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Original Article

The diagnostic value of macrophage migration inhibitory factor, carcinoembryonic antigen, and carbohydrate antigen 19-9 in gastric cancer

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Abstract

Introduction: Gastric cancer is a rather common malignancy worldwide and a major healthcare system issue. Lately, the importance of biomarkers such as macrophage migration inhibitory factor (MIF) has been demonstrated in the diagnosis of various gastrointestinal (GI) malignancies. The present study aimed to evaluate the diagnostic value of MIF, carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 **(**CA 19-9) in gastric cancer.

Methods: In this descriptive-analytical study, 84 patients with gastric cancer referred to the gastroenterology clinic of Tabriz University of Medical Sciences, Tabriz, Iran, for diagnostic and therapeutic procedures, and 80 healthy individuals were enrolled. Serum levels of MIF, CEA, and CA 19-9 were measured in both groups. Further, the grade and stage of the cancer were determined in in the patient group.

Results: Serum levels of all three MIF, CEA, and CA 19-9 biomarkers in patients with gastric cancer were significantly higher than those of the control group (P = 0.001). However, no statistically significant correlations were found between the studied biomarkers with the tumor grade and stage. The MIF cut-off point for the diagnosis of gastric cancer was found to be 7.05 pg/ml and its sensitivity and specificity were 85.7% and 73.8%, respectively.

Conclusion: MIF biomarker may involve in the pathogenesis and development of gastric cancer and it is a potential diagnostic and therapeutic marker in this malignancy.

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Introduction

Gastric cancer is the most common malignancy in men and the second most common malignancy in women in Iran.[1,2] Despite the development of novel diagnostic and treatment strategies, its five-year survival is still low.[3] Gastric cancer is asymptomatic in its early stages and thus is hardly diagnosed; albeit, its early diagnosis and treatment bears a favorable prognosis.[4]

Recently, a wide range of biomarkers has been proposed to diagnose and follow up gastric cancer. The advent of these biomarkers has led to a modest improvement in the

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survival of patients with gastric malignancy.[5,6]

Amongst factors participating in the gastric mucosa inflammation, macrophage migration inhibitory factor (MIF) is of special importance due to its pro-inflammatory features. Being discovered four decades ago, MIF biological role and function were unclear until the discovery of its three-dimensional (3D) structure. MIF primarily inhibits macrophage migration which has role in the chronic inflammation and cytokines production. MIF also involves in the catalytic processes, endocrine functions, signal transduction, and lymphocyte-mediated immunity.[7]

Evidence shows that MIF level increases during malignancies such as melanoma, neuroblastoma, and prostate, liver, and breast cancers.[8] It has been revealed that MIF can have prognostic value in gastric cancer and its expression is higher in malignant tissues than in normal tissues.[9] On the other hand, it has also been stated that MIF does not have a noticeable role in the progression of gastric adenocarcinomas and their grade and stage.[10]

Moreover, carcinoembryonic antigen (CEA) is one of the most commonly used tumor markers to monitor the treatment process of colorectal, breast, pancreas, lung, and cervix cancers. A recent meta-analysis confirmed the link between increased serum levels of CEA with poor prognosis and mortality among patients with gastric cancer.[11] Further, carbohydrate antigen 19-9 (CA 19-9) is a tumor marker being used in the follow-up of colorectal malignancies.[12] A study showed that gastric cancers with higher levels of CA 19-9 bore poor prognosis.[13]

A diagnostic blood sample-based test enabling us to determine the likelihood of developing gastric cancer is of high priority. However, this has not been available until now. In some studies performed in China, Turkey, and Japan, the plasma levels of MIF have been shown to be high in patients with gastric cancer.[14]

Due to the importance of this issue and lack of organised studies in this field in Iran, we were determined to assess the serum levels of MIF, CA 19-9, and CEA in patients with gastric cancer and their association with tumor stage and grade.

Methods

Study design: In a descriptive-analytical study, 84 patients with pathologically-confirmed gastric cancer referred to the gastroenterology clinic of Tabriz University of Medical Sciences, Tabriz, Iran, were included. The duration of the study was one year, from February 2015 to February 2016.

Inclusion and exclusion criteria: Patients with gastric cancer diagnosed using endoscopy and confirmed via pathology report were included in this study. However, patients with metachronous cancer and those with positive history for chemo/radiotherapy were excluded from the study. Also, patients who declined to participate were not enrolled in this study.

