

Immunohistochemical expression of p53 in gastric carcinoma (A Clinicopathological study)

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Abstract

Background: Carcinoma of the stomach is one of the most prevalent cancer types in the world today. P53 is the most notable tumor suppressor gene mutated in human cancers, including gastric cancer. The practical implication of this phenomenon in gastric cancer prognosis or even treatment by restoration of mutated p53 function are yet to be fully exploited.

Objective: 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.

Materials and methods: Forty formalin fixed paraffin embedded gastric carcinoma tissue blocks (partial or total gastrectomy specimens) from the archived materials of the Department of Pathology of Baghdad Teaching Hospital and the Center of Gastrointestinal and Hepatic Diseases, and other private laboratories were included in this study. A four micrometer – thick tissue sections were obtained and three slides had been prepared for each case, one was stained with Hematoxylin and eosin (H&E) and then reviewed, while two sections were stained immunohistochemically for p53. Statistical analysis was done using chi-square test for tables with frequencies, percentages, range, mean and standard deviation. Values were considered statistically significant when $P < 0.05$.

Results: A clinico-pathological assessment revealed that 28 patients were males and 12 patients were females. Male to female ratio was 2.3 /1. The age of patients ranged between 30-80 years with a mean $\pm$ standard error of (55.77 $\pm$ 1.88 year). The majority of the gastric carcinoma cases, in this study (70%) were above 50 years of age. Large proportions of gastric carcinoma cases (80%) were located in the antral region while the remaining cases were located in the cardia region. The

ulcerative gross pattern was the most predominant gross pattern type (72.5%). Whereas the commonest histological type was the intestinal type (75%). The majority of the gastric carcinoma cases (62%) were moderately differentiated. Most of the gastric carcinoma cases (92.5%) fall in stage III disease. The overall expression of p53 in gastric carcinoma cases in the present study was (44%). No statistically significant difference was found between p53 overexpression with age and sex of patients ($P > 0.05$). Although there was no significant correlation in the relationship between p53 overexpression with tumor site and gross pattern type, p53 positivity rate was higher in gastric carcinoma cases located in the antrum and in those cases of ulcerative gross pattern type. P53 overexpression was more commonly seen in gastric carcinoma case of intestinal type compared to diffuse type. However, the results were statistically not significant ($P > 0.05$). P53 overexpression was more common in gastric carcinoma cases of moderately differentiated type compared to poorly differentiated type, with no statistically significant difference ($P > 0.05$). Although the majority of gastric carcinoma cases which showed positive p53 expression were in stage III disease, these results were not significant ($P > 0.05$).

Conclusion: The overall expression of P53 protein in gastric carcinoma cases in this immunohistochemical study was 44%. There was no significant correlation between p53 overexpression and different clinicopathological variables like: age, gender, gross pattern, histological type, tumor grade and stage.

Keywords: P53, gastric carcinoma, immunohistochemical expression

IRAQI J MED SCI, 2008; VOL.6 (2):77-89

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Received: 16th April 2008, Accepted: 8th June 2008.

Introduction

Understanding the molecular basis of gastric cancer is essential to develop more effective methods of primary prevention and secondary prevention (early diagnosis and treatment). Although the molecular mechanisms in gastric carcinogenesis have not been completely delineated, some important advances in the molecular biology of gastric cancer have been made⁽¹⁾.

Several abnormalities in oncogenes, tumor suppressor genes, and growth factor expression have been identified in gastric cancer. P53 is a tumor suppressor gene which is usually mutated up to 67.9% in gastric carcinoma⁽¹⁾; these mutations are usually missense point mutations leading to genetic instability and uncontrolled cell proliferation⁽²⁾. These mutations impair P53 anti-cancer gene inducing effects, so restoring its function would be a major step in curing many cancers, including gastric cancer⁽³⁾, especially the ability of P53 to control apoptosis in response to DNA damage which has important practical therapeutic implications to enhance the effect of radiation and chemotherapy, or even evaluating the effect of adenovirus mediated re-introduction of wild type P53 as a potential clinical utility in gene therapy of gastric cancer⁽⁴⁾. Thus, it is accepted that P53 plays a fundamental role in tumorigenesis and hence is an obvious choice for therapeutic exploitation. However, conflicting evidence and insufficient knowledge about the P53 pathways in detail and the fact that other mechanisms exist to modulate P53 activity leave this useful tool a hope for the future as regards use in the clinic⁽⁵⁾.

So this study aims 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.

