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RESEARCH ARTICLE

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Diagnostic value of macrophage inhibitory cytokine 1 as a novel prognostic biomarkers for early gastric cancer screening

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Abstract

Background: Early diagnosis is very important to improve the survival rate of patients with gastric cancer (GC), especially in asymptomatic participants. However, low sensitivity of common biomarkers has caused difficulties in early screening of GC. In this study, we explored whether MIC-1 can improve the detection rate of early GC.

Methods: We screened 8257 participants based on risk factors such as age, gender, and family history for physical examination including gastroscopy. Participant blood samples were taken for measure MIC-1, CA-199, CA72-4, and PG1/PG2 levels. The diagnostic performance of MIC-1 was assessed and compared with CA-199, CA72-4, and PG1/PG2, and its role in early GC diagnosis and the assessment of the risk of precancerous lesions have also been studied.

Results: Based on endoscopic and histopathological findings, 55 participants had GC, 566 participants had low-grade neoplasia, and 2605 participants had chronic gastritis. MIC-1 levels were significantly elevated in GC serum samples as compared to controls ($P < .001$). The sensitivity of serum MIC-1 for GC diagnosis was much higher than that of CA-199 (49.1% vs 20.0%) with similar specificities. Moreover, receiver operating characteristic (ROC) curve analysis also showed that serum MIC-1 had a better performance compared with CA-199, CA72-4, and PG1/PG2 in distinguishing early-stage GC (AUC: 72.9% vs 69.5%, 67.5%, 44.0%, respectively).

Conclusions: Serum MIC-1 is significantly elevated in most patients with early GC. MIC-1 can serve as a novel diagnostic marker of early GC and value the risk of GC.

KEYWORDS

biomarkers, gastric cancer, MIC-1

1 | BACKGROUND

Although the incidence of gastric cancer (GC) has declined in some developed countries, it continues to be an important healthcare problem from a global perspective.[1] Due to poor standards of hygiene, more than 70% of GCs occur in developing countries, and GC is the third leading cause of cancer-related death in China.[2,3] Despite advances in therapeutic approaches for GC, clinical prognosis with a reported five-year survival rate was less than 30%.[4] Therefore, the most critical means to reduce the incidence of GC is the improvement of early diagnosis rate.

Histological study of gastric mucosa is the most accurate method for diagnosing GC, but the popularity of endoscopic is very low due to the invasive nature, especially in asymptomatic patients. So,

serum tumor biomarkers play an important role in GC screening in the past decades. As the lack of unique biomarkers, combined detection of multiple serological biomarkers was used for predicting risk of GC.[5] Carcinoembryonic antigen (CEA), pepsinogen (PG), carbohydrate antigen 724 (CA 724), and carbohydrate antigen 199 (CA 199) as GC biomarkers have been studied widely.[6–8] However, there are many problems with the specificity and sensitivity of joint diagnosis, so it is particularly important to find a specific biomarker that can improve the diagnosis of early GC.[9]

Macrophage inhibitory cytokine-1(MIC-1), as a member of the transforming growth factor-beta superfamily, is detected in the inflammatory response and tissue repair after acute injury initially.[10] Serum MIC-1 is elevated in many cancer patients and is associated with tumor pathogenesis, progression, and invasion, such as lung cancer, colorectal cancer, and pancreatic cancer.[11–13] There have been related studies between MIC-1 and GC, but they focus on related pathogenic mechanisms, while the role of MIC-1 in early GC screen is still unknown.[12–15]

The purpose of the current study was to determine whether MIC-1 can improve the detection rate of GC in asymptomatic participants compared with other traditional indicators.

2 | MATERIALS AND METHODS

2.1 | Study population

The residents of Hefei City, Anhui Province participated in a medical examination program for GC screening from the west branch of the First Affiliated Hospital of the University of Science and Technology of China from 2014 to 2017. Participants were asymptomatic adults aged over 40 years old and had no prior history of any cancer. For each individual visiting for medical examination, relevant clinical notes, previous operations, family history of all types of cancer in first-degree relatives, and other risk factors for GC including cigarette smoking were documented. All participants were enrolled into the study only after written informed consent.

