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Diagnostic Value of Serum P35 in Comparison with Tissue P35 in Gastric Adenocarcinoma and Their Relationship with Microscopic Prognostic Factors

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Abstract: Early diagnosis of Gastric adenocarcinoma could increase survival of the patients and also remarkably reduce treatment costs. This study aimed at evaluating the diagnostic value of serum P35 in comparison with tissue P35 in gastric adenocarcinoma and their relationship with microscopic prognostic factors. In this descriptive analytical study, 35 patients (74.3% male and 25.7% female with mean age of 63.00±12.75 years with gastric adenocarcinoma were evaluated. Blood samples were taken from all patients before gastrectomy to evaluate serum P35 with ELISA method and after surgery tissue samples were gathered to evaluate tissue P35 with immunohistochemical method. The relation between tissue and serum P35 with severity of the disease and microscopic findings was assessed. Tissue P35 was negative in 57.1%, positive in 22.9% and very positive in 20%. Mean serum P35 was 1.34±0.43 mg dL^{-1}. There was no relation between serum P35 and adenocarcinoma type, tumor grade, vascular and neurological invasion and number of lymph node involved. Serum P35 levels significantly increased by increase in tissue P35 positivity ($p = 0.004$). There was significant correlation between tissue P35 and adenocarcinoma type ($p = 0.006$), neurological involvement ($p = 0.04$) and number of involved lymph nodes ($p = 0.001$). Although serum P35 level was higher in cases with more lymph node involvement and vascular and neural invasion, the marker was not capable to predict the involvement degree of stomach cancer. In comparison with serum P35, tissue P35 plays more significant role in these cases.

Key words: Adenocarcinoma, serum P35, tissue P35, diagnosis, survival

INTRODUCTION

Stomach carcinoma is regarded as the fifth common cause of cancer leading to mortality all over the world (Golfurushan *et al*., 2011; Guo *et al*., 2011). Also, it is respectively regarded as the second and third prevalent cancer in Iranian men and women such that intestinal adenocarcinoma was introduced as the most prevalent type of cancer in Iran. Annual mortality rate has been estimated at about 3.6 and 8.4 per every 100,000 cases in Iran, respectively (Omar *et al*., 2012; Sadighi *et al*., 2011). According to the Lauren categorization, stomach carcinoma can be divided into two main histological group including intestinal and dispersed types (Goldust *et al*., 2012a-d; Ide *et al*., 2012). In addition to substantial morphological difference found between these two types, they have different epidemiological and clinical features and pathogenesis (Takami *et al*., 2012). The important point about digestive system cancers is that their early detection will result in increasing the patients' life span and significant decrease of their treatment expenses (Goldust *et al*., 2012b; Zhang *et al*., 2012). Early detection of kinds of cancer using noninvasive serological tests as a screen test is regarded as one of the one the common subjects and main researches in the research centers (Lv *et al*., 2012). Identifying cancer cells or abnormal proteins of the patients is not a conventional technique. Few markers have already been introduced as accurate tumoral marker and P35 is regarded as one of the most prevalent mutated genes in human tumors serving through stopping cellular growth or developing of apoptosis. (Feng *et al*., 2011; Flejou, 2011; Goldust *et al*., 2012c). It has been recently stated that P35 serum level increases before clinical detection of tumor in some cases and, therefore, act as a premature biomarker (Goto *et al*., 2011; Sadeghpour *et al*., 2011). As mentioned, although P35 have been more evaluated using immunohistochemistry technique in the tissues obtained from stomach adenocarcinoma, serum P35 have been less studied in stomach adenocarcinoma (Goldust *et al*., 2012a; Ichinoe *et al*., 2011). Since serum P35 has not already introduced as a prognostic factor in previous studies

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(Barrezueta *et al*., 2010; Goldust *et al*., 2011; Terada, 2012), the aim of this study was to introduce serum P35 as a new prognostic factor as well as a screen method to early diagnosis and more effective and quick treatment intervention in the affected patients if a correlation can be found between serum P35 and other factors.

MATERIALS AND METHODS

Subjects: This descriptive analytical study evaluated all patients with endoscopy and clinical diagnosis of stomach adenocarcinoma referred to Imam Reza and Amiralmomenin hospitals, Tabriz from July, 2010 to July, 2012. This study was approved by ethic committee of Tabriz university of medical sciences. Written consent was obtained from all the patients.

Exclusion criteria: Patients suffered from other cancers and those underwent radiotherapy and chemotherapy were excluded from the study.

