received were fixed in neutral buffered formalin for 24 to 48 hours and grossly examined. Clinicopathological details such as age, gender, location, gross appearance, histological subtype, grade or degree of differentiation and pathological stage of tumor were obtained from histopathological reports.

Immunohistochemistry

The unstained sections were kept in incubator overnight at 37^{o} Celsius before immunohistochemical staining. Sections were deparaffinized and dehydrated. Antigen retrieval was performed by pressure cooking. They were stained with p53 antibody. Then, the slides were mounted using DPX with cover slip and studied under light microscope (Labomed, 400X) and were cross-examined by senior faculty member of the department.

The findings were recorded accordingly. The Hematoxylin and Eosin (H&E) stained slides were studied for the tumor histology, degree of differentiation, lymph node metastasis and invasion. The immunostained slides were examined for nuclear staining with anti-p53

antibody. Positive p53 results give intracellular (nuclear) dark brownish color, granular or homogenous precipitate (clear cut) with blue cytoplasm. 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.

p53 expression

A positive reaction was considered only in the presence of immunostained nuclei in brown shades. Quantification of the reaction was performed as following:

p53-positive (+): if over 10% of the nuclei of tumor cells, regardless of the intensity of the reaction, show expression.

p53-negative (-): if there is absence of immunostaining overexpression in less than 10% of the tumor nuclei[(110)] .

Pattern of staining

The pattern was considered:

Diffuse pattern if the positive cells were distributed through almost all fields.

Regional pattern if more than one area of the section showed large number of positive cells.

Focal pattern if there were only very few positive cells in the section.

Intensity of staining

The intensity of staining of the brownish coloration were considered as -

(1) Highly strong: if the stain is very dark and seen at low magnification (10X) (score 4+).

(2) Strong: if it could be detected very clearly at low magnification (10X) (score 3+).

(3) Moderate: if it could be detected with difficulty at low magnification (score 2+).

(4) Weak: if it could only be detected at high magnification (×40) (score 1+).

The histologic type was classified according to World Health Organization (WHO) classification and Lauren classification.

The Pathological stage was determined according to the protocol recommended by American Joint Committee on Cancer (AJCC) that includes tumor invasion, lymph node metastasis (pTNM) classification- 8th edition.

The Tumor grading/degree of differentiation was done according to College of American Pathologists (CAP) recommended grading system. The gross appearance was based on Borrmann classification of advanced gastric carcinoma.

Statistical analysis

Data obtained were entered in Microsoft office excel sheet (Microsoft 2007). This was analysed using software Statistical Package for Social Science (SPSS) version16.0. The statistical test used was the Chi -square test. p-value of <0.05 was considered statistically significant in the tests for correlation. Correlation between p53 protein expression and clinicopathological parameters mentioned was studied. The findings are presented in appropriate charts and tables.

Observation and Results

I. Frequencies

1. Histological types

Out of 60 cases of gastric carcinoma, majority were of intestinal type (73% i.e. 44 cases), followed by diffuse type (23% i.e. 14 cases), and one each of mixed type and mucinous type.

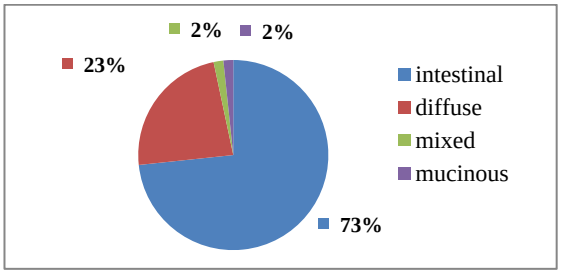

Fig.1 Frequency of histological types

2. Degree of differentiation in intestinal type

Among 44 cases of intestinal type, tumors with moderate differentiation were the commonest comprising 39%.

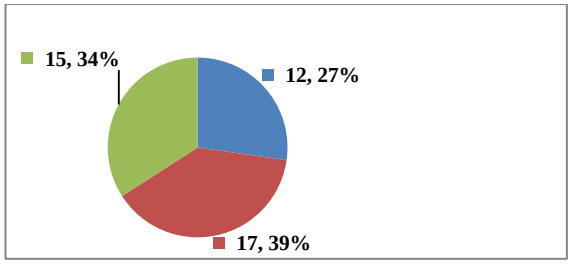

Fig.2 Frequency of degree of differentiation

3. Positive and Negatives cases for p53 immunostaining

Among 60 cases of gastric carcinoma, 87% of cases showed p53 expression. Only 13 % showed negative reaction.

