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Original Article

Correlation of serum exosomal miR-21 with the risk of gastric cancer onset: its value for early diagnosis

Changjiang Li*, Chen Hong*, Guosheng Zhou, Qianzi Chai, Jie Hu, Aihong Lv, Weiping Fang

*Department of Gastroenterology, The First People's Hospital of Jiande, Hangzhou 311600, Zhejiang, China. *Equal contributors and co-first authors.*

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Abstract: Objectives: This research explored the connection between serum exosomal miR-21 expression and the risk of gastric cancer (GC), and evaluated the viability of using this non-invasive marker for early GC diagnosis. Methods: A retrospective evaluation was performed on 539 individuals who had received serum exosomal miR-21 testing. Based on diagnoses within 12 months, patients were categorized into a GC group (n=235) and a non-GC group (n=304). To measure the levels of exosomal miR-21, we used the technique of reverse transcription quantitative polymerase chain reaction (RT-qPCR). Results: The GC group had significantly higher body mass index (BMI), rates of manual labor, smoking, drinking, high-salt/pickled diet, chronic gastritis, *Helicobacter pylori* (Hp) infection, and family history of GC (all P<0.05). The laboratory findings indicated that the GC group exhibited significantly higher levels of white blood cell count, platelet count, alanine aminotransferase, aspartate aminotransferase, blood urea nitrogen, creatinine, carcinoembryonic antigen, carbohydrate antigen (CA) 19-9, CA 72-4, and serum exosomal miR-21 levels, but lower levels of hemoglobin, total cholesterol, and triglyceride (all P<0.05). Logistic regression revealed that high miR-21 constitute an independent risk factor for GC (OR=3.477, P<0.001). Receiver operating characteristic for early GC diagnosis showed an area under the curve of 0.772 for miR-21 alone, and a combined model with CA72-4 increased it to 0.927. The high miR-21 group demonstrated a markedly greater GC incidence (P<0.001). Conclusions: Serum exosomal miR-21 is linked to GC and demonstrates potential as a non-invasive biomarker for early detection, particularly when combined with CA72-4.

Keywords: Serum exosomal miR-21, gastric cancer, correlation, diagnosis

Introduction

Gastric cancer (GC) is a predominant cause of cancer-associated mortality globally. Moreover, the early identification and management of GC have always been challenging in the East Asian region [1]. Although there have been some breakthroughs in treatment in recent years, the long-term survival for most patients is poor. A critical issue is that many patients are diagnosed with GC at an advanced stage, which limits the available treatment options. If GC could be detected early, such as through surgical removal, the treatment outcomes would often be much better. Therefore, early screening and diagnosis are crucial for improving the chances of survival [2, 3].

Currently, the commonly used GC screening methods in clinical practice mainly include gastroscopy and some tumor marker tests, including carbohydrate antigen 19-9 (CA19-9) and carcinoembryonic antigen (CEA). However, the results obtained through these methods are often not accurate enough. In cases where a diagnosis is missed, some healthy individuals may also be misdiagnosed and forced to undergo unnecessary invasive examinations [4].

In recent years, microRNAs (miRNAs), a category of short non-coding RNA molecules, have gained increasing attention in the field of cancer research. These molecules modulate gene expression post-transcriptionally, thereby influencing various critical biological functions like cell proliferation, apoptosis, and metastasis. Consequently, numerous studies have begun to investigate the potential of miRNAs as biomarkers for various types of cancer, including GC [5, 6]. Among the many miRNAs, miR-21 has

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garnered considerable attention owing to its close connection with tumor development and progression, often referred to as an "oncomiR" to highlight its crucial role in promoting tumor growth. This molecule can specifically target and inhibit certain tumor suppressor genes, thereby accelerating tumor progression. Several studies have indicated that miR-21 is significantly upregulated in various cancerous tissues and body fluid samples, making it a promising circulating biomarker. Additionally, miR-21 exhibits relatively stable properties in exosomes, which makes it more suitable for non-invasive detection. Researchers can further analyze the expression levels of miR-21 in exosomes extracted from blood samples to assess the feasibility of using it as a diagnostic biomarker [7, 8].

Exosomes, small vesicles secreted by various cells, can transport proteins, lipids, nucleic acid, and other biologically active molecules, playing a crucial role in cell-cell communication. In addition to mediating intercellular communication, exosomes are actively implicated in the development and progression of cancer [9]. Specifically, miRNAs contained in exosomes can be transferred to neighboring or distant cells and regulate their functional state, thereby affecting the dynamic balance of the entire tumor microenvironment. In recent years, exosomal miRNAs as new markers for diagnosis and prognostic evaluation of diseases have attracted extensive attention. Such markers are expected to offer a more precise picture of disease progression and therapeutic response [10, 11]. Research indicates that exosomal miR-21 levels in the blood of individuals diagnosed with colorectal cancer and lung cancer are substantially elevated compared to those found in healthy people. This result provides strong support for advancing miR-21 as a clinically usable biomarker for cancer [12, 13].

In GC research, there is already evidence that miR-21 is critically involved in disease progression. The expression levels of miR-21 in GC tissues were markedly higher than adjacent healthy tissues. Furthermore, increased miR-21 expression has been associated with adverse clinical outcomes, such as late cancer stage or lymph node metastases. Further studies have also pointed out that miR-21 can regulate PTEN/PI3K/AKT signaling pathways closely related to GC, thereby affecting tumor biological behavior. These findings prompted researchers to begin exploring the potential of serum exosomal miR-21 for the diagnosis of early GC. Although the outcomes of the present study are encouraging, more work is needed to confirm reliability of this method before it is used for clinical diagnosis [14, 15].

Considering the established role of miR-21 in promoting tumor growth and its association with poor clinical outcome in GC, we hypothesized that serum exosomal miR-21 could function as biomarker for GC early detection. Our research aimed to investigate whether serum exosomal miR-21 levels correlate with the risk of developing GC and evaluate its diagnostic value when combined with other markers such as carbohydrate antigen 72-4 (CA72-4). By focusing on miR-21, we aim to leverage its stable presence in exosomes for non-invasive testing, to improve early diagnosis.

Materials and methods

Study patients

A retrospective selection was made of patients who had undergone serum exosomal miR-21 testing at The First People's Hospital of Jiande from May 2020 to April 2024, and whose clinical records and laboratory test results were complete. These patients formed the basis of the study population, totaling 539 cases. According to whether GC occurred within 12 months after exosomal testing, the 539 patients were defined into two groups: a non-GC group (n=304) and a GC group (n=235).

