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Serum VEGF levels in gastric cancer patients: correlation with clinicopathological parameters

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Background/aim: To evaluate the predictive role of the circulating levels of vascular endothelial growth factor (VEGF) in gastric cancer patients.

Materials and methods: This study is a case-control study. We measured serum VEGF levels of 30 patients aged between 34 and 83 years with gastric cancer and 30 patients without malignant pathology, operated on for benign pathologies, with ages ranging from 18 to 69.

Results: Serum levels of VEGF were correlated with the tumor type classification (signet cell adenocarcinoma) and the presence of adjacent tissue invasion. There was also a positive correlation between serum VEGF and carcinoembryonic antigen levels.

Conclusion: In gastric cancer patients, serum VEGF levels may provide additional prognostic information for preoperative evaluation of invasion and tumor type.

Key words: Gastric cancer, vascular endothelial growth factor, angiogenesis

1. Introduction

Gastric cancer is one of the most common cancers worldwide, accounting for about 8% of new cancers (1). The incidence of gastric cancer has declined rapidly over the past few decades in most parts of the world, but it is still one of the most common cancer types worldwide (2). Incidence rates for gastric cancer are highest in East Asia (China, Japan, and Korea), East Europe, and South America, and the 5-year survival rate for gastric cancer is poor. Altogether, gastric cancer still accounts for more than 10% of cancer deaths worldwide, being the second most frequent cause of cancer death following lung cancer (1). The main prognostic factors in gastric cancer are clinicopathological characteristics of the disease including tumor size, stage, and grade. There are prognostic models to predict the outcome of gastric cancer patients (3,4). However, the prognostic factors do not fully predict individual clinical outcomes. Better markers are needed to identify patients with poor prognosis at the time of diagnosis. Researchers have focused on the potential role of new biological factors involved in the carcinogenic process as prognostic markers in patients with gastric cancer (5).

Angiogenesis is defined as the process of new capillary formation from preexisting vasculature (6). In regulating tumor angiogenesis, the vascular endothelial growth factor (VEGF) family plays a determinant role. VEGF induces cell proliferation, differentiation, and migration of vascular endothelial cells (7). VEGF is also required for the establishment of vascularization in malignant tumors, which benefits primary tumor growth and metastasis (8). Recently, targeting constitutive VEGF and/or its receptors has become an attractive approach for cancer therapy (9).

In this study, we investigated, in a consecutive series of 30 gastric cancer patients undergoing surgery, the possible correlation of VEGF with clinicopathological features in an effort to identify gastric cancer patients with different prognoses who could benefit from tailored and targeted treatments.

2. Materials and methods

This prospective study was performed in the Ankara Numune Research and Training Hospital after the regional ethics committee approved the project, and written informed consent was obtained from all patients and controls before their inclusion. Thirty consecutive patients with newly diagnosed and histologically confirmed gastric cancer were included in this study. There were 20 men and 10 women with a median age of 64.3 (min: 34, max: 83) years. Patients who had a second cancer or received

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chemotherapy, radiotherapy, or blood transfusion before surgery were excluded from the study. Tumor staging was based on clinical information, radiologic reports (chest radiography, abdominal ultrasonography, and computerized tomography), operative findings, and pathology reports. The staging was made in accordance with the TNM staging system for gastric cancer and TNM staging was done according to the American Joint Committee on Cancer (AJCC) (10). Tumors were histologically classified as intestinal or diffuse according to their Lauren type and were graded as well, moderately, or poorly differentiated based on the predominant cell type.

The control subjects were 30 healthy volunteers with a median age of 41.3 (min: 18, max: 69) years and consisted of 19 men and 11 women. The absence of disease was confirmed by clinical history, physical examination, and routine laboratory tests, including liver and renal function tests.

Five-milliliter venous blood samples were taken from the 30 healthy volunteers. The gastric cancer patients' blood samples were taken just before operation. The values of VEGF, hemoglobin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alpha-fetoprotein (AFP), and carcinoembryonic antigen (CEA) of each patient who had the diagnosis of gastric cancer were recorded from the patient's file. Information about invasion and metastasis was recorded from the operation notes, and tumor differentiation, Lauren type, histological tumor type, T (depth of the tumor), and N (involvement of dissected lymph nodes) data were taken from pathology results. VEGF levels were compared with these results.

