GMJ 2024;35:416-421

DOI: http://dx.doi.org/10.12996/gmj.2024.4205

The Relationship Between Serum miRNAs and Surgical Prognostic Factors in Gastric Cancers

Mide Kanserlerinde Serum miRNA Düzeyleri ile Cerrahi Prognostik Faktörler Arasındaki İlişki

Çağrı Büyükkasap[1], Engin Küçükdiler[2], Mahir Nasirov[3], Niyazi Samet Yılmaz[4], Hasan Dağlı[5], İlke Önen[6], Furkan Aydoğdu[7], Aydın Yavuz[1], Hüseyin Göbüt[1], Asiye Uğraş Dikmen[8], Kürşat Dikmen[1], Hasan Bostancı[1], Özlem Gülbahar[9], Abdulkadir Bedirli[1]

[1]Department of General Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye
[2]Aydın State Hospital, Aydın, Türkiye
[3]Caspian International Hospital, Baku, Azerbaijan
[4]Polatlı Duatepe State Hospital, Ankara, Türkiye
[5]Department of Medical Biochemistry, Kahramanmaraş Sütçü İmam University Faculty of Medicine, Kahramanmaraş, Türkiye
[6]Department of Medical Biology, Gazi University Faculty of Medicine, Ankara, Türkiye
[7]Bandırma Training and Research Hospital, Balıkesir, Türkiye
[8]Department of Public Health, Gazi University Faculty of Medicine, Ankara, Türkiye
[9]Department of Medical Biochemistry, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: There is a continued need for biomarkers for patients with gastric cancer that can aid in diagnosis, treatment follow-up, and relapse detection. The objective of this study was to investigate the role of serum microRNA (miRNAs) in diagnosing gastric cancer and their relationship with prognostic factors that impact the choice of surgical approach.

Methods: We compared the serum miRNA expression levels of 35 patients who underwent gastrectomy and D2 lymph node dissection for gastric adenocarcinoma between 2015-2018 with those of 33 controls. We also evaluated the relationship between serum miRNA expression levels and pathological prognostic factors.

Results: The serum levels of miR-17-5p, miR-21-5p, miR-27a-3p, miR-146a-5p, miR-148a-3p, and miR-203a-3p were significantly higher in gastric cancer patients. In early gastric cancer patients, serum levels of miR-21-5p, miR-27a-3p, miR-106b-5p, miR-146a-5p, and miR-148a-3p levels were significantly higher. Furthermore, serum levels of miR-106b-5p and miR-146a-5p were associated with tumor localization [area under the curve (AUC): 0.773, 0.797; p=0.049, 0.036], while serum levels of miR-27a-3p and miR-148a-5p were associated with the T-stage of the tumor (AUC: 0.748, 0.729; p=0.036, 0.049).

Conclusion: Serum levels of miR-17-5p, miR-21-5p, miR-27a-3p, and miR-203a-3p were found to be diagnostic biomarkers in gastric

ÖZ

Amaç: Gastrik kanser hastalarında tanı, tedavi takibi ve nüks tespitinde yardımcı olabilecek biyobelirteçlere olan ihtiyaç devam etmektedir. Bu çalışmanın amacı, mide kanserinin teşhisinde serum mikroRNA'larının (miRNA) rolünü ve cerrahi yaklaşım seçimini etkileyen prognostik faktörlerle ilişkilerini araştırmaktır.

Yöntemler: 2015-2018 yılları arasında gastrik adenokanser nedeniyle gastrektomi ve D2 lenf nodu diseksiyonu uygulanan 35 hastanın serum miRNA ekspresyon seviyelerini 33 kontrolle karşılaştırıldı. Ayrıca serum miRNA ekspresyon seviyeleri ile patolojik prognostik faktörler arasındaki ilişkiy değerlendirildi.

Bulgular: Gastrik kanser hastalarında miR-17-5p, miR-21-5p, miR-27a-3p, miR-146a-5p, miR-148a-3p ve miR-203a-3p'nin serum seviyeleri anlamlı derecede daha yüksekti. Erken gastrik kanser hastalarında, miR-21-5p, miR-27a-3p, miR-106b-5p, miR-146a-5p ve miR-148a-3p serum seviyeleri önemli ölçüde daha yüksekti. Ayrıca miR-106b-5p ve miR-146a-5p serum seviyeleri tümör lokalizasyonuyla ilişkilendirildi [eğri altındaki alan (AUC): 0,773, 0,797; p=0,049, 0,036], miR-27a-3p ve miR-148a-5p serum seviyeleri ise tümörün T-evresiyle ilişkilendirildi (AUC: 0,748, 0,729; p=0,036, 0,049).

