

Immunohistochemical Expression of P53 in Gastric Adenocarcinoma

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ABSTRACT:

BACKGROUND:

Gastric carcinoma is the fifth most common cancer worldwide. P53 is tumor suppressor gene, located on chromosome 17p13.1, involved in cell senescence and differentiation. In gastric cancer, P53 expression had shown to be associated with bad prognosis.

AIMS OF THE STUDY:

To determine P53 immunohistochemical expression in gastric adenocarcinoma and correlate it with clinic-pathological variables.

MATERIALS AND METHODS:

Forty formalin fixed, paraffin embedded tissue blocks (24 endoscopic biopsies & 16 gastrectomy cases) included in this retrospective study, collected from archived materials from Baghdad hospitals & laboratories (from January 2019 to December 2020).

Two sections of 5µm thickness were done, one stained with H&E and the other for P53 using mouse monoclonal antibody (clone BP-53-12).

RESULTS:

Immunohistochemical expression of P53 was expressed in 32 cases (80%) (24 cases intestinal type adenocarcinoma & 8 cases diffuse type adenocarcinoma). Five had score +1, eleven had score +2 and sixteen had score +3. Twelve lymph nodes positively expressed p53, eleven of them (91.7 %) involved by metastasis.

CONCLUSION:

P53 significantly expressed in intestinal type adenocarcinoma and directly related with tumor differentiation, depth of invasion and lymph node metastasis but not with tumor location.

KEYWORDS: P53 expression, gastric adenocarcinoma.

INTRODUCTION:

Gastric cancer is the fifth most frequently diagnosed cancer and the third leading cause of cancer death worldwide.⁽¹⁾ Based on GLOBOCAN 2018 estimates, it is responsible for over 1,000,000 new cases and an estimated 783,000 deaths.⁽¹⁾ Two-folds higher in males than in females.⁽²⁾ Gastric cancer is a multifactorial disease.⁽³⁾ Helicobacter pylori is the main risk factor for this cancer & accounts for almost 90% of non-cardia new cancer cases.⁽⁴⁾

P53 is a tumor suppressor gene⁽⁵⁾, usually mutated in up to 67.9% gastric carcinoma⁽⁶⁾ and plays an important role in cell cycle control and apoptosis.

Defective p53 could allow abnormal cells to proliferate, resulting in cancer⁽⁵⁾, which can be studied with immunohistochemical methods⁽⁷⁾.

The majority of changes involving p53 are missense mutations which result in single amino acid substitutions and expression of mutant proteins.^(7,8)

MATERIALS AND METHODS:

A retrospective study including 40 formalin fixed, paraffin embedded tissue blocks for histopathologically diagnosed gastric adenocarcinoma cases including 24 endoscopic biopsies & 16 gastrectomy cases (8 total & 8 partial gastrectomy), collected from archived materials from GIT and Liver Teaching Hospital, Teaching Laboratory Institute and some private laboratories in Baghdad (from January 2019 to December 2020). Clinicopathological parameters were obtained from the available histopathological reports.

Two sections of 5 µm thickness were taken from each block, one stained with H&E, the other stained for p53 using mouse monoclonal IgG2a antibody (clone BP-53-12).

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Colon cancer tissue & reactive follicular hyperplasia of tonsillar tissue were used as positive & negative control for p53 staining, respectively. The assessment of immunohistochemical scoring system was done by counting the percentage of the tumor cell's nuclei and was assigned as 0, 1+, 2+ and 3+ when p53 express nuclear positivity in <10 %, 10-30 %, 31-50 % and >50 %. The intensity of the stain was estimated and the slides were checked to exclude any error.

Statistical analysis:

Statistical package for social sciences version 24 (SPSS v24) used to analyze data.

Chi-square test for independence and Fisher's exact tests were used as appropriate to test the significance of association between discrete variables.

P value < 0.05 was set as level of significance.

RESULTS:

Twenty four out of 40 collected cases (60%) were endoscopic biopsies, 8 cases (20%) were partial gastrectomy while the remaining 8 cases (20 %) were total gastrectomy.

A significant statistical correlation between the histological type of gastric adenocarcinoma and p 53 expression (p value = 0.043) as in table 1.

Table 1: P53 expression in relation to histological type of gastric adenocarcinoma.

Histological Type	P 53 positive				P value
	Positive		Negative		
	N	100.0%	N	100.0%	
Intestinal	24	75.0%	2	25.0%	0.043
Diffuse	8	25.0%	6	75.0%	

The score of p53 expression increases with decreasing the degree of differentiation with highest score (+3) obtained in poorly differentiated cases (71.5%).

Table 2: Score of P53 expression in relation to grade of intestinal type adenocarcinoma.

Grade of intestinal type	P 53 Expression							
	Negative		+1		+2		+3	
	N=2	100.0%	N=3	100.0%	N=8	100.0%	N=13	100.0%
Well differentiated	1	50.0%	2	66.7%	3	37.5%	1	7.1%
Moderately differentiated	1	50.0%	0	0.0%	5	62.5%	3	21.4%
Poorly differentiated	0	0.0%	1	33.3%	0	0.0%	9	71.5%

Gastric cancers located at antrum showed the highest p53 expression (50%) with no significant statistical relation between p53 expression & site of carcinoma, with p value = 0.734.

