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Clinical Study

Diagnostic Significance of Serum Eotaxin-1 Level in Gastric Cancer Patients

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Introduction. Gastric cancer is the second cause of cancer-related deaths worldwide. Delayed diagnosis leads to high mortality rates. Eotaxin-1 was originally discovered as an eosinophil-selective chemoattractant and may play a role in a number of chronic inflammatory diseases, cancer, and other gastrointestinal disorders. The aim of this study was to analyse diagnostic and prognostic significance of serum eotaxin-1 (s-eotaxin-1) levels in gastric cancer. *Methods*. Sixty gastric cancer patients and 69 healthy subjects were included into the study. S-eotaxin-1 levels were compared with clinicopathological features and outcomes in gastric cancer. *Results*. Serum levels of eotaxin-1 in gastric cancer patients were significantly higher than controls (74.51 ± 16.65 pg/mL versus 16.79 ± 5.52 pg/mL, respectively ($P < 0.001$)). The s-eotaxin-1 levels did not differ significantly with histopathological grade, tumor-node-metastasis (TNM) stage, tumor localization, lymph node metastases, positive lymph node ratio, size, perineural and perivascular invasion. So there is no relationship found between s-eotaxin-1 level and prognosis. *Conclusion*. S-eotaxin-1 levels may be used as an easily available biomarker for gastric cancer risk and may alert physicians for early diagnosis. Due to the limited number of patients included in this study, larger cohort studies are warranted to validate the diagnostic value of s-eotaxin-1 level in gastric cancer.

1. Introduction

Gastric cancer is a major public health problem [1]. Every year there are 900000 new cases and 700000 gastric cancer-related deaths in the world [2]. In spite of chemotherapy and complete resection of gastric cancer (R0) via gastrectomy, more than 80% of patients with advanced gastric cancer die of the disease or recurrent disease within 1 year after diagnosis. This situation suggests that standard treatment protocols are ineffective in a considerable number of cases [3]. Early diagnosis is important for the diagnosis and management of disease.

Chemokines and chemokine receptors are shown to play an important role in regulation of tumor growth, migration, and invasion of different types of cancer. CXCL12 (SDF-1)/CXCR4 in ovarian [4] and breast cancers [5], CXCL13 (BCA-1)/CXCR5 in pancreatic and colon cancers in human and mouse [6], and IL-8/CXCR2 in epidermoid carcinoma cells [7] are examples of chemokines which have potentially critical role in cancer. Eotaxin-1 is a member of the CC chemokine family and was originally discovered as an eosinophil-selective chemoattractant. Eotaxin-1 plays a central role in eosinophil trafficking and is mediated by the CC chemokine receptor-3 (CCR-3). CCL11 is a ligand for a receptor, CCR3, which is expressed on eosinophils, basophils, Th2 helper, and T cells. Eotaxin-1 mRNA is expressed at high levels in the small intestine, colon, heart, kidney, and pancreas and at lower levels in other tissues including the lung, liver, ovary, and placenta. In the gastrointestinal tract, eotaxin-1 is expressed in mucosa, and it has been suggested that eotaxin-1 has an important role in the maintenance of normal eosinophil homeostasis [8]. Eotaxin-1 also was shown to induce formation of blood vessels in vivo [9]; proangiogenic molecules such as FGF-1,5,6, IL-6, VEGF A, and VEGF C are upregulated when human airway epithelial cells are exposed to it [10]. These data indicate that eotaxin-1 facilitates tumor

progression and metastases. Eotaxin-1 is a gene product induced by proinflamatory cytokines, such as TNF-α, IL-1 or IFN-α, also may play an important role in chronic inflammatuar diseases such as sinusitis, rhinitis, inflammatory bowel disease, and coronary artery disease [11].

Several studies report that elevated eotaxin-1 levels are diagnostic or prognostic for cancers such as prostate, renal cell cancer, head and neck small cell cancer. The association of eotaxin-1 with gastric cancer or potential eotaxin-1 expression by tumor has not been investigated. In the current study, we try to analyse diagnostic and prognostic significance of preoperative serum eotaxin-1 (s-eotaxin-1) levels in gastric cancer patients.

2. Patients and Methods

Sixty consecutive patients with gastric adenocancer were enrolled in this study. The diagnoses in all patients were confirmed by histopathologic examination of the gastric resection specimens. Informed written consent was obtained before patient enrollment. This study has been approved by the institutional review board of Turkiye Yuksek Ihtisas Education and Research Hospital. Patients under the age of 18, with a history of malignant disease, patients with synchronous tumor, patients with gastric tumor except adenocarcinoma, and patients who had received chemotherapy or radiotherapy before current surgical therapy were excluded from the study.