2.2 | Screening endoscopic

Endoscopic was offered to all individuals who joined GC screening. The individuals were fasted for at least six hours, and one of the lidocaine mucilage took 10 minutes before the endoscopic examination. All participants' blood pressure was guaranteed under 140/90 mm Hg. After endoscope was inserted into the esophagus of examiner, the order of esophageal-cardiac-gastric-pyloric-duodenum examination was performed. Mucosal color, smoothness, mucus, peristalsis, and lumen shape were observed to determine the presence of lesions. If the lesion was found, it was necessary to record the shape, extent, location, and cytology organization. The tissue was further stained with hematoxylin and eosin in the pathology department to determine the nature of the lesion and classification.

2.3 | MIC-1 ELISA

The serum of all participants was collected. The patients diagnosed as GC and high-grade neoplasia were defined as the group of GC, and 52 healthy subjects were randomly selected as the control group. Serum MIC-1 was detected by a sensitive ELISA, which was produced by CICAMS (cancer institute and hospital, Chinese Academy of Medical Sciences) as detailed previously.[16] This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for MIC-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells, and any MIC-1 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for MIC-1 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MIC-1 bound in the initial step. The color development is stopped, and the intensity of the color is measured. All samples were analyzed using ELISA assays on the same day. All serum samples were duplicately assayed.

2.4 | Serum CA-199, CA72-4, PG1, and PG2 assay

The serum levels of CA-199, CA72-4, PG1, and PG2 were tested by electrochemiluminescence immunoassay (ECLIA) kits using Elecsys 601 (Roche). All serum samples were duplicately assayed.

2.5 | Statistics

The chi-square test was used to investigate associations between MIC-1 level and clinical characteristics of patients with GC. ROC curve was used to identify the diagnostic information. Multivariable logistic regression model was conducted to corporate diagnostic performance of biomarkers. The clinical cutoff value for MIC-1 was assigned as the mean value plus two point five standard deviations of healthy individuals, and the clinical cutoff values for CA-199, CA72-4, PG1, and PG2 were based on the manufacturer's protocols. The statistical activity was operated with SPSS 16.0. *P*-value of $<.05$ for a two-sided test was considered to be statistically significant.

3 | RESULTS

3.1 | Clinic pathological characteristics of the study population

Among the 8257 participants (5340 males; 4717 females), 55 had GC including 27 with high-grade neoplasia, 18 with early GC, and 10 with advanced GC. The baseline characteristics of the subjects who underwent an endoscopic examination are presented in Table 1. The features indicative of GC are listed in Table 2.

3.2 | Risk factors for GC development

The risk factors for GC were shown in Table 3. According to a univariate analysis, age, sex, and family history were significant. Smoking history and BMI cannot be risk factors for GC.

3.3 | Early diagnostic value of MIC-1 for GC

In this study, we classified high-grade neoplasia into the GC group, and most GCs were in early stage, so we evaluated the value of serum MIC-1 as a marker for early diagnosis of GC compared with traditional biomarkers. We randomly selected 52 healthy subjects as negative controls from the population participating in the study. Interestingly, the serum level of CA-199 ($P = .097$), PG1/PG2 ($P = .303$) did not differ significantly in GC patients compared with healthy controls but CA72-4 ($P = .022$) and MIC-1 ($P < .001$) level was significantly increased in patients with GC (Figure 1).

Next, receiver operating characteristic curves (ROC) were used to evaluate the advantage of MIC-1 as serum diagnostic markers for early-stage GC. The area under ROC curve (AUC) of CA-199, CA72-4, and PG1/PG2 was 0.695, 0.675, and 0.440, respectively. Compared with traditional biomarkers, MIC-1 (AUC:0.729) had the better ability to distinguish GC cases from healthy subjects (Figure 2).