Patients, materials and methods

From October 2006-May 2007, forty formalin fixed paraffin embedded gastric carcinoma tissue blocks (partial or total gastrectomy specimens) from the archived materials of the Department of Pathology of Baghdad Teaching Hospital and the Center of Gastrointestinal and Hepatic Diseases, and other private laboratories were included in this study.

Clinicopathological parameters as the age and gender, site, gross appearance, histological type, grade and stage of tumor were obtained from histopathological reports.

A four micrometer -thick tissue sections were obtained from representative area, one section was stained with Hematoxylin and eosin (H&E) and then reviewed, while two sections were stained immunohistochemically for p53. The histologic type was classified according to Lauren classification 1965.

The positive control tissue used in the present study was a specimen from poorly differentiated ductal breast carcinoma tissue, which was known to be positive for monoclonal anti p53 protein.

Untreated sections with primary antibody (by omission of the primary antibody) were considered as technical negative control, while normal gastric tissue sections were considered as *tissue negative control* and were used for each set of slides. These tissues should show absence of specific staining.

All the slides were examined by light microscope; a random selection of the fields was used for analysis of all cases. Positive p53 results give

intracellular (nuclear) dark brownish color, granular or homogenous precipitate (clear cut) with blue cytoplasm.

The results of p53 positivity in each individual specimen were analyzed according to these independent variables^(6, 7):

-Intensity of staining: the intensity of staining of the brownish coloration was considered:

1. Strong (S) if it could be detected very clearly at low magnification (x10).

2. Moderate (M) if it was detected with difficulty at low magnification.

3. Weak (W) if it could only be detected at high magnification (x40).

-The pattern of staining: the pattern was considered:

A. Diffuse pattern (D) if the positive cells were distributed through almost all fields.

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

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

-Extent of staining: a minimum of 100 tumor cells were scored (the percentage of positive stained nuclei with p53 protein in malignant cells counted manually at x400 total magnification, in 3-5 neoplastic fields randomly selected, that represent the most positive neoplastic area). P53 immunostaining in at least 10% of the cell nuclei of tumor tissue was regarded as p53 overexpression.

Scoring according to Sophia K.1999⁽⁸⁾ and Roviello F.1999⁽⁹⁾ was done at x40 objective as follows:

*Negative (Score 0) (none of the cells revealed positivity for p53 marker)

*Weak or mild staining (5-<10% positive of tumor cells) (Score +1)

* Moderate staining (<25%) (Score +2)

* Strong staining (>25 %-< 50%) (Score +3)

* Highly strong staining over 50%) (Score +4)

Statistical analysis

Was done using chi-square test for tables with frequencies, percentages, range, mean and standard deviation. Values were considered statistically significant when $P < 0.05$.

Results

A total of (40) forty formalin fixed paraffin embedded gastric carcinoma tissue blocks were included in the present study. Clinicopathological assessment revealed that 28 patients were males and 12 patients were females. Male to female ratio was 2.3/1. The age of patients ranged between 30-80 years with a mean $\pm$ standard error of (55.77 $\pm$ 1.88) years. The majority of the gastric carcinoma cases 28(70%) were above 50 years, while 12 (30%) of the cases were below 50 years. Sex distribution of gastric carcinoma cases, showed male predominance 28(70%) compared with female 12(30%). Large proportion of gastric carcinoma cases 32 (80%) were located in the antral region while the remaining cases 8(20%) were located in the cardia region.

Regarding the gross pattern of gastric carcinoma cases, the ulcerative type constituted 29 (72.5%) , while the fungating type constituted 5(12.5%) of the cases, the least gross pattern types were the stenosing 1(2.5%) and polypoidal 1(2.5%) types.

The histological type showed the predominance of intestinal type 30(75%) compared to the diffuse type 10(25%).

Taking into consideration tumor grade, this study revealed that the majority of cases were moderately differentiated type 25(62%) while 15(38%) of the cases were poorly differentiated type.

According to AJCC (TNM) staging system, the majority of gastric

carcinoma cases 37(92.5%) fall in stage III disease.

Positive p53 staining was detected in 18(44%) of gastric carcinoma cases while negative p53 immunostaining was detected in 22(56%) of the cases. (Figure 1)

Twelve cases of gastric carcinoma were below the age of 50 years and 6 cases (50%) of them showed positive P53 expression, while 28 of gastric carcinoma cases were equal or above 50 years of age and 12 cases (43%) of them showed positive p53 expression.

Thirteen cases of gastric carcinoma (46%) out of 28 cases which were of male gender type showed positive p53 expression, while 5 cases(42%) out of 12 cases which were of female gender type showed positive p53 expression. However there was no statistically significant difference in the relationship between p53 overexpression with age and sex, as shown in (Table 1).