Study procedure: 84 patients with confirmed gastric cancer were randomly selected using convenient sampling method and enrolled in the study. In addition, 80 healthy age- and gender-matched subjects with no specific known disease were selected and studied as the control group.

Blood sample (3 ml) was obtained from all of the patients and control group. One hour after blood collection, the samples were centrifuged at 800 rpm for 10 minutes, and then the serum samples were separated; the CEA and CA 19-9 levels were quickly measured using quantitative luminescence method, and the remaining serum was frozen at -20 °C for the subsequent use. Accordingly, the serum MIF level was measured by enzyme-linked immunosorbent assay (ELISA) method in four steps (to prevent serum MIF reduction).

Ethics: This study was approved by the Medical Ethics Committee of Tabriz University of Medical Sciences. All of the patients' information was confidential, and their personal information was not mentioned anywhere. Informed consent was obtained from all of the included patients and control group. Moreover, the study protocol was consistent with the ethical guidelines of the

Declaration of Helsinki (1975) as reflected in a prior approval by the institution's human research committee.

Statistical analysis: The SPSS software (version 16, SPSS Inc., Chicago, IL, USA) was used for all statistical analyses. Data were expressed as mean ± standard deviation (SD) and/or medium plus minimum or maximum, frequency, and percentage. The normal distribution of data was evaluated using Kolmogorov-Smirnov test (K–S test).

Independent t-test and Mann-Whitney U test were used to compare the quantitative variables. The Spearman correlation coefficient was used to assess the relationship between biomarkers with grade and stage of cancer. The Receiver Operating Characteristic (ROC) curve was used to determine the cut-off point and sensitivity and specificity of the biomarker. $P \leq 0.050$ was considered as statistically significant.

The sample size of the primary data was determined according to Camlica et al. study,[15] using G*Power sample size calculation software, and considering $\alpha = 0.05$ and $1\text{-}\beta = 80\%$. This yielded a minimum sample size of 84 patients which were randomly selected and included in the study.

Results

Of 84 patients studied, 62 (73.8%) patients had low-grade and 22 (26.2%) patients had high-grade stomach tumors. Staging of the tumor in the included population revealed that 7 patients were at stage IB, 40 patients were at stage II A and B, 27 patients were at stage III A and B, and 10 patients were at stage IV.

We found that CEA serum level was significantly higher in patients with gastric cancer than that of the control group (P = 0.001) (Table 1).

Table 1. Comparison of carcinoembryonic antigen (CEA) levels in the two studied groups

Group	Median (mcg/l)	Minimum (mcg/l)	Maximum (mcg/l)
Case group	1.35	0.40	102.50
Healthy controls	0.60	0.20	2.30
P		0.001	

In addition, results revealed that CEA serum level was significantly higher in the case group than that of the control group (P = 0.001) (Table 2).

Table 2. Comparison of carbohydrate antigen 19-9 (CA 19-9) levels in the two studied groups

Group	Median (U/ml)	Minimum (U/ml)	Maximum (U/ml)
Case group	14.30	0.70	239.60
Healthy controls	4.70	0.20	24.90
P		0.001	

Further analysis showed a significantly higher serum level of MIF in the case group compared with the control group (P = 0.001) (Table 3).

Table 3. Comparison of macrophage migration inhibitory factor (MIF) levels in the two studied groups

Group	Median (pg/ml)	Minimum (pg/ml)	Maximum (pg/ml)
Case group	8.40	5.10	38.70
Healthy controls	5.80	3.40	24.40
P		0.001	

Moreover, the serum levels of CEA, CA 19-9, and MIF had no significant correlation with the stage and grade of the tumor in patients ($P < 0.050$ for all comparisons). However, in case of any significance, the correlation was not found to be strong ($rs > 0.50$) (Table 4).

Table 4. The association of the studied biomarkers with stage and grade of gastric cancer in the patients group

Biomarker	Grade	Stage
CEA	Rs = 0.026	Rs = 0.264
	P = 0.812	P = 0.015
CA 19-9	Rs = -0.036	R = -0.407
	P = 0.747	P < 0.001
MIF	R = -0.148	R = -0.134
	P = 0.179	P = 0.225

CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigen 19-9; MIF: Macrophage migration inhibitory factor

In addition, the cut-off point of CEA biomarker was determined to be 0.75 mcg/l for the diagnosis of gastric cancer with a sensitivity of 76.2% and specificity of 70.0%. Accordingly, the positive predictive value of this test was 72.5%, and the negative predictive value was 73.8%. Also, the area under the curve of the graph was calculated

to be 0.800 (Figure 1).

