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Immunohistochemical Analysis of p53, Ki-67, CD44, HER-2/neu Expression Patterns in Gastric Cancer, and Their Association with One Year Survival in North-West of Iran.

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ABSTRACT

Introduction: Gastric cancer remains the second most common cause of cancer-related deaths worldwide. In many malignancies like, lung and breast, multiple prognostic factors are known, such as mutations in Ki-67, HER-2/neu, p53. In this study, we evaluated immunohistochemical protein expression patterns of cell-cycle-regulators p53, proliferation marker Ki-67, surface expression of CD44, HER-2/neu oncogene proposed as useful prognostic factors.

Methods: In this descriptive-analytic study, we evaluate 100 patients with gastric cancer who were referred to Shahid Ghazi Hospital or other oncology clinics of Tabriz University of Medical Sciences in 2005-2010. Patients with pathologic confirmation of gastric cancer were selected. Expression of p53, ki-67, CD-44, HER-2/neu were detected by immunohistochemical staining.

Results: In this study, 100 patients with gastric cancer participated. 76(76%) were men and 24(24%) were women with mean age of 64.02(8.05) years. Seventy two samples were intestinal type and 28 were diffuse type. CD44 was positive in 27(27%) patients. P53 was positive in 35(35%) patients. Ki-67 was positive in 53(53%) patients. HER-2/neu was positive in 51(51%) patients.

Conclusion: The frequency of positive p53, Ki-67, CD44 and HER-2/neu varied in different studies. Positive Ki-67 and HER-2/neu were not associated with changes in survival but positive p53 and CD44 were significantly associated with improved survival.

KEY WORDS: Gastric cancer, Ki-67, P53, HER-2/neu, CD-44, Gastric cancer.

INTRODUCTION

Gastric cancer remains the second most common cause of cancer-related deaths worldwide.[1] However, there are large geographic variations in incidence, which may be related to environmental and genetic factors.[2-3] In addition to dietary factors such as excessive intake of salt and food contaminations with Nitrous compounds, cigarette smoking, and H.pylori infection have been regarded as presumed environmental factors contributing to gastric carcinogenesis.[4-6] The main risk factors for distal gastric cancer include Helicobacter pylori infection and dietary factors; whereas gastro-esophageal reflux disease and obesity play important roles in the development of proximal stomach cancer.[7] The other risk factors for gastric cancer are gastric surgery, gastric ulcer and

adenomatous polyposis, blood type A, positive family history, and hereditary factors.[7] In some regions of the world its incidence is decreasing due to changes in diet, food preservation and environmental factors.[7] Over 95% of gastric malignancies are adenocarcinoma. According to the widely used Lauren's 1965 classification, there are two types of gastric cancer: the intestinal-type of adenocarcinoma, which follows the pathologic sequential steps of atrophic gastritis, intestinal metaplasia, dysplasia, carcinoma; and the less common diffuse-type, with worse prognosis and correlated with chronic gastritis.[8] Distal gastric cancer (non-cardial) is often of the intestinal type and predominates in developing countries, among blacks, and in lower socio-economic groups, whereas proximal tumors (many of which show diffuse-type histology) are more common in developed countries, among whites, and in higher socio-economic classes.[9] While the incidence of the former is declining, that of the latter is not; in particular, the signet-ring subtype has been increasing [9].

At the molecular level gastric tumors arise from multiple genetic and epigenetic alterations that involve oncogene, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules, and its pathogenesis is still unknown.

In this study we evaluated immunohistochemical protein expression patterns of cell-cycle-regulators p53, proliferation marker Ki-67, surface expression of CD44, HER-2/neu oncogene have been proposed as useful prognostic factors.

Abnormalities of the p53 gene have been identified in many malignancies, including gastric carcinomas.[10] This 53-kDa is a tumor suppressor gene and its mutation affects the accurate control of DNA activities in cell cycle.[10] In a study by Azarhoosh et al., on prevalence of p53 mutations in cancers of cardia and antrum of stomach, the mutation of p53 were more prevalent in cardiac tumors than antral tumors.[11] A study by Staib showed that cancers of cardiac region had a worse prognosis compared with those of other regions of stomach.[12] A study by Jovanovic showed that p53 positive patients with gastric cancers of cardia were younger and had poorer prognosis.[13]

The HER-2/neu gene is located on chromosome 17q21, and encodes a 185-kDa transmembrane protein which exhibits tyrosine kinase activity and functions as a growth factor receptor.[7] Some studies suggest that over expression of the HER-2/neu protein correlates inversely with survival, and directly with increased invasiveness and aggressive growth in gastric cancer.[14] In a study by Park et al., HER-2/neu positive patients with gastric cancer had a shorter survival (922 days vs. 3243 days) and had a lower five-year survival rate (21.4%vs.63%).[15] Age, stage of the disease, and expression of HER-2/neu are independent prognostic factors.[15] With regard to gastric carcinomas, several immunohistochemical (IHC) studies have reported different frequencies of HER- 2/neu over expression, over a wide range, from 8 to 91 %.[16]