Regarding the relation of p53 immunostaining with the tumor site, out of 32 gastric carcinoma cases located in the antrum 15 cases of them (47%) were positive for p53 immunostaining, while out of 8 gastric carcinoma cases located in the cardia , 3 cases of them (38%) were positive for p53 immunostaining. Out of 32 cases located in the antrum , 17 cases of them (53%) were negative for p53 immunostaining , while out of 8 cases located in the cardia , 5 cases of them (63%) were negative for p53 immunostaining, the difference was statistically not significant , as shown in (Table 1).

In regard to the ulcerative gross pattern, 15(52%) gastric carcinoma cases out of 29 cases of ulcerative gross pattern type showed positive p53 expression, followed by fungating type, in which out of 5 cases of fungating type, 2 cases of them (40%) showed positive p53 expression. While

out of 29 ulcerative gross pattern gastric carcinoma cases, 14 cases of them (48%) showed negative p53 expression, and out of 5 cases of fungating type, 3 cases of them (40%) showed negative p53 expression (Figure 2).

In consideration to the histological type, out of 10 cases of diffuse type gastric carcinoma, 4 cases of them (40%) showed positive p53 expression (Figure 3), while out of 30 cases of intestinal type gastric carcinoma, 14 cases of them (47%) showed positive p53 expression (Figure 4). Out of 10 cases of diffuse type gastric carcinoma, 6 of them (60%) showed negative p53 expression, while out of 30 cases of intestinal type gastric carcinoma, 16 cases of them (53%) showed negative p53 expression (Figure 5), theses results were also statistically not significant, as shown in (Table 1).

Out of 25 cases of moderately differentiated type gastric carcinoma, 12 cases of them (48%) showed positive p53 expression, while out of 15 cases of poorly differentiated type gastric carcinoma, 6 cases of them (40%) showed positive p53 expression. Out of 25 cases of moderately differentiated type gastric carcinoma, 13 cases of them (52%) showed negative p53 expression, while out of 15 cases of poorly differentiated type gastric carcinoma, 9 cases of them (60%) showed negative p53 expression (Figure 6).

Regarding the relationship between gastric carcinoma cases and stage of disease, out of 37 cases of gastric carcinoma falling in stage III disease, 17 cases of them (46%) showed positive p53 expression, while one case falling in stage IV disease showed positive p53 expression. Out of 37 of gastric carcinoma cases falling in stage III disease, 20 cases of them (54%) showed negative p53 expression; while one case falling in

stage IV disease showed negative p53 expression (Figure 7). There was no statistically significant difference in relationship between p53 protein expression with tumor grade and stage as shown in (Table 1).

P53 immunostaining in at least 10% of the cell nuclei of tumor cells was regarded as p53 overexpression.

The extent of staining of p53 expression in gastric carcinoma in the present study (according to Sophia K. 1999⁽⁸⁾) and Roviello F.1999⁽⁹⁾ was done at x40 objective as follows: (Table 2)

A) 22(55%) of negative cases were within score 0.

B) 7(17.5%) of positive cases were within score 1.

C) 5(12.5%) of positive cases were within score 2.

D) 2(5%) of positive cases were within score 3.

E) 4(10%) of positive cases were within score 4.

The pattern of positive p53 staining was diffuse in 6 (33%), regional in 8(45%), and focal in 4(22%). (Table 2)

The intensity of p53 staining was weak in 6(33%) (Figure8), moderate in 7 (39%), and strong in 5 (28%) (Figure 9) of gastric carcinoma cases (Table 2).

Table 1: Distribution of p53 expression in gastric carcinoma cases in relation to different studied parameters.

		Age		Gender		Site		Histological type		Histological grade		Pathological stage		
		<50	≥50	M	F	Antrum	Cardia	Diffuse	Intestinal	Moderate	Poor	I	III	IV
	positive	6	12	13	5	15	3	4	14	12	6	0	17	1
	negative	6	16	15	7	17	5	6	16	13	9	1	20	1
	total	12	28	28	12	32	8	10	30	25	15	1	37	2
P53		P-value		0.701		0.812		0.709		0.471		0.870		0.653

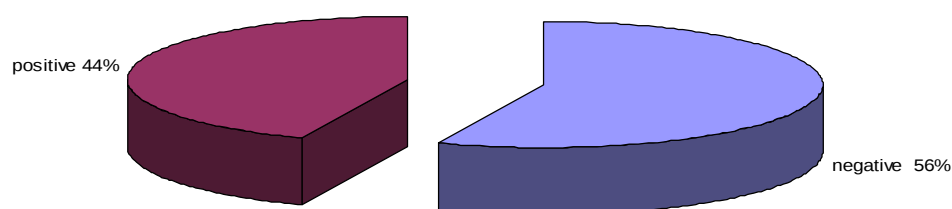


Figure 1: The expression of p53 in gastric carcinoma cases.

