P53 Expression in Gastric Dysplasia and carcinoma in Erbil City

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Summary:

Background: Alteration in the expression of p53 tumor-suppressor protein is an event that occurs frequently in human cancer, but the practical implications of this phenomenon are yet to be fully exploited.

Objectives: to determine the value of p53 expression as a marker of tumor aggressiveness and the relationship between p53 over expression and clinico-pathologic variables in gastric adenocarcinoma.

Material & Methods: The expression of p53 was studied immunohisto-chemically in 10 cases with gastric dysplasia and 85 cases with gastric adenocarcinomas using formalin fixed, paraffin-embedded tissue samples. D07 a monoclonal antibody to p53 protein was used for the immunehistochemical analysis. The correlation between p53 expression in gastric dysplasia, gastric adenocarcinoma and clinicopathologic variables were analyzed.

Results: P53 positivity was found in 48.2% of gastric carcinoma cases, and 40% of gastric dysplasia. There was a significant correlation between the rate of p53 over expression and tumor grade (p = 0.015), and also with carcinomas associated with lymph node involvement (p = 0034). There was no significant association between p53 protein expression and histological type (p = 0.171). The association between p53 protein expression and the depth of tumour invasion was found to be statistically insignificant.

Conclusion: The results obtained suggest that P53 protein expression is a useful biomarker for the assessment of gastric adenocarcinoma aggressiveness. The preoperative assessment of p53 expression in gastric carcinomas can be helpful in identifying patients with higher grades and more advanced tumors. P53 immunoreactivity can predict lymph node status in patients with gastric carcinoma.

Key words: P53, Gastric Dysplasia, Gastric carcinoma

Introduction:

Gastric cancer is one of the major causes of cancer death worldwide Its pattern and incidence varies widely between different parts of the world. The risk of developing gastric cancer is relatively lower in the Middle East and North Africa compared with those of western countries and Japan (1). Environmental factors such as Helicobacter pylori and dietary habits play an important role in gastric carcinogenesis (1, 2). P53 is a cell cycle control gene that prevents cells with DNA breaks from entering DNA synthesis where the breaks could be replicated causing chromosome damage and leading to progressive genetic instability and eventually cancer (3). The p53 gene is located at chromosome 17p (p53 locus). Most people inherit two normal alleles of p53, one from the mother and one from the father. Inactivation of the p53 gene involves a two-step mechanism. One allele is typically inactivated by mutation, whereas the second copy is lost by a mechanism called 17p loss of heterozygosity (LOH) consistent with Knudson's two-hit hypothesis. The p53+/+ cells respond to genotoxic injury by causing cell cycle arrest or programmed programmed cell death. In the presence of p53-/- cells there is no cell cycle arrest in the presence of DNA damage and the

cells continue to proliferate, accumulating genetic lesions that lead eventually to cancer (3). This aim of this study is to evaluate p53 expression status in patients with gastric dysplasia and carcinoma by immunohistochemistry, with the hope of being able to select patients with gastric dysplasia who are a risk of developing carcinoma and to compare the results with similar studies performed abroad; however our study is the first that done in Kurdistan.

Materials and Methods:

In this retrospective study, eighty five cases of histologically confirmed gastric carcinoma and ten cases of gastric dysplasia were retrieved from Rizgary Teaching Hospital and some histopathological private labs in Erbil city between January 2005 to July 2008. The gastric carcinoma specimens comprises 59 gastrectomy specimens either partial or total and 26 endoscopical biopsies. Informations regarding relevant clinico-pathological parameters were collected from the patients case records of the department of histopathology in Rizgary Teaching Hospital and private labs. The most representative tumor tissue block were chosen for each case and new sections were made and stained with Hematoxylin and Eosin (H&E) for histological re-evaluation and additional 4µm sections were made for immunohistochemical studies. The H&E stained slides were reviewed and the tumors were classified according to the Lauren 1965 classification into two main types: intestinal

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type adenocarcinoma and diffuse type (4). Histological grading was performed by assessment of differentiation for intestinal type as G1= Well differentiation, G2=Moderate differentiation, GIII= Poorly differentiation. Pathological staging performed according to TNM system,T1= tumor invade the submucosa, T2 = invades muscularis properia, T3= invades through muscularis properia into subserosa. The lymph node involvement was regarded as either present or absent regardless the number, the gastric dysplasia were divided into two main categories; low grade and high grade dysplasia (5). The immunostained slides were interpreted using a light microscope. The Dako Cytomation. En Vision® +Dual Link System-HRP (DAB$^+$) staining protocol was used for immunostaining to detect P53 expression and was applied to formalin fixed, paraffin embedded tissue (6)

Evaluation of immunohistochemical results: P53 immunohistochemical results: Positive expression of p53 gives clear cut nuclear staining of Brown color. The results of p53 positivity in each individual specimen were analyzed according to:

Selection of field: Random selection of the field was used for analysis of all cases.

