Association between Immunohistochemical Expression of p53 Protein and Histopathological Prognostic Factors in Gastric Carcinoma

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Abstract

Background: Mutation in the *TP53* suppressor gene and accumulation of p53 protein are the common genetic events in gastric carcinomas. This mutation has been associated with abnormalities in cell cycle regulation, DNA repair and synthesis, apoptosis, and suggested to be implicated in the prognosis of gastric carcinoma.

Objectives: To assess the immunohistochemical expression of p53 protein in gastric adenocarcinoma and to study the association between p53 protein expression and different histopathological prognostic factors like histological type, tumour grade, depth of invasion, regional lymph node involvement, lymphovascular and perineural invasion.

M**ethods:** In this cross sectional observational study, 60 paraffin blocks of gastric adenocarcinoma were re-evaluated and above mentioned prognostic parameters were assessed. The p53 expression in the tumour cell nucleus of the submitted blocks were assessed by standard immunoperoxidase method. Appropriate positive control were run for each batch of slides.

Results: In this study, out of 60, forty cases (66.67%) showed high expression of p53 protein. Our study showed statistically significant association of p53 protein expression with tumor grade ($p = 0.001$), depth of invasion ($p < 0.001$), lymphovascular invasion ($p < 0.001$) and regional lymph node involvement ($p < 0.001$). However, there was no significant association of p53 expression with histological type of both Lauren ($p = 0.680$) and WHO classification ($p = 0.786$), and perineural invasion ($p = 0.170$).

Conclusion: There was a significant association between immunohistochemical expression of p53 protein and histopathological prognostic factors in gastric adenocarcinoma (tumour grade, depth of invasion, lymphovascular invasion and regional lymph node involvement) except histological type and perineural invasion. These results indicate that immunohistochemical expression of p53 protein is an important prognostic factor of gastric adenocarcinoma, allowing the selection of a group of patients with an extensive therapeutic indication.

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Keywords: Gastric adenocarcinoma, p53 protein, immunohistochemical expression

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Introduction

Carcinoma of the stomach is one of the leading causes of cancer death. World-wide gastric carcinoma (GC) ranks fourth in frequency and third in cancer mortality rate. The incidence of GC in Bangladesh is 5.2 per 100000.[1] Gastric carcinoma is a multi-factorial disease. Several risk factors have been identified in the etiology of gastric carcinoma.[2] Genetic factors are also important but imperfectly elucidated.[3]

Gastric carcinoma have several prognostic and predictive factors. These prognostic factors are widely used to determine whether to apply neoadjuvant therapy in patient with gastric carcinoma .[4]The *TP53,* tumour suppressor gene is thought to play a crucial role in the process of gastric carcinogenesis.[5]The p53, is a tumour suppressor protein that plays a vital role in regulation of genomic stability by controlling cell cycle, DNA repair and inducing apoptosis or cellular senescence when cell damage is beyond repair.[6] MDM2, a p53 specific E3 ubiquitin ligase, is the principal cellular antagonist of p53. In non-stressed healthy cells MDM2 ubiquitinylates p53, leading to its degradation by the proteasome. As a result, in healthy cell, nuclear accumulation is usually not detectable due to the short half life time (5-10min) of the wild type p53 protein. In stressed cell p53 is released from the inhibitory effect of MDM2. The majority of human carcinoma demonstrate bi-allelic loss of function mutations in *TP53* gene. With loss of p53 function, the cell marches blindly along a dangerous path leading to malignant transformation.

Missense mutation within the *TP53* gene results in protein that is stabilized through post transcriptional modification and accumulation within the cell nucleus. Thus the immunohistochemical detection of mutated protein product can be possible.[7]The nuclear staining of p53 protein can be seen in both intestinal and diffuse type gastric carcinoma though commoner in intestinal type. Previous reports suggested that over expression of p53 in gastric carcinoma is associated with bad prognosis. Furthermore, immunohistochemical evaluation of p53 protein may have an important prognostic value as there was reduced survival at five years for patients with p53 positive gastric carcinoma than p53 negative carcinoma.