Table 2: Distribution of p53 expression in relation to scoring system, staining, and intensity in gastric carcinoma cases.

Scoring of P53 in all cases	0	1	2	3	4	Total
Frequency No. %	22 55%	7 17.5%	5 12.5%	2 5%	4 10%	40 100%
Staining in P53 positive cases	Diffuse	Regional		Focal		Total
Frequency No. %	6 33%	8 45%		4 22%		18 100%
Intensity in P53 positive cases	Weak	Moderate		Strong		Total
Frequency No. %	6 33%	7 39%		5 28%		18 100%

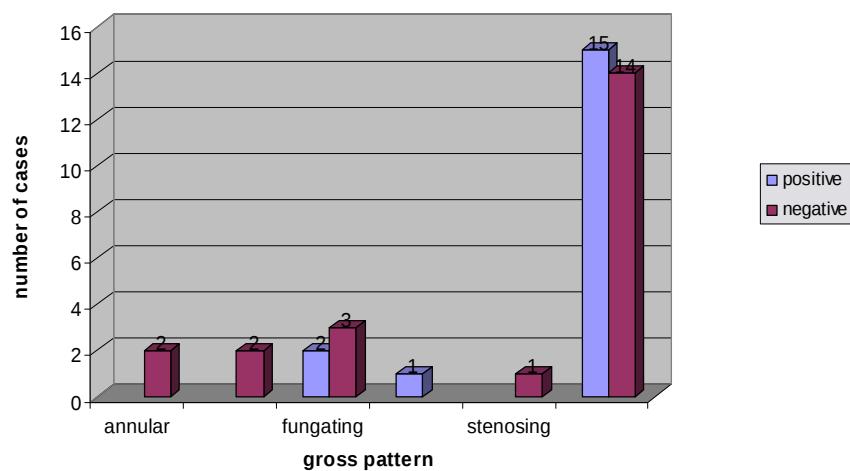


Figure 2: The distribution of p53 expression according to gross pattern.

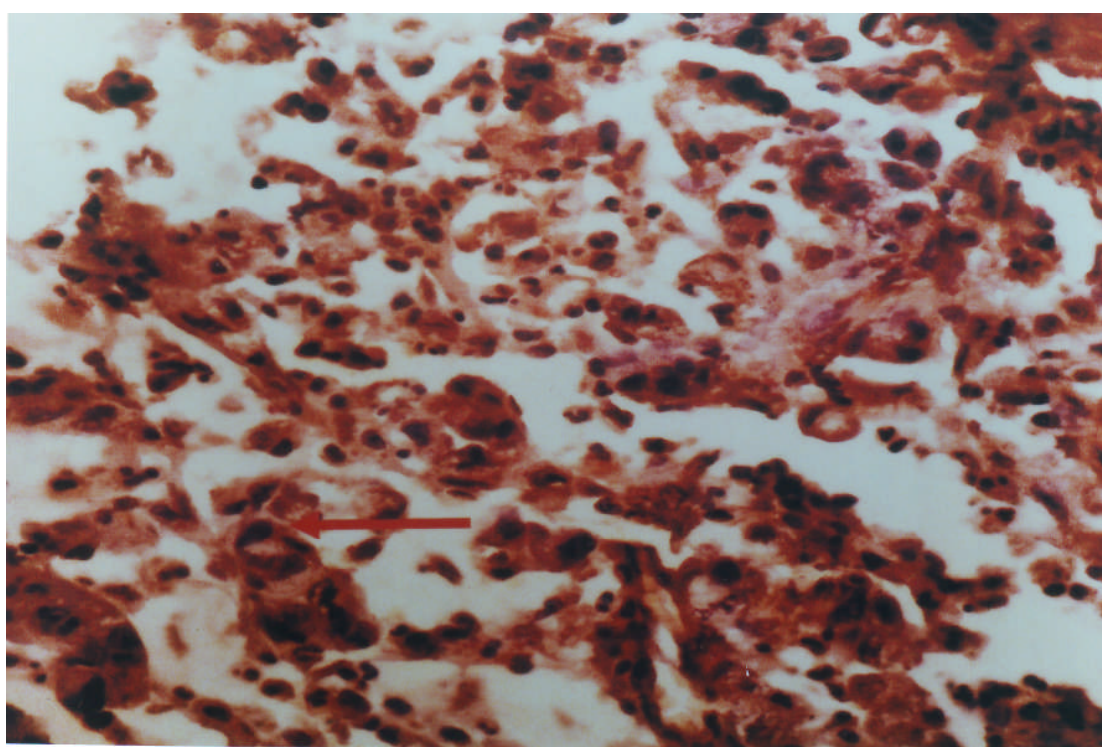


Figure 3: poorly differentiated diffuse type gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method), (Arrow) at (x40).