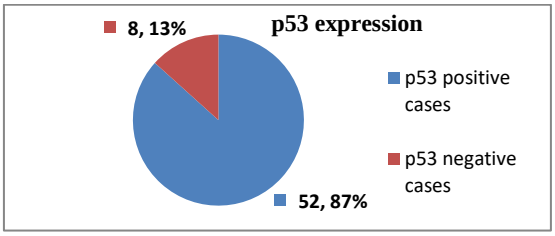

Fig.3 Frequency of Positive and Negatives cases for p53 immunostaining

4. Positive and Negatives cases in Histological subtypes (Lauren-classification)

Among the histological subtypes, maximum number of cases showing p53 expression was of intestinal type. 41 out of 44 cases (93%) of intestinal type gastric carcinoma showed p53 expression whereas only 9 out of 14 cases (64%) of diffuse type showed p53 expression.

Table 1. Frequency of positive and negatives cases in histological subtypes

Histological subtypes	p53 expression		Total
	Positive	Negative	
Intestinal	41 (70.7%)	3 (5.2%)	44(75.9%)
Diffuse	9 (15.5%)	5 (8.6%)	14(24.1%)
Total	50 (86.2%)	8 (13.8%)	58(100.0%)

5. Pathological (pTNM) stages

Out of 60 cases, majority of the cases were in pTNM stage IIIA followed by stage IIIB. One case each of IA, IIA and IIIC was seen.

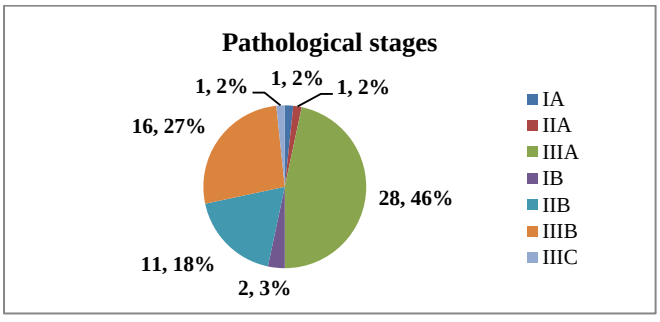

Fig.4 Frequency of Pathological (pTNM) stages

II. Correlation between p53 immunostaining and different clinicopathological parameters

1.Age and Intensity of p53 staining

Mean age = 61.63± 9.6 SD. Study population included age ranging from 40 to 80 yrs. Patients older than 60 years showed increased strong p53 expression. There was no statistically significant correlation between patient's age and intensity of p53 staining.(P-value = 0.682)

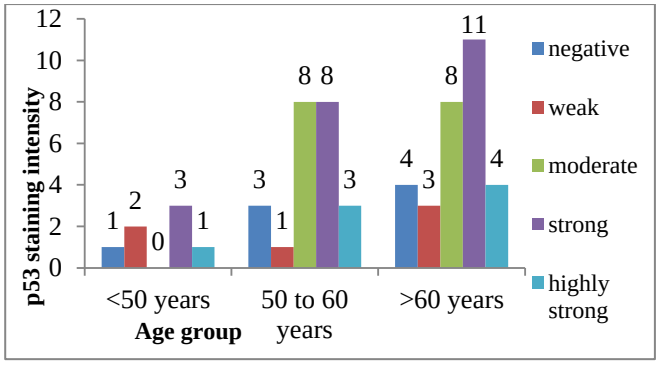

Fig.5 Correlation between age and p53 staining intensity

2. Sex and Intensity of p53 staining

There was no statistically significant correlation between sex and p53 expression. (P value = 0.775)

Table 2 sex and p53 staining intensity

Sex	Negative	Weak	Moderate	Strong	Highly strong	Total
M	6	6	13	18	7	50
F	2	0	3	4	1	10
Total	8	6	16	22	8	60

3.Tumor location and Intensity of p53 staining

Majority of the cases located at pyloric antrum showed strong p53 expression. There was no statistically significant correlation between tumor location and p53 expression.
(P value = 0.622).