Surgery consisted of subtotal or total gastrectomy and D2 (i.e., extended) lymph-node dissection in all patients, except in the cases with peritoneal or distant metastasis. Serial sections from paraffin-embedded tissue blocks were obtained from gastric tumor tissues and used for histopathological diagnosis. Microdissected areas were assessed by an expert pathologist to estimate perineural and vascular invasion, the depth of tumor invasion, lymph-node metastasis and histopathological grade. The American Joint Committee on Cancer (AJCC) TNM Staging Classification of the Stomach (7th ed., 2010) was used for the staging of the tumor [12].

Control serum samples were obtained from 69 individuals who had health examination suffered with minimal gastritis is symptoms or patients who had normal appearance of gastric mucosa on gastroscopic examination. Serum samples were collected before malignancies were treated surgically. All of the serum samples were separated by centrifugation within one hour and plasma was removed and stored at −80°C. Serum eotaxin-1 levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems Europe, UK) following the manufacturer's instructions. The eotaxin-1 levels was expressed as picograms per millilitre (pg/mL). The minimum detectable dose of s-eotaxin-1 is typically less than 45 pg/mL. Reproducibility in intraassay is <10%, inter-assay is <12%.

3. Statistical Analysis

Data were tested for normality and were found to be non-normally distributed. Shapiro-Wilk test was used for assessing normality. All statistical procedures were performed with SPSS 15.0 (SPSS Inc., Chicago, Inc.). Accordingly, all data are presented as median value (interquartile range), with non-parametric analyses being employed to assess differences. The Kruskal-Wallis analysis of variance (ANOVA) and the Mann-Whitney U test were used to evaluate differences between multiple group, and unpaired observations, respectively. Correlations were evaluated using the Spearman rank test. The Kaplan-Meier curves were plotted to calculate 5-year survival curves, and log-rank test was used to estimate the differences. Clinicopathologic factors known to be associated with prognosis were tested in univariate analysis. Variables that were found to be significant in univariate analysis were then entered in a multivariate Cox proportional hazards regression model to identify those with independent prognostic information for overall survival. $P < 0.05$ was considered significant.

4. Results

There were 38 (55.1%) male and 31 (44.9%) female individuals in the control group. Serum eotaxin-1 levels were detectable in all healthy controls. The median s-eotaxin-1 level in healthy controls was 16.79 ± 5.52 pg/mL. There was no significant difference in s-eotaxin-1 levels between the male and female controls (males = 17.7 ± 5.6 pg/mL and females = 15.5 ± 5.2 pg/mL, resp.). No correlation was found between s-eotaxin-1 levels and age ($r = -0.024$; $P = 0.84$).

Serum levels of eotaxin-1 in gastric cancer patients were significantly higher than those in healthy controls (74.51 ± 16.65 pg/mL versus 16.79 ± 5.52 pg/mL, resp. ($P < 0.001$)). Serum levels of Eotaxin-1 in stage I and II gastric cancer patients were significantly higher than those in healthy controls (78.11 ± 26.9 pg/mL versus 12.46 ± 4.76 pg/mL resp. ($P < 0.001$)).

The relationship between s-eotaxin-1 level and clinicopathological variables of gastric cancer was evaluated (Table 1). The s-eotaxin-1 levels did not differ significantly with histopathological grade, tumor-node-metastasis (TNM) stage, tumor localization, lymph node metastases, positive lymph node ratio, perineural invasion, perivascular invasion, size, age and gender. Because of these factors, it is considered that there is no relationship between s-eotaxin-1 level and disease prognosis.

Univariate analysis showed that, TNM stage ($P = 0.006$), the ratios of metastatic to retrieved lymph nodes ($P = 0.002$) invasion are significant factors affecting overall survival. Multivariate regression analysis showed the TNM stage (hazard ratio = 6.65; 95% CI = 2.14–20.41; $P = 0.003$) to be the single significant independent factor for overall survival.

5. Discussion

Gastric cancer is the fourth most frequent cancer and the second cause of cancer-related deaths worldwide [1, 2]. Because of identified at late stages, gastric cancers have high level of morbidity and mortality. The majority of early stages are asymptomatic and only 25% of the patients can undergo resection at diagnosis. Thus, the early diagnosis of gastric cancer is fundamental in decreasing the mortality rates.

Table 1: The relationship between s-eotaxin-1 levels and pathologic variables in gastric cancer patients.