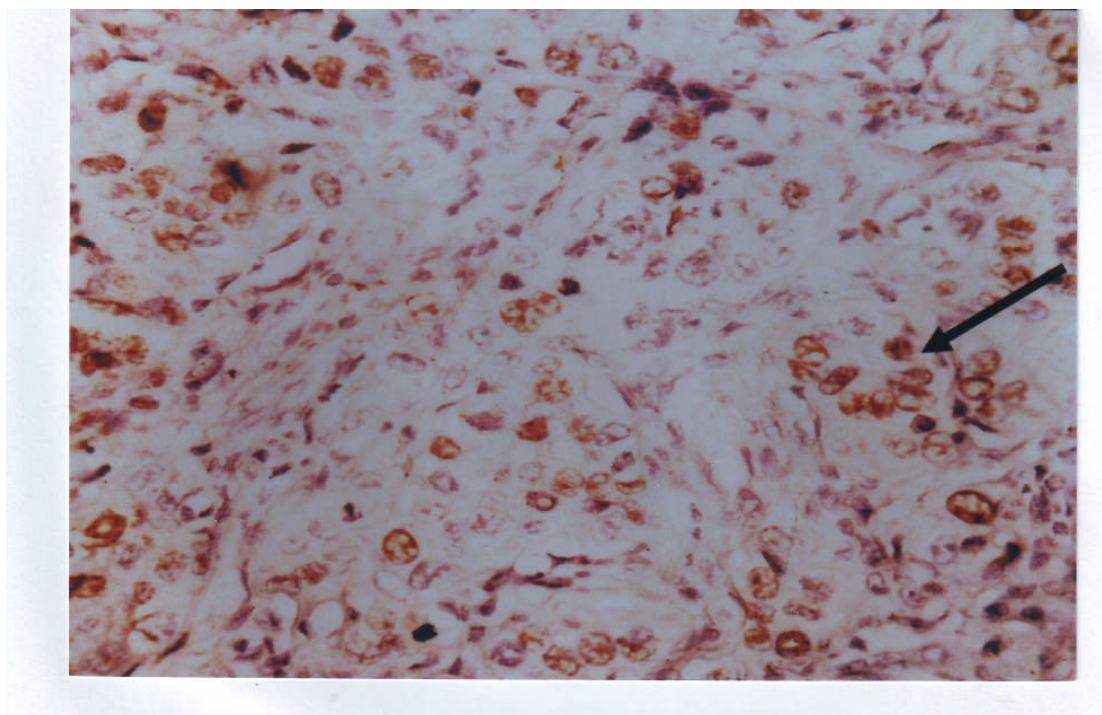


Figure 4: intestinal type gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method), (Arrow) at (x40).

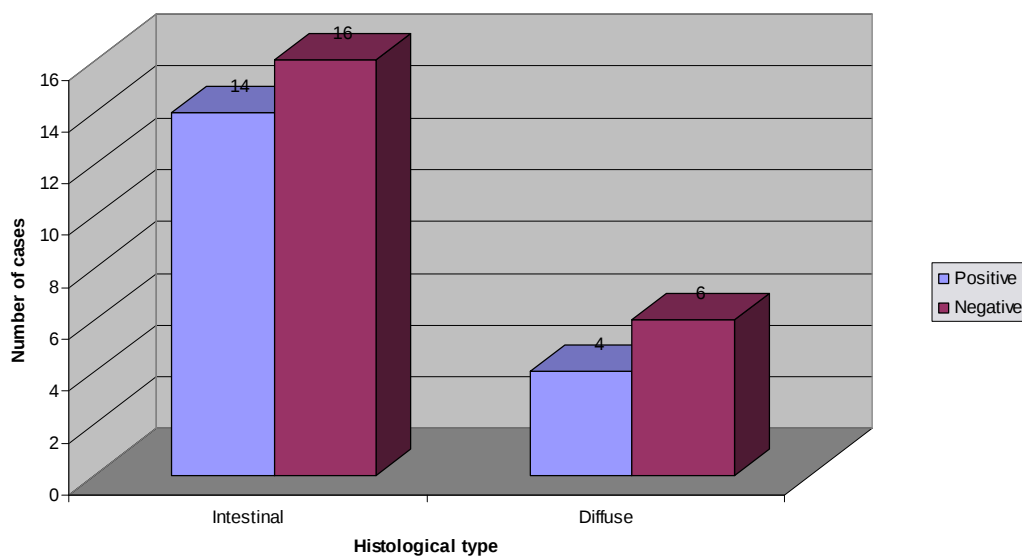


Figure 5: The distribution of p53 expression according to histological type.