The GC group was defined as patients newly diagnosed with gastric cancer within 12 months after exosomal miR-21 testing. Inclusion criteria included: 1) age ≥18 years, no gender restriction; 2) histopathologically confirmed gastric adenocarcinoma [16]; 3) TNM stage I to II; 4) Eastern Cooperative Oncology Group (ECOG) [17] performance status score ≤2; and 5) normal function of vital organs such as heart, liver, and kidneys. Exclusion criteria included: 1) prior treatment with chemotherapy and/or radiotherapy; 2) concurrent malignancies other than gastric cancer; 3) comorbidities such as hypertension and diabetes; and 4) an estimated survival time of less than 3 months.

The non-GC group was defined as individuals who underwent health check-ups at The First People's Hospital of Jiande during the same

timeframe as the GC group and were not diagnosed with gastric cancer within 12 months after exosomal miR-21 testing. Inclusion criteria included: 1) age ≥18 years, matched by age and gender with the GC group; and 2) normal health check-up results (including physical examination, complete blood count, biochemical indicators, tumor markers, abdominal ultrasound, gastroscopy, and other tests showed no abnormalities). Exclusion criteria included: 1) comorbidities such as diabetes and hypertension; 2) a history or family history of malignancies other than gastric cancer; and 3) dysfunction of vital organs such as heart, liver, and kidneys.

Finally, based on the best threshold of serum exosomal miR-21 determined by receiver operating characteristic (ROC) analysis (2.305), an additional 247 patients who met the same inclusion and exclusion criteria were further defined into a low miR-21 group (≤2.305) (n=138) and a high miR-21 group (>2.305) (n=109).

Ethical statement

This research complied with the ethical standards set forth in the World Medical Association's Declaration of Helsinki, as amended in 2022. The Medical Ethics Committee of The First People's Hospital of Jiande City has approved the study protocol. Given the retrospective design and the use of pre-existing, anonymized clinical data from the hospital's records, the ethics committee exempted the need for informed consent. All patient information was de-identified to protect confidentiality.

Data collection

General data: General data, including body mass index (BMI), occupation, educational level, smoking and drinking status, dietary habits, chronic gastritis, *Helicobacter pylori* infection, family history of GC, and physical activity, were collected at the time of serum sampling for exosomal miR-21 testing. For the GC group, this occurred within 12 months before diagnosis; for the non-GC group, data were obtained during routine health check-ups.

Routine laboratory tests: We used a fully automated hematology analyzer (XN-9000, Sysmex Corporation, Japan) to detect blood routine indicators, including white blood cell count (WBC), platelet count (PLT), and hemoglobin (Hb). At the same time, we also used a fully automated biochemical analyzer (Cobas 8000, Roche Diagnostics, Switzerland) to measure blood biochemical indicators, containing alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), creatinine (Cr), total cholesterol (TC), and triglycerides (TG).

Traditional serum tumor marker assays: Using an electrochemiluminescence immunoassay system (Cobas e602, Roche, Switzerland), we detected CEA, CA19-9, and CA72-4.

Exosomal miR-21 testing

Serum exosome extraction and characterization: Blood samples were collected from patients after an overnight fast (minimum 8 hours). Approximately 10 mL of venous blood was drawn into serum separator tubes (SST) without anticoagulants. The blood samples were allowed to clot at room temperature for 30 minutes, followed by centrifugation at 1500×g for 15 minutes at 4°C to separate the serum. The resulting serum was divided into 500 µL aliquots and stored at -80°C in the hospital's biobank until further processing. All samples were processed within 2 hours of collection to minimize RNA degradation. Exosome extraction was performed on thawed aliquots without refreezing to maintain integrity. For exosome extraction, 500 µL of serum was used per sample. Briefly, the serum was subjected to centrifugation at 3000 rpm for 15 minutes to eliminate cellular debris and large particles. Following this, 500 µL of the supernatant was transferred into an EP tube, and 120 µL of exosome precipitation reagent (EXOQ-5A-1, System Biosciences, USA) was added to extract the serum exosomes. The obtained exosomes were immobilized overnight in 2.5% glutaraldehyde, embedded in epoxy resin, and then stained with 9.3% uranyl acetate and lead citrate. Subsequently, these samples were analyzed by transmission electron microscope (Tecnai G2 Spirit BioTWIN, FEI Corporation, USA). The size distribution of the exosomes was assessed using a nanoparticle analyzer (Nano Sight NS300, Malvern Panalytical, UK). Finally, Western blot analysis was employed to detect exosomal marker proteins. Total protein was extracted from exosome samples using RIPA lysis buffer, separated via SDS-PAGE gel elec-