Restoring functions of p53 would be a major steps in curing gastric carcinoma. Evaluating the effect of adenovirus mediated reintroduction of wild type *TP53* or modulation of MDM2 protein for induction of apoptosis as a potential clinical utility in gene therapy of gastric carcinoma, is a promising field in near future in the prevention of gastric carcinoma.[8] A preoperative assessment of p53 expression could be helpful in identifying patients with high risk of higher grade and more advanced tumours. Hence, evaluation of expression of p53 protein or alterations in its encoded *TP53*gene may provide promising applications for diagnosis, prognosis, or therapeutic targets for gastric carcinoma.[7]

Methods

It was a Cross-sectional observational study carried out from September, 2017 to June, 2019 at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. The studied materials were paraffin blocks of gastric tissues, were taken from 60 patients underwent partial or total gastrectomy in the Department of Surgery & were diagnosed as gastric adenocarcinoma in Pathology Department, BSMMU. Cases were re-evaluated and prognostic parameters including histologic type, tumor grading, depth of invasion, lymphovascular invasion, perineural invasion and regional lymph nodes metastases were assessed. Cases were

classified and interpreted according to Lauren (Lauren, 1965) and WHO (2010) classification. The p53 expression in the tumour cell nucleus of the submitted blocks were assessed by standard immunoperoxidase method using an automatic DAKO immunostainer at immunohistochemistry laboratory, Department of Pathology, BSMMU. The result of p53 protein immunohistochemistry was quantified as immunoreaction score (IRS) (Table I). Appropriate positive controls were run for each batch of slides. The statistical analysis was carried out using the Statistical Package for Social Sciences for Windows (SPSS-22). Continuous variable was expressed as Mean ± SD. Categorical variable was presented by frequency and percentage. The association between the histological parameters of malignancy, and nuclear expression of p53 protein was evaluated with Chi-Square (X2) test. A p-value of < 0.5 was considered as statistically significant.

Table I: Immunoreaction scoring for P53 protein[9]

Percentage of p53 positive cells	Score	Staining intensity	Score
≤ 10%	1	Negative	0
11- 49%	2	Weak	1
50- 79%	3	Moderate	2
≥ 80%	4	Strong	3

IRS score = Percentage of p53 positive cells × Staining intensity
Total score = 0 to 12 {≤ 6 = low and >6 = high}

Results

Age of the patients ranged from 22 to 80 years and mean ± SD was 53.43 ± 12.12 years. The highest number of cases (30%) belonged to 51-60 years age group. Among 60 cases, 37 were males and 23 were females with a male/female ratio of 1.6:1 (Table II).

Table II: Age distribution of the patients (n=60)

Age group (years)	Frequency	Percentage (%)
20-30	4	6.7
31-40	6	10.0
41-50	16	26.7
51-60	18	30.0
61-70	13	21.7
71-80	3	5.0
Total	60	100.0

Mean ± SD = 53.43 ± 12.12; Range 22 – 80 years

According to the histological type of Lauren classification, out of 60 cases, 44 were intestinal type gastric carcinoma of which 30(68.2%) cases showed high expression, and 16 cases were diffuse type gastric carcinoma of which 10(62.5%) showed high expression of p53 protein. According to WHO classification, most of the cases, 42 cases were tubular adenocarcinoma, of which 29(69%) cases showed high expression, and 16 cases were poorly cohesive carcinoma including signet ring cell carcinoma and other variants, of which 10(62.5%) cases showed high expression, and only 2 were mucinous adenocarcinoma of which 1(50%) case showed high expression of p53 protein (Figure 1, 2, 3, 4, 5, 6). Nuclear expression of p53 protein among the histological types of gastric adenocarcinoma yielded statistically insignificant result.

Regarding tumour grading of cases, most of them, 35 were poorly differentiated carcinoma, of which 28(80%) showed high expression, and 20 cases were moderately differentiated carcinoma, of which 12(60%) showed high expression, and Only 5 were well differentiated carcinoma, all of which showed low expression of p53 protein(Figure 1,2,3,4,5,6). So, it was observed that with advancing grade of tumor, frequency of high expression of p53 protein was high and yielded statistically significant result (p value = 0.001).

Distribution of cases according to depth of invasion, most of them, 26 had serosal invasion, of which almost all 25(96.2%) cases showed high expression, and subserosal invasion were in 16 cases, of which 12(75%) cases showed high expression, and muscularis propria invasion were in 13 cases, of which majority 12(92.3%) cases showed expression of p53 protein, and remaining 5 cases had submucosal invasion, of which 2(40%) high expression of p53 protein respectively. With increasing depth of invasion, frequency of high expression of p53 protein was high and yielded statistically significant result (p value = 0.001)

Table III: Association between the nuclear expression of p53 protein and histopathological prognostic parameters of gastric adenocarcinoma

