Correlation of P53 Expression with Various Clinicopathological Parameters of Gastric Carcinoma and Its Relationship with Survival

Tushar Hiralal Sankalecha, Sudhir Jagdish Gupta, Nitin Rangrao Gaikwad, Suresh Narayanrao Ughade

AIM: Gastric cancer is the most aggressive type of cancer. The immunohistochemical protein expression of mutant p53 has been proposed as a potential tool to evaluate the biological behavior of gastric cancer. Predictive value of p53 for survival is debatable; hence this study was formulated to know the survival of patients with p53 expression in gastric cancer.

METHODS: It is prospective study from September 2014 - July 2015, included 58 consecutive patients of gastric cancer. Biopsy specimens were treated immuno-histochemically and expression of p53 gene was analyzed by Immunoreactive Score (IRS). These findings were then compared with clinico-pathological parameters like age, gender, tumor location, tumor size, Lauren's classification and TNM staging according to American joint committee for cancer guidelines, using CT scan of abdomen, and histopathological grading and types according to WHO classification.

RESULTS: Mp53 expression was observed in 90% of gastric cancer patients among which 37 (63.8%) patients showed high and 21 (36.2%) patients showed low p53 expression. Level of p53 expression was found significantly associated with age, tumor site, tumor size, histological grade, T stage, M stage and Clinical stage. Multivariate analysis shows that high p53 expression is an independent predictor of survival. On Kaplan-Meier survival analysis, patients with p53 high expression had significantly shorter overall survival than those patients with low p53 expression.

CONCLUSION: Expression of p53 correlates with the survival and is a simple, effective and reproducible modality to determine the prognosis and survival in various grades & stages of gastric cancer.

Key words: Gastric cancer; Gene expression; Immunohistochemistry
Survival correlation of p53 expression in gastric cancer patients

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Immunohistochemistry

Immunohistochemistry was performed on tissues fixed in 10% neutral buffered formalin. The sections were cut serially to 5 μm for immunohistochemical staining. Peroxidase Detection System (Streptavidin-Biotin Detection System HRP-DAB; Product Code: RE7110K, Novo- castra kit) was used. Endogenous peroxidase activity was blocked by treating hydrated sections with 3% H2O2 for 10 min. The slides were immersed in a microwave oven for 10 min in 0.01M sodium citrate buffer (pH6.0) for antigen retrieval. After cooling at room temperature in a humidifying chamber for 60 min, the sections were incubated at 4°C for 12 h in a humidifying chamber for 60 min. The slides were then washed with 0.01M PBS pH 7.4, and the sections were incubated with 10% serum for 10 min. The pre-diluted p53 antibody (clone DO-7; Product code: N1581, Dako, Denmark) was used as a positive control. All patients received secondary biotinylated antibody and streptavidin-peroxidase reagent at room temperature in a humidifying chamber for 30 min. Freshly prepared substrate/chromogen solution of 3, 3’ Diaminobenzidine (mixing 5 ml of concentrated DAB in 50 ml of substrate buffer) was used to detect the antigen–antibody reaction. Finally, the sections were counterstained in Mayer’s hematoxylin.

The IHC staining of mutant (MT) p53 was assessed according to the immunoreactive score (IRS) (Table 1A, 1B), which is based on the percentage of positive cells and the staining intensity. The cells were considered positive for p53 antigen when there was an intranuclear DAB staining (brown color) (Figure 1C, 1D). The percentage of positive cells was assessed with the help of labeling index (P53 Labeling index = Number of IHC Positive Cells X 100/ total number of cells observed). The two scores were multiplied to get IRS score, ranging from 0 to 12 and corresponded to ≤6 as low and >6 as high groups of p53 expression. The counting was done by two observers and the mean was taken as a final count.

Statistical analysis

The Statistical Package for the Social Sciences software version 20 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. The χ2 test and Fisher’s exact test were performed to evaluate the correlation between the clinicopathological features of the patients and the p53 expression level. For the survival analysis, the Kaplan-Meier method with log-rank test was used. Prognostic factors were further evaluated in univariate and multivariate logistic regression analysis using the Cox’s proportional hazards model to know relevant prognostic variables. The risk ratio (RR) with 95% confidence interval (95% CI) was used to assess the relationships between those factors and overall survival. A P value < 0.05 was considered as statistically significant.