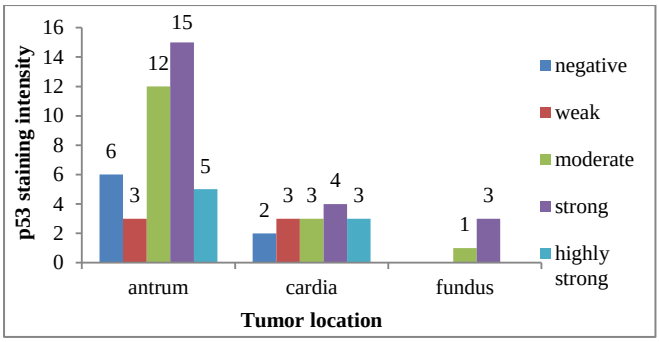

Fig.6 Correlation between tumor location and p53 staining intensity

4.Gross appearance and Intensity of p53 staining

Out of 60 cases, majority of the cases had ulceroproliferative gross appearance. Others included fungating, polypoidal etc. No statistically significant correlation between these two entities. (P value = 0.556)

Table 3. Gross appearance and p53 staining intensity

Gross appearance	P53 Staining Intensity					
	Negative	Weak	Moderate	Strong	Highly strong	Total
Ulceroproliferative	8	6	13	18	7	52
Others	0	0	3	4	1	10
Total	8	6	16	22	8	60

5.Histological subtypes and Intensity of p53 staining

Majority of the intestinal type showed strong p53 expression. However, there was no statistically significant correlation. (P value = 0.06)

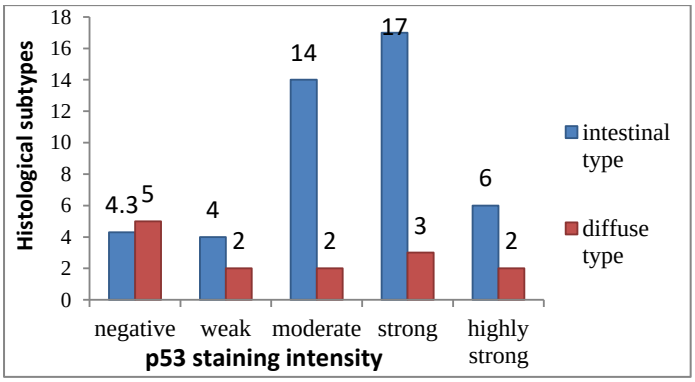

Fig.7 Correlation between histological subtypes and p53 staining intensity

6.Invasion and Intensity of p53 staining

Majority of the cases with serosal invasion showed strong p53 expression. However, there was no statistically significant correlation. (P value = 0.439)

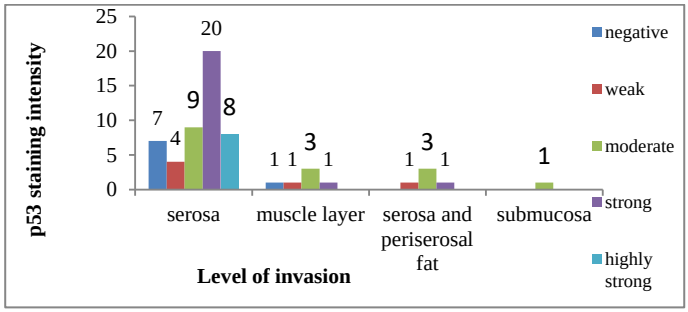

Fig.8 Correlation between invasion and p53 staining intensity p53 staining intensity

7.Pathological staging and Intensity of p53 staining

The cases belonging to pTNM stage IIIA showed maximum p53 positivity. However, p value was 0.825, which is not statistically significant.

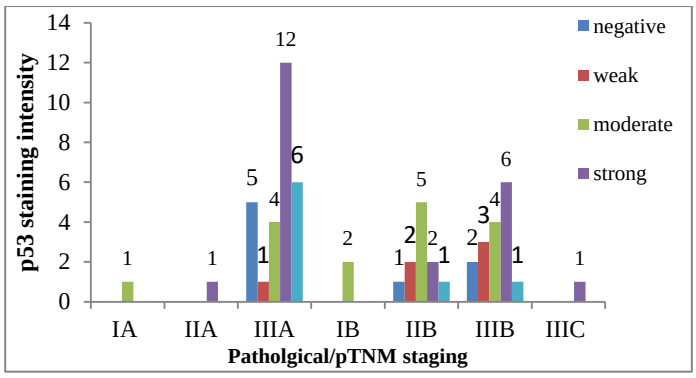

Fig.9 Correlation between pathological staging and p53 staining intensity

8.Pathological staging and Percentage of tumor cells showing positive p53 staining

The cases belonging to pTNM stage IIIA showed tumor cells with maximum p53 positivity. However, p value was 0.825, statistically insignificant.

