Study on the Value of miR6503-5p Combined with PGR in the Diagnosis of Early Gastric Cancer

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Abstract: Objective To investigate the value of serum miRNA 6503-5p (miR6503-5p) combined with pepsinogen ratio (PGR) in the diagnosis of early gastric cancer. Methods: 94 patients (gastric cancer group) with gastric cancer confirmed by pathological examination and 90 patients with chronic atrophic gastritis collected by Department of Pathology in our hospital were selected as the control group, the serum levels of pepsinogen (PG I, PG II) and miR6503-5p were measured in the two groups, and the value of the two indexes in the diagnosis of gastric cancer was analyzed by ROC. Results: The serum levels of miR6503-5p in gastric cancer group were significantly higher than those in control group (P<0.05), the serum levels of PG I and PGR in gastric cancer group were significantly lower than those in control group (P<0.05), the serum levels of miR6503-5p in stage II gastric cancer group were significantly higher than those in stage I patients with statistically significant difference (P<0.05), and the serum levels of PG I and PGR in stage II gastric cancer group were significantly lower than those in stage I patients with statistically significant difference (P<0.05). The serum levels of PG I, PG II and PGR in the patients with highly and moderately differentiated gastric cancer were not significantly different from those in the patients with poorly and undifferentiated gastric cancer, with no statistically significant different (P>0.05); the serum levels of miR6503-5p in the patients with highly and moderately differentiated gastric cancer were significantly lower than those in the patients with poorly and undifferentiated gastric cancer, with statistically significant difference (P<0.05); the sensitivity of miR6503-5p in diagnosing gastric cancer was 81.33%, the specificity was 71.09%, the area under the ROC curve was 0.767; the sensitivity of PGR in diagnosing gastric cancer was 85.81%, the specificity was 78.40%, and the area under the ROC curve was 0.827. The sensitivity of serum miR6503-5p combined with PGR was 96.40%, the specificity was 85.44%, and the area under the ROC curve was 0.920. Conclusion The miR 6503-5p combined with PGR has high sensitivity and specificity in the diagnosis of gastric cancer and is worthy of clinical application in the screening of patients with early gastric cancer.

Keywords: miRNA6503-5p; Pepsinogen ratio (PGR); Gastric cancer; Diagnosis
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Gastric cancer is the third most common cause of cancer-related death. The prognosis of patients is closely related to the stage. The 5-year survival rate of early gastric cancer is >95%, while that of late gastric cancer is usually <30%[1]. Periodically individualized screening and assessment of risk of gastric cancer in high-risk population is the focus of the study. The determination of serum pepsinogen (PG) is simple and rapid. Combined PGI, PGII and PGI/PGII (PGR) can help to determine whether gastric mucosa is atrophic[2]. Domestic screening in high risk areas of gastric cancer indicated that PGI≤70μg/L and PGR≤7.0 were the threshold values for the diagnosis of atrophic gastritis and the criteria for screening of gastric cancer[3], but there was no large sample follow-up data to support this. miRNA not only has significant tumor-related, tissue-specific, and

expression stability characteristics in gastric cancer tumor tissue, but also has a characteristic miRNA expression profile in the serum of gastric cancer patients[4,5]. To investigate the value of serum miRNA6503-5p (miR6503-5p) and PGR in the diagnosis of early gastric cancer, we studied gastric cancer patients who were diagnosed by pathological examination in our hospital.

DATA AND METHODS

Data

94 cases of gastric cancer (gastric cancer group) and 90 cases of chronic atrophic gastritis (CAG) confirmed by pathological examination in our hospital were selected as control group. The study objects were selected from April 2017 to July 2019. Inclusion criteria: (1) Diagnosis criteria of gastric cancer refer to the criteria in the "Guidelines for Diagnosis and Treatment of Primary Gastric Cancer of CSCO (2017 Edition)"; (2) Diagnosis criteria of chronic atrophic gastritis refer to the criteria in the eighth edition of "Diagnosis Science" of People's Medical Publishing House[6]; (3) Patients with gastric cancer underwent endoscopic biopsy for pathological examination for confirmation; (4) The study protocol was implemented after the approval of the Medical Ethics Committee. Exclusion criteria: (1) Patients received previous radiotherapy or chemotherapy; (2) Patients with cancer at other sites; (3) Patients with recurrence of previously diagnosed gastric cancer after surgery; (4) Patients with immune function or rheumatoid disease.