-In cases that showed variable staining, the areas of greatest nuclear staining were chosen.

-In cases that showed patchy distribution of p53, the areas that showed the highest staining were chosen for assessment. Percentage of positive p53 cells**:** The extent of p53 immunostaining was assessed as follows:

Negative = no staining or the amount of stained cells are less than 10%.

+ = the number of stained cells are equal or more than 10% but less than 20% of the total examined cells (mild positive). ++ = the number of stained cells are more than 20% but less than 50% of the total examined cells (moderate positive). +++ = the amounts of stained cells are more than 50% (strong positive) (7).

One thousand tumor cells were counted.

Statistical analysis: Statistical analysis was done by using statistical package for social sciences (SPSS) version 16. The results were considered significant when the p value was less than 0.05.

Results:

A total of 95 cases were included in this study, 10 cases (11%) were gastric dysplasia either low or high grade and 85 cases (89%) were gastric adenocarcinoma.

Table 1: Histological grading of gastric dysplasia with p53 expression

Grading of dysplasia	Negative p53 expression	Positive p53 expression	Total
Low grade	4 (100%)	0 (0%)	4 (100%)
High grade	2 (33.3%)	4 (66.7%)	6 (100%)
Total	6 (60.0%)	4 (40.0%)	10 (100%)

There was statistically significant relationship between grading of dysplasia and p53 expression (p value 0.035) (Table 1).

Table 2: Histological type of gastric adenocarcinoma with p53 expression:

Histological type of GC	Negative p53	Positive p53	Total
Intestinal	35 (48.6%)	37 (51.4%)	72 (100%)
Diffuse	9 (69.2%)	4 (30.8%)	13 (100%)
Total	44 (51.8%)	41 (48.2%)	85 (100%)

There was no significant correlation between histological types of gastric adenocarcinoma (intestinal and diffuse) with p53 expression. (p value 0.171) (Table 2). Out of 85 cases with gastric adenocarcinoma, 41 cases (48.2 %) of them were positively expressed, while 44 cases (51.8%) were negatively expressed.

Table 3: Histological grading of gastric adenocarcinoma with p53 expression:

Histological type (Lauren classification)	Negative p53	Positive p53	Total
DIFFUSE	9 (69.2%)	4 (30.8%)	13 (100%)
INTESTINAL			
G I	14 (63.6)	8(36.4%)	22 (100%)
G II	13 (61.9%)	8(38.1%)	21 (100%)
G III	8 (27.6%)	21(72.4%)	29 (100%)
TOTAL	44 (51.8%)	41 (48.2%)	85 (100%)

There was a statistically significant positive correlation between the tumour grade and p53 expression; when the tumour grade increases more p53 expression. (P value 0.015). (Table 3).
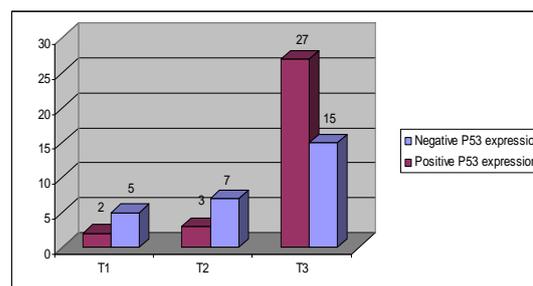

Figure 1: Depth of tumor invasion and p53 expression

The p53 expression according to depth of tumor invasion in this study showed that out of 42 (T3) cases 27 (64.3%) cases of them were positive for p53 expression, and out of 10 (T2) cases 3 (30%) cases were positive for p53 expression, while out of 7(T1) cases only 2 (28.6%) cases were positive for p53 expression. this assessment was not significant statistically (p value 0.051). (Figure 1). Out of 59 gastrectomized patients, lymph node involvement

was seen in 39 patients only. Positive p53 expression was seen in 25 (64.1%) of patients with lymph node involvement and 7 (35%) of patients with no lymph node involvement.(Figure2).
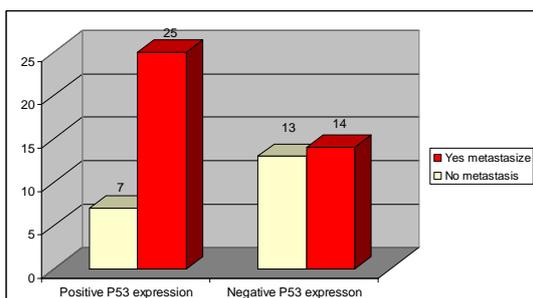

Figure 2: P53 expression in Gastric Carcinoma cases in relation to lymph node metastasis:

