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Original Research

EpCAM is a new potential serum biomarker for early gastric cancer

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Abstract

Aim: Overexpression of epithelial cell adhesion molecule (EpCAM) has been shown in a wide spectrum of epithelial cancers. In this study we aimed to examine the utility of serum EpCAM levels as a new marker for early diagnosis of gastric cancer, and to determine its association with different stages of gastric cancer.

Methods: A total of 88 patients who underwent resection due to gastric cancer and 44 healthy subjects as the control group were included in the study. The diagnosis of gastric cancer was confirmed by a combination of upper gastrointestinal endoscopy, endoscopic ultrasonography, and histopathology. EpCAM levels were evaluated using the enzyme-linked immunosorbent assay (ELISA) method following the instructions of the manufacturer.

Results: The mean preoperative EpCAM level was found as 235.7 ± 34.2 pg/mL in the patient group and 42.5 ± 2.38 pg/mL in the control group. The mean preoperative serum EpCAM level was found to be significantly higher in gastric cancer patients compared to the control group ($P < .001$). In addition, mean EpCAM levels were higher in patients with class T4 and T2 compared to T1. Mean EpCAM levels were also significantly higher in N1 and N2 classes compared to N0, and in M1 compared to M0 (all, $P < .001$).

Conclusion: The results of this study indicate that the elevated expression of the EpCAM levels can predispose to the development of gastric cancer. Thus, the analysis of this marker could be a useful biomarker for screening patients with gastric cancer.

Keywords

gastric cancer, epCAM, biomarker, TNM

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Introduction

Gastric cancer (GC) is the fourth most common cancer and the second most common cause of mortality among all cancers worldwide.[1] Patients with GC have a poor prognosis, especially those with advanced stage disease. Although the treatment outcomes have been improved, the recurrence rate following curative resection remains approximately 30% in patients with stage II-III GC.[2] The main cause of death is invasion and metastasis of tumors. Tumor invasion and metastasis are a very complex and continuous process involving multiple steps. Invasion and metastasis are regulated by cellular growth factors, adhesion molecules, protein catabolic enzymes, and various angiogenic factors. Surgery is the only curative method in localized advanced GC, although chemotherapy is the only therapeutic approach for metastatic or recurrent GC patients.[3] Therefore, it is important to identify patients at high risk for recurrence before surgery. In recent years, numerous drugs have become available for the treatment of GC. However, even if these agents are used for recurrent and metastatic GC, the prognosis of GC is still poor.[4]

Recently studied targeted treatments that are related to the expression of a certain gene may result in new opportunities in the treatment of cancer. Epithelial cell adhesion molecule (EpCAM) is a target, which is currently being explored in GC. EpCAM is a type I transmembrane glycoprotein with 39 - 42 kDa, and 314-amino-acid.[5] EpCAM was discovered for the first time as a superficial antigen in colon cancer.[6] EpCAM functions as a homotypic intracellular adhesion molecule. EpCAM's effects are not limited to cell adhesion and also include diverse processes such as signaling, proliferation, differentiation, and cell migration. Overexpression of EpCAM has been demonstrated in a variety of epithelial cancers.[7] Furthermore, EpCAM was found to be overexpressed in breast cancer, ovarian carcinomas, and most human adenocarcinomas. In addition, EpCAM has been considered as a target antigen for a large number of specific immunotherapies because of its frequent and high-level expression.[8]

In this study, we aimed to examine the potential role of serum EpCAM levels as a new marker in the early diagnosis of GC and to determine its association with different stages of GC.

Materials and Methods

Patients and Samples

This study was designed as a retrospective observational study. At the initiation of this study, the necessary approval was received from the Van Yuzuncu Yıl University Ethics Committee (Decision No: 2022/11-11). All patients were informed about the procedure and gave written informed consent during the collection of serum and clinical samples. This study was performed in accordance with the ethical principles of the Declaration of Helsinki, revised in 2013.

A total of 88 patients who underwent resection due to GC in the General Surgery Clinic of our hospital and 44 healthy subjects as the control group were included in the study. Patients with diagnosis of gastric cancer who were candidates for surgery and not receiving neoadjuvant therapy were included in the study. Patients with inflammatory gastric and bowel diseases, atherosclerosis, diabetes, autoimmune diseases, infections, smokers, and those receiving immunosuppressive or immunomodulator therapy were excluded. The diagnosis of GC was confirmed by a combination of upper gastrointestinal endoscopy, endoscopic ultrasonography, and histopathology. Chest radiography, whole-body positron emission tomography (PET), and abdominal pelvic computed tomography (CT) were utilized to identify the tumor/node/metastasis (TNM) classification for GC as described in the 7th TNM guidelines for GC issued by the International Union Against Cancer.[9] EpCAM levels were evaluated using the enzyme-linked immunosorbent assay (ELISA) method following the instructions of the manufacturer.