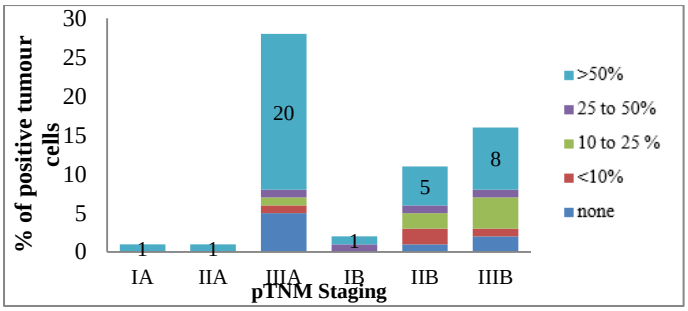

Fig.10 Correlation between Pathological staging and percentage of positive tumor cells

9.Degree of tumor differentiation and Intensity of p53 staining

Majority of Intestinal type well differentiated cases showed strong p53 expression. No statistically significant correlation between Degree of differentiation and p53 expression was found. (P value = 0.98)

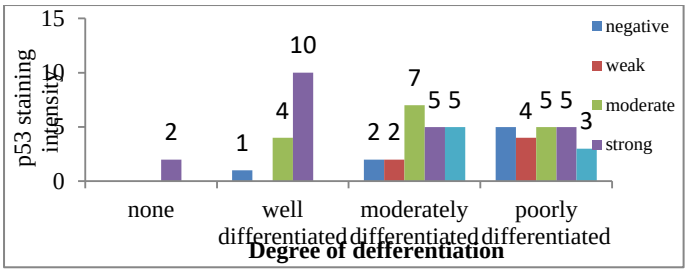

Fig.11 Correlation between degree of tumor differentiation and p53 staining intensity

10.Degree of tumor differentiation and Percentage of tumor cells showing positive p53 staining

Tumors with moderate differentiation showed maximum p53 expression. There was no statistically significant correlation between the mentioned entities. (P value = 0.396)

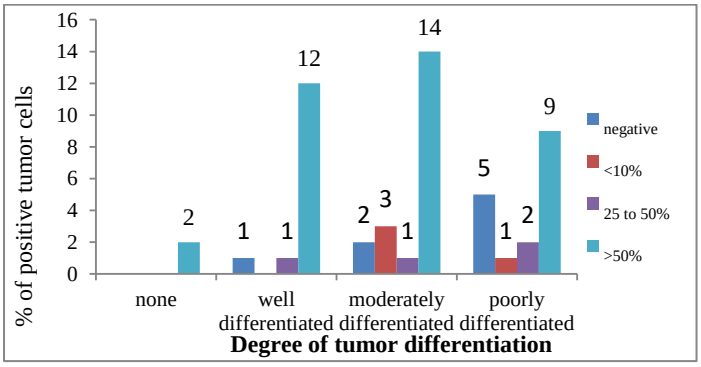

Fig.12 Correlation between degree of tumor differentiation and percentage of positive tumor cells

11.Lymph node involvement and Intensity of p53 staining

Cases with no lymph nodes metastasis also showed p53 expression with majority showing moderate intensity. There was no statistically significant correlation between lymph node involvement and p53 staining intensity. (P value = 0)

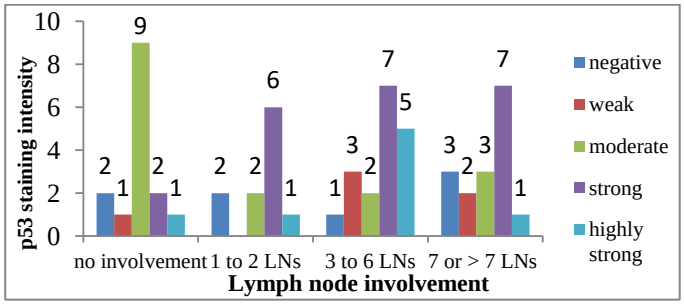

Fig.13 Correlation between lymph node involvement and p53 staining intensity

Photomicrographs

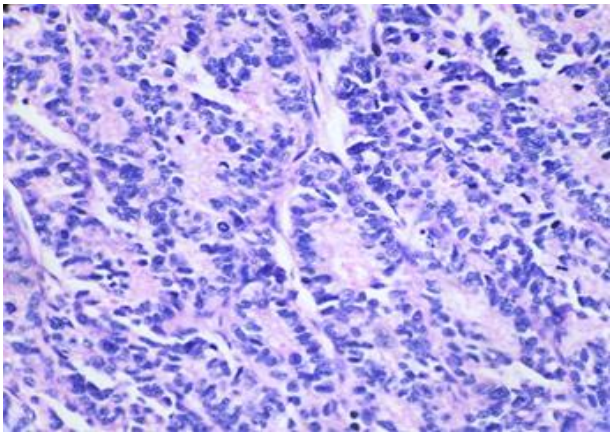

Fig 14: Intestinal type-well differentiated (H&E, 40X)