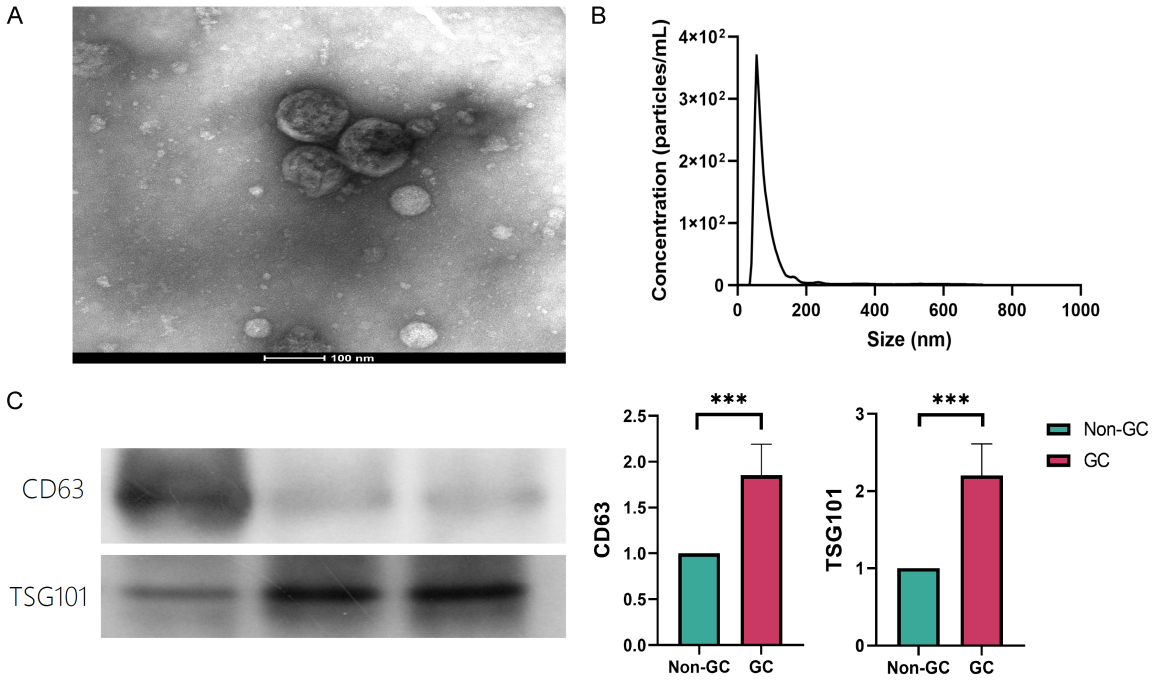

Figure 1. Characterization of isolated exosomes. Notes: Under transmission electron microscopy, lipid bilayer membrane particles with a diameter of approximately 70 nm were observed (A). Nano Sight analysis showed that the mean particle diameter in the samples was approximately 56 nm (B). Western blot results (C) demonstrated the expression of exosomal marker proteins CD63 and TSG101 in the exosome samples. GC, gastric cancer; ***: P<0.001.

trophoresis, and transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk for 2 hours at room temperature, followed by overnight incubation at 4°C with primary antibodies CD63 (ab13045, Abcam, UK) and TSG101 (ab133586, Abcam, UK). On the second day, the membrane was rinsed and subsequently incubated with the corresponding secondary antibody for 1 hour at room temperature. After soaking in ECL ultra-sensitive luminescent solution, the membrane was developed using a protein imaging system (ChemiDoc XRS+, Bio-Rad, USA). The grayscale results were then analyzed using Image J software (version 1.8.0; developed by Wayne Rasband at the National Institutes of Health, USA). Normalization was performed using the protein expression levels of the Non-GC group as the reference: the grayscale values of the CD63 and TSG101 protein bands in the Non-GC group were set to 1, and the expression levels in the GC group were calculated as relative ratios (**Figure 1**).

Extraction and quantification of miR-21 from exosome: First, the exoRNeasy Serum/Plasma Maxi Kit (77064, Qiagen, Germany) was used to extract RNA from exosomes strictly following the manufacturer's instructions. To account for variations in RNA extraction and amplification, 5 fmol of synthetic cel-miR-39 (Qiagen, Germany) was spiked into each sample during the lysis step as an exogenous control. Subsequently, we further employed an endogenous reference gene for data normalization. Specifically, we selected miR-16-5p, which is relatively stably expressed in serum exosomes, as the candidate reference. The expression stability of miR-16-5p across all samples in this study, including both GC and non-GC groups, was verified using the geNorm algorithm. It was confirmed that the mean expression stability value M was <1.5, meeting the criteria for a stable reference. Finally, the PrimeScript™ RT-PCR Kit (RR037A, Takara, Japan) was employed to reverse transcribe the extracted exosomal RNA into cDNA. Subsequently, real-time quantitative polymerase chain reaction (RT-qPCR) was conducted using the StepOnePlus Real-Time PCR System (Thermo Fisher Scientific, USA) with SYBR Green detection method. The primer sequences were as follows: for miR-21, forward 5'-AAC CGG TAG ATC TTG GAT CCT G-3', reverse 5'-CAA GAU CAU CUA

CGG UUU GGG U-3'; for cel-miR-39, forward 5'-UCA CCG GGU GUA AAU CAG CUU G-3', reverse 5'-GTG CAG GGT CCG AGG T-3'; miR-16-5p, forward 5'-UAG CAG CAC GUA AAU AUU GGC G-3', reverse 5'-CGA CAG GCC AGG GAA AAG A-3'. The relative expression level of miR-21 was determined utilizing the 2^-ΔΔCt method.

Statistical analysis

Data analyses were conducted utilizing SPSS software (version 29.0; developed by SPSS Inc., Chicago, IL, USA). A two-sided *P* value of less than 0.05 was considered significant. The normality of continuous variables in this study was validated using the Shapiro-Wilk test, with results presented as mean ± standard deviation (M ± SD). Inter-group comparison was conducted employing an independent sample t-test. Categorical variables were presented as frequencies and percentages [n (%)] and were compared between groups using χ^2 test. Pearson correlation analysis was employed to analyze the relationship between GC risk and the variables with significant intergroup differences ($P<0.05$). Multivariate logistic regression was then used to identify independent risk factors, with GC occurrence as a dependent variable (occurrence=1, non-occurrence =0). The $P<0.05$ in regression analysis was analyzed by receiver operator characteristic curve (ROC), and the diagnostic value of area under the curve (AUC), sensitivity, specificity, Youden index and F1 score was evaluated. Based on the diagnostic efficacy of a single index, biomarker parameters with high AUC (serum exosome miR-21 and CA72-4) were selected to construct a joint diagnostic model. Finally, based on ROC analysis, a serum exosomal miR-21 level of 2.305 was determined as the best threshold for differentiating between the high-level and low-level miR-21 groups.

For this retrospective study, the sample size was determined by the number of patients meeting the inclusion criteria over the four-year period (May 2020 - April 2024). Given the exploratory nature of research on exosomal miR-21 in GC, we aimed to include all eligible cases to maximize statistical power. The study's post hoc power analysis, performed using G*Power software (version 3.1), indicated that with n=539, the study achieved greater than 80% power to identify a medium effect size (d=0.5) in miR-21 levels between groups (α=0.05), based on preliminary data. Subgroups for miR-21 analysis (n=247) were defined using the best threshold from ROC curves to ensure balanced comparisons.

Results

General data among patients with and without GC

The comparison of general data among patients with and without GC (**Table 1**) showed several significant differences. BMI was elevated in the GC group (23.87 ± 3.02 vs 23.15 ± 2.95, t=2.787, P=0.006). More patients in the GC group were engaged in manual labor (58.30% vs 47.37%, χ^2=6.345, P=0.012), while fewer had a college degree or above (39.15% vs 49.34%, χ^2=5.566, P=0.018). Smoking (P=0.007), drinking (P=0.002), and consumption of high salt/pickled diet (P=0.005) were more prevalent in the GC group. Chronic gastritis (P=0.008), Hp infection (P=0.006), and family history of GC (P=0.002) were also more common in the GC group. Regular physical activity was less frequent in the GC group (49.79% vs 59.87%, χ^2=5.453, P=0.020). The age and gender distributions were comparable between the two groups, with no notable differences observed for either variable (both P>0.05).