CD-44 is an intra membranous cellular glycoprotein first indentified in lymphocytes. It facilitates adhesiveness and implantation of cells. Its presence in lymphatic malignancies and other tumors is associated with higher cellular cleavage and proliferation, stronger tumoral cells' adhesiveness, and finally led to cellular metastasis.[17] Most positive CD44 gastric cancer is originated from cardia region.[17] A study by Hsieh, showed that the presence of some variants of CD44 in gastric cancer were associated with more lymph node involvement.[18] A study by Sneath, showed that the level of CD44 expression in cell cycle of gut tumors resulted in cell-cycle dependent changes in their adhesiveness to endothelium.[19] The Ki-67 protein is a cellular marker for proliferation and its prognostic value has been confirmed for breast cancer.[14]

The objectives of this study were to determine the frequency and the relationships between immunohistochemical expressions of p53, HER-2/neu, CD-44, Ki-67,with demographic and clinic pathologic findings, stages of gastric carcinoma, location of stomach cancer, and one- year survival of patients with gastric cancer. The identification of these factors helps the clinicians to modify the therapeutic regimens and hopefully achieve improved survival.

MATERIALS AND METHODS

In this descriptive-analytic study, 100 patients with gastric cancer who were referred Shahid Ghazi Hospital or other oncology clinics of Tabriz University of Medical Sciences in 2005-2010 were evaluated. Patients with pathologic confirmation of gastric cancer were selected. All participants signed an informed written consent for using their biopsy samples. The demographic data including age, sex, and the cancer stage at diagnosis were extracted from the patients' records. The patients admitted on clinic underwent chemotherapy and followed for one year.

Immunohistochemistry

The tissue samples were cut into 3 micrometer slices and mounted on polyethylene coated slides and incubated in 36° c for 24 hours. The samples then were dewaxed by passing them through a xylol solution and rehydrated by treating them with a graded series of progressively diluted ethanol. The sample were treated with protease and tripsin then they were stained with rabbit polyclonal antibody of p53, HER-2/neu, Ki-67, CD44. Then after immune peroxidase reaction the samples are counter stained with hematoxilyn. The stained samples were evaluated based on the number of stained cells and the densities of staining .The result were reported based on the following definition:

CD44 positive: when more than 50% of cells were stained

Ki-67 positive: when more than 10% of cells were stained

P53 positive: any staining of cells

HER-2/neu: when reported ++ or higher

The data were reported as frequency, rate (%) and mean (+SD). Chi -square and Fisher's exact test were carried out using SPSS-16. Kaplan-Meier curves were used for assessing one year survival and Cox regression model were used for exploring the effect other variable on survival.

RESULTS

In this study 100 patients with gastric cancer examined. Of all subjects 76(76%) were men and 24(24%) were women with mean age of 64.02(8.05) years. Two patients were staged as II, 46 as III and 52 as IV. Seventy two samples were intestinal type and 28 were diffuse type. There were no significant association between sex and stage of the gastric cancer (p=0.08) and between sex and pathology type (p=0.05).

Sixty three (63%) patients had a cancer on body of stomach, 33(33%) on cardia, 3(3%) on antrum and one (1%) in pylorus. There were no significant association between sex and anatomic site of the gastric cancer (p=0.45).

CD44 was positive in 27(27%) patients. There were no significant association between positive CD44 and sex (p=0.19), stage (p=0.18), pathology type (p=0.22) and anatomic site (p=0.54).

P53 was positive in 35(35%) patients. There were no significant association between positive p53 and sex (p=0.84), stage (p=0.36) pathology type (p=0.87), and anatomic site (p=0.92).

Ki-67 was positive in 53(53%) patients. There were no significant association between positive Ki-76 and sex (p=0.2), stage (p=0.15), pathology type (p=0.96), and anatomic site (p=0.33).

HER-2/neu was positive in 51(51%) patients. There were no positive association between positive HER-2/neu and sex (p=0.72) stage (p=0.16), pathology type (p=0.14) and anatomic site (p=0.31).

Fifty six (56%) patients died in first year, while 44(44%) survived at one year (Fig-1).

There were significant association (p≤0.0001) between, the type of pathology and overall survival (Fig- 2).The positive CD44, p53 were significantly associated with overall survival (p=0.02 and p=0.014, respectively).The overall survival had no significant association with positive Ki-67, HER-2/neu.