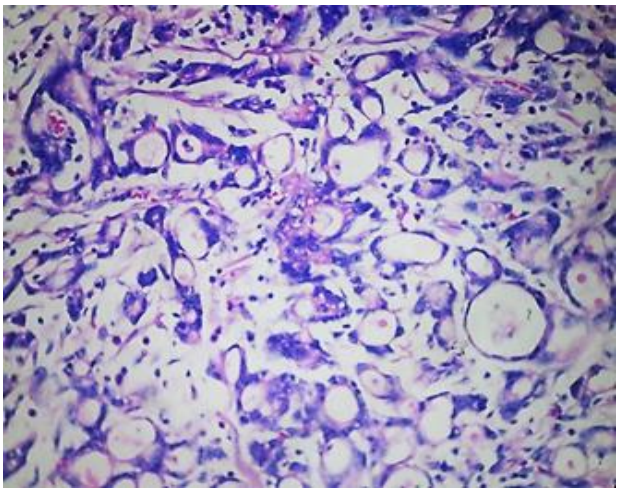

Fig 15: Intestinal type-Moderately differentiated (H&E, 40X)

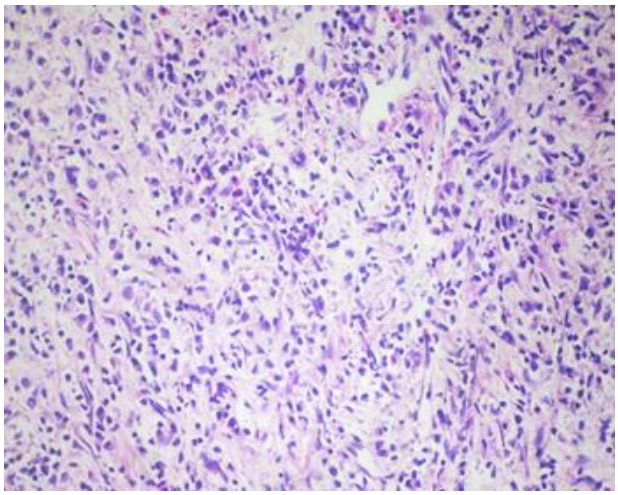

Fig 16: Intestinal type – Poorly differentiated (H&E, 40X)

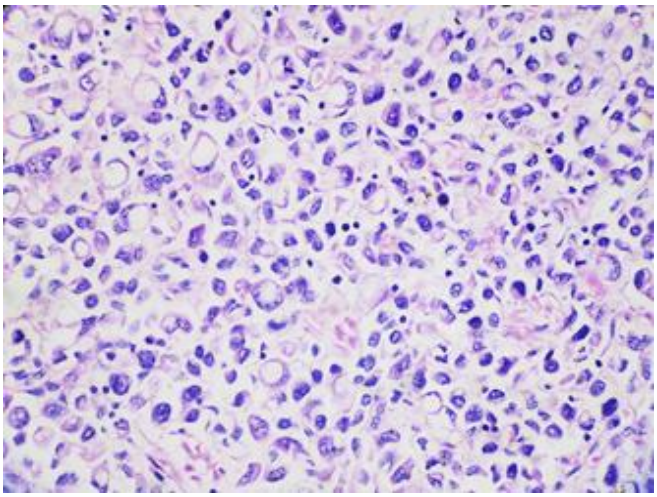

Fig 17: Diffuse type (H&E, 40X)