RESULTS

Total 58 patients of gastric cancer were enrolled in our study. 38 (65.5%) were male and 20 (34.5%) were female with M: F ratio 1.9: 1. Age range in study population was 35-80 years with mean age of 59.63 (SD ± 6.2) years. Mp53 expression was observed in 90% of gastric cancer patients. Among the total of 58 patients 90% of patients show positive Mp53 expression and remaining 10% of patients show no expression. According to IRS scoring system 0 to 6 score is considered under low expression group. Therefore the 10% of patients showing no expression are considered under low expression group as per IRS system. Therefore low expression group numbers were up to 21 and high expression group numbers up to 37 patients.
Level of p53 expression was found significantly associated with age, tumor site, tumor size, histological grade, T stage, M stage and clinical stage, whereas it was not associated with gender, N stage, Lauren classification and histopathological type of tumor (Table 2A, 2B).

On Kaplan-Meier survival analysis, patients with high p53 expression group had significantly shorter survival than patients with low p53 expression group (log-rank $P < 0.00001$). (Figure 1) After 12 months of follow up, 56.76% (CI 0.39-0.70) of patients with p53 high expression group and 95.24% (CI 0.70-0.99) patients with p53 low expression group were alive. Multivariate analysis by Cox regression model further showed that high p53 expression was independent predictor of overall poorer survival (HR = 9.34; 95% CI 1.003-90.90, $P = 0.049$). However, gender, tumor location, tumor size, histological grade, histopathological type, Lauren classification, T stage, N stage, M stage and clinical stage were not significant predictors of survival in gastric cancer patients (Table 3 and 4).

**DISCUSSION**

Tumorigenesis of gastric cancer is a complex process which is affected by environmental as well as genetic factors. The exact pathogenesis of gastric cancer remains unclear; however various studies indicate it to be multifactorial.

The p53 is a tumor suppressor gene, localized to chromosome 17q13.1 and is classically considered as the “guardian of the genome”. P53 protein is a product of p53 gene, composed of 393 amino acids, which functions in G1 phase of cell cycle arrest to allow the repair of DNA damage and to prevent the cell from dividing.

**Table 2A** Comparison of clinicopathological parameters with p53 expression.

| Characteristics | Total | Low Expression | High Expression | P Value |
|-----------------|-------|---------------|----------------|---------|
| Gender          |       |               |                |         |
| male            | 38    | 15            | 23             | 0.476   |
| female          | 20    | 6             | 14             |         |
| age             |       |               |                |         |
| < 60            | 25    | 14            | 11             | 0.006*  |
| ≥ 60            | 33    | 7             | 26             |         |
| Tumour location |       |               |                |         |
| proximal        | 18    | 2             | 16             | 0.008*  |
| distal          | 40    | 19            | 21             |         |
| Tumour size     |       |               |                |         |
| ≤ 5 cm          | 25    | 18            | 7              | 0.0001* |
| > 5 cm          | 33    | 3             | 30             |         |
| Pathological grade | |           |                |         |
| well diff.      | 17    | 13            | 4              | 0.0001* |
| mod diff.       | 21    | 6             | 15             |         |
| poorly diff.    | 20    | 2             | 18             |         |
| Lauren classification | |         |                |         |
| intestinal      | 30    | 15            | 15             |         |
| diffuse         | 18    | 3             | 15             | 0.06    |
| intermediate    | 10    | 3             | 7              |         |
| Histological type | |           |                |         |
| Tubular         | 28    | 13            | 15             |         |
| Pappilary       | 10    | 5             | 5              |         |
| Signet ring     | 10    | 3             | 7              | 0.09    |
| Mucinous        | 5     | 0             | 5              |         |
| Mixed           | 5     | 0             | 5              |         |

* = Statistically significant

The $\chi^2$ test was used to evaluate the association between p53 expression and clinicopathological parameters.