2.1. Biochemical analysis

The 5-mL blood sample was put in an EDTA-Na tube. Serum samples were kept at –20 °C for more than 2 weeks after centrifuging for 10 min at 3000 rpm. VEGF levels from sera were determined with the enzyme-linked immunosorbent assay method (CytELISA Human VEGF, CytImmune Science, College Park, MD, USA). The measuring range for the assay was 40–5000 pg/mL.

2.2. Statistical analysis

Obtained data were evaluated statistically by SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The evaluation between the control group and the group who had a diagnosis of gastric cancer was conducted with a Fisher's exact chi-square test in terms of age and sex. VEGF levels between the gastric cancer and control groups were evaluated with a 2 independent-samples t-test. VEGF levels and parameters that contained 2 varieties were evaluated with a Mann–Whitney U test. Both nonparametric one-way ANOVA and the Kruskal–Willis test were applied between groups that had VEGF levels and 3 varieties or more. The relations between varieties were evaluated by using the Spearman correlation test and $P < 0.05$ was considered statistically significant.

3. Results

In total, 39 patients were men (65%) and 21 patients were women (35%). In the gastric cancer group, 20 patients were men (66.7%) and 10 patients were women (33.3%). In the control group, 19 were men (63.3%) and 11 were women (36.7%). The mean age in the gastric cancer group was 64.3 (min: 34, max: 83) years and the mean age in the control group was 41.3 (min: 18, max: 69) (Table 1).

The mean VEGF value of the gastric cancer patients was 142 pg/mL (min: 40, max: 542.1), and the mean VEGF value of the control group was 104 pg/mL (min: 40, max: 542). This difference between the control and the gastric cancer groups was not statistically significant ($P > 0.05$) (Table 1).

The mean hemoglobin value in the gastric cancer group was 10.453 g/dL (min: 4.2, max: 14.6), the mean AST value was 29.48 IU/L (min: 12, max: 79), and the mean ALT value was 25.93 IU/L (min: 3, max: 93).

CEA and AFP levels were obtained from 11 of 30 patients in the gastric cancer group. The mean CEA level was 714.391 µg/L (min: 1.5, max: 7480), and the mean AFP value was 15.527 µg/L (min: 1.2, max: 112.2). There was a significant positive correlation between CEA and high VEGF levels ($P < 0.05$). When the relation between VEGF and CEA was evaluated with Spearman correlation, the R parameter was found to be 0.80, and this shows a strong relationship.

The distribution of the tumor types in the pathology results of 30 patients operated on with gastric cancer were adenocarcinoma in 24 patients (80%), signet ring cell adenocarcinoma in 4 patients (13.3%), early gastric cancer in 1 patient (3.3%), and malignant tumor showing neuroendocrine differentiation in 1 patient (3.3%). The mean VEGF levels in signet ring cell adenocarcinoma, early gastric carcinoma, and well-differentiated neuroendocrine carcinoma cases were 110, 384, and 40 pg/mL respectively. This difference in the signet ring cell adenocarcinomas was statistically significant ($P > 0.005$) (Table 2).

Table 1. Demographic features.

	Gastric cancer group, n (% or min–max)	Control group, n (% or min–max)
Sex		
Male	20 (67%)*	19 (63%)*
Female	10 (33%)*	11 (37%)*
Age	64.3 (34–83)*	41.3 (18–69)*
VEGF levels	142 (40–542.1)*	104 (40–542)*

*: The differences are not significant ($P > 0.05$).

Table 2. The relation between preoperative serum VEGF and clinicopathological parameters in gastric cancer patients.