Routine laboratory parameters among patients with and without GC

The comparison of routine laboratory parameters among patients with and without GC (**Table 2**) showed significant differences. In blood routine tests, the GC group had higher WBC (6.98 ± 2.31 vs 6.52 ± 1.45, t=2.632, P=0.009), PLT (261.92 ± 71.45 vs 245.67 ± 55.33, t=2.883, P=0.004), and lower Hb (137.84 ± 18.69 vs 142.36 ± 12.58, t=3.186, P=0.002). In blood biochemistry, the GC group showed higher levels of ALT (25.65 ± 8.24 vs 23.76 ± 6.91, t=2.826, P=0.005), AST (27.17 ± 8.56 vs 24.92 ± 6.83, t=3.305, P=0.001), BUN (5.65 ± 1.68 vs 5.28 ± 1.25, t=2.789, P=0.006), Cr (76.96 ± 16.54 vs 72.64 ± 14.27, t=3.188, P=0.002), and lower levels of TC (4.45 ± 0.89 vs 4.68 ± 0.82, t=3.075, P=0.002) and TG (1.43 ± 0.37 vs 1.52 ± 0.31, t=3.234, P=0.001).

Serum biomarkers among patients with and without GC

The comparison of serum biomarkers among patients with and without GC (**Figure 2**) show-

Table 1. Comparison of general data among patients with and without GC

Item	Non-GC group (n=304)	GC group (n=235)	t/χ^2	P
Demographic characteristic				
Age (years)	58.26 ± 8.43	59.62 ± 8.74	1.822	0.069
Male/Female [n (%)]	167 (54.93%)/137 (45.07%)	133 (56.6%)/102 (43.4%)	0.148	0.700
BMI (kg/m^2)	23.15 ± 2.95	23.87 ± 3.02	2.787	0.006
Occupation [n (%)]			6.345	0.012
Manual labor	144 (47.37%)	137 (58.30%)		
Mental work	160 (52.63%)	98 (41.70%)		
Educational level [n (%)]			5.566	0.018
High school or below	154 (50.66%)	143 (60.85%)		
College degree or above	150 (49.34%)	92 (39.15%)		
Personal lifestyle habits				
Smoking [n (%)]	106 (34.87%)	109 (46.38%)	7.329	0.007
Drinking [n (%)]	84 (27.63%)	94 (40.00%)	9.167	0.002
Medical history				
Chronic gastritis [n (%)]	102 (33.55%)	105 (44.68%)	6.939	0.008
Hp infection [n (%)]	96 (31.58%)	101 (42.98%)	7.427	0.006
Family history of GC [n (%)]	11 (3.62%)	24 (10.21%)	9.492	0.002
Dietary history in the past year				
High salt/pickled diet [n (%)]	74 (24.34%)	83 (35.32%)	7.736	0.005
Physical activity				
Regular (≥3 times/week) [n (%)]	182 (59.87%)	117 (49.79%)	5.453	0.020

GC, gastric cancer; BMI, body mass index; Hp, helicobacter pylori.

Table 2. Comparison of routine laboratory data among patients with and without GC

Item	Non-GC group (n=304)	GC group (n=235)	t	P
Blood routine				
WBC (×10^9/L)	6.52 ± 1.45	6.98 ± 2.31	2.632	0.009
PLT (×10^9/L)	245.67 ± 55.33	261.92 ± 71.45	2.883	0.004
Hb (g/L)	142.36 ± 12.58	137.84 ± 18.69	3.186	0.002
Blood biochemistry				
ALT (U/L)	23.76 ± 6.91	25.65 ± 8.24	2.826	0.005
AST (U/L)	24.92 ± 6.83	27.17 ± 8.56	3.305	0.001
BUN (mmol/L)	5.28 ± 1.25	5.65 ± 1.68	2.789	0.006
Cr (μmol/L)	72.64 ± 14.27	76.96 ± 16.54	3.188	0.002
TC (mmol/L)	4.68 ± 0.82	4.45 ± 0.89	3.075	0.002
TG (mmol/L)	1.52 ± 0.31	1.43 ± 0.37	3.234	0.001

GC, gastric cancer; WBC, white blood cell count; PLT, platelet count; Hb, hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; TC, total cholesterol; TG, triglyceride.

ed significant differences across all indices. The GC group had higher levels of CEA (4.07 ± 1.32 vs 3.15 ± 0.78, t=9.497, P<0.001), CA19-9 (40.54 ± 5.13 vs 37.36 ± 3.42, t=8.219, P<0.001), and CA72-4 (10.41 ± 2.86 vs 4.29 ± 1.05, t=31.256, P<0.001). Furthermore, the level of miR-21 in serum exosomes was notably elevated in the GC group (3.58 ± 1.06 vs 1.26 ± 0.37, t=32.003, P<0.001).

Correlation analysis

The correlation analysis of factors for GC onset (**Table 3**) revealed several significant associations. BMI (r=0.128, P=0.006), occupation (manual labor vs mental work) (r=0.108, P=0.012), educational level (high school or below vs college degree or above) (r=0.102, P=0.018), smoking (r=0.117, P=0.007), drinking (r=0.130, P=0.002), chronic gastritis (r=0.113, P=0.008), Hp infection (r=0.117, P=0.006), family history of GC (r=0.133, P=0.002), and high salt/pickled diet (r=0.120, P=0.005) all showed positive associations with GC onset. Serum biomarkers, including CEA

