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Evaluation of the Serum Levels of TNF-α and Interleukin-6 as Potential Diagnostic Biomarkers in the First-Degree Relatives of Patients with Gastric Cancer: A Case-Control Study

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Abstract

Background: Gastric cancer is one of the most prevalent ones with high incidence and mortality worldwide. The aim of this study was to determine the serum levels of tumor necrosis factor alpha (TNF-α) and Interleukin-6 (IL-6) as potential diagnostic biomarkers in the first-degree relatives of patients with gastric cancer.

Method: The present work is a case-control study (case, n = 90; control, n = 90). The cases were selected from the first-degree relatives of the gastric cancer patients referred to Kosar Hospital of Semnan city, Iran, during 2019, consecutively. The controls were; however, a random sample from all the healthy people referred to Blood Transfusion Center of Semnan city in the same year. Demographic, laboratory, and pathological variables were collected from both groups. The levels of IL-6 and TNF-α were measured with ELISA method via platinum ELISA-8-11 kits. STATA 14 software was used for data analysis.

Results: In the case and control groups, 74.44 and 78.79% were male, respectively. Their mean age (SD) was 39.17 (10.75) and 36.16 (10.19) in the case and control groups, respectively. Based on the multivariable logistic regression model, there was a statistically significant difference between the two groups in terms of TNF-α (OR = 1.15; 95% confidence interval (CI): 1.02 – 1.30) and smoking (odds ratio (OR) = 3.57; 95% CI: 1.48 – 8.64). However, no statistically significant difference was seen between them concerning the level of IL-6 (OR = 1.0004; 95% CI: 0.97 – 1.03).

Conclusion: Increased levels of TNF-α might be useful as diagnostic biomarkers in the first-degree relatives of patients with gastric cancer; nonetheless, studies with larger sample size are required.

Keywords: Tumor necrosis factor alpha, Interleukin-6, First-degree relatives, Gastric cancer, Case-control studies

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Introduction

Cancer is the second leading cause of death in many countries. Among the types of cancers, gastric cancer is one of the most prevalent ones with high incidence and mortality worldwide. Despite the decline in the incidence trend of gastric cancer in most parts of the world, it is still the fifth most common type of this disease and the third leading cause of cancer-caused death worldwide, with 1,033,700 new cases and 783,000 deaths in 2018 according to GLOBOCAN data. According to the same report, in Iran, this cancer was the second most common cancer with 110,000 new cases and the first leading cause of cancer death with 55,800 deaths.[1-3] Gastric cardia cancer occurs in the adjoining region of the esophageal-gastric junction and usually has a number of common factors and epidemiological features associated with esophageal adenocarcinoma (EAC). The non-cardia type, also known as distal stomach cancer, is more common in lower parts of the stomach and pyloric valve.[4, 5] Histologically, gastric cancer is divided into intestinal (epidemic with better prognosis) and diffuse (endemic with poor prognosis) types.[6, 7]

One of the important risk factors associated with gastric cancer is Helicobacter pylori (H. pylori) infection. The bacterium is known to be an important pathogen in gastritis, gastric ulcer, and duodenum. H. pylori has the ability to colonize in the human stomach for a long time and create a chronic inflammatory response, which varies depending on the host's genetic background, bacterial virulence, and environmental factors. Understanding the natural history of this bacterium, it may be conducive to determining the potential biomarkers in the rapid diagnosis and timely treatment of gastric cancer.[8, 9] Studies have shown that the interaction between H. pylori and the gastric epithelium leads to production of cytokines (interleukin (IL)-1β, IL-6, IL-8 and TNF-alpha) and chemokines involved in the chronic inflammatory response, which are carcinogenic.[10, 11] Cytokines leads to a proliferative response with a dense infiltrate of neutrophils and macrophages in the gastric mucosa, eventually contributing to chronic gastritis.[12] Additionally, H. pylori induces gastric mucosa through dendritic cells and T and B cells, eventually leading to the secretion of macrophage chemotactic protein (MCP) -1, tumor

