Research Article

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Increased miR-25 expression in serum of gastric cancer patients is correlated with CA19-9 and acts as a potential diagnostic biomarker

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Abstract: Objective: To evaluate miR-25 expression in serum of gastric cancer (GC) patients and whether it can be a potential biomarker for GC diagnosis. Methods: Forty one pathology confirmed GC patients and 41 healthy controls were included in this study. 10 mL peripheral venous blood were collected from GC patients and healthy controls. miR-25 relative expression and CA19-9 level in serum of GC patients and healthy controls were measured by real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) and enzyme linked immunosorbent assay (ELISA), respectively. The diagnostic sensitivity, specificity and receiver operating characteristic (ROC) curve of serum miR-25 and CA19-9 were calculated by STATA11.0 software. Results: The relative expression of miR-25 was 0.47±0.53 and 0.05±0.36 in serum of GC patients and healthy controls respectively with significant statistical difference (P<0.05). The serum level of CA19-9 for GC patients and healthy controls were 11.91±6.17 U/mL and 7.40±3.97 U/mL, indicating GC patients were much higher than those of healthy controls (P<0.05). The diagnostic sensitivity and specificity were 78.05% and 60.98% with the cut-off value of 0.32 for serum miR-25. Under this cut-off value, the area under the ROC curve was 0.75. For serum CA19-9, the diagnostic sensitivity and specificity were 70.73% and 56.10% with the cut-off value of 9.22 U/mL. Under this cut-off value, the area under the ROC curve was 0.73 with the 95% confidence interval of 0.62-0.84. Positive correlation was found between serum miR-25 relative expression and CA19-9 concentration of GC patients (r=0.75, P<0.05). Conclusion: According to our present study, serum miR-25 was elevated in GC patients which may serve as a diagnostic biomarker for GC.

Keywords: Gastric cancer; miR-25; CA19-9; Diagnosis; Serum biomarker

1 Introduction

Gastric cancer (GC) is rampant almost in every country all over the world [1]. In Japan GS is the most diagnosed malignant carcinoma in men [2]. In China mainland, GC is the 3rd leading cause of cancer related death [3]. More new cases of GS are found in China more than any other country each year and comprises nearly fifty percent of the global total GC patients. The general prognosis of GC is poor especially for China because of low early diagnosis rate [4]. Therefore, early detection of GC or effective screening for high risk patients is important to improve GC prognosis. Endoscopic biopsy and histopathological evaluation are known as the gold standard for GC diagnosis. However, highly expensive and relatively complex operating procedures makes these methods unsuitable for screening on a population basis, particularly for asymptomatic subjects.

Serum biomarkers are easy to access and usually used for GC diagnosis such as CEA, CA19-9, CA72-4 and et al [5, 6]. Recently, several studies have reported that some microRNAs were elevated or decreased in the serum of GC patients compared to healthy controls [7, 8]. These different expressed microRNAs may be potential biomarkers for GS diagnosis [7, 9, 10]. miR-25 is part of the miR-106b-25 cluster that was previously reported to be upregulated in several cancers tissues. However, its clinical value for gastric cancer diagnosis has not yet been reported. The objective of this study was to evaluate the serum miR-25

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as a biomarker for gastric cancer diagnosis. Moreover, we further discussed the correlation between miR-25 level and CA19-9 concentration in serum of GC patients.

2 Material and methods

2.1 Patients and controls

Forty one GC patients with pathology confirmation were recruited in this study from Feb 2014 to Jan 2016. All the patients had pathology confirmation of gastric cancer. The patients did not receive any treatment such as chemotherapy, radiation or surgery before blood collection. For the included 41 GC patients, there was 24 male and 17 female subjects with the median age of 61(36 to 76) years old. The median age of the 41 healthy controls was 58 (33 to 81) years old with 22 male and 19 female. The general character of the 41 GC patients is demonstrated in Table 1. The research was approved by the Ethics Committee of 1st Affiliated Hospital of Shihezi University School of Medicine. Written informed consents were obtained from all of the patients.

Table 1: The clinical character of the included 41 GC patients

Character	n=41	%
Gender		
Male	24	58.5
Female	17	41.5
Age(years)		
<50	18	43.9
≥50	23	56.1
Tumor location		
Cardia	16	39.0
Body of stomach	11	26.8
Pylorus	14	34.1
Localregional lymph node metastasis		
Yes	16	39.0
No	25	61.0
Pathology grading		
Well differentiation	10	24.4
Moderate differentiation	14	34.1
Poor differentiation	17	41.5

2.2 Serum collection

10mL peripheral venous blood was collected from GC patients and healthy controls from the basilic vein using clotting tubes, centrifuged at 3,000 rpm for 10 minutes at 4°C, and aliquoted into separate Eppendorf tubes. All samples were immediately frozen by liquid nitrogen and stored at -80°C until used.