In the gastric cancer group, the patients aged between 41 and 76 years (mean 63.7±7.1 years); 53 patients were male and 41 were female; the pathological stage was stage I (n=28) and stage II (n=66); the accompanying diseases were hypertension (n=18), diabetes mellitus (n=8), history of coronary heart disease (n=2), and positive result of Helicobacter pylori (Hp) (n=67). In the control group, the patients aged between 38 and 75 years (mean 61.8±8.5 years);46 patients were male and 44 were female, including 23 patients with hypertension,13 patients with diabetes mellitus, 4 patients with history of coronary heart disease, and 62 patients with positive Hp results. The difference of above baseline data between the two groups had no statistical significance (P>0.05).

Methods

Index detection method

5mL venous blood was collected from all patients on an empty stomach, centrifuged at 3000rmp for 5min, and the separated serum was stored in a freezer at -80°C. Serum PG I, PG II, and PGR levels were quantified by ELISA using a gastric secretion kit from Biohit, Finland. Make doubling dilution on the standard, set the sample wells to be tested, standard wells and blank wells, and add sample; firstly, add 40μl of sample diluent in the sample wells to be tested on the enzyme-labeled coating plate, and then add 10μl of the sample to be tested (the final dilution of sample is 5 times), and seal the plate with sealing film and incubate at 37℃ for 30min. Dilute the 30-fold concentrated washing solution with distilled water by 30-fold for later use. Carefully remove the sealing film, discard the liquid, shake to dryness, fill each well with cleaning solution, allow to stand for 30 seconds before discarding, repeat 5 times, and pat dry. Add 50μ LTMB color rendering base solution (brand: YSRIBIO, Huzhou Innoreagents Biotechnology Co., Ltd.) into each well (except blank wells). Incubate and wash. 50μl stop solution was added into each well to stop the color development. The color development was protected from light at 37℃ for 15 min. The solution changed from blue to yellow. The absorbance (OD value) of each well was measured at 450nm wavelength in sequence. BioTek enzyme-labeled analyzer was purchased from BioTek Instruments, Inc.

Detection of serum miR6503-5p: total RNA extraction: add the serum into the centrifuge tube without RNA, add lysing solution to fully mix, and use spectrophotometry to determine the RNA concentration and purity. Reverse transcription: RNA template 2μL, RT primer 2μL, 5×RT buffer 5μL, dNTP (2.5mm) 2μL,40U/μL RNase Inhibitor 0.5μL, 20 U/μL RT enzyme 0.5μL, ddH 2 O 24μL were operated according to reverse

transcription kit. Detection by qRT-PCR: reverse transcription was performed using cDNA as template and β-actin as internal reference gene. Reaction system: cDNA 2μL, 2×SYBR Green qPCR mixture 10μL, forward primer 1μL, reverse primer 1μL, diethyl pyrocarbonate 6μL of diethoxybicarbonate (DEPC) water, total reaction volume 20μL, each target gene repeated 3 times, reaction conditions: 95℃ 10min; 95℃ 15min; 60℃ 1 min; 40 cycles, 72℃ 30s. The qRT-PCR instrument was started for PCR amplification and the relative expression of miR6503-5p was calculated using the 2-ΔΔCT method.

Statistical processing

SPSS 21.0 was used for the statistical software of this study. The of measurement data of PGR and miR6503-5p in both groups used (±s) for statistical description. t test was used for the comparative analysis of the data; x,2 test was used for the comparison of gender and accompanying diseases between the two groups; ROC curve was drawn, and the related diagnostic indexes were obtained; P<0.05 was significant for the difference.

RESULTS

Comparison of PGR and miR6503-5p values in the two groups

The serum miR 6503-5p value was higher in gastric cancer group than that in control group, and the difference was statistically significant (P<0.05). The serum PG I and PGR value in gastric cancer group was lower than that in control group, and the difference was statistically significant (P<0.05). See Table 1.

Table 1
Comparison of PGR and miR6503-5p Values Measured in the Two Groups (±s)

Group	n	PGI (μg/L)	PGII (μg/L)	PGR	miR6503-5p (Relative expression)
Gastric cancer group	94	44.9±15.2	14.3±2.9	3.14±1.25	2.88±0.71
Control group	90	97.1±23.0	13.6±3.1	7.14±1.88	1.73±0.64
t value		-18.236	1.582	-17.063	11.524
P value		0.000	0.115	0.000	0.000

Comparison of serum PGR and miR6503-5p measured values of patients with gastric cancer in different pathological stages

The serum levels of miR6503-5p in patients with different TNM stages of gastric cancer in stage II were significantly higher than those of gastric cancer in stage I, with statistically significant difference (P<0.05). The serum levels of PG I and PGR in patients with stage II gastric cancer were significantly lower than those in patients with stage I gastric cancer, and the difference had statistical significance (P<0.05); see Table 2.