Sonuç: miR-17-5p, miR-21-5p, miR-27a-3p ve miR-203a-3p'nin serum düzeylerinin gastrik kanserlerde tanısal biyobelirteçler olduğu bulundu. Serum miR-106b-5p ve miR-146a-5p'nin ifade düzeylerinin tümör

Address for Correspondence/Yazışma Adresi: Çağrı Büyükkasap, MD, Department of General Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: cagribk@gmail.com
ORCID ID: orcid.org/0000-0002-9141-4289

Received/Geliş Tarihi: 05.05.2024
Accepted/Kabul Tarihi: 16.07.2024

©Copyright 2024 The Author. Published by Galenos Publishing House on behalf of Gazi University Faculty of Medicine. Licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 (CC BY-NC-ND) International License.

©Telif Hakkı 2024 Yazar. Gazi Üniversitesi Tıp Fakültesi adına Galenos Yayınevi tarafından yayımlanmaktadır. Creative Commons Atıf-GayriTicari-Türetilemez 4.0 (CC BY-NC-ND) Uluslararası Lisansı ile lisanslanmaktadır.

ABSTRACT

cancers. The expression levels of serum miR-106b-5p and miR-146a-5p were found to be related to tumor location, whereas the expression levels of serum miR-27a-3p and miR-148a-5p were found to be related to the T-stage of the tumor. These findings can impact the surgical approach for gastric cancer patients.

Keywords: Gastric cancer, microRNA, prognosis

ÖZ

lokasyonuyla ilişkili olduğu, serum miR-27a-3p ve miR-148a-5p'nin ifade düzeylerinin ise tümörün T-evresiyle ilişkili olduğu bulundu. Bu bulgular mide kanseri hastalarına yönelik cerrahi yaklaşımı etkileyebilir.

Anahtar Sözcükler: Gastrik kanser, mikroRNA, prognoz

INTRODUCTION

Gastric cancers remain a significant type of cancer worldwide. According to the data of the year 2020, it is ranked fifth in the world in terms of incidence and fourth in terms of cancer-related deaths (1). Although mortality rates from gastric cancer have decreased over time, most gastric cancer patients receive a diagnosis at advanced stages due to insufficient clinical symptoms and delayed presentation.

Gastroscopy and biopsy remain the gold standard for diagnosing gastric cancer. However, unwanted results can occur due to low sensitivity, high cost, and dependency on the individual (2). Non-invasive serological markers, such as serum pepsinogen, gastrin-17, cancer embryonic antigen (CEA), and carbohydrate antigen (CA19-9), which are used in the diagnosis and prognosis of gastric cancer, have low sensitivity and specificity (3,4). Hence, there is a need for valuable biomarkers that can be utilized for diagnosis, monitoring treatment, and detecting recurrence.

In recent years, microRNA (miRNAs) have been shown to function as tumor suppressors or oncogenes in various cancer types, including gastric cancer (5,6). After their roles in cancer pathogenesis, progression, and migration were proven, they have been considered potential biomarkers for diagnosis and prognosis. During this process, studies on the use of circulating miRNAs as diagnostic and prognostic biomarkers have increased due to their stability compared to tissue miRNAs, ease of non-invasive collection, and ability to repeat measurements. It has been shown that the serum levels of some miRNA species can be useful biomarkers for diagnosis, prognosis, and treatment monitoring in most cancer types, including gastric cancer. Prognostic factors in gastric cancer include age, gender, tumor location, invasion depth, number of metastatic lymph nodes, and TNM stage, and these factors can affect decisions about surgical treatment (7,8). However, limited studies have shown the relationship between serum miRNA levels and these prognostic factors (9,10). The purpose of this study was to investigate the role of serum miRNAs in diagnosing gastric cancer and their correlation with prognostic factors that influence the choice of surgical approach.