We further calculated the detection sensitivity of MIC-1 for GC at various specificity cutoffs and set 435.66 pg/mL as the cutoff value for warranting acceptable specificity. Table 4 showed

TABLE 1 Clinical characteristics of study population

Characteristic	n	(%)
Age		
≤Median age	4348	52.66
>Median age	3909	47.34
Sex		
Female	4717	57.13
Male	3540	42.87
Smoking status		
Never smokers	5208	63.07
Current smokers	1990	24.10
Former smokers	540	6.54
Passive smoking	519	6.29
BMI, kg/m^2		
<25	5614	67.99
≥25	2643	32.01
Family history (lung cancer)		
Yes	177	2.14
No	8080	97.86
Chronic gastritis	2605	31.55
Low-grade neoplasia	566	6.85
Gastric cancer	55	0.67

TABLE 2 Clinical characteristics of gastric cancer

Gastric cancer (n = 55)	n	(%)
Age		
≤Median age	15	27.27
>Median age	40	72.73
Sex		
Female	15	27.27
Male	40	72.73
Smoking status		
Never smokers	34	61.82
Current smokers	13	23.64
Former smokers	4	7.27
Passive smoking	4	7.27
BMI, kg/m^2		
<25	32	58.18
≥25	23	41.82
Family history (lung cancer)		
Yes	13	23.64
No	42	76.36
Stage		
High-grade neoplasia	27	49.09
Early gastric cancer	18	32.73
Advanced gastric cancer	10	18.18

TABLE 3 The risk factors for gastric cancer

	Univariate			
Variables	Control (n, %)	Cancer (n, %)	χ^2	*P*-value
Age			14.313	**.000**
≤Median age	4333	15		
>Median age	3869	40		
Sex			20.149	**.000**
Female	4702	15		
Male	3500	40		
Smoking status			0.453	.939
Never smokers	5174	34		
Current smokers	1977	13		
Former smokers	536	4		
Passive smoking	515	4		
BMI, kg/m^2			2.448	.118
<25	5582	32		
≥25	2620	23		
Family history (lung cancer)			111.833	**.000**
Yes	164	13		
No	8038	42		

P value is less than .001, the difference is statistically significant.

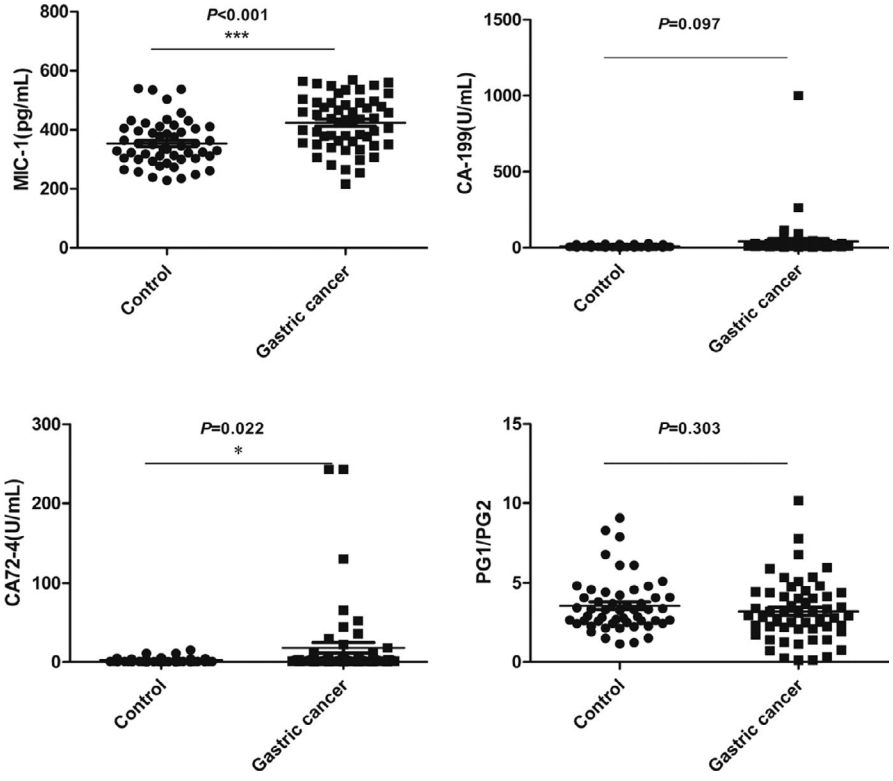
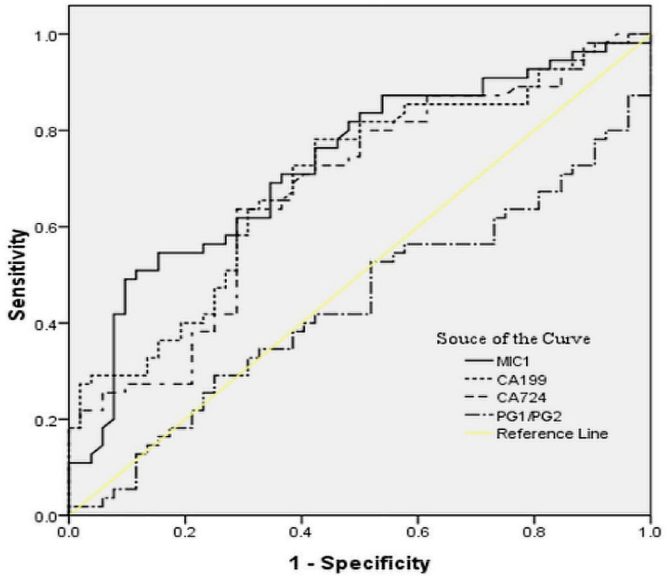