Table 2
Comparison of Serum PGR and miR6503-5p Values in Patients with Gastric Cancer at Different Pathological Stages (±s)

TNM STAGES	n	PGI (μg/L)	PGII (μg/L)	PGR	miR6503-5p (Relative expression)
Stage I	28	53.7±14.0	13.9±2.3	3.86±1.13	2.50±0.68
Stage II	66	40.3±11.8	14.5±2.8	2.77±1.04	3.05±0.66
t value		4.759	-0.999	4.529	-3.662
P value		0.000	0.320	0.000	0.000

Comparison of serum PGR and miR6503-5p values in patients with gastric cancer of different differentiation degree

The serum levels of PG I, PG II and PGR in the patients with highly and moderately differentiated gastric cancer were not significantly different from those in the patients with poorly and undifferentiated gastric cancer (P> 0.05). The serum levels of miR6503-5p in the patients with highly and moderately differentiated gastric cancer were significantly lower than those in the patients with poorly and undifferentiated gastric cancer, with statistically significant difference (P<0.05). See Table 3.

Table 3.
Comparison of Serum PGR and miR6503-5p Values in Gastric Cancer Patients with Different Degrees of Pathological Differentiation (±s)

Degree of differentiation	n	PGI (μg/L)	PGII (μg/L)	PGR	miR6503-5p (Relative expression)
Highly and moderately differentiated	54	55.1±14.2	14.1±2.6	3.91±1.10	2.62±0.66
Poorly and undifferentiated	40	51.6±13.8	14.4±2.5	3.58±1.15	3.13±0.62
t value		1.196	-0.562	1.411	-3.800
P value		0.235	0.575	0.162	0.000

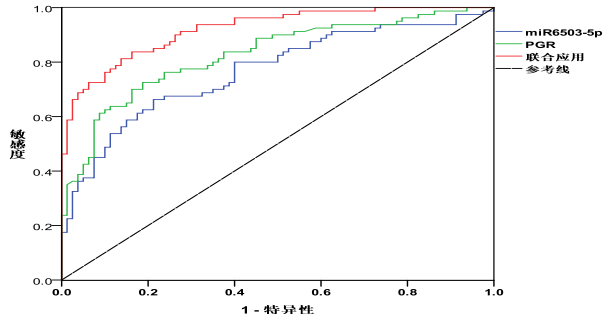
Diagnostic evaluation

ROC curve was drawn. When the optimal diagnostic threshold value of serum miR6503-5p was selected, the corresponding diagnostic value indexes of gastric cancer were 81.33% sensitivity, 71.09% specificity and 0.767 area under ROC curve. The sensitivity, specificity and area under the ROC curve of PGR value were 85.81%, 78.40% and 0.827 for the diagnosis of gastric cancer. The sensitivity, specificity and area under ROC curve of miR6503-5p combined with PGR value in the diagnosis of gastric cancer were 96.40%, 85.44% and 0.920, respectively. See Table 4 and Figure 1.

Table 4
Diagnostic Value of Serum PGR and miR6503-5p Alone and Combined in the Diagnosis of Gastric Cancer

Index	Cutoff value	Sensitivity (%)	Specificity (%)	Missed diagnosis rate (%)	Misdiagnosis rate (%)	AUC value
PGR	4.88	85.81	78.40	14.19	21.60	0.827
miR6503-5p (Relative expression)	2.19	81.33	71.09	18.67	28.91	0.767
Combined application	-	96.40	85.44	3.60	14.56	0.920

Figure 1
ROC Curves of Serum PGR and miR6503-5p Alone and Combined in the Diagnosis of Gastric Cancer