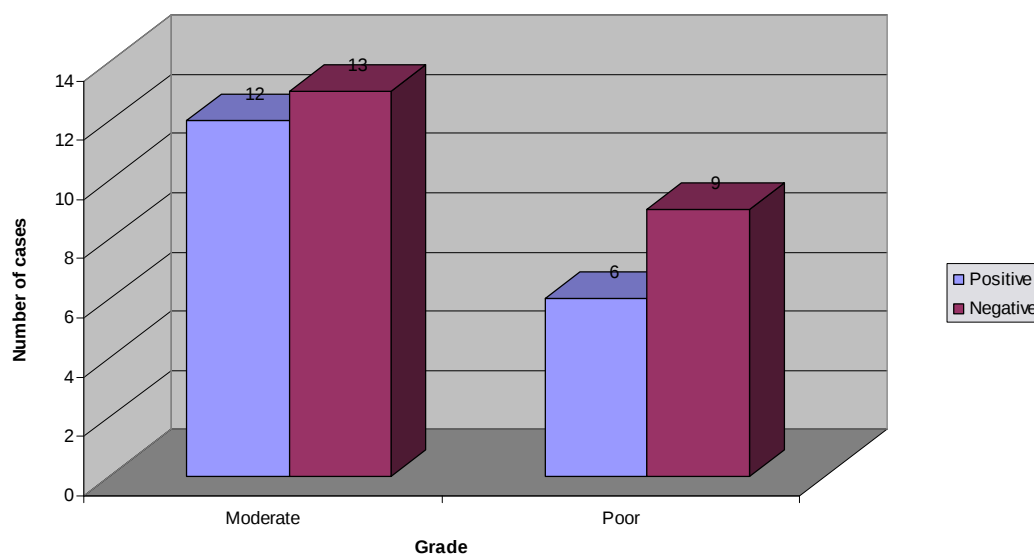


Figure 6: The distribution of p53 expression according to histological grade.

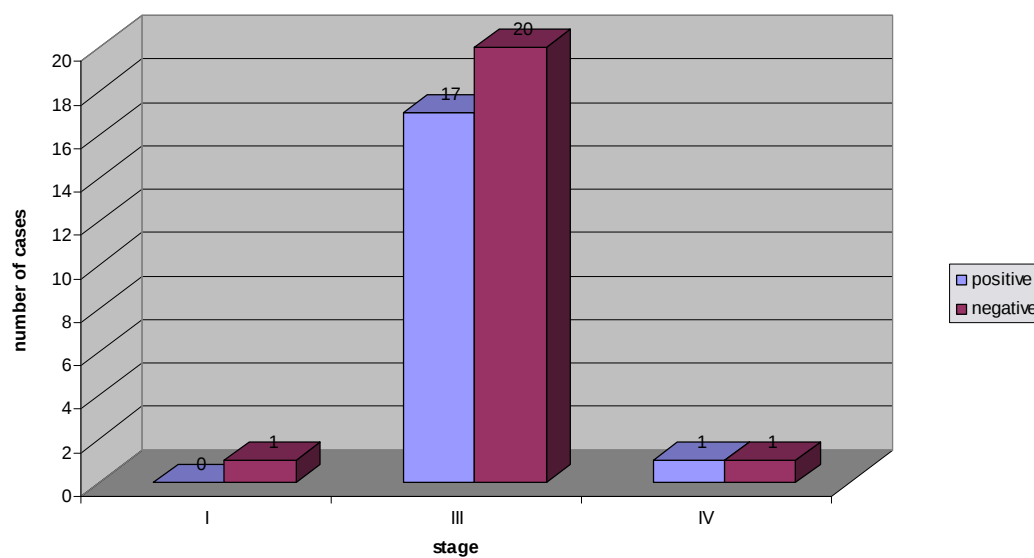


Figure 7: The distribution of p53 expression according to stage.