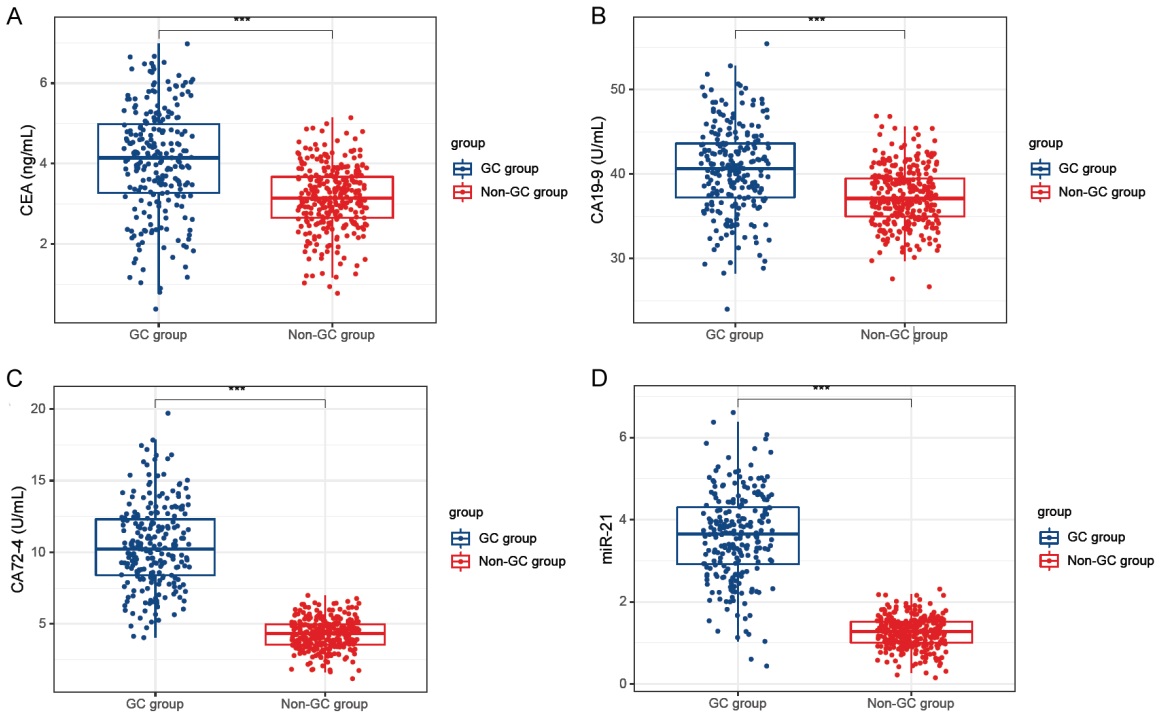

Figure 2. Comparison of serum biomarkers among patients with and without GC. (A) CEA; (B) CA19-9; (C) CA72-4; (D) miR-21. GC, gastric cancer; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4; miR-21, microRNA-21; ***: P<0.001.

(r=0.399, P<0.001), CA19-9 (r=0.345, P< 0.001), CA72-4 (r=0.817, P<0.001), and miR-21 (r=0.837, P<0.001), demonstrated strong positive correlations with GC onset.

Regular physical activity (≥3 times/week) showed a negative association (r=-0.101, P= 0.020). Among laboratory parameters, WBC (r=0.115, P=0.009), PLT (r=0.107, P=0.004), ALT (r=0.115, P=0.005), AST (r=0.145, P= 0.001), BUN (r=0.119, P=0.006), and Cr (r= 0.139, P=0.002) were positively correlated, while Hb (r=-0.148, P=0.002), TC (r=-0.120, P=0.002), and TG (r=-0.150, P=0.001) were negatively correlated.

Multivariate logistic regression analysis

The multivariate logistic regression analysis for GC onset (**Table 4**) revealed several significant risk factors. Hp infection was identified as a risk factor exhibiting an odds ratio (OR) of 1.113 (P=0.011, 95% confidence interval [CI] [1.025, 1.208]). Smoking was identified as a notable risk factor, with an OR of 1.880 (P=0.016, 95% CI [1.127, 3.136]). Drinking showed a similar pattern, with an OR of 1.687 (P=0.049, 95% CI [1.001, 2.843]). Family history of GC presented as a notable risk factor, having an OR of 3.001 (P=0.001, 95% CI [1.536, 5.863]). High salt/pickled diet was another significant risk factor, with an OR of 1.616 (P=0.046, 95% CI [1.010, 2.586]). CA72-4 and miR-21 were also significant, with ORs of 1.684 (P<0.001, 95% CI [1.509, 1.880]) and 3.477 (P<0.001, 95% CI [2.361, 5.121]), respectively. In contrast, occupation, educational level, chronic gastritis, Hp infection, regular physical activity, WBC, PLT, Hb, ALT, AST, BUN, Cr, TC, TG, CEA, and CA19-9 did not show significant associations with GC onset (all P>0.05).

ROC analysis

The ROC analysis of the diagnostic value in the early diagnosis of GC (**Table 5**) revealed modest performance for most data. Smoking, drinking, Hp infection, family history of GC, and high salt/pickled diet had AUC values ranging from 0.514 to 0.574. These factors exhibited sensitivities between 0.543 and 0.594 and specificities from 0.512 to 0.642. In contrast, CA72-4 showed improved diagnostic capability with an AUC of 0.698, a sensitivity of 0.673, and a specificity of 0.702. Moreover, miR-21 demon-

Table 3. Correlation analysis of factors for GC onset

Item	r	P
BMI	0.128	0.006
Occupation (Manual labor/Mental work)	0.108	0.012
Educational level (High school or below/College degree or above)	0.102	0.018
Smoking	0.117	0.007
Drinking	0.130	0.002
Chronic gastritis	0.113	0.008
Hp infection	0.117	0.006
Family history of GC	0.133	0.002
High salt/pickled diet	0.120	0.005
Regular (≥3 times/week)	-0.101	0.020
WBC	0.115	0.009
PLT	0.107	0.004
Hb	-0.148	0.002
ALT	0.115	0.005
AST	0.145	0.001
BUN	0.119	0.006
Cr	0.139	0.002
TC	-0.120	0.002
TG	-0.150	0.001
CEA	0.399	P<0.001
CA19-9	0.345	P<0.001
CA72-4	0.817	P<0.001
miR-21	0.837	P<0.001

GC, gastric cancer; OR, odds ratio; CI, confidence interval; BMI, body mass index; Hp, helicobacter pylori; WBC, white blood cell count; PLT, platelet count; Hb, hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; TC, total cholesterol; TG, triglyceride; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4; miR-21, microRNA-21.

strated superior diagnostic precision, achieving an AUC of 0.772, a sensitivity of 0.753, and a specificity of 0.796.

Joint diagnostic model

The two biomarkers with the highest AUC values, miR-21 (AUC=0.772) and CA72-4 (AUC= 0.698), were selected to construct a joint predictive model (**Figure 3**). The combined ROC analysis showed an AUC of 0.927, surpassing the AUCs of either biomarker alone.