MATERIALS AND METHODS

Between September, 2015 and December, 2018, serum samples were collected from 35 patients who underwent gastrectomy and D2 lymph node dissection for histologically confirmed gastric adenocarcinoma. Patients who received neoadjuvant chemotherapy or radiotherapy, had distant metastases, and had peritoneal carcinomatosis were excluded from the study. Patients were divided into gastric cancer group (GCG) and control group (CG). Based on the AJCC/UICC TNM classification 8th edition, the clinical stage of gastric adenocarcinoma was determined (11). Patients' demographic and clinicopathological characteristics were prospectively collected. Thirty-three healthy participants served as controls. This study was funded by the Gazi University Department of Projects of Scientific Inquiry and was approved by the Institutional Review Board of the Gazi University Faculty of Medicine Ethics Committee (approval number: 666, date: 24.09.2018). Informed consent was obtained from all patients and healthy volunteers who participated in the study.

Collection of Samples, RNA Extraction, and qRT-PCR

Peripheral venous blood was collected (18 cc) together with blood samples taken preoperative from each patient and healthy volunteer. The samples were centrifuged, and serum samples were stored at -80 °C. Subsequently, total RNA was isolated from serum samples using Qiagen miRNeasy following the manufacturer's guide. Complementary DNA reactions were prepared using a Qiagen miScript II Kit. Afterwards, preamplification reaction and RT-PCR mix were prepared using a Qiagen miScript microfluidics PreAMP Kit, Qiagen, miScript Microfluidic PCR kit, respectively. Expression of miR-17-5p, miR-21-5p, miR-27a-3p, miR-101-3p, miR-106b-5p, miR-146a-5p, miR-148a-3p, miR-200a-3p, miR-203a-3p by quantitative RT-PCR (qRT-PCR) with Biomark according to the instructions of the manufacturer (Fluidigm, South San Francisco, CA). The expression cel-mir-39 was used as the internal control. The cycle threshold (CT) value is defined as the number of PCR cycles at which the fluorescent signal crosses the threshold (Figure 1). The difference in CT values between the internal control and each miRNA was presented as -ΔCT. ΔΔCT is the difference of ΔCT values between serum samples. $2^{\Delta\Delta CT}$ represents the exponential value of ΔCT, which means fold change in expression.

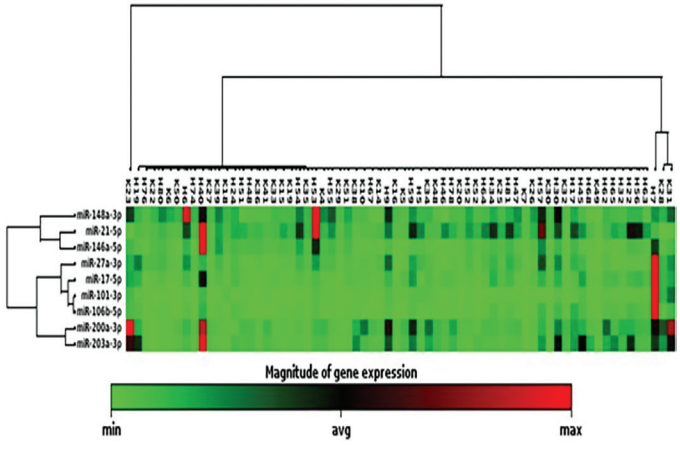

Figure 1. Distribution of miRNA expression levels.
miRNAs: microRNA, min: Minimum, max: Maximum, avg: Average.

Statistical Analysis

Raw data from the RT-PCR device (Biomark HD, Fluidigm, USA) were loaded into the "Qiagen miScript miRNA PCR Array Data Analysis" software to compare with the CG. Values were standardized using "Global mean normalization" (12). Data comparing patient and CGs were used. ΔCT and $2^{-\Delta CT}$ values for each group were calculated using IBM SPSS Statistics v.22. Demographic qualitative variables are presented as numbers and percentages, and the chi-square test was used for comparisons between the two groups. Quantitative variables were presented as median and interquartile range (IQR; 25-75 percentiles). Non-parametric tests were used according to the analysis of visual and operational normal distribution compliance. Mann-Whitney U test was used for comparisons between the two groups. The relationship between serum miRNA expression levels determined by the $2^{-\Delta CT}$ method and prognostic factors was evaluated by receiver operating characteristic (ROC) analysis. The variable considered as the primary endpoint in the ROC analysis was taken as a dichotomous variable, and the area under the curve (AUC), sensitivity, and specificity were calculated. When the calculated form of type-1 error, p-value, is <0.05, it was considered significant.

