

## Evaluation of correlation between the expression of p53 as an immunohistochemical marker & prognosis of gastric cancer

Nastaran Ranjbari <sup>1</sup>, Neda Hojatpanah <sup>2,\*</sup>

<sup>1</sup> Assistant Professor of Pathology, Department of Pathology, Imam Khomeini Hospital, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

<sup>2</sup> MD, Resident of Pathology, Department of Pathology, Imam Khomeini Hospital, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

\*Corresponding author: Neda Hojatpanah, MD, Resident of Pathology, Department of Pathology, Imam Khomeini hospital, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. Tel: +989106006032, E-mail: hojatpanahn@gmail.com

Received: Apr 10, 2014; Revised: Jun 16, 2014; Accepted: Aug 13, 2014

**Background:** Gastric cancer is the most common form of gastro intestinal malignancy in certain part of the world and the most common type is Adenocarcinoma. The tumor suppressor gene p53 is a nuclear protein which plays a key role in tumor progression. Therefore in this study, we investigate p53 expression in gastric cancer specimens and possible relationship between this marker and clinical-pathological factors of disease.

**Material and methods:** This study included 54 patients with primary Adenocarcinoma of stomach whom underwent gastrectomy at the Ahvaz Emam Khomeini hospital. Tumor specimens examined by immuno-histochemical staining with monoclonal antibody against p53. For evaluate value of p53, A stain was considered positive, when %20 of cancer cells showed a positive nuclear staining.

**Result:** P53 marker was positive in 40 cases (%74) of all cases. Stronger expression of p53 was related with higher grade ( $p=0/035$ ) and lymph node metastasis ( $p=0/033$ ). No significant differences were seen between value of p53 marker with age, sex, tumor size, depth of invasion and tumor type. Expression of p53 was not correlate with vascular and neural invasion or involvement of margins.

**Conclusion:** In gastric cancer, the expression of p53 provides significant information about prognosis and evaluation of p53 level could be a useful tool in identification of patients with more aggressive disease.

**Keywords:** p53, Adenocarcinoma, Immunohistochemical staining

### Background

In the last several years, developments in molecular genetics have shown that activation of oncogenes and inactivation of tumor-suppressor genes, results in cancer development in various organs (1). It has been shown that the p53 gene is a tumour-suppressor gene and its mutations play an important role in the development of human malignancies (2). Mutations of p53 are common and found in various human cancers (3, 4). Although, gastric cancer is the most common form of gastro-intestinal malignancy in certain parts of the world, relatively little is known about the molecular occurrences leading to its development (1). Immunohistochemical studies show that antibodies against p53 proteins may be used as screening method for the presence of p53 (5). Therefore, the intent of the present immuno-histochemical study is to determine to what extent histopathological findings are identified in the p53 tissue status of patients with resectable gastric cancer.

### Materials and Method

**Patients:** This study included 54 patients with primary gastric cancer, all of whom underwent gastrectomy at the Department of Surgery of Emamkhomeini hospital of the Ahvaz jundishapur University of Medical sciences, from 2003-2012.

**Immunohistochemical staining:** All tumour specimens were fixed in 10% formaldehyde and em-

bedded in paraffin. The 4  $\mu$ m thick sections were cut from the paraffin blocks containing representative histological features of the tumors. Paraffin was removed from the sections in xylene after hydration the sections were placed in Phosphate Buffered Saline (PBS) at pH 7.4 and 3% H<sub>2</sub>O<sub>2</sub> was applied in order to abolish nonspecific binding of the primary antibody. Then the slides were incubated with the monoclonal antibody p53- Do7 overnight at 4°C. In the next step, the slides were incubated with rabbit anti-mouse antibody. Weak nuclear counterstaining was performed with hematoxylin. Incidences of nuclear accumulation of p53 protein were determined for each specimen (figure 1), also depth of tumour invasion, Grade, microscopic vessels and neural invasion, lymph node status and margins and lauren type. For evaluate value of p53, A stain was considered positive, when %20 of cancer cells showed a positive nuclear staining (figure2).

**Statistical analysis:** The kendalls tau-b test was used for testing differences about tumor size, depth of invasion and Grade. The pearson chi-square test was used for other factors such as lauren type, lymph node status. All calculations were performed using the statistical software SPSS version 15.

## Results

In 54 primary gastric carcinomas, we compared common prognostic markers such as age, sex, tumor size, Lauren type, depth of invasion, tumor grading, lymph node status, vascular and neural invasion, involvement of surgical margins and p53 expression. The tumors examined in this study were separated into two groups, based upon the percentile of p53-protein-positive cell nuclei. A total of 40 tumours (74%) stained positive for p53; 14 tumors with a p53 protein expression of <20% were treated as negative results.

There was no obvious relation between p53 staining and the age or sex of the patients. No significant correlations were found between p53 tissue status and tumor size, lauren type, depth of invasion, vascular and neural invasion and involvement of surgical margins.

In contrast, there was a significant association between p53 tissue status and the metastatic spread to lymph nodes. p53 positive tumors were associated with a higher incidence of metastasis to lymph nodes (88.5%) than were p53 negative tumors (11.5%;  $p < 0.05$ ). Furthermore, differences were also noted in tumor Grading. Stronger expression of p53 was related with higher grade ( $p < 0.05$ ).