General data among patients with different serum exosomal miR-21 levels

The analysis of general patient data across different serum exosomal miR-21 levels (**Table 6**) showed several significant differences. BMI was greater in the High miR-21 group (24.21 ± 3.13 vs 23.18 ± 2.94, t=2.674, P=0.008). More patients in the High miR-21 group were engaged in manual labor (59.63% vs 44.20%, χ^2=5.802, P=0.016), while more in the Low miR-21 group had a college degree or above (52.17% vs 37.61%, χ^2=5.201, P=0.023). Smoking (P=0.001), drinking (P=0.008), chronic gastritis (P=0.026), Hp infection (P=0.004), family history of GC (P=0.005), and high salt/pickled diet consumption (P=0.004) were more prevalent in the High miR-21 group. Regular physical activity was less frequent in the High miR-21 group (46.79% vs 61.59%, χ^2=5.395, P=0.020). There were no notable differences in age and gender distribution between the two groups (both P>0.05).

Laboratory tests among patients with different serum exosomal miR-21 levels

The comparison of laboratory tests among patients with different serum exosomal miR-21 levels (**Table 7**) revealed several significant dif-

Table 4. Multivariate logistic regression analysis of risk factors for GC onset

Item	Coefficient	Std Error	Wald Stat	P	OR	OR CI Lower	OR CI Upper
BMI	0.305	0.244	1.563	0.211	1.357	0.841	2.190
Occupation (Manual labor/Mental work)	0.218	0.240	0.825	0.364	1.244	0.777	1.991
Educational level (High school or below/College degree or above)	-0.192	0.251	0.585	0.444	0.825	0.505	1.349
Smoking	0.631	0.261	5.847	0.016	1.880	1.127	3.136
Drinking	0.523	0.266	3.870	0.049	1.687	1.001	2.843
Chronic gastritis	0.708	0.237	8.923	0.059	2.030	1.276	3.229
Hp infection	0.107	0.042	6.505	0.011	1.113	1.025	1.208
Family history of GC	1.099	0.342	10.326	0.001	3.001	1.536	5.863
High salt/pickled diet	0.480	0.240	4.000	0.046	1.616	1.010	2.586
Regular (≥3 times/week)	-0.226	0.248	0.830	0.362	0.798	0.491	1.297
WBC	0.038	0.050	0.578	0.447	1.039	0.942	1.146
PLT	0.004	0.003	1.778	0.182	1.004	0.998	1.010
Hb	-0.012	0.008	2.250	0.134	0.988	0.973	1.003
ALT	0.015	0.017	0.778	0.378	1.015	0.981	1.050
AST	0.018	0.016	1.265	0.261	1.018	0.987	1.062
BUN	0.098	0.077	1.620	0.203	1.103	0.949	1.282
Cr	0.009	0.008	1.265	0.261	1.009	0.993	1.025
TC	-0.158	0.126	1.575	0.210	0.854	0.667	1.093
TG	-0.241	0.301	0.642	0.423	0.786	0.436	1.417
CEA	0.198	0.158	1.571	0.063	1.219	0.894	1.662
CA19-9	0.087	0.038	5.246	0.072	1.091	0.913	1.175
CA72-4	0.521	0.056	86.517	<0.001	1.684	1.509	1.880
miR-21	1.246	0.198	39.586	<0.001	3.477	2.361	5.121

GC, gastric cancer; OR, odds ratio; CI, confidence interval; BMI, body mass index; Hp, helicobacter pylori; WBC, white blood cell count; PLT, platelet count; Hb, hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; TC, total cholesterol; TG, triglyceride; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4; miR-21, microRNA-21.

Table 5. ROC analysis of the diagnostic value in early diagnosis of GC

Item	Best_threshold	Sensitivities	Specificities	AUC	Youden_index	F1_score
Smoking	0.050	0.558	0.602	0.514	0.116	0.578
Drinking	0.050	0.562	0.588	0.536	0.124	0.562
Hp infection	0.050	0.543	0.512	0.574	0.086	0.528
Family history of GC	0.050	0.579	0.623	0.535	0.158	0.591
High salt/pickled diet	0.050	0.594	0.642	0.546	0.188	0.612
CA72-4	6.850	0.673	0.702	0.698	0.375	0.659
miR-21	2.305	0.753	0.796	0.772	0.549	0.741

ROC, receiver operating characteristic; GC, gastric cancer; AUC, area under the curve; CA72-4, carbohydrate antigen 72-4; miR-21, microRNA-21.

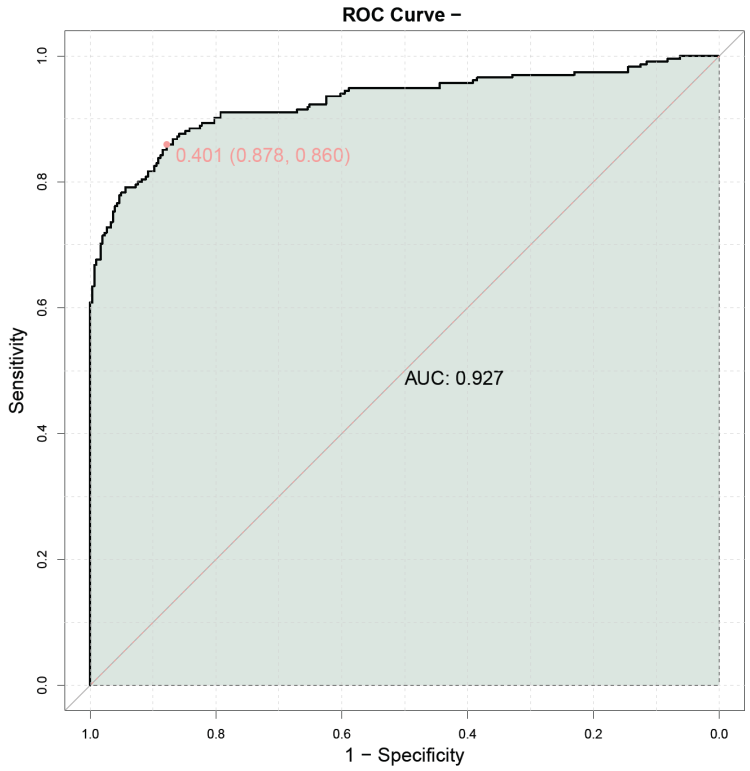

Figure 3. ROC curve of the early diagnostic value of serum exocrine miR-21 combined with CA72-4 for GC. ROC, receiver operating characteristic; GC, gastric cancer; miR-21, microRNA-21; CA72-4, carbohydrate antigen 72-4.