RESULTS

Serum miRNA expression levels were determined for a total of 68 subjects, including 35 patients and 33 controls. There were no significant difference in terms of gender, smoking history, family history, and blood groups between the GCG and CG groups. Age was statistically different between the two groups (p<0.005). Pathological features of GCG are presented in Table 1.

All miRNAs in the GCG showed higher serum expression levels than in CG. The serum expression levels of miR-17-5p, miR-21-5p, miR-27a-3p, miR-146a-5p, miR-148-3p, and miR-203a-3p were significantly higher in the GCG than in the CG (p=0.031; 0.001; 0.011; 0.04; 0.019; 0.041, respectively). Although the expression of miR-106b-5p was 3.44 times higher in the GCG than in the CG, the increase was not statistically significant (p=0.074) (Table 2) (Figure 2a).

Compared with the CG, significant increases were observed in the serum expression levels of miR-21-5p, miR-27a-3p, miR-106b-5p, miR-146a-5p, and miR-148-3p in patients with early gastric cancer (p=0.001; 0.003; 0.05; 0.011; 0.002, respectively) (Table 2) (Figure 2b).

In patients with tumor located in the cardia, significant increases were observed in the serum expression levels of miR-17-5p, miR-21-5p, miR-27a-3p, miR-106b-5p, miR-146a-5p, miR-148-3p, and miR-203a-3p compared with CG (p=0.002; 0.001; 0.001; 0.001; 0.001; 0.001; 0.021, respectively). Although serum expression levels of miR-200a-3p were 2.67 times higher in patients with tumor located in cardia compared to the CG, this increase was not statistically significant (p=0.236) (Table 2).

In patients with tumor located outside the cardia, increases in serum expression levels of miR-21-5p and miR-27a-3p were statistically significant when compared with CG (p=0.001 and p=0.03, respectively). Despite the 2.16-fold increase in miR-17-5p expression, 2.93-fold increase in miR-106b-5p expression, and 2.31-fold increase in miR-203a-3p expression in patients with non-cardia localization, these increases were not statistically significant (p=0.059; 0.122; 0.07, respectively) (Table 2).

Compared with the CG, significant increases were observed in the serum expression levels of miR-17-5p, miR-21-5p, miR-27a-3p, miR-106b-5p, and miR-203a-3p in patients with diffuse type gastric cancer (p=0.005; 0.001; 0.003; 0.007; 0.038, respectively) (Table 2).

In patients with intestinal type gastric cancer, significant increases were observed in the serum expression levels of miR-17-5p, miR-21-5p, miR-27a-3p, miR-146a-5p, and miR-148a-3p (p=0.005; 0.001; 0.019; 0.016; 0.008, respectively). Although serum expression levels of miR-106b-5p and miR-203a-3p were 3.61 and 2.33 times higher, respectively, in patients with intestinal type gastric cancer than in the CG, this difference was not statistically significant (p=0.058 and p=0.091, respectively) (Table 2).

Table 1. Pathologic characteristics of gastric cancer group

Characteristics	Values
Tumor size (cm), median (IQR[a])	4.0 (0.9-10.0)
Tumor localization, n (%)	
Cardiac	5 (14.3%)
Non-cardiac	30 (85.7%)
Gastrectomy type, n (%)	
Total gastrectomy	23 (65.7%)
Proximal gastrectomy	2 (5.7%)
Distal gastrectomy	10 (28.6%)
Differentiation type, n (%)	
Low grade differentiated	19 (54.3%)
Well differentiated	16 (45.7%)
Lauren classification, n (%)	
Intestinal type	26 (74.3%)
Diffuse type	9 (25.7%)
Borrmann classification, n (%)	
Type 1-2	7 (20%)
Type 3-4	28 (80%)
2 median (IQR[a])	41 (31-57)
Metastatic lymph nodes, median (IQR[a])	5 (0-9)
pT, n (%)	
pT1	7 (20.0%)
PT2	1 (2.9%)
pT3	12 (34.3%)
pT4	15 (42.9%)
pN, n (%)	
pN0	9 (25.7%)
pN1	7 (20.0%)
pN2	2 (5.7%)
pN3	17 (48.6%)
Angiolymphatic invasion, n (%)	28 (80.0%)
Staging, n (%)	
Stage 1-2	17 (48.6%)
Stage 3	18 (51.4%)

[a]Interquartile range.