**Table 2B** Comparison of TNM and Clinical stage with p53 expression.

| Characteristics | Total | Low Expression | High Expression | P Value |
|-----------------|-------|---------------|----------------|---------|
| T1              | 10    | 9             | 1              |         |
| T2              | 14    | 8             | 6              | 0.0001* |
| T3              | 21    | 3             | 18             |         |
| T4              | 13    | 1             | 12             |         |
| N STAGE         |       |               |                |         |
| N0              | 2     | 1             | 1              |         |
| N1              | 15    | 9             | 6              | 0.138   |
| N2              | 32    | 9             | 23             |         |
| N3              | 9     | 2             | 7              |         |
| M STAGE         |       |               |                |         |
| M0              | 45    | 20            | 25             | 0.015*  |
| M1              | 13    | 1             | 12             |         |
| Clinical stage  |       |               |                |         |
| I               | 8     | 6             | 2              |         |
| II              | 14    | 10            | 4              | 0.001*  |
| III             | 23    | 4             | 19             |         |
| IV              | 13    | 1             | 12             |         |

* = Statistically significant

The $\chi^2$ test was used to evaluate the association between p53 expression and clinicopathological parameters.

**Table 1** Immunoreaction score (IRS)hii.

| Percentage 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 = Table 1A × Table 1B

Total score = 0 to 12 [≤ 6 = low and > 6 = high]

**Chart 1** Survival analysis using Kaplan meier method between p53 low and high expression group.

The Kaplan Meier survival curve of gastric cancer patients (n=58). Patients having high levels of p53 protein expression are associated with a poor survival.
entering into the S phase or alternatively to guide damaged cells to apoptosis. So p53 played major role in cell cycle regulation, DNA repair and cell apoptosis. Mutation in p53 results in the loss of its ability to induce cell death leading to uncontrolled cell growth which promotes tumorigenesis. Normally p53 gene is not detected immunohistochecmically but when mutated p53 becomes stabilized and has increased half life, thus it accumulates in the cell nucleus and can be detected immunohistochemically using monoclonal antibodies[24-27].

Mutations of the p53 gene have been observed in many malignancies and are found in ~30%-50% of lung, colorectal, head and neck, ovarian cancers and esophageal cancer and in ~5% of leukemia, sarcoma, melanoma, testicular cancer, and cervical cancer patients[28-29]. This lead to many observers to study p53 mutation profile meticulously in gastric cancer patients also. Laboratory analysis of p53 gene is done by three methods: (1) Polymerase chain reaction (PCR) (2) Detection of serum p53 antibody and (3) Immunohistochemistry (IHC)[30]. In comparison to DNA sequencing, immunohistochemical methods are cheaper, easier, widely available throughout the world and more familiar to pathologists. P53 protein accumulation not only represent mutated p53 gene but also represent effect of other genes on its expression, so expression of p53 needs to be assessed separately for survival prediction.

P53 protein expression is found to be variable, may be because of using different antibody and different techniques of analysis by different studies. P53 expression is found in about 19% to 90% patients of gastric cancer. A study done by Akshatha C et al, Fenoglio-Preiser et al, Brito et al and Ghaffarzadegan et al [31-34] noted p53 positivity in 62.5%, 19%, 35% and 75% of gastric carcinoma patients respectively. In our study we found 90% patients of gastric cancer showing p53 expression.

Daniela lazar et al in 2010[35] showed that there was insignificant association with gender, in spite of having higher incidence of gastric cancer in male patients. Similar results were seen in our study.

Risk of carcinogenesis increases with increase in age, a study done by Honda T et al[36] confirmed that age group of > 60 years has significantly higher risk for gastric cancer. Similar results were seen in our study i.e. p53 expression is significantly higher in age group of > 60 years. But these results are not confirmed by Daniela lazar et al[36].

One of the important parameter to assess prognosis in stomach cancer patients is histopathologic grade of tumor. As grade increases prognosis become poorer. When p53 expression was compared with histopathological grading we found that its expression increases significantly with the increasing histopathological grades. So overexpression of p53 can be linked with histological aggressiveness of the tumor. Similar results were seen by Sasaki I et al[37] while some other studies like study done by Akshatha C et al[31] showed no correlation with histological grade.