FIGURE 1 T test of gastric cancer patients compared with healthy controls



Area under receiver operating characteristic curve (AUROC)					
	AUC	SE	P value	95% CI	
				lower	up
MIC1	0.729	0.049	<0.001	0.633	0.825
CA199	0.695	0.051	0.001	0.595	0.795
CA724	0.675	0.052	0.002	0.573	0.777
PG1/PG2	0.440	0.056	0.281	0.330	0.549

FIGURE 2 The area under ROC curve (AUC) of CA-199, CA72-4, and PG1/PG2

TABLE 4 Performance characteristics of tumor markers

	True positive	False negative	False positive	True negative	Sensitivity (%)	Specificity (%)
CA199	11	44	1	51	20.00	98.08
CA724	12	43	3	49	21.82	94.23
PG1/PG2	28	27	19	33	50.91	63.46
MIC1	27	28	5	47	49.09	90.38
CA199 + CA724	19	36	3	49	34.55	94.23
CA199 + CA724 + PG1/PG2	32	23	21	31	58.18	59.62
PG1/PG2 + MIC1	41	14	22	30	74.55	57.69
CA199 + CA724 + PG1/PG2 + MIC1	43	12	24	28	78.18	53.85

TABLE 5 Characteristics of the gastric cancer patients according to MIC1 status

	MIC1			
Variables	**(≤435.66)**	**(>435.66)**	χ^2	***P***
Age				
≤Median age	12	6	2.658	.103
>Median age	16	21		
Sex				
Female	6	9	0.982	.322
Male	22	18		
Smoking status				
Never smokers	18	16	2.082	.586
Current smokers	6	7		
Former smokers	1	3		
Passive smoking	3	1		
BMI, kg/m^2			0.025	.874
<25	16	16		
≥25	12	11		
Family history (lung cancer)			0.154	.695
No	22	20		
Yes	6	7		
Stage				
High-grade neoplasia	15	12	0.538	.764
Early gastric cancer	8	10		
Advanced gastric cancer	5	5		
CA199				
≤27	24	20	1.164	.281
>27	4	7		
CA724			1.897	.168
≤8.2	24	19		
>8.2	4	8		
PG1/PG2			0.019	.891
≤3	14	14		
>3	14	13		

the diagnostic value of MIC-1, CA-199, CA72-4, PG1/PG2, and combined biomarkers. The sensitivity of MIC-1 was 49.09% and significantly higher than that of CA-199 (20.00%) and CA72-4 (21.82%). Although PG1/PG2 showed similar sensitivity to MIC-1, its specificity (63.46%) was much lower than MIC-1(90.38%). These data revealed that serum MIC-1 separate detection was superior to other biomarkers in GC detection, especially early-stage GC.