ferences. In blood routine tests, the High miR-21 group had higher WBC (7.12 ± 2.24 vs 6.61 ± 1.52, t=2.040, P=0.043), PLT (264.15 ± 70.38 vs 247.82 ± 56.14, t=1.976, P=0.049), and lower Hb (136.72 ± 18.54 vs 141.95 ± 12.67, t=2.514, P=0.013). Blood biochemistry results indicated higher ALT (26.18 ± 8.31 vs 24.03 ± 6.85, t=2.174, P=0.031), AST (27.69 ± 8.63 vs 25.14 ± 6.91, t=2.507, P=0.013), BUN (5.71 ± 1.65 vs 5.32 ± 1.27, t=2.002, P=0.047), Cr (77.84 ± 16.49 vs 73.15 ± 14.36, t=2.385, P=0.018), and lower TC (4.48 ± 0.87 vs 4.72 ± 0.84, t=2.281, P=0.023) and TG (1.42 ± 0.35 vs 1.51 ± 0.32, t=2.114, P=0.036). Traditional serum tumor markers also differed significantly, with higher CEA (3.73 ± 1.04 vs 3.34 ± 0.81, t=3.245, P=0.001), CA19-9 (39.27 ± 5.22 vs 37.59 ± 3.48, t=2.891, P=0.004), and notably higher CA72-4 (9.96 ± 2.91 vs 5.41 ± 1.07, t=15.514, P<0.001) in the High miR-21 group.

GC onset among patients with different serum exosomal miR-21 levels

The comparison of GC onset among patients with different serum exosomal miR-21 levels (**Table 8**) revealed significant differences. In high miR-21 group, overall GC incidence was markedly higher (68.81% vs 34.78%, χ^2=28.201, P<0.001). In terms of GC stage, the incidences of stage I (27.52% vs 15.22%, x^2=5.628, P=0.018) and II (41.28% vs 19.57%, x^2=13.91, P<0.001) were both notably higher than those in miR-21 low expression group.

Discussion

Globally, gastric carcinoma (GC) causes many deaths, underscoring an urgent need for effective early diagnostic tools. This study focuses on miR-21 in serum exosomes, aiming to

Table 6. Comparison of general data among patients with different serum exosomal miR-21 levels

Item	Low miR-21 group (n=138)	High miR-21 group (n=109)	t/χ^2	P
Demographic characteristic				
Age (years)	58.91 ± 8.52	59.37 ± 8.69	0.414	0.679
Male/Female [n (%)]	75 (54.35%)/63 (45.65%)	62 (56.88%)/47 (43.12%)	0.158	0.691
BMI (kg/m^2)	23.18 ± 2.94	24.21 ± 3.13	2.674	0.008
Occupation [n (%)]			5.802	0.016
Manual labor	61 (44.20%)	65 (59.63%)		
Mental work	77 (55.80%)	44 (40.37%)		
Educational level [n (%)]			5.201	0.023
High school or below	66 (47.83%)	68 (62.39%)		
College degree or above	72 (52.17%)	41 (37.61%)		
Personal lifestyle habits				
Smoking [n (%)]	45 (32.61%)	58 (53.21%)	10.632	0.001
Drinking [n (%)]	36 (26.09%)	46 (42.20%)	7.131	0.008
Medical history				
Chronic gastritis [n (%)]	43 (31.16%)	49 (44.95%)	4.958	0.026
Hp infection [n (%)]	41 (29.71%)	52 (47.71%)	8.402	0.004
Family history of GC [n (%)]	4 (2.90%)	13 (11.93%)	7.745	0.005
Dietary history in the past year				
High salt/pickled diet [n (%)]	31 (22.46%)	43 (39.45%)	8.373	0.004
Physical activity				
Regular (≥3 times/week) [n (%)]	85 (61.59%)	51 (46.79%)	5.395	0.020

miR-21, microRNA-21; GC, gastric cancer; BMI, body mass index; Hp, helicobacter pylori.

Table 7. Comparison of laboratory tests among patients with different serum exosomal miR-21 levels

Item	Low miR-21 group (n=138)	High miR-21 group (n=109)	t	P
Blood routine				
WBC ($\times 10^9/L$)	6.61 ± 1.52	7.12 ± 2.24	2.040	0.043
PLT ($\times 10^9/L$)	247.82 ± 56.14	264.15 ± 70.38	1.976	0.049
Hb (g/L)	141.95 ± 12.67	136.72 ± 18.54	2.514	0.013
Blood biochemistry				
ALT (U/L)	24.03 ± 6.85	26.18 ± 8.31	2.174	0.031
AST (U/L)	25.14 ± 6.91	27.69 ± 8.63	2.507	0.013
BUN (mmol/L)	5.32 ± 1.27	5.71 ± 1.65	2.002	0.047
Cr (μmol/L)	73.15 ± 14.36	77.84 ± 16.49	2.385	0.018
TC (mmol/L)	4.72 ± 0.84	4.48 ± 0.87	2.281	0.023
TG (mmol/L)	1.51 ± 0.32	1.42 ± 0.35	2.114	0.036
Traditional serum tumor markers				
CEA (ng/mL)	3.34 ± 0.81	3.73 ± 1.04	3.245	0.001
CA19-9 (U/mL)	37.59 ± 3.48	39.27 ± 5.22	2.891	0.004
CA72-4 (U/mL)	5.41 ± 1.07	9.96 ± 2.91	15.514	<0.001

miR-21, microRNA-21; WBC, white blood cell count; PLT, platelet count; Hb, hemoglobin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine; TC, total cholesterol; TG, triglyceride; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; CA72-4, carbohydrate antigen 72-4.

explore its use as a biomarker for predicting GC risk and for early screening purposes. The findings obtained were encouraging, providing insight into the underlying mechanisms and offering new prospects for future clinical application.

Table 8. Comparison of GC onset among patients with different serum exosomal miR-21 levels [n (%)]

Item	Low miR-21 group (n=138)	High miR-21 group (n=109)	χ^2	P
Total GC	48 (34.78%)	75 (68.81%)	28.201	<0.001
I stage	21 (15.22%)	30 (27.52%)	5.628	0.018
II stage	27 (19.57%)	45 (41.28%)	13.91	<0.001

miR-21, microRNA-21; GC, gastric cancer.