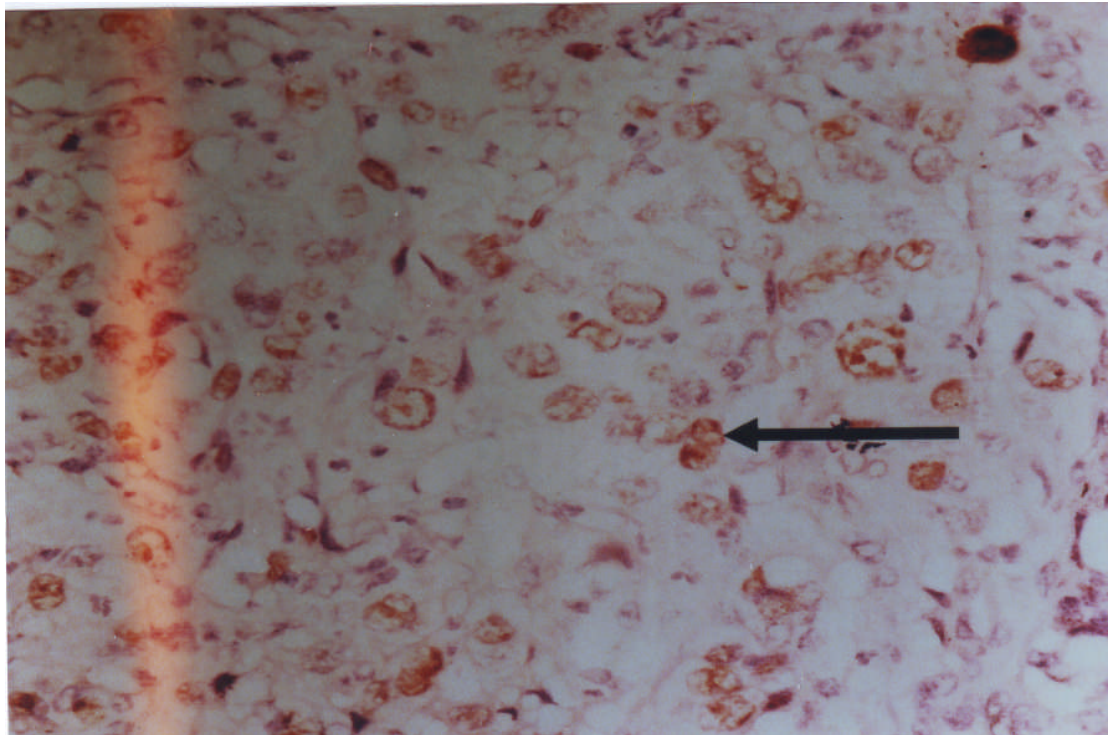


Figure (8) moderately differentiated gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method),(weak expression), (Arrow) at (x40).