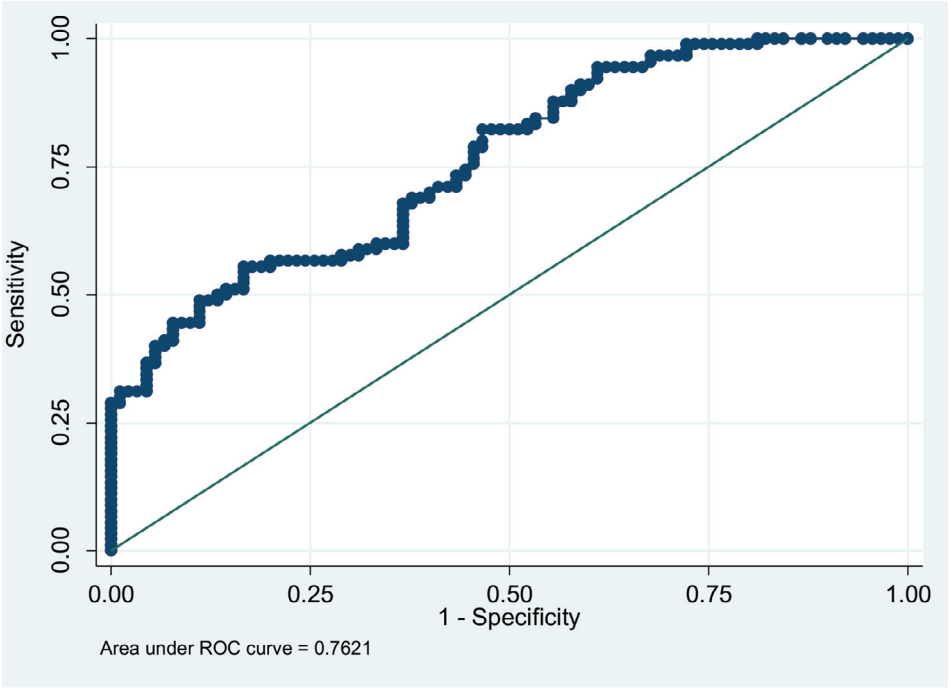

Figure 1. This figure shows the area under the curve for the variables entered in multivariable logistic regression model.
ROC: Receiver operating characteristic

Table 1. Demographic and clinical characteristics of the cases and controls under study

Quantitative variables	Groups	Number	Mean	SD	Min	Max
Age (Year)						
	Case	90	39.17	10.75	22	64
	Control	90	36.16	10.19	20	59
IL-6	Case	90	14.17	11.96	0.12	48.62
	Control	90	14.10	11.55	0.87	49.75
TNF-α	Case	90	2.73	5.48	0.30	32
	Control	90	1.02	2.50	0.10	16.90
Qualitative Variables			**Groups**			
		Case (%)		**Control (%)**		
Sex						
Male		67 (74.44)		71 (78.79)		
Female		23 (25.56)		19 (21.11)		
Education						
Academic		23 (25.56)		16 (17.78)		
Non-academic		67 (74.44)		74 (82.22)		
Smoking						
No		64 (71.11)		82 (91.11)		
Yes		26 (28.89)		8 (8.89)		
Family dependency						
Offspring		66 (73.33)		-		
Other		24 (26.67)		-		
Histological type of cancer						
Intestinal		81 (90.00)		-		
Diffuse		9 (10.00)		-		
Type of cancer based on anatomical location						
Cardia		28 (31.11)		-		
Non-cardia		62 (68.89)		-		
Differentiation of pathology						
Poor		22 (24.44)		-		
Moderate		59 (65.55)		-		
Well		9 (10.01)		-		

SD: Standard deviation; TNF-α: Tumor necrosis factor α; IL-6: Interleukin-6

necrosis factor (TNF)-α, IL-12, IL-10, transforming growth factor (TGF) -β, and interferon (IFN) –γ.[9]

The inflammatory mediators are produced in the gastric mucosa into the bloodstream and can be detected in plasma samples; identifying them can provide a basis for early diagnosis and early treatment of gastric cancer in the early stages.[10] Although studies have shown the levels of these biomarkers in patients with gastric cancer to be higher than those in healthy people,[13] there is scarce research on the level of these factors in relatives of gastric cancer patients. Therefore, given the fact that the inflammatory mediators could function as indirect indicators of tissue damage, the present study aimed to determine the serum levels of TNF-α and IL-6 as potential diagnostic biomarkers in the first-degree relatives of patients with gastric cancer.