Histopathological parameters		No of cases	Nuclear expression of p53 protein		p-value
			High No. (%)	Low No. (%)	
Histological type – Lauren classification	Intestinal type gastric carcinoma	44	30(68.2%)	14(31.8%)	0.680*
	Diffuse type gastric carcinoma	16	10(62.5%)	6(37.5%)	
WHO classification	Tubular adenocarcinoma	42	29(69.0%)	13(31.0%)	0.786*
	Mucinous adenocarcinoma	2	1(50.0%)	1(50.0%)	
	Poorly cohesive carcinoma including signet ring cell carcinoma and other variants	16	10(62.5%)	6(37.5%)	
Tumour grading	Well differentiated	5	0(0.0%)	5(100.0%)	0.001**
	Moderately differentiated	20	12(60.0%)	8(40.0%)	
	Poorly differentiated	35	28(80.0%)	7(20.0%)	
Depth of invasion	Up to sub mucosal invasion	5	2(40.0%)	3(60.0%)	<0.001**
	Muscularis propria invasion	13	1(7.7%)	12(92.3%)	
	Subserosal invasion	16	12(75.0%)	4(25.0%)	
	Serosal or adjacent structure invasion	26	25(96.2%)	1(3.8%)	
Regional lymph node metastasis	Present	45	38(84.4%)	7(15.6%)	<0.001**
	Absent	15	2(13.3%)	13(86.7%)	
Lymphovascular invasion	Present	40	37(92.5%)	3(7.5%)	<0.001**
	Absent	20	3(15.0%)	17(85.0%)	
Perineural invasion	Present	19	15(78.9%)	4(21.1%)	0.170*
	Absent	41	25(61.0%)	16(39.0%)	

** significant at $p<0.05$; *insignificant at $p\geq0.05$

Regarding regional lymph node metastasis, most of the cases, 45 had regional lymph node metastasis, of which 38(84.4%) cases showed high expression and in the remaining 15 cases which had no lymph node metastasis, majority 13(86.7%) cases showed low expression of p53 protein. The result was statistically significant.

Among the cases, 40 showed lymphovascular invasion of which 37(92.5%) cases showed high expression, and 20 showed no lymphovascular invasion of which only 3(15%) cases showed high expression. So, nuclear expression of p53 protein was statistically significant (p value < 0.001) in respect of lymphovascular invasion.

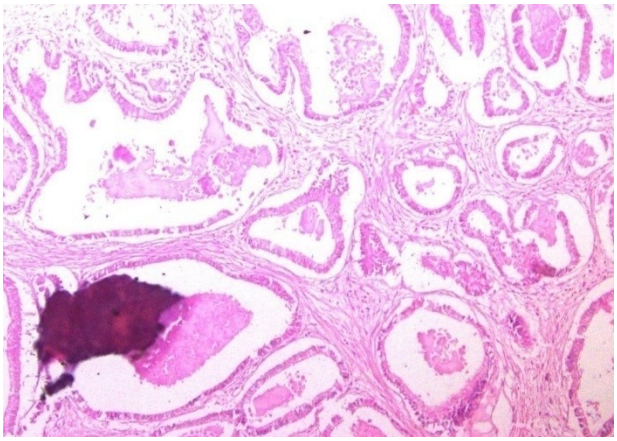

Figure 1. Photomicrograph of a case of intestinal type of well differentiated adenocarcinoma (Case no 38, H&E stain, x100)

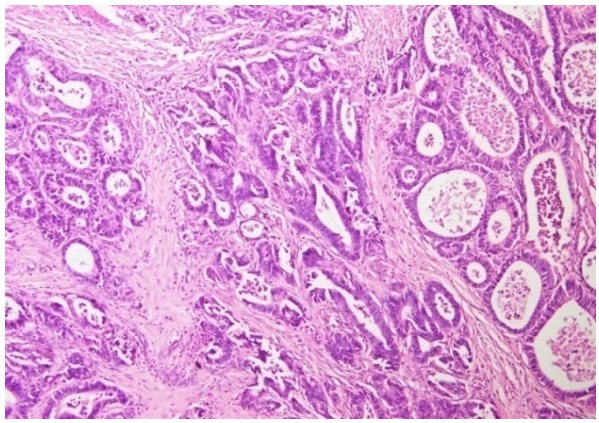

Figure 2. Photomicrograph of a case of intestinal type moderately differentiated adenocarcinoma (case no.26, H&E stain, x200)