Of 16 gastrectomy cases, 12 lymph nodes were positively expressing p53, 11 of them (91.7 %)

were lymph nodes involved by metastatic gastric adenocarcinoma while the remainder (8.3 %) was lymph node without metastasis, as shown in figure 1. However, no significant statistical relation was present and the p value was 0.135.

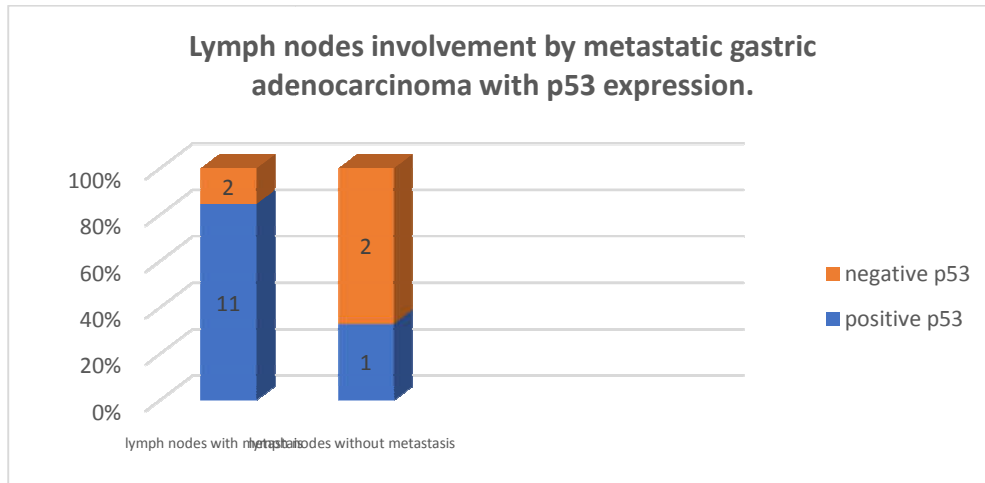


Figure 1: Lymph nodes involvement by metastatic gastric adenocarcinoma with p53 expression.

P53 expression increased in score with increasing depth of tumor invasion, by going from T0 to T4, however there was no significant statistical relation with p value 0.286 as shown in figure 2.

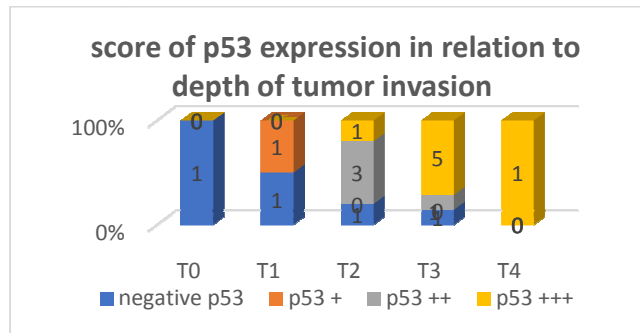


Figure 2 : Score of p53 expression in relation to depth of tumor invasion.

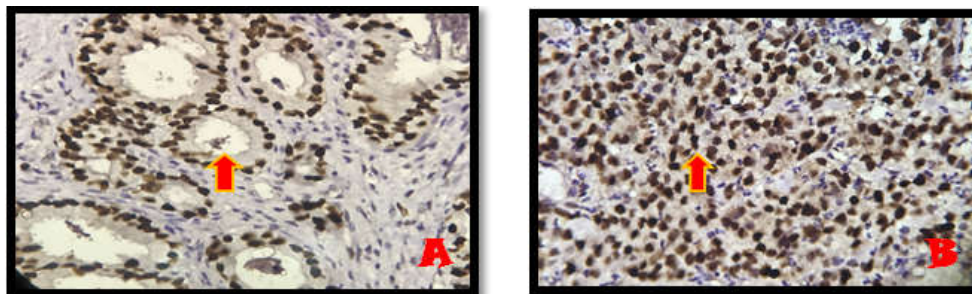


Figure 3: Well differentiated intestinal type gastric adenocarcinoma (A) , diffuse type gastric adenocarcinoma (B) with positive nuclear staining for p53, score 3+ (H&E X 40).



Figure 4: Intestinal type gastric adenocarcinoma, well differentiated with negative nuclear staining for p53. (H&E X 40).

DISCUSSION:

This study involved 40 patients diagnosed as gastric adenocarcinoma, 26 cases (65 %) were diagnosed as intestinal type & the remainder 14 cases (35 %) were diagnosed as diffuse type gastric adenocarcinoma

The age range of gastric carcinoma cases in this study was from 24 to 82, the commonest age affected was 60-69 years (25%), which was comparable to the study done by **Ragheed Sameer et al 2008** ⁽⁹⁾. This was in contrast to **Rayan A. Kasim 2014** ⁽¹⁰⁾ who found that commonest affected age by gastric carcinoma was 50-60 years (28%).