Material and Methods

Study design and subjects

The present work was a case-control study aiming to determine the serum levels of IL-6 and TNF-α in the first-degree relatives of patients with gastric cancer (case, n = 90) compared with those of healthy subjects (control, n = 90). We recruited the cases from the first-degree relatives of the patients with gastric cancer referred to Kosar Hospital of Semnan city, Iran, during 2019, consecutively. Subsequently, for each case, one control was selected. The controls were a random sample from all the healthy people referred to Blood Transfusion Center of Semnan city in the same year. The inclusion criteria for the cases

Table 2. The relationship of TNF-α and IL-6 levels with demographic and pathological variables in the first-degree relatives of patients with gastric cancer by Mann–Whitney U test

Qualitative variables	IL-6		TNF-α	
	Mean ± SD	*P*-value	Mean ± SD	*P*-value
Age				
<50	12.98 ± 11.40	0.052	2.71 ± 5.93	0.381
≥50	18.21 ± 13.22		1.75 ± 3.12	
Sex				
Male	13.96 ± 12.19	0.615	2.31 ± 4.81	0.804
Female	14.75 ± 11.54	3.01 ± 6.97		
Education				
Academic	12.76 ± 11.42	0.380	3.17 ± 6.82	0.800
Non-academic	14.67 ±12.20		2.25 ± 4.87	
Smoking				
No	13.75 ±11.30	0.582	2.18 ± 4.76	0.454
Yes	15.21 ± 13.70		3.30 ± 6.85	
Family dependency				
Offspring	13.49 ± 12.42	0.160	2.78 ± 6.01	0.955
Other	15.97 ± 10.70		1.72 ± 2.96	
Histological type of cancer				
Intestinal	14.31 ± 12.58	0.462	1.90 ± 2.80	6.78
Diffuse	16.52 ± 11.17		3.45 ± 7.26	
Type of cancer based on anatomical location				
Cardia	15.08 ± 13.82	0.664	2.50 ± 2.30	0.496
Non-cardia	15.99 ± 12.71		4.06 ± 9.27	

SD: Standard deviation; TNF-α: Tumor necrosis factor α; IL-6: Interleukin-6

were having a patient with a definite diagnosis of gastric cancer in the first-degree relatives (offspring, parents, sister, and brother), absence of lesions in other parts of the body on examination and the patient's history, and informed consent for participation in the research. Inclusion criteria for the controls were absence of gastrointestinal malignancies, not taking anti-inflammatory drugs (ibuprofen, cortisone), and informed consent for participation in the research.

Data collection

We used a checklist for collecting the data, which consisted of demographic (age, sex, education, smoking, and family dependency), laboratory (IL-6 and TNF-α), and pathological variables (histological type of cancer, type of cancer based on anatomical location). Demographic data was obtained through interviews with the participants. Pathological data was also obtained from the medical records of the subjects with gastric cancer. To measure IL-6 and TNF-α, 5 CC of intravenous blood was taken from all the participants under fasting conditions. After one hour, they were placed at a temperature of -4 °C and then centrifuged at 2,000 rpm for 10 minutes. They were then kept at -25 °C until the final immunological evaluations. Finally, the levels of IL-6 and TNF-α were measured using ELISA method via platinum ELISA-8-11 kits (made in Australia) according to the kit instructions.

Statistical analysis

Data were analyzed using Stata version 14 (StataCorp, College Station, TX, USA). For descriptive analyses, the mean, standard deviation (SD), and number (%) were calculated. Afterwards, independent samples t-test or Mann–Whitney U test were utilized for determining the relationship of TNF-α and IL-6 levels with the variables under study in the case group. In addition, univariate and multivariable logistic regression models were used for comparing the demographic and clinical variables in the case and control groups, followed by which the crude and adjusted odds ratio (OR) with a 95% confidence interval (CI) was estimated. A *P*-value

Table 3. Comparison of demographic, laboratory-related, and pathological variables in the case and control groups via univariate logistic regression model

Variable	Crude OR	95% confidence interval	*P*-value
Age (Year)	1.03	0.99 – 1.05	0.057*
IL-6	1.0004	0.97 – 1.03	0.971
TNF-α	1.16	1.02 – 1.31	0.028
Sex			
Female	Reference	-	0.481
Male	0.78	0.39 – 1.56	
Education			
Academic	Reference	-	0.197
Non-academic	0.63	0.31 – 1.29	
Smoking			
No	Reference	-	0.001
Yes	4.16	1.77 – 9.81	

*P-values ≤ 0.20 were considered significant to enter the multivariable logistic regression model; OR: Odds ratio; TNF-α: Tumor necrosis factor-α; IL-6: Interleukin-6

<0.05 was considered to indicate the statistical significance.