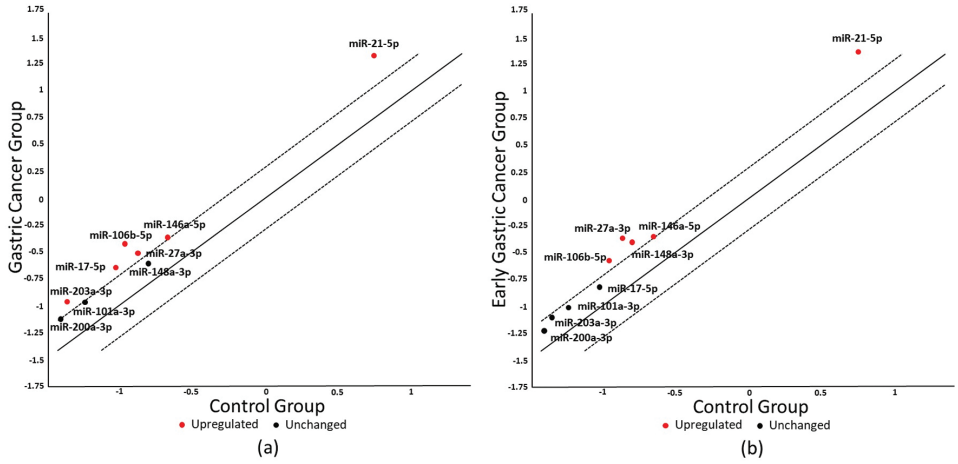

Figure 2. (a) Graphical distribution of fold change ratios of serum miRNA expression levels compared to the control group (b) Graphical distribution of fold change ratios of serum miRNA expression levels compared to the control group in early gastric cancer patients.

miRNAs: microRNA

Table 2. Comparison of serum miRNA expression levels between groups

	Gastric cancer vs. control groups		Early gastric cancer: control group		Cardiac carcinoma and control groups		Non-cardiac carcinoma vs. control group		Diffuse-type carcinoma vs. control group		İntestinal type carcinoma vs. control group	
miRNA	**FC***	**p**	**FC***	**p**	**FC***	**p**	**FC***	**p**	**FC***	**p**	**FC***	**p**
miR-17-5p	2.393	**0.031**	1,59	0.163	4.413	**0.002**	**2.161**	0.059	2.458	**0.005**	2.371	**0.041**
miR-21-5p	3.82	**0.001**	4.248	**0.001**	10.114	**0.001**	3.248	**0.001**	3.087	**0.001**	4.112	**0.001**
miR-27a-3p	2.358	**0.011**	3.182	**0.003**	5.733	**0.001**	2.034	**0.03**	2.558	**0.003**	2.293	**0.019**
miR-101-3p	1.857	0.44	1.647	0.863	1.72	0.862	1.881	0.415	1.669	0.925	1.927	0.361
miR-106b-5p	**3.443**	0.074	2.468	**0.05**	9.017	**0.001**	**2.932**	0.122	2.986	**0.007**	**3.617**	0.058
miR-146a-5p	2.037	**0.04**	2.067	**0.011**	8.066	**0.001**	1.619	0.085	1.242	0.978	2.417	**0.016**
miR-148a-3p	1.569	**0.019**	2.398	**0.002**	3.355	**0.001**	1.383	0.056	1.05	0.577	1.803	**0.008**
miR-200a-3p	1.914	0.468	1.493	0.78	**2.671**	0.236	1.811	0.69	1.883	0.51	1.925	0.582
miR-203a-3p	2.479	**0.041**	1.738	0.486	3.776	**0.021**	**2.311**	0.07	2.951	**0.038**	**2.333**	0.091

*FC: Fold change, miRNA: MicroRNA.