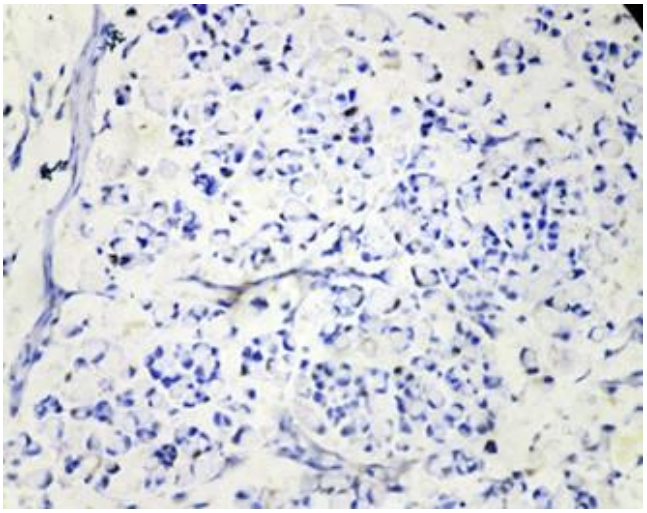

Fig 18: Negative staining- Diffuse type (IHC, 40X)

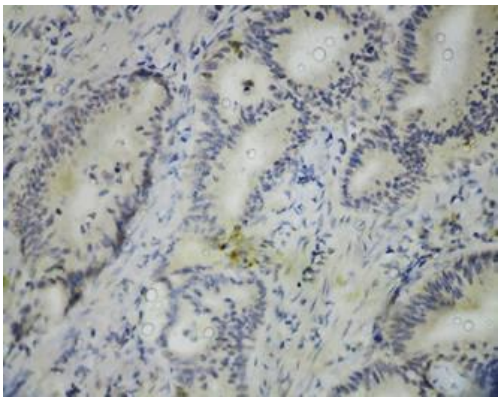

Fig 19: Negative staining -Intestinal type (IHC, 40X)

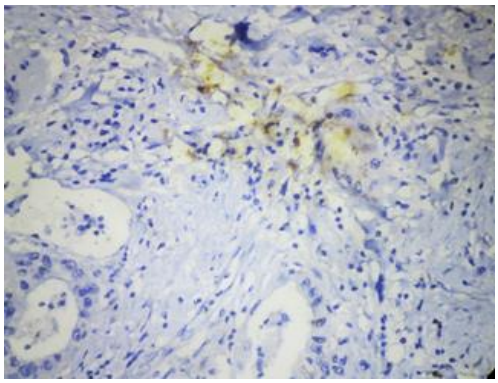

Fig 20: Weak staining (IHC, 40X)

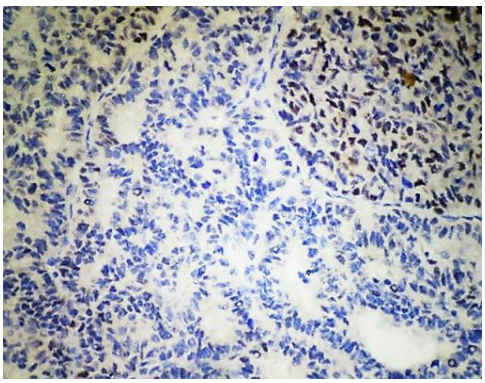

Fig 21: Moderately staining (IHC, 40X)

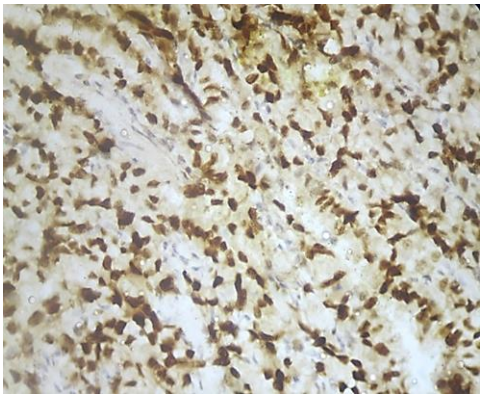

Fig 22: Strong staining (IHC, 40X)

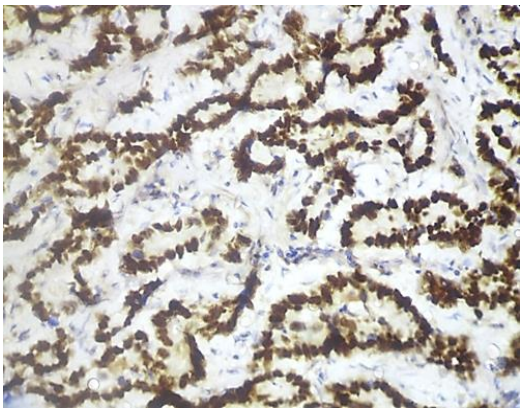

Fig 23: Highly strong staining (IHC, 40X)

Discussion

Worldwide, gastric carcinoma is one of the commonest cancers after lung cancer and a major cause of mortality and morbidity, especially in developing countries[10] . It is one of the leading causes of cancer deaths making it the 5th and 6th cause among males and females respectively in India. The etiology of gastric carcinoma includes both genetic and environmental factors such as H. Pylori. The prognosis for gastric cancer depends on its stage; so, detection in the early stage of disease is important, when complete and curative removal is possible.