In the current study, it had been found that there was no significant correlation between age group of patients and p 53 expression (p value > 0.05) , this similar finding was reported by **Li-Jun Xiao et al 2013** ⁽¹¹⁾ and **Rayan A. Kasim 2014** ⁽¹⁰⁾.

Regarding patients' gender, 24 patients were male (55%) and 18 were female (45%) with the male: female ratio equals to 1.2:1. Absence of significant relationship between p53 expression & the gender of the patients with p value 0.28, which was similar to **Ando K et al 2013** ⁽¹¹⁾ and **Rayan A. Kasim**

2014 ⁽¹⁰⁾. This contrasts from the results obtained by **Bianca Grosser et al 2020** ⁽¹²⁾ which showed significant association with male patients.

P53 expression in gastric cancer

This study showed that p53 was expressed in 32 cases (80%) while 18 cases (20%) were negative for p53, this result was close to the rate (75 %) detected by **Bianca Grosser et al 2020** ⁽¹²⁾ while it contrasts from the results obtained by **Ragheed Sameer et al 2008** ⁽⁹⁾ who detected positive p53 in 40 % of the cases.

The difference in rate of p53 expression can result from differences in the sample's number, the method used in immunohistochemical staining or the type of the antibody used in the technique and the duration of the study.

P53 expression with gastric cancer's histological types.

Significant statistical correlation was present between p53 expression in intestinal type gastric carcinoma than to diffuse type which showed a p value 0.043. Comparable findings were reported in previous studies as shown in following table:

P53 expression in diffuse type gastric cancer %	P53 expression in intestinal type gastric cancer	Year of the study	Study
43.6	56.4	2020	Bianca Grosser et al ⁽¹²⁾
16.1	83.9	2014	Rayan A. Kasim ⁽¹⁰⁾
40.5	68.9	2013	Li-Jun Xiao et al ⁽¹³⁾

Ghaffarzadegan et al 2004 ⁽¹⁴⁾ found that p53 was expressed in 17.7% of intestinal type gastric cancer while expressed in 82.3 % diffuse type gastric carcinoma & this result disagrees with the result obtained in this study.

Higher frequency of p53 expression in intestinal type adenocarcinoma perhaps attributes to the proliferative rate of the intestinal type which tends to be higher than the proliferative rate of gastric carcinoma of diffuse type and this indicates that progression & gastric cancer differentiation may be related to p53 expression and also the *H. pylori* strain that tends to predispose to intestinal type adenocarcinoma may also play a role in this difference.

P53 expression & its relation to gastric carcinoma grade

Significant statistical relation between p53 expression & the grade of intestinal type adenocarcinoma was found with p value 0.042 in which p53 increased in expression from well to poorly differentiation. This significant association was also found in studies done by **Al-Badri BA and Gayllan QA 2011** ⁽¹⁵⁾ and **Rayan A. Kasim 2014** ⁽¹⁰⁾.

Studies done by **Li-Jun Xiao et al 2013** ⁽¹³⁾ and **Ando K et al 2013** ⁽¹¹⁾ showed nonsignificant correlation and this can be attributed to the technique of immunohistochemistry used or to the antibody used.

P53 expression and depth of tumor invasion.

A direct relation between p53 expression & the depth of tumor invasion was present with increased positivity when the tumor invades deeper through the gastric wall. Similar conclusion was found in studies done by, **Salih BA et al 2012** ⁽¹⁶⁾ and **Ando K et al 2013** ⁽¹¹⁾, while disagrees with study reported by **Li-Jun Xiao et al 2013** ⁽¹⁰⁸⁾.

This increase in expression supports that tumor progression and aggressiveness or poor prognosis are related to p53 expression.

P53 expression with lymph node involvement by metastatic malignancy.

Lymph nodes involved by metastatic gastric carcinoma, in this study, expressed higher rates of p53 expression than those free from malignant metastasis.

This result agreed with **Ghaffarzadegan et al 2004** ⁽¹⁴⁾ and **Ando K et al 2013** ⁽¹¹⁾ while disagreed with **Al-Badri BA and Qayllan QA 2011** ⁽¹⁵⁾, **Rayan A. Kasim 2014** ⁽¹⁰⁾, and **Bianca Grosser et al 2020** ⁽¹¹⁶⁾.

P53 expression in relation to tumor location in the stomach.

Higher rates of p53 positivity shown to be in cancers located in gastric antrum and this study failed to show significant statistical relation in regarding to site of carcinoma (p value 0.734), this agreed with result reported by **Ragheed Sameer et al 2008** ⁽⁹⁾, while **Bianca Grosser et al 2020** ⁽¹²⁾ reported that proximally located tumors has higher p53 expression (51.4%). **Fléjou et al 1999** ⁽¹⁷⁾ found that tumors located in the cardia expressed p53 positivity in a higher rate than tumors located elsewhere and concluded that higher rates of aneuploidy were present in tumors of cardia location than of antral location which can be ascribed to different molecular mechanisms responsible for malignant transformation in cardia located carcinoma and the antrum & that environmental factors were are more responsible for development of antral gastric cancers.

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