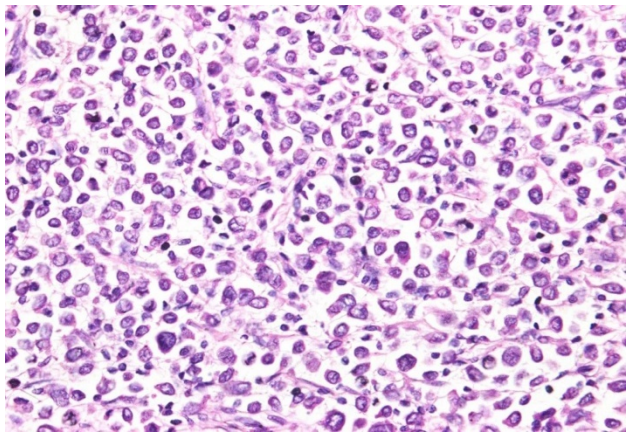

Figure 3. Photomicrograph of a case of diffuse type (Lauren) adenocarcinoma (Case no 13, H&E stain, x400)

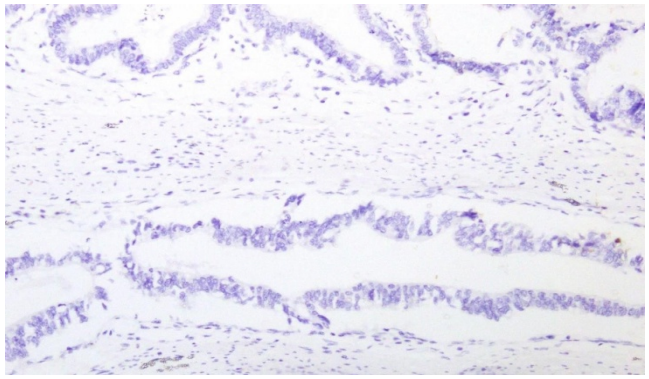

Figure 4. Photomicrograph shows low expression of p53 protein in well differentiated adenocarcinoma , intestinal type (Case no 15, IHC, x400)

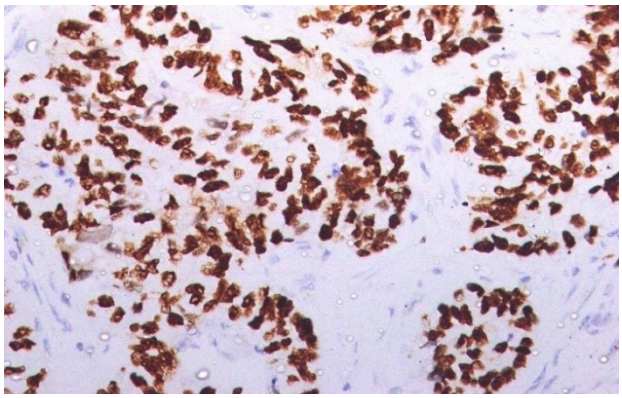

Figure 5. Photomicrograph shows high expression of p53 in moderately differentiated adenocarcinoma , intestinal type (Case no 26, IHC, x400)

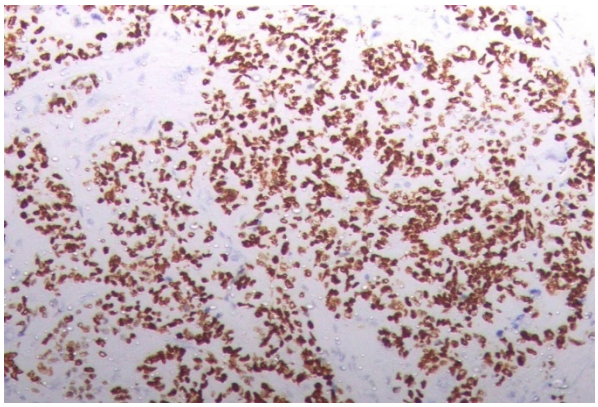

Figure 6. Photomicrograph shows high expression of p53 in poorly differentiated adenocarcinoma, intestinal type (Case no 42, IHC,x400)

Discussion

Gastric carcinoma is a common tumour in the world. The *TP53* is a tumor suppressor gene, mutation in which plays an important role in tumorogenesis and progression of many carcinomas including gastric carcinoma. However, the relationship between p53 expression and histopathological prognostic parameter including histologic type, tumor grading, depth of invasion, lymphovascular invasion, perineural invasion and regional lymph nodes metastases remains ambiguous.In this study, cases were classified and interpreted according to Lauren and WHO (2010) classification.[10]Out of 60 cases, 40(66.7%) cases showed high expression of p53 protein, while 20(33.3%) cases showed low expression.