ELISA Procedure

In all participants, the blood samples were put into a vacuum collection tube three days before the operation. After centrifugation at 1000 × g for 20 minutes, the supernatants were aliquoted in 1.5 mL Eppendorf tubes and stored at -80 °C. Serum EpCAM concentrations were assayed using the ELISA kit (MyBioSource; Catalog No: MBS2020817; Quantitative Sandwich ELISA; Sensitivity: 11.77 pg/mL; Assay range: 31.2 - 2000 pg/mL; Intra-Assay: CV 10%; Inter-Assay: CV 12%). The samples were pre-coated with an antibody specific to EpCAM and put into the microplate wells containing an EpCAM-specific biotin-conjugated antibody. Horseradish peroxidase conjugated with Avidin was then added to the microplate wells and incubated. Following the addition of substrate solution (3,3',5,5'-Tetramethylbenzidine), only the wells containing avidin conjugated with enzyme, antibody conjugated with biotin, and EpCAM changed in color. Then, the reaction of the substrate with the enzyme was terminated by the addition of sulphuric acid and the change in color was spectrophotometrically measured at a wavelength of 450 nm. The optical density (OD) of the samples was compared with the standard curve to determine the concentrations of EpCAM in the samples. EpCAM findings were compared between patient and control groups and between TNM classes.

Ethical Approval

Ethical approval for this study was obtained from the Van Yüzüncü Yıl University Ethics Committee (Decision No: 2022/11-11).

Statistical Analysis

Data obtained in this study were statistically analyzed using the NCSS v.10 (NCSS, LLC 2015, Kaysville, Utah, USA) software. Normal distribution of the variables was tested using the Shapiro-Wilk method, histogram, box plot, and Q-Q plot graphs. Continuous variables were presented as mean ± SD, median (minimum and maximum) values according to the distribution, while categorical variables were expressed as frequency and percentage (*n*, %). Variables that were normally distributed were compared between two groups with the independent t-test and non-normally distributed variables with the Mann-Whitney U test. Variables with three or more categories were compared with the Kruskal-Wallis one-way analysis of variance. Multiple comparisons were made with Dunn's test. Nominal variables were evaluated using the Yates corrected Chi-square test. $P = .05$ was considered statistically significant.

Reporting Guidelines

This study was reported in accordance with the STROBE guideline.

Results

The mean age was 59.18 ± 4.74 years in the patient group and 60.82 ± 4.47 years in the control group. No statistically significant difference was found between the two groups in terms of age. The patient group consisted of 52 (59.09%) males and 36 (40.91%) females, and the control group consisted of 23 (52.27%) males and 21 (47.73%) females. The gender distribution was similar between the two groups.

According to the Sandwich ELISA assay results following the diagnosis, the mean preoperative EpCAM level was 235.7 ± 34.2 pg/mL in the patient group and 42.5 ± 2.38 pg/mL in the control group. The mean preoperative serum EpCAM level was significantly higher in GC patients compared to the control group ($P < .001$). Table 1 shows the demographic and clinical findings of the groups. Figure 1 shows the EpCAM levels of the groups.

The mean serum EpCAM concentration of the patients with class T4 (244.27 ± 15.98 pg/mL) was statistically significantly higher compared to those with class T1 (175.04 ± 39.14 pg/mL) ($P < .001$).

Table 1. Demographic Features and Preoperative Epcam Levels of the Groups

Parameter	Control group (*n* = 44)		Patient group (*n* = 88)		*P* value
	n (%) / Mean ± SD	Median (min–max)	n (%) / Mean ± SD	Median (min–max)	
Gender (F/M)	21/23 (44.73/52.27)		36/52 (40.91/59.09)		.558
Age, y	60.82 ± 4.47	60 (54–69)	59.18 ± 4.74	60 (54–69)	> .05
EpCAM , pg/mL	42.5 ± 2.38	43.15 (38–47.8)	235.7 ± 34.2	237.8 (120.5–290.7)	< .001

Table 2. Preoperative Serum Epcam Concentrations According to the TNM Classification