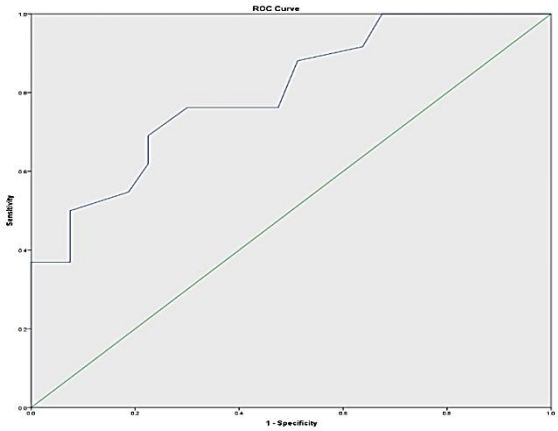

Figure 1. Receiver operating characteristic (ROC) curve for carcinoembryonic antigen (CEA) levels in patients with gastric cancer

The cut-off point of CA 19-9 was found to be 4.35 U/ml for the diagnosis of gastric cancer with a sensitivity of 81.0% and specificity of 47.5%. Accordingly, the positive predictive value of this test was 61.6% and the negative predictive value was 70.6%. Also, the area under the curve of the graph was calculated to be 0.696 (Figure 2).

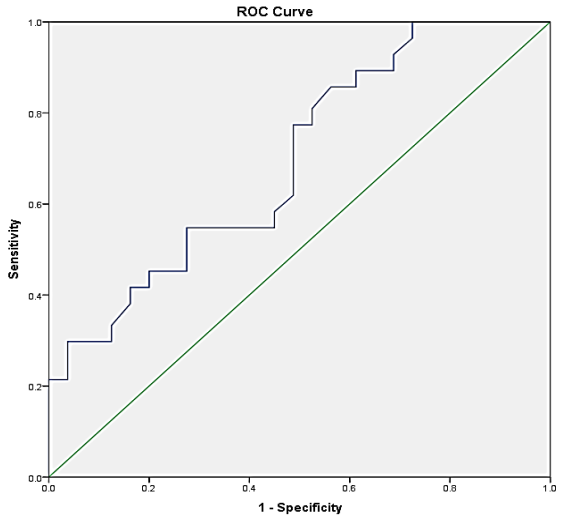

Figure 2. Receiver operating characteristic (ROC) curve for carbohydrate antigen 19-9 (CA 19-9) levels in patients with gastric cancer

We also found that the cut-off point of MIF was 7.05 pg/ml for the diagnosis of gastric cancer with a sensitivity of 85.7% and specificity of 73.8%. Accordingly, the positive predictive value of this test was 77.2% and the negative predictive value was 83.2%. Also, the area under the curve of the graph was calculated to be 0.774 (Figure 3).

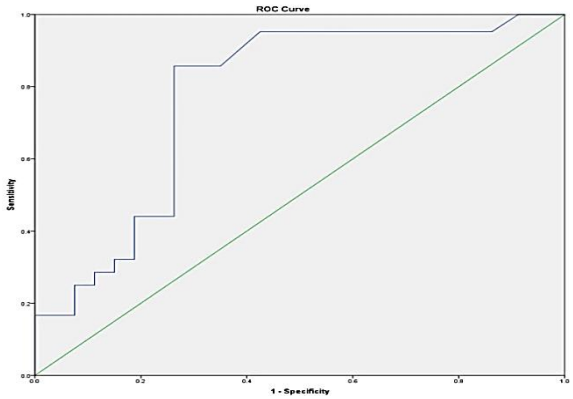

Figure 3. Receiver operating characteristic (ROC) curve for macrophage migration inhibitory factor (MIF) levels in patients with gastric cancer

Discussion

Recently, the importance of biomarkers in the treatment and diagnosis of gastrointestinal (GI) malignancies has been revealed. CEA is a glycoprotein that is normally produced in the GI tissues during the fetal period, but its production is halted right after the birth. However, its serum levels increase in several malignancies and thus is used as a tumor marker.[16] Further, CA 19-9 is a tumor marker and its serum levels commonly increase in pancreatic cancer.[17] MIF is produced by T lymphocytes and inhibits macrophage migration. The increased serum level of MIF has been shown in some malignancies.[15,18]

Due to the importance of biomarkers in early diagnosis of gastric cancer, we compared the serum levels of MIF, CEA, and CA 19-9 in the gastric cancer patients with those of healthy controls. Based on the finding of the present study, the serum levels of CEA, CA 19-9, and MIF were significantly higher in the respected patients compared with the control group. However, there was no association between the serum levels of these biomarkers and tumor stage and/or grade.