Ethics statement

Prior to data collection, the objectives of the research were explained to the participants, and informed consent was then obtained. We conducted this study according to the principles expressed in the Declaration of Helsinki. Moreover, the Deputy of Research and Ethics Committee of Semnan University of Medical Sciences, Semnan, Iran approved the present research under the code IR.SEMUMS.385.

Results

The current study aimed to determine the serum levels of IL-6 and TNF-α in the first-degree relatives of patients with gastric cancer (case group; n=90) compared with those in a healthy group (control group; n = 90). Table 1 shows the demographic and clinical characteristics of the population under study. As shown, the numbers of men and women were 67 (74.44%) and 23 (25.56%) vs. 71 (78.79) and 19 (21.11) in the case and control groups, respectively. The mean of the subjects (SD) was 39.17 (10.75) and 36.16 (10.19) in the case and control groups, respectively. Furthermore, the number (%) of smokers was 26 (28.89) in the case group vs. 8 (8.89) in the control group. In addition, the means (SD) of IL-6 and TNF-α levels were respectively 14.17 (11.96) vs. 14.10 (11.55) and 2.73 (5.48) vs. 1.02 (2.50), for the case and control groups. Among the cases, the most common type of gastric cancer based on histologic classification and anatomical location were intestinal (90%) and non-cardia (68.89%), respectively. Other details concerning the clinical and demographic variables can be seen in table 1.

Table 2 represents the association of TNF-α and IL-6 levels with demographic and pathological variables in the first-degree relatives of the patients with gastric cancer through Mann–Whitney U test. As seen, the results of this test showed no significant statistical relationship between their levels and age, sex, education, smoking, family dependency, histological type of cancer, or the type of cancer based on anatomical location ($P > 0.05$).

Univariate logistic regression model was used to compare the demographic, laboratory-related, and pathological variables in the case and control groups (Table 3). As seen, a significant difference was found between the case and the control groups regarding TNF-α (OR = 1.16; 95% CI: 1.02 - 1.31), age (OR = 1.03; 95% CI: 0.99 – 1.05), education (OR = 0.63; 95% CI: 0.31 – 1.29), and smoking (OR = 4.16; 95% CI: 1.77 – 9.81) ($P \leq 0.20$). The crude OR for TNF-α was 1.16 (95% CI: 1.02 - 1.31), meaning that the TNF-α was 1.16 times higher among the case subjects compared with that in the controls.

Table 4 depicts OR and 95% CI derived from multivariable logistic regression model. According to the table, after adjusting for the confounding

Table 4. OR and 95% confidence interval derived from multivariable logistic regression model

Variable	Adjusted OR	95% Confidence interval	*P*-value
Age (Year)	1.03	0.99 – 1.06	0.077
TNF-α	1.15	1.02 – 1.30	0.027
Education			
Academic	Reference	-	0.267
Non-academic	0.65	0.30 – 1.39	
Smoking			
No	Reference	-	0.005
Yes	3.57	1.48 – 8.64	

OR: Odds ratio; TNF-α: Tumor necrosis factor-α

variables, the TNF-α (OR = 1.15; 95% CI: 1.02 – 1.30) and smoking (OR = 3.57; 95% CI: 1.48 – 8.64) showed a statistically significant difference between the case and the control groups ($P < 0.05$). The adjusted OR for TNF-α was 1.15 (95% CI: 1.02 - 1.30), suggesting that the TNF-α was 1.16 times higher among the case group in comparison with that in the control group after adjusting for the confounding variables.

Figure 1 illustrates the area under the receiver operating characteristic (ROC) curve for significant variables included in the final model, which was 0.7621; this value demonstrates the high discriminative power of this model distinguishing between the case and control subjects.

Discussion

According to the results herein, the numbers of men and women were 67 and 23 vs. 71 and 19 in the case and control groups, respectively. The mean age of the participants (SD) was 39.17 (10.75) and 36.16 (10.19) in the case and control groups, respectively. Moreover, there was no significant statistical relationship between TNF-α and IL-6 levels and the demographic and pathological variables under study in the case groups ($P > 0.05$). The multivariable logistic regression model also showed a statistically significant difference between the case and control groups in terms of TNF-α (OR=1.15; 95% CI: 1.02 – 1.30) and smoking (OR = 3.57; 95% CI: 1.48 – 8.64). However, there was no statistically significant difference concerning the level of IL-6 (OR = 1.0004; 95% CI: 0.97 – 1.03) between the groups. Additionally, the area under the ROC curve was 0.7621 for multivariable logistic regression model, demonstrating the high discriminative power of this model distinguishing between the case and control subjects.