Evaluation of the Relationship between Serum miRNA Expression Levels and Prognostic Factors

The relationship between serum miRNA expression levels determined by the $2^{-\Delta CT}$ method and prognostic factors was evaluated by ROC analysis. The serum expression levels of miR-106b-5p and miR-146a-5p were found to be associated with tumor location (AUC: 0.773; 0.797; p=0.049; 0.036, respectively) (Table 3) (Figure 3a, b). The serum expression levels of miR-27a-3p and miR-148-5p were found to be associated with the T-stage of the tumor (AUC: 0.748; 0.729; p=0.036; 0.049, respectively) (Table 3) (Figure 3c, d).

DISCUSSION

Due to the low sensitivity and specificity of current non-invasive biomarkers for diagnosing and prognosing gastric cancer, there is a need for more useful biomarkers. It has been shown that the serum expression levels of some miRNA species can be a useful biomarker for the diagnosis and prognosis of gastric cancer (13). However, studies on their relationship with the location and T-stage, which can affect surgical management are limited. The aim of this study was to evaluate the relationship between serum miRNA levels and pathological features that affect the diagnosis, surgical management, and prognosis of gastric cancer.

Previous studies have shown that the serum expression levels of the miRNAs evaluated in our study can be used as diagnostic biomarkers for gastric cancer (10,14-17). Consistent with the literature, our study showed that miR-17-5p, miR-21-5p, miR-27a-3p, miR-146a-5p, miR-148a-5p, and miR-203a-3p serum expression levels can be used as diagnostic biomarkers for gastric cancer. In addition, the statistically significant differences in the serum expression levels of miR-21-5p, miR-27a-3p, miR-106b-5p, miR-146a-5p, and miR-148a-5p in early-stage gastric cancer patients compared to the CG indicate that these miRNAs can also be used for diagnosis in early-stage gastric cancer patients.

Table 3. Evaluation of the relationship between serum miRNA expression levels and prognostic factors

	Lymph node metastasis		Differentiation		Localization		T-stage		Tumor size		Angiolymphatic invasion		Stage		c-erb-B2	
	AUC*	p	AUC*	p	AUC*	p	AUC*	p	AUC*	p	AUC*	p	AUC*	p	AUC*	p
miR-17-5p	0.397	0.365	0.563	0.527	0.600	0.480	0.616	0.326	0.460	0.689	0.510	0.934	0.598	0.322	0.470	0.867
miR-21-5p	0.415	0.450	0.430	0.484	0.700	0.157	0.625	0.289	0.425	0.453	0.520	0.869	0.618	0.235	0.545	0.802
miR-27a-3p	0.494	0.955	0.523	0.816	0.640	0.322	**0.748**	**0.036**	0.560	0.549	0.518	0.885	0.621	0.222	0.682	0.316
miR-101-3p	0.476	0.836	0.550	0.617	0.543	0.759	0.600	0.398	0.587	0.386	0.584	0.496	0.578	0.428	0.477	0.900
miR-106b-5p	0.385	0.308	0.537	0.714	**0.773**	**0.049**	0.514	0.906	0.453	0.641	0.469	0.805	0.637	0.166	0.515	0.933
miR-146a-5p	0.532	0.777	0.553	0.594	**0.797**	**0.036**	0.662	0.169	0.563	0.527	0.589	0.470	0.492	0.934	0.530	0.867
miR-148a-3p	0.412	0.439	0.375	0.211	0.610	0.437	**0.729**	**0.049**	0.397	0.301	0.472	0.821	0.658	0.109	0.758	0.155

*AUC: Area under the curve.