	Number of patients	Serum VEGF (pg/mL)	P
Tumor type			
Adenocarcinoma	24	110	ns
Signet ring cell adenocarcinoma	4	380	<0.05*
Early gastric cancer	1	40	ns
Malign neuroendocrine tumor	1	40	ns
Tumor histology			
Intestinal	13	116	ns
Diffuse	17	16	ns
Tumor differentiation			
Well	4	170	ns
Moderately	10	87	ns
Poorly	16	168	ns
Tumor class			
T_1	1	40	ns
T_2	2	89	ns
T_3	9	159	<0.05*
T_4	18	268	<0.05*
Adjacent tissue invasion			
Absent	16	105	<0.05*
Present	14	174	
Lymph node metastases			
N_0	7	114	ns
N_1	14	145	ns
N_2	8	173	ns
N_3	1	40	ns
Distant metastases			
Absent	10	128	ns
Present	20	152	ns
TNM stage			
IA	1	40	ns
IB	2	268	ns
II	2	40	ns
IIIA	7	112	ns
IIIB	7	180	ns
IV	11	141	ns

*: $P < 0.05$ (statistically significant), ns = nonsignificant.

Distant metastasis was not determined in 24 (80%) patients by ultrasound examination before the operation, but distant metastasis was seen in 6 (20%) patients. The mean VEGF value of the patients who had no radiological metastasis before the operation was 155 pg/mL. In patients who had radiological metastasis before the operation, the mean VEGF value was 89 pg/mL. There was no significant correlation between these obtained VEGF values and radiologically proven metastasis ($P > 0.005$).

The number of patients who had metastasis noted during the operation was 10 (33.3%) and the number of patients who had no metastasis was 20 (66.7%). This difference was not significant ($P > 0.05$).

Sixteen patients (53.3%) had invasion of adjacent tissues and organs noted during the operation and the mean VEGF level of these patients was 174 pg/mL; the mean VEGF level of the 14 patients (46.7%) who had no invasion was 105 pg/mL. This difference was statistically significant ($P < 0.05$).

According to the differentiations of tumor types, 4 (13.3%) patients had well-differentiated tumors, 10 (33.3%) patients moderately differentiated, and 16 (53.3%) were

poorly differentiated. The mean VEGF levels were 170, 87, and 168 pg/mL, respectively, in the well-differentiated, moderately differentiated, and poorly differentiated tumors. There was no statistically significant correlation between VEGF values and tumor differentiation ($P > 0.05$).

According to Lauren classification, 17 (56.7%) patients had diffuse-type and 13 (43.3%) patients had intestinal-type gastric cancer in pathologic analysis of all gastric cancers. The mean VEGF value of the patients who had diffuse-type gastric cancer was 16 pg/mL and the mean VEGF value of the patients who intestinal-type gastric cancer was 116 pg/mL. This difference was not statistically significant ($P > 0.05$).

When the 30 patients were examined in terms of the depth of the tumor, 1 was T_1 (3.3%), 2 were T_2 (6.7%), 9 were T_3 (30%), and 18 were T_4 (60%). The mean VEGF values of these groups were 40, 89, 159, and 268 pg/mL, respectively. Differences between VEGF values and the depth of the tumor were statistically significant ($P < 0.05$).

Distribution of patients according to stage of tumor, lymph nodes in the pathology specimen, and VEGF levels of each group is shown in Table 2. There were no statistically differences between VEGF levels ($P > 0.05$).

4. Discussion

In 1971, Folkman suggested that solid tumor development and metastasis formation depend on new vessels (11). Based on this hypothesis, the role of angiogenesis has been well recognized in the processes of tumor development, infiltration, and metastasis formation. Tumor cells must have a vascular structure that carries the required oxygen and other micronutrients to their fields in order to provide the unlimited growth and clearance of the residual materials in their fields (12). While angiogenesis is in balance within very strict rules in normal organisms, angiogenesis in tumor tissue is uncontrolled and immature (12). Serosal invasion, adjacent tissue invasion, peritoneal dissemination, and metastasis are common in gastric cancer. We examined the serum VEGF levels of the patients in terms of clinicopathologic characteristics and differences between healthy control groups in this study.