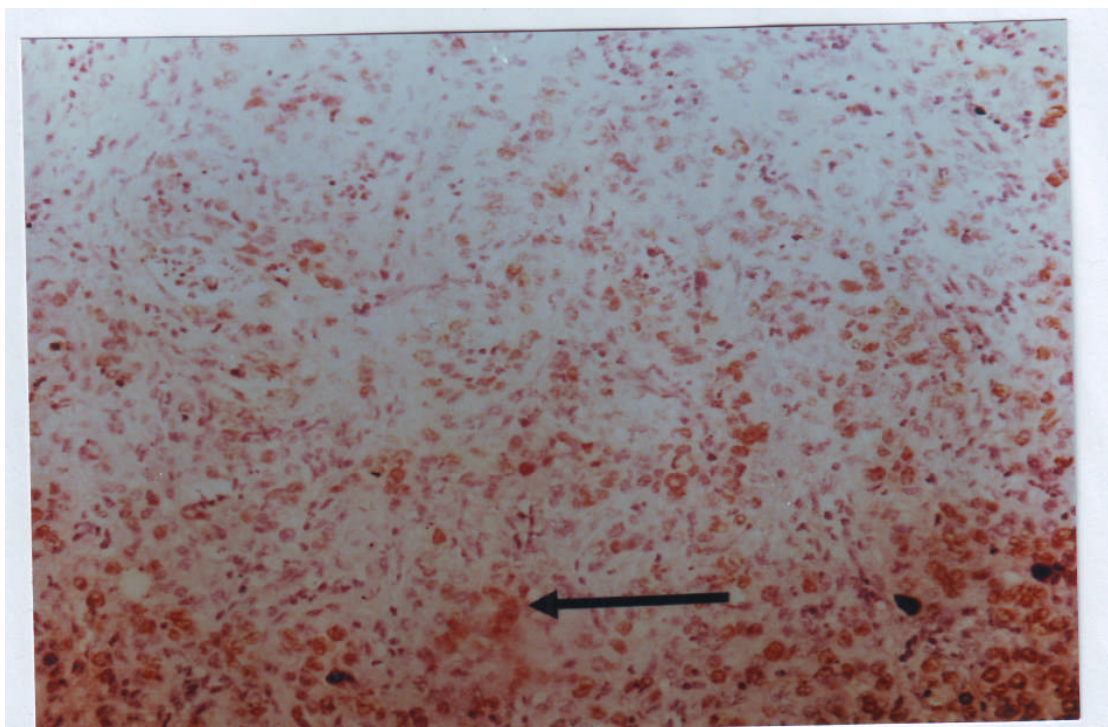


Figure 9: poorly differentiated gastric adenocarcinoma showing positive p53 expression as brown stained nuclei (DAB method),(strong expression), (Arrow) at (x10)

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⁾.

The etiology of gastric carcinoma includes both genetic factors and environmental factors such as H.pylori. Multiple genetic alterations are detected not only in gastric carcinoma, but also in tumors at other sites⁽¹¹⁾. 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⁽¹²⁾.

In this study, p53 overexpression was positive in (44%) of gastric carcinoma cases, which is compatible with other studies in Iraq⁽¹³⁾, Iran⁽¹⁴⁾, and Turkey⁽¹⁵⁾.

This study showed that there was no significant correlation between p53 overexpression and patient's age. This result is nearly compatible with the results from Iraq⁽¹³⁾, Iran⁽¹⁴⁾, Turkey⁽¹⁵⁾, and also with other studies^(11, 16, 17). Also no significant correlation was found between p53 overexpression and sex of the patient. The slightly higher expression rate in males than females could be attributed to the higher incidence rate of gastric carcinoma in males compared to females, similar results were seen in different studies^(11, 13, 14, 15, 16, 17).

Regarding the relation of p53 immunostaining with the tumor site, although the results were statistically not significant, a higher rate of p53 positivity was seen in gastric carcinoma cases located in antrum, this could be explained by the fact that majority of gastric cancers cases (80%) were located in antrum. Fléjou et al found p53 positivity rate was higher in

cases located in the cardia and concluded that the tumors located in the cardia exhibited higher rates of aneuploidy than those located in the antrum. They ascribed this difference to different molecular mechanisms leading to malignant transformation in carcinomas located in the cardia and the antrum and proposed that antral tumors developed mostly in response to environmental factors⁽¹⁸⁾. In other studies, no correlation was found between tumor location and the rate of p53 positivity^(16,17, 19).

In the literature, it was reported that there was no significant relationship between p53 positivity rate and growth pattern^(11, 16, 17). This result is similar to a study in Turkey⁽¹⁵⁾, while in this study p53 positivity rate was higher in ulcerative growth pattern type.

In various studies, the rate of p53 positivity was found to be different in varying histological types of gastric carcinoma. The positivity rates were higher in intestinal type gastric carcinoma, varying between (50% and 70%). This rate was lower in diffuse type gastric carcinoma, being (12-27%)^(11, 16, 17, 18, 20). However, some of these studies had found a significant relationship between p53 overexpression and histological type^(11, 18, 20); others did not^(16, 17, 21). These figures are congruent with those in the literature and suggest that p53 may play a part especially in the formation of intestinal type carcinoma. In a study in Turkey⁽¹⁵⁾, the positivity rates were higher in intestinal type carcinoma, whereas in Iran⁽¹⁴⁾, they found that the positivity rates were higher in diffuse type carcinoma. In the present study, p53 positivity was higher in intestinal type compared to diffuse type.

Regarding the correlation between p53 overexpression and tumor grade,

no significant correlation was found in this study and other studies^(14, 17, 18, 20). In a previous study done in Iraq, p53 protein accumulation was higher in poorly differentiated gastric carcinoma than in moderately and well differentiated ones⁽¹³⁾, similar results were also found in other studies^(22, 23, 24), this difference in the results obtained from different studies could be due to sample size limitation and different techniques used during work.

In concordance with other studies, there was no statistical significant difference between p53 overexpression and stage of gastric cancer^(17, 21, 25).

In conclusion, the overall expression of p53 protein in gastric carcinoma cases in the present study was 44% and there was no significant correlation between p53 overexpression and different clinicopathological variables like: age and sex of patients, site, gross pattern, histological type, tumor grade and stage. However, Intestinal type gastric carcinoma showed a higher p53 expression rate in comparison to diffuse type and also P53 overexpression was more common in moderately differentiated type gastric carcinoma cases than in poorly differentiated type.

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