Discussion:
Gastric carcinoma is the second most common tumor in the world (8). 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 immunehistochemical staining is a mutated form of the p53 gene product (9) Forty percent of the gastric dysplasia cases were +ve for p53 expression (Table 1), which lies within the range previously reported (15–63.2%) (10,11). Results showed that 48.2% of gastric adenocarcinomas had +ve expression of p53 protein , which was comparable with Fukunaga et al (12) found that 49% of gastric carcinomas expressed positive expression of p53 protein, while others found it to be 51.5%, 60%, 62%, 66%, 75% respectively (7,13,14,15,16) The positivity rates for p53 protein expression in gastric carcinoma in this study lie in the range of (23%-75%) and this is similar to the figures reported by others(17,18,19). Because of the type of fixation, processing and pretreatment, the results were found to be higher in studies using frozen sections (12, 17, 18, and 19). They emphasized that microwave fixation yielded particularly higher results compared to others and were superior. This study showed p53 positivity rate of 51.4% in intestinal type and 30.8% in diffuse type gastric adenocarcinoma (Table 2). These results agree with the following studies; Fukunaga *et al*,(12) found that the p53 accumulation in intestinal type (56%) was higher than in diffuse type (27%) gastric adenocarcinoma, Brito *et al*,(15) found that the frequency of p53 positivity in intestinal type was 46% ,and only 10% in diffuse type gastric adenocarcinoma,   Craanen *et al*,(14)demonstrated that p53 positivity was 70% in intestinal type and 52% in diffuse type gastric adenocarcinoma, Roviello *et al*,(13) found that p53 positivity in intestinal type was 51.8%, and 50.9% of diffuse type gastric adenocarcinoma, while Filiz *et al*, (16) found that p53 was positive in 85% in intestinal type carcinomas, 59% of diffuse type carcinomas, Liu *et*

al, (20)found that over expression of p53 was observed in 50% intestinal type and 34.6% in diffuse type, however in a study done by Ghaffarzadegan *et al*, (7) disagree with above mentioned results, he found that the p53 over expression was in 67.7% of intestinal type, and in 81.2% of diffuse type. P53 nuclear staining can be seen in both intestinal and diffuse type gastric carcinoma, although it is more common in intestinal than in diffuse type tumors. The degree of p53 expression correlates with the proliferative rate of the tumors, perhaps explaining the higher incidence of p53 positivity in intestinal vs. diffuse GC (diffuse GC tends to have low proliferative rates) in this study. Results demonstrated that there was over expression in intestinal type than diffuse type GC but no statistically significant correlation was found between the level of p53 expression and tumor histological type (p = 0.171) (Table 2).Similarly Flejou et al,(16); Rugge *et al*, (21) found that no significant correlation of p53 expression with histological type of GC. This study showed that there was significant correlation between p53 expression and tumour grade (p value = 0.015) (Table 3). Studies done by (Martin *et al*, (22) and Do *et al*,(23) agreed with the results of this study , while (Flejou et *al*,(18); Muller and Borchard et al,(24); and Filiz et *al*,(16)found no significant correlation between   p53 and tumour grade. Although the rate of p53 over expression showed positive correlation with the depth of the tumour invasion , however this relation was not statistically significant (p value = 0.051) (Figure 1). Similar conclusion were found by   Flejou   *et al*,(18); Fukunaga *et al*(12), while other studies done by Roviello *et al*,(13) ; and Ghaffarzadegan *et al*,(7).This observation supports the suggestion that over expression of p53 is associated with tumor progression and may be related to the prognosis. Results also showed that there was a significant correlation of p53 expression with lymph node metastasis (p value = 0.034) (Figure 2). Motojima *et al* , (25); Roviello *et al*, (13);Kaye *et al* ,(26)agreed with results of present study, while studies done by Hurlimann and Saraga et al,(19);and Ghaffarzadegan *et al*,(7)found that there was no significant correlation between p53 expression with lymph node metastasis.

References:
1- *Kamal E. Bani-Hani, Nidal M Combined Evaluation of expressions of Cyclin E   and p53 Proteins as Prognostic Factors for patients with Gastric Cancer.Clinical Cancer Research. (2005) ;(11): 1447-1453.*
2- *Porock D and Palmer D Cancer of Gastrointestinal Tract.A handbook for nurse practitioners   .Whurr   publishers.London &Philadelphia: (2004);115.*
3- *Karaman A, Pirim Ipredictor of progression in Gastric   Carcinoma.The Internet   Journal   of genomics and Proteomics; (2007); 3(1): 1540-1630.*

4- Lauren P. The two histological main types of Gastric carcinoma,Diffuse and so called intestinal type carcinoma. Acta Pathol Microbiol.Scand.1965; 64:31-49.