Variables	*n*	S-eotaxin, pg/mL median (interquartile range)	*P*
Age, y			
<50	9	81.2 (±13.70)	0.681
50–60	12	76.8 (±19.56)	
>60	39	72.2 (±16.20)	
Sex			
Male	40	76.08 (±16.74)	0.845
Female	20	71.38 (±16.44)	
Tumor localization			
Cardia/Fundus	25	74.35 (±15.00)	0.354
Corpus	7	79.49 (±18.54)	
Antrum	28	73.41 (±17.95)	
Differentiation			
Well	9	72.08 (±18.53)	0.299
Moderate	12	80.26 (±11.62)	
Poor	33	73.65 (±18.35)	
Undifferentiated	6	71.38 (±12.86)	
Tumor size			
≤4 cm	15	78.25 (±14.83)	0.271
4 cm<	39	74.00 (±17.64)	
Lymph node metastases (N)			
0	9	75.82 (±19.19)	0.823
I	8	80.15 (±14.26)	
II	17	74.09 (±14.51)	
IIIA	11	70.51 (±20.02)	
IIIB	11	75.59 (±19.03)	
Positive lymph node ratio (%)			
0	9	75.82 (±19.19)	0.547
1–9	10	80.30 (±13.78)	
10–25	12	69.67 (±11.74)	
26<	25	74.75 (±19.39)	
TNM Stage			
I/II (early gastric cancer)	17	78.29 (±15.47)	0.427
III/IV (late gastric cancer)	43	73.02 (±17.04)	
Perineural invasion			
Positive	46	74.40 (±16.67)	0.831
Negative	9	74.12 (±18.14)	
Vascular invasion			
Positive	46	74.77 (±16.76)	0.898
Negative	9	72.23 (±17.45)	

Routine clinical application requires easily implementable tests for biomarker analyses. Biomarkers for the early detection of gastric cancer are the central component of ongoing efforts to deal with the disease. Serum markers ever used in the diagnosis of gastric cancer such as CEA and CA19-9 are not specific or sensitive to the disease, so more useful and feasible screening methods should be provided [13].

Eotaxin-1 is an eosinophil-selective chemoattractant and is a member of subfamily of chemokines. And as known, chemokines are responsible for promoting leukocyte attraction to sites of inflammation and cancer. Also some of the chemokines may promote and regulate metastasis and angiogenesis [11]. Until recently, eotaxin-1 was considered to be just an eosinophil-specific chemoattractant and consequently was studied mostly in diseases characterized by an accumulation of eosinophils in tissues, notably allergic conditions, such as asthma, rhinitis, and atopic dermatitis, and other inflammatory disorders, such as inflammatory bowel disease, eosinophilic gastroenteritis and pneumonia [14]. Since eotaxin-1 has been poorly investigated outside the realm of

lymphoid cells, little is known about its biological significance in different cell types, normal or malignant. Eotaxin-1 seems to play a potential role in a number of diseases such as gastrointestinal disorders [15] and it has been suggested that eotaxin-1 could play a protective role against tumour progression [16] probably as a factor in tumour immune surveillance [17].

The function of eotaxin-1 and its receptors have been examined in several types of cancer. CCR3, the primary receptor of eotaxin-1, was found to be overexpressed in renal cell cancer and found to be associated with the tumour grade [18]. In a recent study, Agarwal et al. examined the eotaxin-1 as a diagnostic marker for prostate cancer, it was shown that serum levels of eotaxin-1 were significantly elevated among men with prostate cancer regardless of prostate size and concluded that Eotaxin-1 levels may provide a useful diagnostic tool to help distinguish between prostatic enlargement and prostate cancer. However, other cytokines that were evaluated in the study did not exhibit significant correlation with prostate cancer [19]. In another study, Wagsater et al. determined the role of eotaxin-1 in colorectal cancer. They found that eotaxin-1 levels in tissues with colorectal cancer were significantly higher than those in normal tissue; however plasma eotaxin-1 levels in patients with colorectal cancer were lower compared with control group and also patients classified as Dukes' stages B and C had lower levels than patients with Dukes' stage A [11]. On the other hand, eotaxin-1 level was found significantly lower in patients with epithelial ovarian cancer and endometrial cancer compared with healthy volunteers [20, 21]. Levina et al. demonstrated that serum concentrations of eotaxin-1 levels in patients with ovarian cancer were significantly lower compared with healthy women, and patients with early stages were found to have significantly lower concentrations of eotaxin-1 compared with patients with late stages. No correlation was found between the serum eotaxin-1 level and the histology of ovarian cancer. Recently, it was shown that cultured ovarian carcinoma cells absorbed soluble eotaxin-1 indicating that absorption by tumor cells could be responsible for the observed decrease of eotaxin-1 in patients with ovarian cancer compared to healthy women and benign patients [22].

Our data showed that eotaxin-1 levels in patients with gastric cancer were significantly higher compared with healthy controls. However, we could not find an association between serum eotaxin-1 level and any of the investigated clinicopathological factors. Taken together, results obtained in this study emphasized the utility of serum eotaxin-1 level as marker for early detection of gastric cancer. However, larger prospective studies are warranted to validate the diagnostic value of serum eotaxin-1 level in gastric cancer.

As described, the role of eotaxin-1 in tumorigenesis included cell proliferation, angiogenesis, cell migration, novel therapeutic agents call attention to targeting this chemokine. The ideal agent would specifically target chemokines or their receptors, there are some agents clinical or preclinical use are defined. But none of them are approved for the treatment of malignancy yet [23].

In conclusion, serum eotaxin-1 level may provide a useful diagnostic tool for screening gastric cancer; despite of this data, larger prospective studies are needed to confirm this result.

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