Mutations of the p53 gene are the most common genetic alteration known to occur in a wide range of human cancers. Frequency of p53 overexpression in gastric adenocarcinoma is 26 to 65%. In gastric carcinoma, p53 is present solely in tumor cells while it is not so in adjacent normal stomach mucosa. It is also present in dysplastic epithelium surrounding tumor in varying degrees. Joypaul et al reported that there was 20% staining with p53 in severe dysplasia [20] .

p53 gene is considered “guardian of the genome” and represents a tumor suppressor gene located on the 17p chromosome, coding a protein of 53 kD. p53 protein is essential for control of tumor growth, apoptosis and maintaining genome stability. Losing of the p53 function caused by genome alterations or interactions with products from the environment and bacterial products is considered to represent a critical stage in gastric carcinogenesis [11] .

Most of the mutations alter the conformation of the nuclear protein product, which can inactivate any wild-type p53 protein present. The half-life of the wild-type p53 gene product is short, whereas the half-life of some mutant forms is prolonged. Therefore, most of the protein detected by immunohistochemical staining is a mutated form of the p53 gene product. Elevated expression of the p53 protein, or mutational inactivation of the p53 gene, has been shown in various human malignant tumors, including carcinomas of the colon and rectum, breast, esophagus, and stomach [10] .

The purpose of this study was to assess the immunohistochemical expression of p53 protein in gastric carcinoma and to study the correlation between p53 protein expression and different clinicopathological variables like: age, gender, site, gross pattern, histological type, grade, and stage of the tumor in gastric carcinoma cases.

p53 Expression and Clinicopathogical Correlation

Histological subtypes and p53 expression

The present study demonstrated that 93% of the intestinal type gastric adenocarcinomas showed p53 expression, the majority of which were strong p53 expression. As far as diffuse type was concerned only 64% showed p53 expression. While most of the studies in the literature showed higher expression in intestinal gastric carcinoma, the rate of positivity in this study is much higher when compared to other studies in the literature. However, there was no statistically significant correlation. Fukunaga et al. found that the p53 accumulation in intestinal type (56%) was higher than in diffuse type (27%) gastric adenocarcinoma[12], Brito et al. found that the frequency of p53 positivity in intestinal type was 46%, and only 10% in diffuse type gastric adenocarcinoma[13] , Craanen et al. demonstrated the closest p53 positivity rates compared to this study. He got 70% in intestinal type and 52% in diffuse type gastric adenocarcinomas[14].The only

study that showed a reversal pattern was by Ghaffarzadegan et al who found that the p53 over expression was in 67.7% of intestinal type and 81.2% in diffuse type [15] .

Degree of differentiation/ grading and Percentage of tumor cells showing positive p53 expression

As per the present study, tumors with moderate differentiation showed maximum p53 expression. However, there is no statistically significant correlation between degree of tumor differentiation and percentage of tumor cells showing positive p53 staining (P value = 0.98). Study by Al-Badri BA, Ali FG[16] showed that there was significant correlation between p53expression and tumor grade (p value = 0.015). Studies done by Martin et al, and Doet al. agreed with the results of this study [17]. Flejou et al.[25] ; Muller and Borchard et al[18] found no significant correlation between p53 and tumor grade.

Age and p53 expression

In the present study, mean age of the patients was found to be 61.63 ± 9.6 SD. Study population included age ranging from 40 to 80 years (P value = 0.682). It was also found that gastric carcinoma is more frequent in elderly age groups (>60 years) with majority showing strong p53 expression. The study conducted by Hermiz RS, Hussain AG showed that there was no statistically significant correlation between patient's age, sex and p53 expression[10] . Rugge M et al. reported that young patients (aged less than 40 years) have a lower incidence of p53 mutations than older individuals. Honda T et al. have also shown that p53 immunoreactivity rate is significantly lower in young patients [19].

Gender and p53 expression

Hermiz RS, Hussain AG found that sex distribution of gastric carcinoma cases, showed male predominance 28 (70%) compared with female 12 (30%) while this study showed a prevalence of 83% versus 17%. According to the present study, the intensity of p53 staining was moderate in 36% and 40% in males and females respectively. There was no statistically significant correlation between sex and p53 expression (P value = 0.775).