Methods: Before gastrectomy, blood samples of all patients were taken, their serum was isolated and frozen. Additionally, tissue samples slides of the patients were reviewed and prognostic cases of every patient was recorded. Following collection of all patients' serum, pathological and block samples, ELIZA method (photometric immunoassay ELISA, ROCHE Molecular Biochemicals, Manheim, Germany) was used to evaluate serum P35. It was studied using immunohistochemistry method in tissue samples. Finally, correlation or non-correlation between serum P35 and tissue P35 and their relationship with other prognostic factors were studied. The understudy variables included age; gender; grade, type and location of adenocarcinoma, serum and tissue P35.

Statistical analysis: All data was analyzed using SPSS16 statistical software. Descriptive statistical methods (frequency, percentage, mean and standard deviation) were used to statistically evaluate the data. Chi-square test was used to compare qualitative variables while quantitative ones were compared using independent t-test and one-way ANOVA test. Pearson correlation test was used to evaluate the potential relations found between quantitative variables. In this study, $p<0.05$ was regarded meaningful.

RESULTS

Mean age of the understudy patients was 63.00±12.75. The oldest and youngest patients were 86 and 31 years old, respectively. The study was consisted of 26 (74.3%) male and 9 (25.7%) female patients. Histological studies indicated to 26 (74.3%) cases of intestinal and 9 (25.7%) cases of dispersed adenocarcinoma. The tumor was localized at anteroom in 4 cases (11.4%), pilar in 2 cases (5.7%), trunk in 20 cases (57.1%) and cardia in 9 cases (25.7%). There were tumor in 20 cases (57.1%) of low grade and 15 cases (42.9%) of high grade. Vascular and neural invasions were seen in 29 (82.9%) and 25 (71.4%) cases, respectively. The invasion was limited to serosa in 31 (88.6%), periopera muscularis in 3 (8.6%) and sub mucosa in one (2.9%) cases. Mean size of the tumor was 7.04±3.72 cm. The smallest and biggest tumors were 2 and 15 centimeters, respectively. Mean number of the involved lymph node was 8.94±4.91 such that there were 2 and 24 involved lymph node as the least and most, respectively. Mean P35 serum level was 1.34±0.43 mg dL^{-1} with the average of 0.6 mg dL^{-1} in the understudy patients. The lowest and highest P35 serum level was zero and 14.40 mg dL^{-1}, respectively. Tissue P35 was negative in 20 (57.1%), positive in 8 (22.9%) and double positive in 7 (20%) cases (Table 1). Serum P35 level was, respectively 1.40±0.56 and 1.17±0.39 mg dL^{-1} in cases with intestinal and dispersed adenocarcinoma. There was not any statistically meaningful difference between these two groups ($p = 0.82$). Serum P35 level was 1.38±0.7 and 1.28±0.39 mg dL^{-1} in cases with low and high grade tumor, respectively. In this case, there was not any significant statistical difference between the groups ($p = 0.9$). Serum P35 level was 1.54±0.51 and 0.36±0.12 mg dL^{-1} in cases with and without vascular invasion. Although the serum P35 level was higher in cases with vascular invasion,

Table 1: Demographic data of study population

Variable	No.	%
Gender		
Male	26	74.3
Female	9	25.7
Histological type		
Intestinal	26	74.3
Dispersed adenocarcinoma	9	25.7
Localization		
Anteroom	4	11.4
Pilar	2	5.7
Trunk	20	57.1
Cardia	9	25.7
Grade		
Low grade	20	57.1
High grade	15	42.9
Invasion		
Serosa	31	88.6
Periopera muscularis	3	8.6
Sub mucosa	1	2.9
Tissue P35		
Negative	20	57.1
Positive in	8	22.9
Double positive	7	20.0

Table 2: The relationship between serum P35 and other variables

Variable	Serum P35	p-value
Histological type		
Intestinal	1.40±0.56	0.82
Dispersed adenocarcinoma	1.17±0.39	
Grade		
Low	1.38±0.70	0.9
High grade	1.28±0.39	
Vascular invasion		
Positive	1.54±0.51	0.31
Negative	0.36±0.12	
Neural invasion		
Positive	1.62±0.59	0.31
Negative	0.64±0.21	
Involved lymph node		
1-6	0.55±0.12	0.19
7-15	1.45±0.74	
More than 15	3.20±0.94	