DISCUSSION

The generation and development of gastric cancer is a multistep and progressive process in which the normal mucosa-chronic inflammatory response-atrophy-intestinal metaplasia-dysplasia-ultimately cancerates[6]. Currently, the primary treatment for gastric cancer is surgery. The study data showed that[7] the 5-year survival rate of patients with stage I gastric cancer is up to 90%, while the 5-year survival rate of patients with stage IV disease is less than 10%. Early screening and early clinical treatment for high-risk gastric cancer are important to improve patient survival. Serum PG is a good index of the shape and function of gastric mucosa and an inactive precursor of pepsin. PGI is secreted by the main cells of the fundic gland and PGII is secreted by the antrum and pyloric gland[8]. The results of this study showed that the serum levels of PG I and PGR in patients with gastric cancer were lower than those

in patients with chronic atrophic gastritis, and the serum levels of PG I and PGR in patients with stage II gastric cancer were significantly lower than those in patients with stage I gastric cancer in gastric cancer group. The reason is that in the process of mucosa carcinogenesis, the PG gene of cells is destroyed and the ability to secrete PG I decreases gradually, which leads to the continuous decrease of PG I level. PG II is secreted by glands and metamorphic pyloric glands at different sites. The PG II level is usually not significantly decreased, and the higher the pathological stage is, the more serious the damage of gastric mucosa is, so the lower the PG I and PGR value is. However, there was no significant difference between the serum levels of PG I, PG II and PGR in patients with highly and moderately differentiated gastric cancer and those in patients with poorly or undifferentiated gastric cancer (P>0.05). Because it is a less invasive diagnostic method, ROC curves have been established through large-scale clinical studies and are expected to improve detection sensitivity and specificity through joint test. Although PG has good sensitivity and specificity in the diagnosis of gastric cancer, new gastric cancer tumor markers still need to be urgently studied. In recent years, the correlation between miRNA and tumor has become one of the research hotspots. The known miRNA is an endogenous non-coding micromolecule RNA with the length of about 18-25 nucleotides[9,10], which can completely or incompletely inhibit the synthesis of protein or induce the degradation of target gene. This negatively regulates the expression of target genes at the post-transcriptional level and then regulates the physiological processes of cell differentiation, proliferation, growth and apoptosis[11,12]. miRNA have the effects of oncogenes and tumor suppressor genes. The expression of miRNA is tissue-specific, and the expression profiles of miRNA vary in different tumors[13]. After repeated freezing and thawing, the stability of miRNA in peripheral blood was not significantly affected by normal temperature, strong acid and strong alkali, and there was a good correlation between plasma or serum and miRNA in tissues, which made miRNA a new biomarker for the diagnosis of tumors[14,15,20.] Study showed[16] that miRNA was associated with the occurrence, development, invasion and metastasis of gastric cancer. The relationship between miRNA and epigenetics in gastric cancer constitutes a complex network for regulation of gene expression.[17] It can be used as a new non-invasive biomarker for early detection of gastric cancer. However, few studies have been done on miRNA for early diagnosis of gastric cancer. In this study, the expression level of miRNA-6503-5p in peripheral blood of patients with gastric cancer was studied, and the value of miRNA-6503-5p in the auxiliary diagnosis of gastric cancer was discussed. We found that the expression level of miRNA-6503-5p in the serum of patients with gastric adenocarcinoma was significantly higher and its stability was higher, which could be used for the early diagnosis of gastric cancer. The serum levels of miR6503-5p in patients with well-differentiated and moderately-differentiated gastric cancer was lower than those in patients with poorly differentiated and undifferentiated gastric cancer. The reason for this analysis is that it may play a role as a potential oncogene, over-expression of which promotes tumorigenesis, and the higher the cancer stage, the more pronounced its expression increases. In this study, ROC curve was used to analyze the detection values of different detection methods, and it was found that the sensitivity and specificity of serum miR6503-5p combined with PGR to gastric cancer were significantly improved by selecting the optimal diagnostic threshold.

Currently, clinical studies on miRNA in patients with gastric cancer only reveal specific expression in patients' peripheral blood. If miRNA is used as serum markers for screening and early diagnosis of clinical gastric cancer, large-scale sample studies are still needed [18,19] , and there are few studies on early detection of gastric cancer patients by combining miRNA with gastric mucosal serology. This study combined serum miR6503-5p and PGR for initial screening of gastric cancer to achieve early detection and early treatment.

In conclusion, miR6503-5p combined with PGR has high sensitivity and specificity in the diagnosis of gastric cancer, and it is worthy of clinical application in the screening of patients with early gastric cancer.