The Lauren classification is frequently used in gastric cancer patients. It is based on how the gastric tissue looks and behaves when examined under a microscope. It divides adenocarcinoma of the stomach into 3 main types: intestinal, Diffuse and mixed type.

When a Lauren study which is similar to the observation noted by Nabi the stomach into 3 main types: intestinal, Diffuse and mixed type. when examined under a microscope. It divides adenocarcinoma of patients. It is based on how the gastric tissue looks and behaves with increasing grades of T, M & clinical stages (I to IV) [Table 2]. Hence p53 expression can also be linked with invasiveness & clinical aggressiveness of the tumor.

Hence p53 expression can also be linked with invasiveness & clinical aggressiveness of the tumor. Similar finding were seen in Akshatha C et al, Daniela lazar et al[31].

Other important parameters are T, N, M & clinical stage. As the stage increases patients survival decreases. On comparison with these parameters, we found that p53 expression was significantly increased with increasing grades of T, M & clinical stages (I to IV) [Table 2]. Hence p53 expression can also be linked with invasiveness & clinical aggressiveness of the tumor.

Whereas our analysis also shows p53 expression was not correlated with N stage. Similar finding were seen in Akshatha C et al, Daniela lazar et al and Filiz et al[31,35,41].

The results of Kaplan meier analysis demonstrates that patients with high p53 expression show significantly poor survival than the patients with low p53 expression. This result contradicts many previous studies, which fails to show association between p53 expression & survival[31,35,41]. The results of univariate analysis showed that tumor size, histopathological grading, T stage, M stage, clinical

Table 3 Univariate analysis to identify the factors that affect the survival

| Variables | Odds Ratio (Or) | Confidence Interval (CI) | P Value |
|-----------|----------------|--------------------------|---------|
| Age       | 2.2            | 0.60-9.7                 | 0.175   |
| Gender    | 0.46           | 0.124-1.77               | 0.194   |
| Tumour location | 1.9 | 0.48-7.24               | 0.282   |
| Tumour size | 9.5       | 1.7-93                  | 0.0019* |
| Hp grading | 4.3          | 0.80-43                 | 0.05*   |
| Hp type   | 0.465          |                          |         |
| Lauren classification | 1.8 | 0.56-6.8                | 0.301   |
| T stage   | 4.9            | 1.09-29.7                | 0.01*   |
| N stage   | 0.354          |                          |         |
| M stage   | 10.4           | 2.1-55.7                 | 0.0003* |
| Clinical stage | 7.14 | 1.3-70.1                | 0.0082* |
| P53 expression | 15   | 1.9-670                | 0.002*  |
| *: Statistically significant |
| **: Statistically significant |

**: P <0.05 was considered as a statistically significant difference.

* OR: odds ratio; CI: confidence interval.

Table 4 Multivariate analysis to identify the factors that independently affect the survival

| Variables | Odds Ratio (Or) | Confidence Interval (CI) | P Value |
|-----------|----------------|--------------------------|---------|
| Gender    | 2.64           | 0.58-11.96               | 0.206   |
| N stage   | 0.366          | 0.102-1.31               | 0.125   |
| M stage   | 0.224          | 0.045-1.10               | 0.066   |
| P53 expression | 9.34 | 1.003-90.9            | 0.049** |
| *: Statistically significant |
| **: Statistically significant |

* The Cox proportional hazards model was used to find out the factors that had a significant influence on overall survival

* OR: odds ratio; CI: confidence interval.
stage and p53 expression were significantly correlated with the survival. Additionally, multivariate analysis revealed p53 expression was found to be independent variable affecting gastric cancer patient’s survival.

CONCLUSION

Significant numbers of gastric cancer patients demonstrated increased expression of p53 and is found to be independent variable affecting survival. Finally we arrived at conclusion; Immunohistochemical analysis of p53 is simple & effective modality which can be used to determine the prognosis and survival in various grades & stages of gastric cancer.

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