3.4 | Risk factors for GC according to MIC-1 level

Analysis by chi-square test, no significant correlation was identified between MIC-1 level and other factors (Table 5). Relevant data suggested MIC-1 can be an independent risk factor for early GC.

4 | DISCUSSION

Gastric cancer is one of the most malignant tumor, and its mortality and morbidity are increasing year by year in China. Despite the variation of practice pattern worldwide, surgical resection of early- and mid-stage GC patients is still recognized as the most effective method. However, the lack of typical clinical symptoms and the intolerance of endoscopy have led to delay in GC diagnosis. Most patients with clinical signs have been diagnosed as advanced GC and have lost optimal surgical timing. Therefore, early detection and diagnosis of GC are extremely important in improving the survival of the patients.

Tumor biomarkers are highly correlated with tumorigenesis, diagnosis, recurrence, and prognosis. Finding a specific serum biomarker for tumors has always been the focus of research such as the specificity of alpha-fetoprotein for liver cancer diagnosis. There are many serum biomarkers of GC currently used internationally, and the common ones are CEA, CA-199, CA72-4, and PG1/PG2. However, the sensitivity and specificity are not satisfactory, especially for early-stage GC. The serum levels of CEA, CA19-9, and CA72-4 may be elevated in patients with GC at various stages.[17] The National Comprehensive Cancer Network guidelines do not recommend serum marker testing for preoperative evaluation and staging of GC.

Therefore, an additional biomarker favoring early detection and diagnosis of GC is still urgently needed.

Macrophage inhibitory cytokine 1 (MIC-1/GDF15), a 25-kDa secreted growth factor of transforming growth factor-β (TGF-β) superfamily, was originally discovered in macrophage cells.[10,18] In previous studies, overexpression of MIC-1 in GC tissues was associated with tissue differentiation, and high levels of MIC-1 predicted high potential invasiveness.[19] Although previous studies have reported that the serum MIC-1 level in patients with GC was higher than healthy people, they have concluded that MIC-1 is associated with GC progression and prognosis.[20,21] The diagnostic value of MIC-1 in early GC has not been investigated comprehensively.

The present study is the first to evaluate the clinical value of MIC-1 in early GC based on large-scale investigation. We analyzed the expression of MIC-1 in GC serum and found that MIC-1 showed a significant difference in levels between GC patients and healthy controls ($P < .001$), with no noticeable difference observed for CA-199, CA72-4 or PG1/PG2 ($P > .10$). Compared with the biomarkers of CA-199, CA72-4, and PG1/PG2, MIC-1 appears to hold the most individual promise in differentiating patients with GC from those without, giving sensitivities of 49.09%, specificities of 90.38%, and areas under the curve (AUCs) of 0.729. Interestingly, there is no correlation between MIC-1 and high-risk factors of GC, suggesting that high MIC-1 level can be used as an independent risk factor for early GC.

5 | CONCLUSIONS

In summary, our research confirms that MIC-1 can be a new ideal tumor biomarker for early diagnosis of GC. Based on our present findings, future study is warranted for the development of relationship between MIC-1 and GC staging, treatment effect detection, recurrence, and metastasis. In this manner, MIC-1 may be evaluated for its superiority to serum as a source for biomarkers in GC.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

GX performed the study, analyzed the results, and drafted the manuscript. XL Zhang designed the study and edited the manuscript. ZW Chen and SH Chen performed sample collection. LM contributed to strategic development decisions. All authors have read and approved this version of the article. Neither the entire article nor any part of its content has been published or has been accepted elsewhere.

CONSENT FOR PUBLICATION

Not applicable.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Medical Ethical Committee of the First Affiliated Hospital of USTC. All patients had signed informed consent.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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