Several studies have shown that VEGF plays a key role in angiogenesis in gastric cancer (13–15). In our study, VEGF levels in the gastric cancer group were higher than in the control group. There was no meaningful difference statistically, but this may be due to the small size and heterogeneity of our study. There was no contribution of hemoglobin, AST, or ALT values in terms of staging and prognosis in the preoperative period of our study, and this has been shown in the literature as well. The mean of these values in this study was within the normal limits, and there was no correlation between clinical and pathological features of the patients.

Although Dittrich et al. said that it is early to suggest CEA levels as a reliable routine prognosis and treatment parameter before and after the operation for gastric cancer patients in their study (16), Gaspar et al. observed that CEA levels increased when liver and peritoneal metastasis appeared in gastric cancer (17). Ishigami et al. showed that high levels of CEA depend on liver metastasis in 549 patients (18). A strong and statistically significant correlation between CEA levels and high VEGF levels was determined in our study. Yoshikawa et al. determined that there is a relation between increased blood VEGF levels and disease recurrence (19). When the biological role of VEGF is considered in metastasis formation, high blood CEA levels can provide information in terms of prognosis before the operation.

Huang et al. did not find a difference between serums VEGF levels of patients who had early-diagnosed gastric cancer in their study, which consisted of 107 patients (20). They used the Lauren classification of intestinal, diffuse, and mixed types in the same study and did not find any meaningful difference between intestinal and diffuse types. However, they found that serum VEGF levels were higher in the mixed type than in the other 2 groups. Salgado et al. found that the tissue VEGF expression was higher in diffuse-type than in intestinal-type gastric cancer (21). Takahashi et al. found that tissue VEGF levels were higher in the intestinal type in their study, where 38 of the patients had diffuse-type and 51 had intestinal-type gastric cancer, and they concluded that angiogenesis is more necessary for metastasis in the intestinal type (22). There was no significant difference between intestinal- and diffuse-type gastric cancers according to serum VEGF levels in our study.

Kitamura et al. showed that serum VEGF levels were higher in patients who had serosal invasion, and there was no relation between tumor depth and VEGF levels in their study carried out in 281 patients (23). In our study, serum VEGF levels in the patients who had serosal invasion were higher. It is clear that tumors need vascularity to extend out of serosa. For this reason, it is possible that the VEGF level of tumors that do not extend to the serosa is lower.

Shi et al. did not determine a relation between VEGF levels and tumor differentiation in the cancer tissue in their study carried out in 281 patients (24). Du et al. determined that increased VEGF values were seen when tumor differentiation worsened in their study (12). Kitamura et al. did not determine a significant relation between tissue VEGF levels and tumor differentiation (23). There was also no significant relation between serum VEGF levels and tumor differentiation in our study. When tumor types and serum VEGF levels were compared, statistically significant high levels of VEGF were seen in signet ring cell adenocarcinoma. Indeed, VEGF is associated with tumor

invasion rather than differentiation. In signet ring cell tumors, peritoneal implants, hematogenous metastasis, and early recurrence are seen in most cases. Our study also supports these findings. Maeda et al., and many other researchers, showed that the higher levels in both serum and tissue VEGF levels were associated with peritoneal, hematogenous, and lymphatic metastasis (25).

The above comparisons between VEGF levels and lymph node involvement come from Japan, where D1 and D2 lymph node dissections have been precisely carried out. The results from these studies clearly show that VEGF is necessary for lymphatic metastasis. However, we found no relation between lymph node involvement and serum VEGF levels in our study. One of the reasons for this is that palliative surgical procedures were chosen for the majority of our patients because most of them were stage IIIB and IV; therefore, complete lymph node dissections were not conducted. The numbers of obtained lymph nodes were low because the numbers of patients who had tumors in early stages were low.

Serum VEGF levels in the gastric cancer group were higher than in the control group in our study, but this difference was not statistically significant. There was no relation between the distribution of patients according to the stage and serum VEGF values. This situation may have resulted from the low number and the heterogeneity of the patients.

In conclusion, in gastric cancer patients, serum VEGF levels may provide additional prognostic information for preoperative evaluation of invasion and tumor type. The benefits of the clinical use of VEGF in choosing treatment methods and antiangiogenesis treatments will become clearer after results of further clinical studies are obtained with an increased number of patients.

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