2.3 MiR-25 detection

Total RNAs of the included GC patients and healthy controls were isolated by TRIzol reagent according to the manufacturer's protocol. The isolated RNA was reverse-transcribed into gene cDNAs or miR-25 cDNAs by reverse transcription kit from Promega by using oligo(dT) n primer or miR-25 specific transcriptional primer. Real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) assay was performed by SYBR Green Realtime PCR Master Mix from Toyobo. The results were normalized against a-tubulin expression for genes and U6B expression for microRNAs. The RT-qPCR assay was performed in triplicate.

2.4 CA19-9 detection

Serum CA19-9 concentration of the two groups were determined by using commercial enzyme immunoassay kits (CA19-9; Dainabbot, Tokyo, Japan).

2.5 Statistical analysis

Data expressed as the mean±sd were analyzed by STATA11.0 software. The difference between GC patients and healthy controls was assessed by student-t test. Diagnostic sensitivity and specificity were calculated by the equation of sensitivity=true positive/(true positive + false negative), specificity = true negative / (true negative + false positive). The area under the ROC curve was used to evaluate the feasibility of serum miR-25 and CA19-9 as biomarkers for GC diagnosis. Pearson's correlation test was performed to evaluate the relationship between the miR-25 relative expression and CA19-9 concentration in serum of GC patients. Two tailed $P<0.05$ was considered statistically significant.

3 Results

3.1 MiR-25 and CA19-9 expression in serum of GC patients and healthy controls

The serum level of MiR-25 and CA19-9 was detected by RT-qPCR or enzyme linked immunosorbent assay. As shown in Figure 1a, the relative expression level of miR-25 in serum of GC patients or healthy controls was 0.47±0.53 and 0.05±0.36, respectively, with significant difference (P<0.05). The serum CA19-9 level for GC patients and healthy controls was 11.91±6.17 U/mL versus 7.40±3.97 U/mL respectively, which indicates that the level in GC patients was much higher than that of healthy controls (P<0.05, Figure 1b).

3.2 Diagnostic value of serum miR-25 and CA19-9

The diagnostic sensitivity and specificity were 78.05% and 60.98% with the cut-off value of 0.32 for serum miR-25. Under this cut-off value, the area under the ROC curve were was 0.75 with the 95% confidence interval of 0.64-0.85 (Figure 1a). For serum CA19-9, the diagnostic sensitivity and specificity were 70.73% and 56.10% with the cut-off value of 9.22 U/ml. Under this cut-off value, the area under the ROC curve were was 0.73 with the 95% confidence interval of 0.62-0.84, (Figure 1b).

3.3 Positive correlation or serum miR-25 and CA19-9

The diagnostic sensitivity and specificity were 78.05% and 60.98% with the cut-off value of 0.32 for serum miR-25. Under this cut-off value, the area under the ROC curve was 0.75 with the 95% confidence interval of 0.64-0.85 (Figure 1a). For serum CA19-9, the diagnostic sensitivity and specificity were 70.73% and 56.10% with the cut-off value of 9.22 U/ml. Under this cut-off value, the area under the ROC curve was 0.73 with the 95% confidence interval of 0.62-0.84 (Figure 1b).

4 Discussion

Gastric cancer (GC) is one of the most diagnosed malignant tumors world-wide especially in Japan and China. The general prognosis for GC is relatively good in Japan because of its effective methods for early diagnosis [11]. However, only a small percentage of patients, about 9 percent, are diagnosed with GC in the early stages even in some large medical centers. Early diagnosis of GC is even lower in some rural poor areas [4]. Therefore, cheap and convenient methods for early diagnoses of gastric cancer are badly needed which may improve the general prognosis of GC. The National Comprehensive Cancer Network (NCCN) gastric cancer guidelines describe several GC screening or early diagnosis procedures such as gastroscopy, barium meal test and other tests [12]. However, the highly expensive and relatively complex operating procedures make these methods unsuitable for screening on a population basis, particularly for asymptomatic subjects. So, the ideal gastric cancer screening or early diagnosis methods should be of high sensitivity, high specificity,

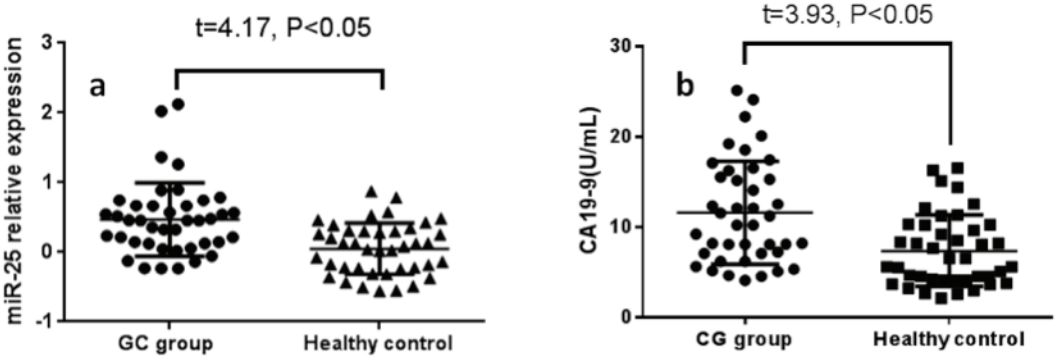

Figure 1: MiR-25 and CA19-9 expression in serum of GC patients and healthy controls (a: mirR-25 relative expression in serum of GC and healthy controls; b: CA19-9 relative expression in serum of GC and healthy controls)