Location and p53 expression

In the present study, majority of the cases were located at pyloric antrum and showed strong p53 expression. However, there was no statistically significant correlation between tumor location and p53 expression (P value = 0.622). Azarhoush et al. showed that the frequency of p53 gene expression or p53 protein immunoreactivity was higher in adenocarcinomas of the cardia compared with adenocarcinomas of the antrum[20] .

Gross appearance and p53 expression

In the present study, among 60 cases, majority of the cases were ulceroproliferative grossly showing strong to moderate p53 expression (P value = 0.556). Others included fungating, polypoidal gross pattern. Hermiz RS, Hussain AG observed that p53 positivity rate was higher in ulcerative growth pattern type [10] .

Invasion and p53 expression

Majority of the cases in this study showed serosal invasion exhibiting strong p53 immunostaining. But no statistically significant correlation was found between the intensity of p53 expression and tumor histological type (P value = 0.439). Kim et al. [21] and Roviello et al[22] have reported that p53 accumulation positivity correlated with the depth of invasion. Filizet al found no relation between p53 staining and parameters such as invasion depth and lymph node involvement.

Lymph node metastasis with p53 expression

Hurlimann and Saraga et al [23] and Ghaffarzadegan et al [115] found that there was no significant correlation between p53 expression with lymph node metastasis. Present study also did not show any statistically significant correlation between lymph node involvement and p53 expression (P value = 0). It also shows that gastric carcinoma cases even with no lymph node metastasis showed p53 expression with majority showing moderate intensity.

pTNM staging with p53 expression

In the present study, majority of the cases belonging to stage IIIA showed strong p53 expression. However, no statistically significant correlation between pTNM staging and intensity of p53 staining was found. (P value = 0.60). Hermiz RS, Hussain AG reported that out of 37 of gastric carcinoma cases falling in stage III disease, 20 cases (54%) showed negative p53 expression. Lazar D et al. reported that there is no significant correlation between p53 immunoreactions and the pTNM stage. In stage IIIA only 18.2% of tumors were positive.

False Negatives and False Positives

Immunohistochemical demonstration of p53 protein may not always correspond to p53 mutation and absence of staining does not exclude mutation. 'False negatives' have been reported in a range of tumors when mutation results in a truncated protein or a weakly stabilised product which is not detected by immunohistochemistry. 'False positives' may occur if there is non-mutational stabilization of wild type p53, perhaps because of interruption of the normal degradative pathway of p53 or increased transcription in rapidly proliferating cells. This appears to occur with some frequency in breast carcinomas, but not in colorectal cancer. The situation in gastric adenocarcinoma is unknown and future studies should address this issue [(24)] .

Conclusion

60 gastrectomy specimens (partial/ total gastrectomies) with histological diagnosis of gastric carcinoma were studied and the correlation of p53 immunohistochemical expression with various clinicopathological parameters was analysed.

The mean age of the patients in this study was found to be 61.63 ± 9.6 SD, age ranging from 40 to 80 years. It was observed that gastric carcionoma is more frequent among elderly patients (>60 years) with majority showing strong p53 expression.

In the present study, the overall p53 immunohistochemical positivity was observed in 87% of cases including mixed and mucinous carcinoma irrespective of the intensity. Only 13% showed negative reaction. As per the WHO and Lauren classification, the predominant histological subtype was found to be intestinal type moderately differentiated. Strong p53 expression was observed in almost all the cases of intestinal type while negative expression was more in diffuse type. Majority of the cases belong to pTNM stage IIIA, followed by stage IIIB. Stage IIA and IIIC also showed strong positivity, thus, diminishing the prognostic significance of p53 expression.

Increased p53 expression was observed in ulceroproliferative tumors located at pyloric antrum with serosal invasion. Diffuse pattern of immunostaining was observed among majority of the cases. It was found that cases even with no lymph node metastasis showed p53 expression with majority showing moderate intensity, thus, not supporting the hypothesis of predicting lymph node status by p53 expression described in the literature.

p53 mutation plays a vital role in development of gastric carcinoma. IHC is simple, fast, cheaper, and convenient compared to detection methods. However, the present study showed no significant correlation between p53 protein expression and different clinicopathological parameters in gastric carcinoma cases. Most of the findings were in concordance with other studies in the literature while few were contradictory. A prolonged follow up study on a larger number of cases would not only provide statistically significant results but also add to its prognostic value.

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