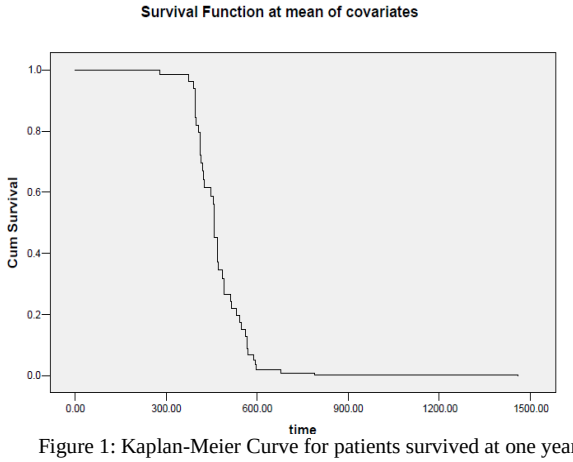

Figure 1: Kaplan-Meier Curve for patients survived at one year

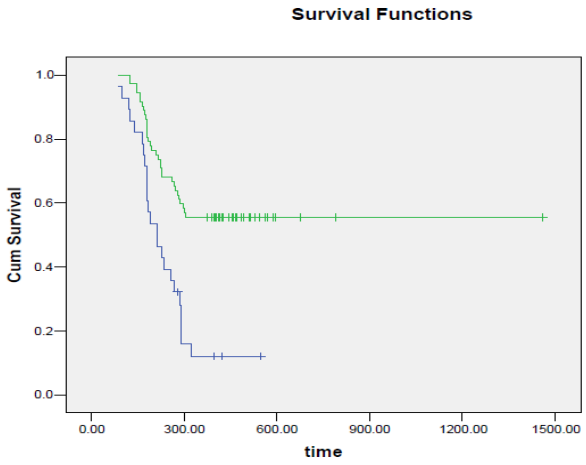

Figure 2: Kaplan-Meier Curve of survival based on pathology type of gastric cancer

DISCUSSION

Gastric carcinoma is a major cause of morbidity and mortality in the worldwide. The most reliable prognostic factors are tumor stage and completeness of excision. Tumor grade and histological type may be also useful factors. In this study we evaluated immunohistochemical protein expression patterns of cell-cycle-regulators p53, proliferation marker Ki-67, surface expression of CD44, HER-2/neu oncogene have been proposed as useful prognostic factors.

In our study, CD-44 was found positive in 27% of patients with gastric cancer while in a study by Wakamatus, CD44 was positive in 62% of their patients with gastric cancer.[20] In a study by Jang and coworker the frequency of positive CD44 in patients with gastric cancer from different regions of the world varied with 65% in Iran based on a study by Ghaffarzadeh, 19% in Korea and 47%, 62%, and 84% in China based on different studies.[21] Sheigeo in their study on patients with positive CD44 gastric cancer found a resistance to chemotherapy and shorter survival compared with patients with negative CD44 gastric cancer.[22]

In our study, p53 was positive in 35% of patients. A study by Shafigh, showed that in patients with gastric cancer who were younger than 60 years, p53 was positive in 14% of patients while in patients over 60 years of age it increased to 19%.[23] A study by Zheng, reported p53 in 54.05% of their patients with gastric cancer. In another study by Shiao, 60% of their patients with gastric cancer were p53 positive.[25] A study by Li and co-worker, reported 43% of patients with gastric cancer positive for p53.[26] Al-Mondhri, reported the prevalence of p53 in their patients with gastric cancer, 54%. They also noticed that the expression of p53 associated with more aggressive tumor behavior and is an independent prognostic factor.[14] The study by Tzanaskis, suggested that more pronounced expression of p53 was a prognostic factor.[27] In our study the positive p53 patients with gastric cancer were more likely to have longer survival.

In our study 53% of participants were Ki-67 positive. In the study by Al-Mondhri, 70% of patients with gastric cancer were Ki-67 positive.[14] In the study by Zheng, 75.68% of patients with gastric cancer were Ki-67 positive.[24] Shafigh, reported 75.9% of patients with gastric cancer Ki-67 positive.[23]

In our study, 51% of participants were HER-2/neu positive. In study by Tai, 27% of patients with gastric cancer were HER-2/neu positive.[28] In the study by Al-Mondhri, 12% of patients with gastric cancer were HER-2/neu positive.[14] In another study by Moelance, 2%-45% of patients with gastric cancer were HER-2/neu positive.[29]

In our study no significant association were found between sex and positive p53, Ki-67, CD44, and HER-2/neu. In studies by Peterson, no significant association were found between p53 expression and sex, age, disease stage and pathology type of tumor [30].

In our study Sixty three (63%) patients had a cancer on body of stomach, 33(33%) on cardia, 3(3%) on antrum and one (1%) in pylorus. The study by Shafigh, reported the frequent site s of gastric cancer as follows: cardia (50%), fundus (33%), antrum (17%).[23]

In our study patients with intestinal type pathology, were more likely to have longer survival ($P<0.001$). There was no significant association between overall survival and positive HER-2/neu and Ki-67.

In study by Wakamatus, suggested that p53 and Ki-67 might be prognostic factors and recommended that they were routinely assessed in patients with gastric cancer.[31]

In study by Cidone, suggested positive HER-2/neu as a prognostic factor for gastric carcinoma.[32]In a study by Yan , positive HER-2/neu was not significantly associated with age, tumor size, the original site of tumor, and type of pathology but it was associated with the stage of the disease.[33] In our study positive HER-2/neu patients with gastric cancer were more likely to have shorter survival but the association was not significant.

The frequency of positive p53, Ki-67, CD44and HER-2/neu in our patients were 35%, 53%, 27%, and 51%, respectively. These results, varied in different studies. Positive Ki-67 and HER-2/neu were not associated with changes in survival but positive p53 and CD44 were significantly associated with improved survival. Our results were consistent with those of other studies.

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