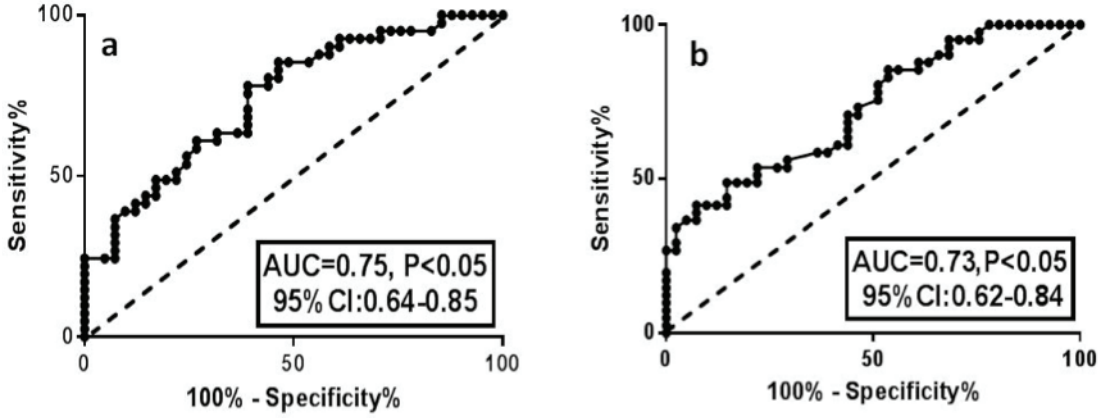

Figure 2: The ROC analysis show the sensitivities and specificities of serum miR-25 and CA19-9 for GC diagnosis (a: serum miR-25 for diagnosis of GC; b: serum CA19-9 for diagnosis of GC).

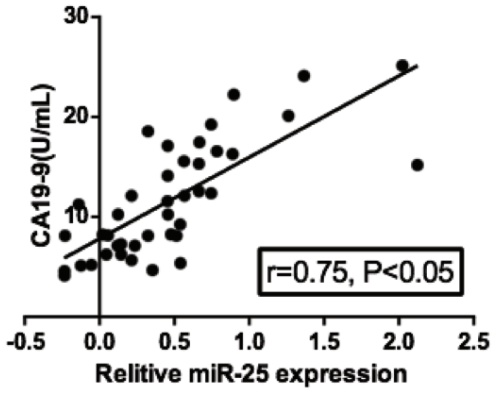

Figure 3: Scatter plot demonstrates the positive correlation between miR-25 expression and CA19-9 concentrations in serum of GC patients.

cheap, non-invasive, and easy to operate [13]. Serum biomarkers are easy to access and widely used for GC diagnoses such as CEA, CA19-9, CA72-4, etc.[14-17]. However, the diagnostic sensitivity and specificity for a single biomarker is limited.

Mir-25 was a microRNA which was found to be differently expressed in serum of several kinds of malignant carcinomas compared to that of healthy people. This different expression between cancer and healthy controls makes it a potential biomarker for cancer diagnosis or screening. Deng T et al [18] identified circulating miR-25 as a potential biomarker for pancreatic cancer diagnosis. They found that miR-25 had significant diagnostic value for the differential diagnosis of pancreatic cancer from normal controls with an AUC of 0.915 (95% CI: 0.893-0.937) which was significantly higher compared with an AUC of 0.725 for serum tumor marker carcinoembryonic antigen (CEA).

We have searched the database of Pubmed and did not find studies about the diagnostic value of serum miR-25 for GC detection. Thus, we performed this work in order to evaluate the serum miR-25 as a biomarker for GC diagnosis. Moreover, we further discussed the correlation between miR-25 level and CA19-9 concentration in serum of GC patients. In our present study, we found that the diagnostic sensitivity and specificity were 78.05% and 60.98% with the cut-off value of 0.32 for serum miR-25. Under this cut-off value, the area under the ROC curve was 0.75. Positive correlation was found between serum miR-25 relative expression and CA19-9 concentration of GC patients ($r=0.75$, $P<0.05$). This study indicates that serum miR-25 is elevated in GC patients which may serve as a diagnostic biomarker for GC. However, the sample size is small with 82 participants which may decrease the statistical power. So, prospective multicenter diagnostic trials are need to further explicate the diagnostic value of serum miR-25 as a biomarker for GC diagnosis.

Conflict of interests: No authors report any conflict of interest.

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