Class	EpCAM, pg/mL		*P* value
	Mean ± SD	Median (min–max)	
T2 (*n* = 14)	175.04 ± 39.14	180.5 (120.5–220)	< .001
T2 (*n* = 13)	247.91 ± 31.04	241.7 (201.7–290.7)	
T3 (*n* = 22)	242.46 ± 29.2	233.7 (208.5–290.5)	
T4 (*n* = 38)	244.27 ± 15.98	244.4 (210.9–280.5)	
N0 (*n* = 15)	190.98 ± 46.00	207.5 (120.5–285.4)	< .001
N1 (*n* = 26)	241.79 ± 29.18	252.5 (201.7–290.7)	
N2 (*n* = 47)	241.79 ± 17.13	241.7 (208.5–280.5)	
M0 (*n* = 14)	184.24 ± 39.32	199.5 (120.5–235.0)	< .001
M1 (*n* = 74)	245.44 ± 22.71	243.75 (201.7–290.7)	

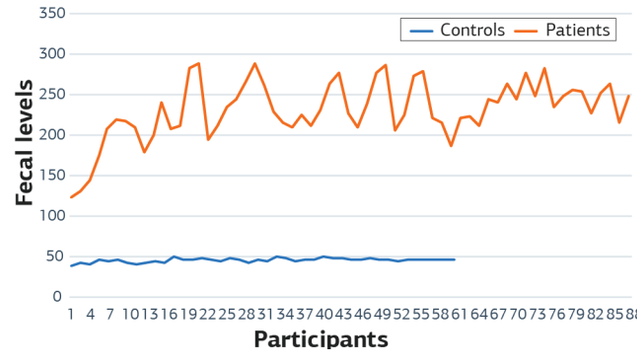

Figure 1. *Epcam Levels of the Groups*

Similarly, the mean serum EpCAM concentration of the patients with class T2 (247.91 ± 31.04 pg/mL) was statistically significantly higher compared to those with class T1 disease (175.04 ± 39.14 pg/mL) (*P* < .001) (Figure 2).

The mean serum EpCAM concentrations of the patients with class N2 (241.79 ± 17.13 pg/mL) and class N1 (250.49 ± 29.18 pg/nL) were statistically higher compared to those with class N0 (190.98 ± 46 pg/nL) (both, *P* < .001). There was no significant difference between the N1 and N2 class patients in terms of the serum EpCAM levels (*P* > .05).

The mean serum EpCAM concentration of the patients with class M1 (245.44 ± 22.71 pg/mL) was statistically significantly higher compared to the patients with class M0 (184.24 ± 39.32 pg/mL) (*P* < .001). Table 2 shows the mean, median, minimum and maximum serum EpCAM values according to the classes.

Discussion

In this study, we investigated preoperative serum concentrations of EpCAM in patients with gastric cancer (GC) in comparison with the healthy control group. Also, we compared the serum EpCAM levels between the TNM classes. Preoperative serum concentrations of EpCAM were significantly higher in the patient group compared to the controls (*P* < .001). In addition, EpCAM levels increased in parallel with the TNM classes.

The incidence of GC increases with aging. In the present study, the mean age of the patients was 59.18 ± 4.74 years. In a study by Niziol et al. evaluating the expression of EpCAM in GC, 69.32% of the patients aged ≥60 years.[10] In another study by Wenqi et al. on EpCAM in GC, 74% of the patients aged ≥50 years.[11] Within this context, our finding is within the range reported in the literature. In the same study, 61% of the patients were male and 39% were female.[11] Similarly, in the present study, 59.09% of the patients were male and 40.91% were female.

EpCAM is secreted in the primary stages of development of tremendously proliferating cells, and its expression is reduced in differentiated cells. EpCAM expression is qualified to epithelial tissues, specifically at the basolateral cell membranes. Nevertheless, its expression increases under pathological conditions such as inflammation and the development of cancer cells.[12] EpCAM is thought to be the primary marker of malignancy. In malignant cells, EpCAM is expressed both in the apical and basolateral membranes.[13]

Overexpression of EpCAM has been shown in numerous malignancies, including pancreatic carcinomas, small intestine, ovary, breast, esophagus, and gastric cancers.[14] In a study by Zhou et al., elevated preoperative levels of EpCAM were associated with early recurrence of hepatocellular carcinoma.[15] In another study by Sun et al., preoperatively, EpCAM+ circulating tumor cells were detected in 82 of 123 patients with hepatocellular carcinoma.[16]

In a study by Niziol et al. investigating EpCAM in GC, the positive expression of this protein was associated with cancerous cells infiltrating to the blood vessels, suggesting that EpCAM may play a role in the early stages of cancer metastases.[10] Supporting this finding, in the present study there was a significant increase in preoperative serum levels of EpCAM as the TNM classes increased.