5- Ming SC, Bajtai A, Corre P, et al.Gastric dysplasia, significance and patho. Criteria.Cancer 54;1984 : 1794-1801.

6- Dako Cytomation(2003). Instructions of DAKO LSAB®2 System Alkaline Phosphatase; Universal. Carpinteria, California, DAKO corporation

7- Ghaffarzadegan K, Zali M, Ahmadi Kh ,Asadzadeh H, and Abbaszadegan M.Correlation of Nuclear P53 Immunoreactive with the Histopathologic feature in Gastric Cancer .Archieves of Iranian Medicine; (2004); 7 (4):279-283.

8- Chew-Wun W, Chin-Wen C and Wen-Chang LGastric cancer, prognostic and diagnostic advances. Cambrige journal ; (2002); (4):1-12.

9- Okusa Y, Ichikura T, Tamakuma S Immunohistochemical staining for the p53 protein and proliferating cell nuclear antigen in familial clustering of gastric cancer. J Surg Oncol; (1996); 62: 253 – 257.

10- Ranzani GN, Luinetti O, Padovan LS, Calistri D, Renault B,Burrel M, Amadori D, Fiocca R, Solcia E p53 gene mutations and protein nuclear accumulation are early events in intestinal type cancer but late events in diffuse type. Cancer Epidemiol Biomarkers Prev (1995); 4:223–231.

11- Imatani A, Sasano H, Asaki S, Toyota T, Saito M, Masuda T,Nagura HAnalysis ofp53 abnormalities in endoscopic gastric biopsies. Anticancer Res .(1996); 16:2049–2056.

12- Fukunaga M, Monden T, Nakanishi H Immunohis-tochemical study of p53 in gastric carcinoma. Am J Clin Pathol; (1994);101: 177-80.

13- Roviello F, Marrelli D, Vindigni C, De Stefano A, Spina D,Pinto E. p53 accumulation is a prognostic factor in intestinal-type gastric carcinoma but not in diffuse type. Ann Surg Oncol (1999);6:739–745.

14- Craanen ME, Blok P, Dekker W, Offerhaus GJA, Tytgat GNJ.Chronology of P53 protein accumulation in gastric carcinogenesis.Gut (1995);36:848-852

15- Brito MJ, Williams GT, Thompson H, Filipe MU.Expression of p53 in early (T1)gastric carcinoma and precancerous adjacent mucosa. Gut (1994);35:1697–1700.

16- Filiz O, Semsi A, and Kemal KDetection of P53 In Gastric Carcinoma .The Turkish Journal of Gastroenterology ;(2000);11(4).

17- Hirohashi s, Sugimura TGenetic alterations in human gastric cancer. Cancer Cells; (1991); 3:49-52.

18- Flejou JF, Muzeau F, Potet F Overexpression of the P53 tumor suppressor gene product in esophageal and gastric carcinomas. Path Res Pract; (1994); 190: 1141-8.

19- Hurlimann J, Saraga EPExpression of p53 protein in gastric carcinomas. Association with histologic type and prognosis. Am J Surg Pathol; (1994);18: 1247 – 1253.

20- Liu XP, Tsushimi K, Tsushimi M, Oga A, Kawauchi S, Oga A, Furuya T, Sasaki K. Expression of p21 (WAF1/CIP1) and p53 proteins in gastric carcinoma: its relationship with cell proliferation activity and prognosis. Cancer Lett(2001);170:183–189.

21- Rugge M, Shiao, Y-H, Busatto G, Cassaro M, Strobbe C, Russo VM, Leo G, Parenti AR,Scapinelli A, Arslan P, Egarter-Vigl Fenglio Preiser et al. The p53 gene in patients under the age of 40 with gastric cancer: mutation rates are low, but are associated with a cardiac location. Mol Pathol(2000); 53:207–210.

22- Martin HM, Filipe M, Morris RW, Lane DP, Silvestre F.p53 expression and prognosis in gastric carcinoma. Int J Cancer (1992); 50:859–862.

23- Do I, kim Y, and Park Y Expression of the GLUT, and P53 protein in Atypical Mucosal Lesions obtained from Gastric Biopsy specimens. The Korean journal of Pathology(2006) ;40:32-8.

24- Muller W, Borchard F Prognostic significance of p53 expression in gastric cancer. J Pathol ,(1996);178:255–258.

25 Motojima K, Furui MD, Kohara N, Ito T, Kanematsu T . Expression of p53 protein in gastric carcinomas is not independently prognostic.Surgery(1994); 116:890–895.

26 Kaye PV, Radebold K, Isaacs S, Dent DM.The expression of p53 and p21waf1/cip1in gastric carcinoma:lack of inter-relationship or correlation with prognosis. Eur J Surg Oncol (2000);26:39–43.