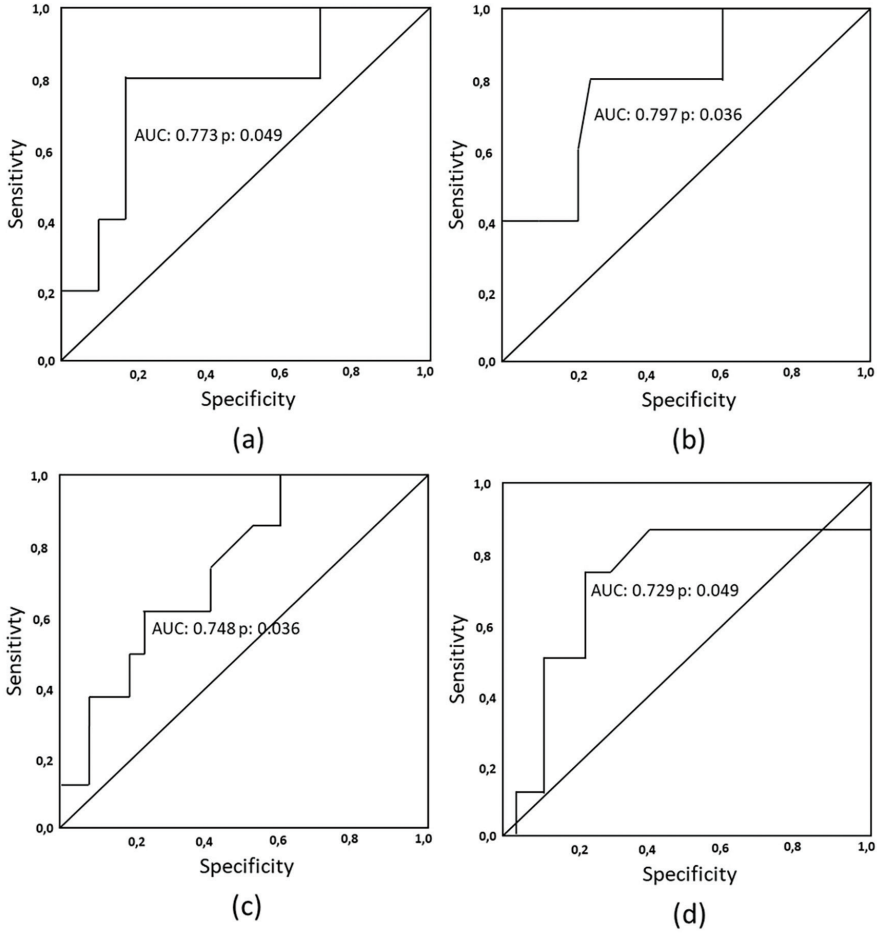

Figure 3. (a) ROC analysis of miR-106b-5p serum expression level in tumor localization discrimination, (b) ROC analysis of miR-146a-5p serum expression level in tumor localization discrimination, (c) ROC Analysis of miR-27a-3p serum expression level in T-extension discrimination, (d) ROC analysis of miR-148a-5p serum expression level in T-extension discrimination.

ROC: Receiver operating characteristic.

Study Limitations

Previous limited studies have indicated that serum miR-27a-3p expression levels could be a prognostic biomarker for gastric cancer (10,17). However, these studies have focused on lymph node metastasis and TNM staging rather than the T-stage of the tumor. In addition to these studies, our study showed that miR-27a-3p and miR-148a-5p serum expression levels are related to T -stage and can be prognostic biomarkers. Furthermore, we believe that miR-106b-5p and miR-146a-5p are potential prognostic biomarkers because of their significant variations in serum expression levels in gastric cardia cancers based on tumor location, another important prognostic factor.

CONCLUSION

Serum miRNA expression levels can play an important role in addressing the need for non-invasive biomarkers for evaluating prognostic factors that can affect surgical management and the diagnosis of gastric cancers. Our study showed that serum miR-17-5p, miR-21-5p, miR-27a-3p, miR-146a-5p, miR-148a-5p, and miR-203a-3p expression levels are diagnostic biomarkers for gastric cancer. Additionally, we observed that miR-21-5p, miR-27a-3p, miR-106b-5p, miR-146a-5p, and miR-148a-5p serum expression levels are diagnostic for early-stage gastric cancers. The relationship of serum miR-106b-5p and miR-146a-5p expression levels with tumor location and serum miR-27a-3p and miR-148a-5p expression levels with the T-stage of the tumor can prevent radical surgical interventions in patients with near-normal serum expression levels of these miRNAs during the preoperative period. However, further comprehensive studies are required to confirm our hypothesis.

Ethics

Ethics Committee Approval: This study was funded by the Gazi University Department of Projects of Scientific Inquiry and was approved by the Institutional Review Board of the Gazi University Faculty of Medicine Ethics Committee (approval number: 666, date: 24.09.2018).

Informed Consent: Informed consent was obtained from all patients and healthy volunteers who participated in the study.