The functions of EpCAM depend on its extracellular domains for intercellular adhesion with molecules. EpCAM adheres to some proteins and Wnt pathway and regulates T-cell tolerance and evacuation.[17] This also suggests that the expression of EpCAM is more beneficial in the diagnosis and is important for profiling during the diagnostic examination.

The EpCAM expression level was significantly higher in GC samples, pointing out that EpCAM expression elevated during the early stages of GC and increased with the progression of the disease. Therefore, EpCAM has regulation effects on cell migration and proliferation. EpCAM has a critical effect on the invasion, proliferation, and metastasis of tumor cells.

EpCAM was also associated with cellular signals through the Wnt pathway to elevate the characteristics of cancer cells associated with chemotherapy resistance.[18] High levels of EpCAM expression in GC are associated with the clinicopathological features of the disease. Hence, the examination of the therapeutic agent targeting EpCAM for GC is presumed to advance tumor resistance to treatment. This supports EpCAM role in the occurrence and metastasis of GC.

Limitations

Major limitations of this study are the relatively small number of patients and its retrospective design. However, as a strength, we demonstrated the relationship between preoperatively measured levels of EpCAM in patients with GC. We believe that our findings will provide contribution to the existing literature on the role of EpCAM protein in GC.

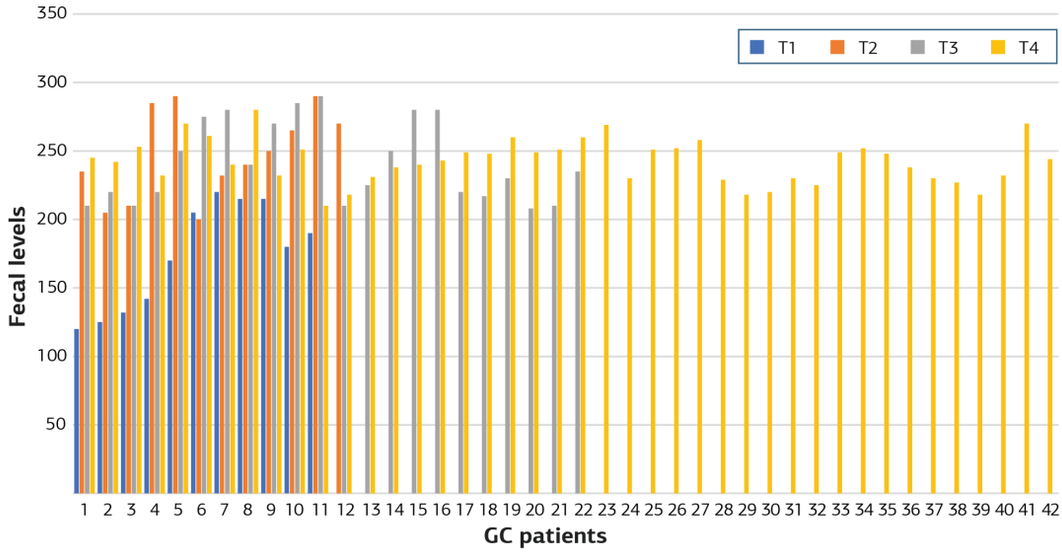

Figure 2. *Epcam Levels of GC Patients According to T Classes*

Conclusion

The results of this study indicate that the EpCAM expression level was significantly higher in GC samples, pointing out that EpCAM expression elevated during the early stages of GC and increased with the progression of the disease. Thus, the analysis of this marker could be a useful biomarker for screening patients with GC. Further, more comprehensive multi-center studies are needed to clarify the potential role of EpCAM in the early diagnosis of GC and predicting the prognosis.

Declarations

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Informed Consent

Written informed consent was obtained from all participants.

Data Availability

The datasets used and/or analyzed during the current study are not publicly available due to patient privacy reasons but are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that there is no conflict of interest.

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None.

Abbreviations

CT: Computed tomography
ELISA: Enzyme-linked immunosorbent assay
EpCAM: Epithelial cell adhesion molecule
GC: Gastric cancer
OD: Optical density
PET: Positron emission tomography
STROBE: Strengthening the reporting of observational studies in epidemiology
TNM: Tumor-node-metastasis

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