There are certain differences in the basic characteristics between patients diagnosed with GC and those without GC. The data shows that patients with GC typically have a Hp infection, are more likely to engage in physical labor, and have a relatively lower level of education. This group also has a higher prevalence of smoking and alcohol consumption, and tends to consume more high-salt or preserved foods in their daily diet. Furthermore, chronic gastritis, Hp infection, and a family history of GC are also more common among individuals with GC. These factors have largely been identified as risk factors for GC, reflecting the combined effect of individual lifestyle habits and environmental factors on health. Examples include smoking and alcohol consumption, which can exacerbate oxidative stress and inflammatory responses in the body. These behaviors not only may directly damage DNA but also interfere with normal cellular repair functions, thereby increasing the underlying risk of tumor development [18, 19]. Long-term high-salt diet can damage the gastric mucosa, creating conditions for Hp colonization and subsequent malignant changes [20, 21]. These associations suggest that modifiable risk factors have practical significance in the prevention of GC.

In terms of laboratory indicators, the two groups of patients also showed significant differences. WBC, PLT and ALT, AST in GC group were higher. Elevated WBC and PLT are usually indicative of a systemic inflammatory response, and have been noted in various types of cancers, including GC. When chronic inflammation persists, multiple cytokines and growth factors are released to create a local microenvironment that promotes tumor cell proliferation and survival [22, 23]. The increase in liver enzyme levels may be related to tumor liver metastasis or systemic inflammation involving the liver [24]. In addition, lower level of Hb in the GC patients often indicate anemia, which is more common in patients with chronic diseases and malignant tumors, usually due to limited red blood cell production or increased destruction [25-27].

The levels of CEA, CA19-9, and CA72-4 in the serum of patients with GC are higher than those in non-GC populations. These biomarkers have long been used for tumor screening, but have low specificity for GC [28]. The expression level of miR-21 was significantly increased in the GC group. MicroRNAs such as miR-21 play a crucial role in gene regulation and are involved in various tumor related pathways. Previous studies have confirmed, as reported by Farazati et al. [14], that miR-21 can promote tumor cell proliferation, migration, and invasion through the targeting of tumor suppressor genes like PTEN and PDCD4. Due to its stable presence in circulating exosomes, miR-21 can be transmitted between cells and may affect distal sites and participate in tumor progression [29, 30]. These characteristics make miR-21 a potential non-invasive diagnostic biomarker and therapeutic target.

Correlation analysis and multiple logistic regression analysis further confirmed the link between multiple risk factors and the occurrence of GC, with miR-21 showing the strongest correlation among all biomarkers. miR-21 is involved in the pathogenesis of GC and can affect various aspects such as cell apoptosis, cycle regulation, and angiogenesis. Research has shown that this molecule can suppress the expression of important tumor-suppressor genes such as PTEN, interfere with the normal functioning of cells, and thereby contribute to the abnormal proliferation and malignant transformation of tumors. It is worth noting that miR-21 is highly stable within exosomes and is less prone to degradation. This stability allows it to persist within the body for an extended period and exert a continuous influence on other cells. As a result, researchers consider it a reliable biomarker for disease diagnosis and prognosis prediction [31, 32]. These findings align with those reported by Wang et al. [10], who also suggested that miR-21-5p in serum might be a promising biomarker for both the diagnosis and prognosis prediction of GC.

We evaluated the diagnostic efficacy of several biomarkers using ROC analysis. From the re-

sults, the diagnostic accuracy of most of the indicators was only moderate. However, miR-21 performed better than traditional biomarkers. It has shown a higher diagnostic potential and could be a new tool for early screening of stomach cancer. Further studies found that when miR-21 and CA72-4 were combined, diagnostic accuracy could be improved even further. This combination is particularly useful for screening high risk groups, allowing for earlier interventions and thus better outcomes.

By detecting the expression levels of miR-21 in the serum exosomes of patients, we can gain a deeper understanding of the clinical significance of this biomarker. In general, high miR-21 expression is associated with unhealthy lifestyle, GC progression, and poor prognosis. Rising levels may indicate increased tumor invasion and metastasis capacity. In addition, miR-21 in exosomes mediates cell-to-cell communication, facilitates interactions between tumor cells and the microenvironment, and even helps cancer cells escape surveillance by the immune system [33, 34]. It can be said that this molecule is not only involved in the local development of tumors, but also plays a role in systemic metastasis, further highlighting its value as a prognostic biomarker.

There are a number of limitations in this study. First, this retrospective study could not establish causal relationships and may have introduced bias. Therefore, prospective investigations are essential to further validate current findings and clarify the chronological sequence of risk factors and GC occurrence. Second, although miR-21 has shown promise as a diagnostic biomarker, its specificity for GC is still unclear compared to other gastrointestinal tumors, and further testing of miR-21's applicability in different cancer types is needed in the future. Lastly, the biological mechanisms behind the related phenomena still need to be further explored through functional experiments and larger scale sample validation.

Our study focused on detecting miR-21 derived from serum exosomes rather than total circulatory RNA, offering several clinical advantages. First, exosomal miR-21 is highly stable due to its encapsulation within lipid bilayers, reducing pre-analytical variability and enabling retrospective studies [9, 10]. Second, although serum exosomes originate from multiple cell types, tumor cells actively secrete exosomes enriched with specific oncogenic cargo. Measuring miR-21 within the exosomal fraction thus provides a selective enrichment of tumor-associated signals, effectively reducing the background noise prevalent in total circulatory RNA (which includes RNA from cell lysis and routine turnover). This leads to a higher signal-to-noise ratio for cancer detection. Third, exosomal miR-21 is not a passive leakage product but is actively packaged and delivered to recipient cells. Its level reflects active intercellular communication within the tumor microenvironment, such as promoting angiogenesis and immune evasion [33, 34]. This functional dimension provides deeper biological insight into GC progression, beyond what total RNA levels can offer. Finally, our results show that exosomal miR-21 alone or combined with CA72-4 achieves high diagnostic accuracy (AUC=0.772-0.927), supporting its use as a robust, non-invasive biomarker for early GC screening.

Conclusion

Higher levels of serum exosomal miR-21 were associated with a greater risk of gastric cancer, and this indicator is a candidate non-invasive biomarker. Combining miR-21 with the traditional biomarker CA72-4 can help improve the diagnostic ability for early gastric cancer. These findings indicate that serum exosomal miR-21 has potential for risk assessment and early screening in gastric cancer.

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Disclosure of conflict of interest

None.

Address correspondence to: Weiping Fang, Department of Gastroenterology, The First People's Hospital of Jiande, No. 599, Yanzhou Avenue, Xin'anjiang Street, Jiande, Hangzhou 311600, Zhejiang, China. E-mail: licj2050@163.com

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