## Discussion

Recent data have shown that mutations of the p53 gene are common in certain human tumors, including lung and colon tumors (5, 6). In contrast, the significance of p53 expression in gastric carcinomas remains a point of controversy (7). Therefore in this study, immunohistochemical examination methods were employed to investigate p53 expression in resected gastric cancer specimens. In the present study, 74% of the gastric cancers were considered p53 positive (>20% of cancer cells staining positive) that was different between various studies (1-6, 8, 9). One explanation for these results can be derived from differences in immunohistochemical techniques employed and p53 antibodies used. The correlation of p53 positive tissue status and age or gender was confirmed in the study of Lee KE et al (13) but in our study and Chakavarthi et al was not (1). As Chakavarthi et al (1), no correlation was found between positive p53 tissue status and type of tumor, in contrast to Lazar D and Lee KE et al (14, 13) that expression of p53 was more in intestinal type. The correlation of p53 positive and size of tumor was confirmed in the study of Tzanakis, Shiroko and Lee et al (8, 11, 13) but in our study was not. As Lazar D et al (14), correlation was found between positive p53 tissue status and histological grade of tumor differentiation but Chakavarthi et al and Muller (1, 9) did not find. Positive expression of p53 correlated with depth of tumor invasion in the study of Seo et al, Shiroko et al and Lazar et al (2, 11, 14) but was not confirmed in our study and Chakavarthi et al and Muller (1, 9). In the own collective, lymph node metastases were found more frequently among p53 positive

tumors ( $p < 0.05$ ). This observation was confirmed in studies by Chakavarthi et al (88.3%) (1), Tzanakis (8), Yonemura et al ( $p < 0/001$ ) (10), Shiroko et al and Pinto-de-Sousa et al (11, 12). In the Muller et al study was not such finding (9). No correlation was found between positive p53 tissue status and vascular and neural invasion and involvement of margins in our study, but Pinto-de-Sousa et al and Lazar et al (12, 14) found correlation with vascular invasion.

## Conclusion

Immunohistochemical analysis of primary gastric cancer appears to be a simple method screening the presence of mutant p53 protein. This study was able to demonstrate that p53 immunoreactivity in gastric cancer is closely associated with Grade of tumor and lymph node metastasis. Therefore, data on the immunohistochemistry of p53 expression in gastric cancer may be clinically useful in obtaining information on the metastatic potential to lymph nodes and additionally in determining the treatment of these patients.

## Acknowledgement

Thanks of pathology department staffs of Imam Khomeini Hospital affiliated to medical school of Ahvaz Jundishapur University of medical sciences.

## References

1. Chakavarthi S, Seng Long A, Hannein B, Pasupati T. The expression of p53 as a reliable immune histochemical marker for clinicopathological correlation of gastric adenocarcinoma. *Research journal of medical sciences*. 2010;4(1) 15-19.
2. Seo YH, Joo YE, Choi SK, Rew JS, Park CS, Kim SJ. Prognostic significance of p21 and p53 expression in gastric cancer. *Korean J Intern Med*. 2003;18(2):98-103.
3. Schneider PM, Casson AG, Levin B, et al. Mutations of p53 in Barrett's esophagus and Barrett's cancer: a prospective study of ninety-eight cases. *J Thorac Cardiovasc Surg*. 1996;111:323-331.
4. Kim JH, Takahashi T, Chiba I, Park JG, Birrer MJ, Roh JK, De Lee H, Kim JP, Minna JD, Gazdar AF. Occurrence of p53 gene abnormalities in gastric carcinoma and cell lines. *J Natl Cancer Inst* 1991;83(13):938-43.
5. Carnerio F, David L, Simoes MS, Seruca R, Nesland JM. Oncogenes and oncosuppressor genes in gastric carcinoma. *surg. Pathol*. 1994;5(3):225-238.
6. Strazynska T, Bromery M, Ghosh A, Stern P. Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. *Br J Cancer* 1992;66(3):558-62.
7. Okuyama T, Maehara Y, Kabashima A, Takahashi I, Kakeji Y, Sugimachi K. combined evaluation of expression of p53 and p21 as prognostic factors for patient with a gastric carcinoma. *Oncology*. 2002; 63:353-361.
8. Tzanakis NE, Peros G, Karakitsos P, Giannopoulos GA, Ef-stathiou SP, Rallis G, Tsigris C, Kostakis A, Nikiteas NI. Prognostic significance of p53 and Ki 67 proteins expression in greek gastric cancer patient. *Acta Chir Belg*. 2009;109(5):606-11.
9. Muller w, Bochar F. Prognostic influence of p53 expression in gastric cancer. *J Pathol*. 1997;181(3):255-8.
10. Yonemura y, fushida a, tsugawa K, ninomiya L, fonseca L, et al. correlation of p53 expression and proliferative activity in gastric cancer. *Anal cell pathol*. 1993;5:277-288.
11. Shiroko T, Saji S, Kawaguchi Y, Kageyama T, Kunieda K,

- Umamoto T. A study on the relationship between clinico pathological findings of gastric cancer and its biological behavior such as DNA ploidy pattern and immunohistochemical staining of PCNA, laminin, p53 and nm 23. *Gan To Kagaku Ryoho*. 1995;22 Suppl 2:118-23.
12. Pinto-de-Sousa J, Silva F, David L, Leitão D, Seixas M, Pimenta A, Cardoso-de-Oliveira M. Clinicopathological significance and survival influence of p53 protein expression in gastric carcinoma. *Histopathology*. 2004;44(4):323-31.
13. Lee KE, Lee HJ, Kim YH, Yu HJ, Yang HK, Kim WH, Lee KU, Choe KJ, Kim JP. Prognostic significance of p53, nm23, PCNA and c-erbB-2 in gastric cancer. *Jpn J Clin Oncol* 2003;33(4):173-9.
14. Lazăr D, Tăban S, Sporea I, Dema A, Cornianu M, Lazăr E, Goldiș A, Rațiu I, Vernic C. The immunohistochemical expression of the p53-protein in gastric carcinomas. Correlation with clinicopathological factors and survival of patients. *Rom. J Morphol Embryol*. 2010; 51(2):249-57.