Authorship Contributions

Concept: Ç.B., Design: Ç.B., H.D., A.U.D., Supervision: Ö.G., A.B., Resources: Ç.B., A.B., Material: Ç.B., E.K., M.N., A.Y., H.G., H.B., Data Collection or Processing: Ç.B., E.K., M.N., A.Y., H.G., H.B., Analysis or Interpretation: N.S.Y., H.D., İ.Ö., Ö.G., Literature Search: Ç.B., F.A., K.D., Writing: Ç.B., Critical Review: Ö.G., A.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: This study was funded by the Gazi University Department of Projects of Scientific Inquiry.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71: 209-49.
2. Abad MRA, Shimamura Y, Fujiyoshi Y, Seewald S, Inoue H. Endocytoscopy: technology and clinical application in upper gastrointestinal tract. Transl Gastroenterol Hepatol. 2020; 5: 28.
3. Zhang A, Zou X, Yang S, Yang H, Ma Z, Li J. Effect of NETs/COX-2 pathway on immune microenvironment and metastasis in gastric cancer. Front Immunol. 2023; 14: 1177604.
4. Zhu XD, Zhang LX, Luo PQ, Zhu H, Wei ZJ, Xu AM. Prognostic significance of post-preoperative tumor markers increments in patients with non-metastatic gastric cancer. J Cancer Res Clin Oncol. 2023; 149: 12191-201.
5. Chen Y, Shen Y, Lin P, Tong D, Zhao Y, Allesina S, et al. Gene regulatory network stabilized by pervasive weak repressions: microRNA functions revealed by the May-Wigner theory. Natl Sci Rev. 2019; 6: 1176-88.
6. Qin Y, Liang R, Lu P, Lai L, Zhu X. Depicting the Implication of miR-378a in Cancers. Technol Cancer Res Treat. 2022; 21: 15330338221134385.
7. Wu D, Lu J, Xue Z, Zhong Q, Xu BB, Zheng HL, et al. Evaluation of dynamic recurrence risk for locally advanced gastric cancer in the clinical setting of adjuvant chemotherapy: a real-world study with IPTW-based conditional recurrence analysis. BMC Cancer. 2023; 23: 964.
8. Kulig P, Pach R, Majewska O, Kulig J. Clinicopathological Prognostic Factors Determining Outcomes of Treatment in Gastric Cancer Surgery. In Vivo. 2022; 36: 2927-35.
9. Jiang X, Wang W, Yang Y, Du L, Yang X, Wang L, et al. Identification of circulating microRNA signatures as potential noninvasive biomarkers for prediction and prognosis of lymph node metastasis in gastric cancer. Oncotarget. 2017; 8: 65132-42.
10. Kim SY, Jeon TY, Choi CI, Kim DH, Kim DH, Kim GH, et al. Validation of circulating miRNA biomarkers for predicting lymph node metastasis in gastric cancer. J Mol Diagn. 2013; 15: 661-9.
11. Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin. 2017; 67: 93-9.
12. Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res. 2014; 42: D68-73.
13. Schwarzenbach H, Nishida N, Calin GA, Pantel K. Clinical relevance of circulating cell-free microRNAs in cancer. Nat Rev Clin Oncol. 2014; 11: 145-56.
14. Cholewinski G, Garczorz W, Francuz T, Owczarek AJ, Kimsa-Furdzik M, Blaszczynska M, et al. Expression of let-7a, miR-106b and miR-29b is changed in human gastric cancer. J Physiol Pharmacol. 2022; 73.
15. Sierzega M, Kaczor M, Kolodziejczyk P, Kulig J, Sanak M, Richter P. Evaluation of serum microRNA biomarkers for gastric cancer based on blood and tissue pools profiling: the importance of miR-21 and miR-331. Br J Cancer. 2017; 117: 266-73.
16. Park JL, Kim M, Song KS, Kim SY, Kim YS. Cell-Free miR-27a, a Potential Diagnostic and Prognostic Biomarker for Gastric Cancer. Genomics Inform. 2015; 13: 70-5.
17. Juzėnas S, Saltenienė V, Kupcinskas J, Link A, Kiudelis G, Jonaitis L, et al. Analysis of Deregulated microRNAs and Their Target Genes in Gastric Cancer. PLoS One. 2015; 10: e0132327.