To the best of our knowledge, this research is among the first studies examining TNF-α and IL-6 levels as potential diagnostic biomarkers in the first-degree relatives of patients with gastric cancer. Due to the lack of similar papers in this field, we were forced to compare our results with some less similar studies. Herein, although the level of IL-6 in the cases was higher than that in the controls, this difference was not statistically significant, which was inconsistent with the results of somewhat similar studies in this area;[10, 14, 15] for example, Norma Sánchez-Zauco et al. investigated circulating cytokines and chemokines as potential diagnostic biomarkers for gastric cancer. They reported that IL-1β, IL-6, IFN-α, and IL-10 levels were significantly higher, whereas MCP-1 level was lower in gastric cancer patients compared with healthy controls. However, IL-8 and TNF-α levels did not show a statistically significant difference between the two groups. Finally, the study concluded that increased levels of IL-6, IFN-α, and IL-10 may be useful as diagnostic biomarkers in gastric cancer.[10] Evidence suggests that IL-6 causes upregulation of DNA methyltransferases and subsequently leads to modification of the methylation status of the genes associated with tumor suppression.[16] Studies on mice with colitis have indicated that IL-6 stimulates the survival and proliferation of premalignant intestinal epithelial cells, whose main cause has been reported to be the increase in signal transducer and activator of transcription

(STAT) 3 signaling. IL-6 also has an adverse effect on the expression of genes associated with tumor suppression and anchorage dependence growth in colonic epithelial cells under laboratory conditions.[17] In addition, studies have shown that the expression of IL-6 and STAT3 is increased in gastric tumor tissue in patients with gastric cancer, and that IL-6 level is directly related to the stage of gastric cancer.[18] Therefore, various studies have highlighted an increase in IL-6 levels and its association with gastric cancer as a risk factor. Perhaps the important reasons behind the incompatibility of our results with those of these studies are different case groups and sample size.

In our study, TNF-α level in the case was higher than that in the control, with the difference being statistically significant, which was consistent with the results of previous studies in this regard.[19] TNF-α, as one of the important mediators of inflammation, is involved in various stages of cellular transformation, survival, proliferation, invasion, angiogenesis, and metastasis.[20, 21] This biomarker is one of the systemic cytokines and belongs to the group of acute inflammatory phase cytokines, which are mainly produced by macrophages. Its primary role is to regulate the immune system. It can cause fever, cell death, sepsis (along with IL-6 and IL-1), cachexia, inflammation and inhibition of tumorogenesis, and viral replication. Evidence attributes the lack of regulation of TNF-α to conditions such as Alzheimer's, neoplasm, osteoporosis, deep depression and inflammatory bowel disease, cardiovascular diseases, and severity of rheumatoid arthritis.[22-23] Yajuan Xu et al. assessed the associations of TNF-α-308 (rs1800629) and -238 (rs361525) with gastric cancer, whose results demonstrated that TNF-α-G308A (rs1800629) polymorphisms increase the susceptibility to gastric cancer in Chinese population (OR: 2.07; 95% CI: 1.34-3.21).[24] In another study by Jung Hwan Yoon et al., the TNF-α -308 polymorphism of the TNF-α gene was associated with susceptibility to gastric cancer in the Korean population.[25] Accordingly, the majority of studies have identified this biomarker as a potentially important factor in the diagnosis and screening of gastric cancer; therefore, attention to this biomarker can be helpful in the early detection of gastric cancer in the first-degree relatives of patients with gastric cancer.

The study has several strengths and limitations. Being one of the first studies examining the levels of TNF-α and IL-6 as potential diagnostic biomarkers in the first-degree relatives of patients with gastric cancer, might be the most important strenght of this study. Secondly, accurate measurements of the level of these biomarkers were directly from blood samples, while many other studies have extracted their levels from medical records. Thirdly, the randomly selected controls from the general population minimized the possibility of selection bias. Nevertheless, the significant weakness of this study might be the relatively small sample size and low number of studied biomarkers.

Conclusion

The present study suggested that the increased levels of TNF-α may be potentially useful as diagnostic biomarkers, as well as a starting point for screening the first-degree relatives of patients with gastric cancer. However, further research with larger sample size and more inflammatory mediators are required.

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Conflict of Interest

None declared.

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