there was not statistically meaningful difference between these two groups ($p = 0.31$). Serum P35 level was 1.62±0.59 mg dL^{-1} and 0.64±0.21 mg dL^{-1} in cases with and without neural invasion. Although the serum P35 level was higher in cases with neural invasion, there was not statistically meaningful difference between these two groups ($p = 0.31$). In this study, the higher the positivity of tissue P35, the higher the serum P35 level ($p = 0.004$). Following classifying the involved lymph node surface to three groups of 1-6, 7-15 and more than 15, serum P35 level was 0.55±0.12, 1.45±0.74 and 3.20±0.94 mg dL^{-1}, respectively. Although serum P35 level increases due to increase of number of the involved lymph node, the difference between these three groups was not statistically meaningful ($p = 0.19$) (Table 2). Evaluating the potential relationship between serum P35 level and quantitative variables including age, tumor size and number of the involved node lymph using correlation test does not indicated to any significant statistical relationship in none case ($p>0.05$). There are more positive and double positive cases in higher grades. However, it was not possible to evaluate the difference found between these two groups because of inappropriate distribution of data. In this study, there were significantly more negative cases in intestinal and cases with double positive tissue P35 in dispersed adenocarcinoma ($p = 0.006$). There were significantly more involved lymph node in cases with double positive P35 ($p = 0.001$). Mean size of tumor was 6.52±3.52, 7.62±4.56 and 7.85±3.56 cm in negative, single positive and double positive cases, respectively. There was not any significant statistical difference in these cases ($p = 0.64$).

DISCUSSION

Evaluating 35 patients with stomach cancer underwent gastrectomy in this study, diagnostic and their relation with microscopic prognostic factors. Most available studies have referred to the relationship found between tissue P35 level evaluated through immunohistochemistry and stomach adenocarcinoma involvement and different markers (Chu *et al*., 2011; Jing *et al*., 2011). The study stated that P35 serum level ascends in some cases prioritized over clinical diagnosis of tumor. Therefore, it can serve as an early biomarker (Luo *et al*., 2011). The present study dealt with the relationship found between tissue P35 level and microscopic findings of stomach cancer. It was observed that tissue P35 was negative in 57.1%, positive in 22.9% and double positive in 2% of cases. In a previous study, authors indicated to positive tissue P35 in 52 cases (54%) of stomach carcinoma (Marvast *et al*., 2011). In the present study, there was a significant relationship between tissue P35 and type of adenocarcinoma (intestinal or dispersed), neural invasion and number of the involved lymph node such that there was more negative cases of tissue P35 in intestinal adenocarcinoma and increase of number of the involved node lymph resulted in increase of the positive cases. However, there was not any relation between tumor size and tissue P35. Maehara *et al*. (2000) suggested that stomach adenocarcinoma with P35 mutation is associated with high probability for metastasis to node lymph (Maehara *et al*., 2000). Contrary to the present study, however, the study conducted by Nakayama *et al*. (2010) referred to positive P35 in 70% of intestinal and 52% of the dispersed adenocarcinoma (Nakayama *et al*., 2010). Similar to the present study, most positive cases considering P35 were associated with more advanced cases and association with metastasis to lymph glands in the study conducted by Andre *et al*. (2010). Contrary to the present study where there was not observed any relationship with tumor size, most positive cases were associated with tumors>5 cm in this study. Evidently, serum level of P35 accurately reflects tissue changes of P35 at genetic or protein level. Also, serum level of P35 increases before than clinical detection time in some cases (Sugai *et al*., 2010). There are few studies on serum P35 in stomach adenocarcinoma as well as its relation with other prognostic factors (Zhang *et al*., 2010). Sometimes, there were obtained contradictory results in these limited studies. In the present study, there was not seen any relation between serum P35 level and type of adenocarcinoma, tumor grade, vascular and neural invasion and number of the involved node lymph. However, serum P35 level was higher in cases with more node lymph involvement and higher vascular-neural invasion. In the study conducted by Wang *et al*. (2003) there was not any meaningful relation between serum P35 variations and tumor size, depth of tumor invasion, metastasis to lymph glands and survival rate of the

patients (Wang *et al*., 2003). Also, the study conducted by Wu *et al*. (1999) suggested that presence of antibodies against P35 is significantly associated with several factors such as tumor size, depth, invasion to lymph canal and metastasis to lymph nodes (84% of patients with positive serum antibodies against P35 suffered from metastasis to lymph nodes). In this study, there was a significant relation between serum and tissue P35 such that the higher the positivity of tissue P35, the higher the serum P35 level. Following comparing of tissue sample with serum sample of the patients, Wang *et al*. (2005) demonstrated that 36.4% of cases have a meaningful relation between tissue and serum P35 level.

CONCLUSION

Although serum P35 level was higher in cases with more lymph node involvement and vascular and neural invasion, the marker was not capable of predicting involvement degree of stomach cancer. In comparison with serum P35, tissue P35 plays more significant role in these cases.

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