In our study, according to Lauren classification,Nuclear expression of p53 protein among two types of gastric adenocarcinoma revealed statistically insignificant results (p-value = 0.680) (TableIII). Malini et al., (2016) found 32 cases of intestinal type, of which 18 (56.3%) showed over expression, and 12 cases of diffuse type, of which 8 (66.6%) showed over expression. There was no statistically significant association between p53 expression and histological type of gastric adenocarcinoma.[11] According to Lauren classification, Joypaul et al., (1994); Azarhoush et al., (2008); Starzynska et al., (1992) similarly found statistically insignificant differences in relationship to high expression of p53 protein and histological types.[12,13,14]

According to WHO classification,in this study, there was no significant association between nuclear expression of p53 protein and histological type according to WHO classification (Table III). Lazar et al., (2010) found positive p53 expression in 12(42%) out of 28 cases of tubular adenocarcinoma, 3(60%) out of 5 papillary, 4(50%) out of 8 mucinous, 5(29.4%) out of 17 cases of signet ring cell carcinoma and 1(33.3%) out of 3 anaplastic carcinoma. There was no statistically significant association between p53 expression and histological type according to WHO classification (p-value: 0.152) similar to this study.Here it should be noted that intestinal type gastric adenocarcinoma of Lauren classification system classified as Papillary, tubular and mucinous adenocarcinoma in WHO classification system and similarly, diffuse type of Lauren system considered as poorly cohesive carcinomas including signet ring cell

carcinoma and other variants in WHO classification system.[15]

In consideration to tumor grade, this study showed that with advancing grade of tumor, frequency of high expression of p53 protein was high and yielded statistically significant result (Table III). This result is similar to the study conducted by Lazar et al., (2008) in which out of 39 cases of poorly differentiated adenocarcinoma, 18(46.1%) cases showed high expression of p53 protein, 20 moderately differentiated adenocarcinoma, of which 7(35%) cases showed high expression of p53 protein and 2 well differentiated adenocarcinoma, none of which showed high expression of p53 protein.They observed a significant association between the tumor grade and p53 expression (p-value = 0.039).[5] Another study done by Malini et al., (2016) also found statistically significant association between high expression of p53 protein and tumour grade (p-value = 0.049).[11]

In this present study, with increasing depth of invasion, frequency of high expression of p53 protein was high and yielded statistically significant result (p-value <0.001) (Table III). This result is similar to the study conducted by Ghaffarzadegan et al., (2004) which showed high expression of p53 protein in 29(80.5%) out of 36 cases with subserosal invasion, 8(72.7%) out of 11 cases with muscularis propria invasion and 2(40%) out of 5 cases with mucosa and submucosal invasion. Statistical analysis showed significant result (p-value = 0.037).[16] Another study done by Malini et al., (2016) also found statistically significant result (p-value <0.001) which is consistent with this present study.[11]

Regarding lymph node metastasis, there was a significant association between lymph node metastasis and p53 expression (p-value <0.001) (Table III). This result is similar to the study conducted by Starzinska et al., (1992) which showed high expression of p53 protein in 20(71%) cases, out of 28 cases with lymph node metastasis and only 2(13%) cases, out of 23 cases without lymph node metastasis. Statistical analysis revealed a significant association (p-value: <0.001) similar to this study.[14] Malini et al. (2016) and Kakezi et al. (1993) also found significant association between nuclear expression of p53 protein and lymph node metastasis.[11, 17]

In consideration to lymphovascular invasion, in this study, nuclear expression of p53 protein was statistically significant in respect of lymphovascular invasion (p-value <0.001) (Table III). A study done by Gabbert et al., (1995) found 199 cases with positive lymphovascular invasion, of which 125(62.8%) showed high expression of p53 protein and out of 219 cases with negative lymphovascular invasion, 116(53%) cases showed high expression of p53 protein. Statistical analysis yielded a significant result (p-value 0.047) which is consistent with our study.[18]

In our study, nuclear expression of p53 protein was statistically non-significant in respect of perineural invasion (Table III). Jung et al., (2014) found 50 cases of positive perineural invasion and 183 cases of absent perineural invasion. Among the cases with perineural invasion, 45(90%) cases showed high expression. Among the cases with absent perineural invasion, 165(90.2%) cases showed high expression.[19] Statistical analysis yielded insignificant results (p-value = 0.973) which is consistent with this present study. Similarly, Ugras et al., (2014) also found statistically insignificant result.[20]

Conclusion

In this present study, there was a significant association of p53 protein expression with tumor grade, depth of invasion, lymphovascular invasion and regional lymph

node involvement, but no significant association with histological types (both Lauren and WHO classification) and perineural invasion. These results indicate that immunohistochemical evaluation of p53 protein is an important prognostic factor of gastric carcinoma, allowing the selection of a group of patients with an extensive therapeutic indication.

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