REFERENCES

1. 戴兵. 胃蛋白酶原 1 对晚期胃癌临床预后的预测价值. 中国现代医学杂志. 2019;29(6):83-87.
2. Kaji K, Hashiba A, Uotani C, et al. Grading of atrophic gastritis is useful for risk stratification in endoscopic screening for gastric cancer. Official journal of the American College of Gastroenterology| ACG. 2019;114(1):71-79.
3. Castro C, Dinis-Ribeiro M, Rodrigues AN, et al. Western long-term accuracy of serum pepsinogen-based gastric cancer screening. European journal of gastroenterology & hepatology. 2018;30(3):274-277.
4. Sjomina O, Pavlova J, Daugule I, et al. Pepsinogen test for the evaluation of precancerous changes in gastric mucosa: a population-based study. Journal of Gastrointestinal & Liver Diseases. 2018;27(1).
5. Kalach N, Legoedec J, Wann A-R, Bergeret M, Dupont C, Raymond J. Serum levels of pepsinogen I, pepsinogen II, and gastrin-17 in the course of Helicobacter pylori gastritis in pediatrics. Journal of pediatric gastroenterology and nutrition. 2004;39(5):568-569.
6. Flood L. BALLENGER'S OTORHINOLARYNGOLOGY HEAD AND NECK SURGERY, 18th edn PA Wackym, JB Snow People's Medical Publishing House, 2016 ISBN 978 1 60795 177 3 pp 1523 Price£ 275.00. The Journal of Laryngology & Otology. 2017;131(10):937-938.
7. Fujiyoshi T, Miyahara R, Funasaka K, et al. Utility of linked color imaging for endoscopic diagnosis of early gastric cancer. World journal of gastroenterology. 2019;25(10):1248.
8. Ning Y, Qian K, Qi C. Role of vascular endothelial growth factor receptor in the pro-proliferation activity of CD40-CD40L in AGS gastric cancer cells. Asian Biomedicine. 2010;4(5):797-802.
9. Hu K, Wang S, Wang Z, et al. Clinicopathological risk factors for gastric cancer: a retrospective cohort study in China. BMJ open. 2019;9(9):e030639.
10. Xin L, Yang W-F, Zhang H-T, Li Y-F, Liu C. The mechanism study of lentiviral vector carrying methioninase enhances the sensitivity of drug-resistant gastric cancer cells to Cisplatin. British journal of cancer. 2018;118(9):1189-1199.
11. Wu B, Gao X, Smith J. Optical Biopsy for Prostate Cancer Diagnosis Using Fluorescence Spectroscopy. International Journal of High Speed Electronics and Systems. 2018;27(03n04):1840026.
12. Kanomata H, Seyama Y, Kudo H, et al. A case of double cancer of intrahepatic cholangiocarcinoma and gastric carcinoma with difficult diagnosis of the primary tumor with peritoneal metastasis. Gan to kagaku ryoho Cancer & chemotherapy. 2018;45(4):746-748.
13. Wang Y, Li Z, Shan F, et al. Current status of diagnosis and treatment of early gastric cancer in China--Data from China Gastrointestinal Cancer Surgery Union. Zhonghua wei chang wai ke za zhi= Chinese journal of gastrointestinal surgery. 2018;21(2):168-174.
14. Morais S, Castro C, Antunes L, Peleteiro B, Bento MJ, Lunet N. Second primary cancers and survival in patients with gastric cancer: association with prediagnosis lifestyles. European Journal of Cancer Prevention. 2019;28(3):159-166.
15. Gao Z, Li Y, Jiang K, Ye Y, Wang S. Progress and controversy on diagnosis and treatment of gastric stump cancer. Zhonghua wei chang wai ke za zhi= Chinese journal of gastrointestinal surgery. 2018;21(5):588-592.
16. Hwang GY, Baek DW, Cho HJ, et al. Elevated Neutrophil–to–Lymphocyte Ratio Predicts Survival in Patients with Advanced Gastric Cancer Treated with Trastuzumab Combination Chemotherapy. Anticancer research. 2018;38(5):3151-3156.
17. Li J, Xu L, Run Z-C, et al. Multiple cytokine profiling in serum for early detection of gastric cancer. World journal of gastroenterology. 2018;24(21):2269.
18. Liu X, Xu D, Huang C, et al. Regulatory T cells and M2 macrophages present diverse prognostic value in gastric cancer patients with different clinicopathologic characteristics and chemotherapy strategies. Journal of translational medicine. 2019;17(1):1-11.
19. Jiang H-P, Qi C-B, Chu J-M, Yuan B-F, Feng Y-Q. Profiling of cis-diol-containing nucleosides and ribosylated metabolites by boronate-affinity organic-silica hybrid monolithic capillary liquid chromatography/mass spectrometry. Scientific reports. 2015;5(1):1-9.
20. Vermeulen, H., Gouse, M., Delport, M., Louw, M., & Miller, T. Consumer Acceptance of Sugar Derived from Genetically Modified Sugarcane in South Africa. AgBioForum, 2020; 22(1).