In line with that, Yuasa conducted a study to assess the MIF serum levels in different stomach pathologies. The expression of MIF was 12.0% in the normal epithelial cells, 52.0% in gastritis, 66.0% in intestinal

metaplasia (IM), and 96.0% in gastric adenocarcinoma. Also, the serum level of MIF in normal subjects was low (576 pg/ml). However, it was higher in patients with gastritis (2100 pg/ml) and IM (4498 pg/ml). The highest level of MIF was found in patients with gastric cancer (9737 pg/ml). The results of this study showed the increased serum levels of MIF in patients with gastric cancer.[19]

Further, Xia et al. performed a study to evaluate the serum levels of MIF in patients with Helicobacter pylori (H. pylori)-induced gastritis. This study showed a lower expression level of MIF in messenger ribonucleic acid (mRNA) (antrum: 11.6 ± 1.0% and body: 10.6 ± 1.1%) and in glandular epithelial cells in H. pylori-negative patients compared with patients who were positive for H. pylori in the gastric antrum and body (42.4% ± 4.5% and 37.9.0% ± 3.9%, respectively). There was no association between the expression of MIF in the gastric epithelial cells with the density of H. pylori and severity of gastritis.[20]

In another study, Shun et al. assessed the MIF levels and its association with gastric cancer in 90 patients. This study found no statistically significant association between MIF levels with tumor location, histologic subtypes, lymph node metastasis, and expression of p53. However, the expression level of MIF was found to be lower in early versus late gastric cancers.[21] Similarly, our study failed to show an association between MIF serum levels and stage/grade of cancer.

Xia et al. performed a study on 97 patients with gastric adenocarcinoma and 222 patients with dyspepsia. The expression levels of MIF in cancer and dyspeptic patients were 6554.0 ± 204.1 pg/ml and 1453.7 ± 79.9 pg/ml, respectively. This study showed that the expression of MIF increased as gastric pathology worsened, and MIF was a better predictor of gastric cancer in patients with dyspepsia than CEA.[22] However, our study did not prove the latter.

Lai et al. conducted a study to assess and compare the serum level of CEA and CA 19-9 in patients with gastric cancer and normal control subjects. The sensitivities of CEA and CA 19-9 as biomarkers for gastric cancer were 31.4% and 16.1%, respectively.[23] In our study, the sensitivity and specificity of CEA as a biomarker for gastric cancer in values higher than 0.75 mcg/l were 76.2% and 70.0%, respectively. These numbers were 81.0% and 47.5%, respectively for CA 19-9 in values higher than 4.35 U/ml.

Additionally, Kochi et al. conducted a study to evaluate the prognostic value of CEA and CA 19-9 in gastric cancer. An increase in the CEA and CA 19-9 levels was seen in 19.0% and 21.8% of the patients, respectively. Only 6.7% of the patients had an increase in both of the markers. This study also showed that the increase in these biomarkers was in association with lymph node and vascular metastasis and invasion depth. In addition, increased level of CA 19-9 correlated with peritoneal and distant metastasis.[24] The findings of our study, however, did not show a relationship between these biomarkers and stage/grade of gastric cancer.

Conclusion

Based on the findings of the present study and other works, the serum levels of MIF, CEA, and CA 19-9 increase in patients with gastric cancer. However, controversy exists over the usefulness of these biomarkers as prognostic factors in this regard. More studies are needed for better decision making.

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Authors' Contribution

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Acquisition of data: Gilda Ghazisoltani, Mahzad Azimpouran, Sepideh Fattahi, Jalal Farshbafinezhad-Zoghi

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Critical revision of the manuscript for important intellectual content: Rasoul Estakhri, Kourosh Masnadi-Shirazi

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Study supervision: Rasoul Estakhri, Kourosh Masnadi-Shirazi.

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Conflict of Interest

Authors have no conflict of interest.

Ethical Approval

This study was approved by the Medical Ethics Committee of Tabriz University of Medical Sciences (No. 3/93-1/5).

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