

## **The value of combined detection of serum gastrin-17 and pepsinogen in the early screening of gastric cancer.**

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### **Abstract**

**Objective:** To investigate change of pepsinogen I (PG I), pepsinogen II (PG II) and gastrin-17 (G-17) in different gastric diseases and to determine the value of PG and G-17 in screening and diagnosis of early gastric cancer.

**Methods:** All patients were divided into 5 groups, non-gastric atrophy group, gastric atrophy (GA) group, peptic ulcer group, non-early gastric cancer group, early gastric cancer group. 90 healthy individuals were recruited as the control group. Using Enzyme Linked Immunosorbent Assay (ELISA) to determine expression of PG I, PG II and G-17 in difference groups of patients. ROC curve was used to determine the diagnostic value of PG I, PG II, G-17 and PRG. The value of PG I, PG II and G-17 in screening and diagnosis of gastric cancer was analyzed through statistical methods.

**Results:** Results showed that expression of G-17 and PG I all showed significant difference compared with the control group,  $P < 0.05$ . Among the indexes, G-17 showed highest specification and sensitivity. However expression of PG II and the ratio of PG I/PG II (PRG) were not significantly different from the control group. All G-17 and PG II showed good sensitivity and specificity in the gastric cancer group. However PRG and PG I showed lower sensitivity and specificity compared with other markers. In early gastric cancer group, G-17 demonstrated high sensibility and specificity which were significant higher than other indexes. Combination of G-17 and PRG showed significant high sensitivity and accuracy in screening of gastric cancer which could enhance the sensitivity to 96.2%.

**Conclusion:** G-17 and PG can be potent markers in screen of diagnosis of gastric cancer, especially for early cancer. G-17 combined with PG can improve the sensitivity and accuracy of the diagnosis of gastric cancer.

**Keywords:** Early gastric cancer, Diagnosis, Gastrin-17 (G-17), Pepsinogen I (PG).

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### **Introduction**

Gastric cancer (GC) is a serious threat to human health. It is one of the most common malignant tumors of digestive tract [1]. Although with the development of the basic medical and clinical treatment, the morbidity and mortality of gastric cancer decreased gradually, but in recent decades, the global annual increase in more than one million cases still ranked [2]. Because of non-invasive, simple and low cost, PG I, PG, and G-17 serological detection has been widely applied in many countries [3-7].

Gastrin was first discovered by Edkins in 1906, and there are many subtypes of gastrin, of which G-17 is the most abundant (about 90%). Rakic et al. found that the serum gastrin levels in patients with gastric cancer changed [8]. Ghee et al. detected *Helicobacter pylori* in patients with ELISA and the results showed that high level of G-17 could promote the occurrence

and development of gastric cancer [9]. Zhuang et al. have shown that gastrin could promote the migration of gastric cancer cells through the  $\beta$ -catenin/tcf-4 pathway [10]. Kikuchi et al. showed that the high expression of G-17 and low expression of PG I and PG II could be used as the basis for early gastric cancer screening [11]. However, there are some drawbacks in each conventional technique for each sole marker has its limitation and can't give the satisfying results. Moreover, the association between the combined detection of serum gastrin-17 plus pepsinogen and gastric cancer is still unclear.

In this study we discussed the clinical value of PG I, PG II and G-17 in the diagnosis and differential diagnosis of gastric cancer to improve the diagnostic precision of gastric cancer. In this study, a total of 192 patients with gastric diseases were selected, including early gastric cancer, non-early gastric cancer, gastric ulcer, chronic atrophic gastritis, chronic atrophic

gastritis. ELISA was used to detect the concentration of PG I, PG II and G-17, recording the changes of PG, PG II and G-17 in benign and malignant gastric diseases.

## Materials and Methods

### Patients

This cross-sectional study was done in Dongtai Hospital Affiliated to Medical College of Nantong University of China

**Table 1.** Demographic characteristics in study groups.

Groups	Healthy (n=90)	Non-chronic atrophic gastritis (n=39)	Chronic atrophic gastritis (n=49)	Gastric ulcer (n=41)	Non-early gastric cancer (n=33)	Early gastric cancer (n=30)
Age (y)	59.91 ± 10.45	59.78 ± 10.91	60.93 ± 9.62	62.84 ± 9.70	65.06 ± 7.83	65.60 ± 9.61
M-F (male:female)	59:31	18:21	22:27	32:9	29:4	6:4

### ELISA assay

ELISA was performed using 96 well microtiter plates (Nunc) that were coated with 50 µL/well of different substrates diluted in coating buffer (100 mmol/L Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub>, pH 9.6). After overnight incubation at 4°C, the coated wells were washed three times after which the nonspecific binding sites were blocked with 1% Bovine Serum Albumin (BSA) in PBS for 1 h at 37°C. All subsequent detection antibodies were diluted in PBS containing 0.05% Tween-20 and 1% BSA (PBS-T-BSA) unless otherwise stated, and each step was followed by washing three times using PBS-0.5% Tween-20. Subsequently, the serum was incubated at indicated dilutions for 1 h at 37°C. After washing, bound C3c was detected using diluted rabbit polyclonal anti-human PG I (PG II or G-17) antibodies (Abs) (Shanghai Long Island Biotec. Co., Ltd. D-3004), followed by Horseradish Peroxides (HRP)-conjugated anti-rabbit Abs (Shanghai Long Island Biotec Co., Ltd., P-0047) diluted in PBS-T-BSA, for 1 h at 37°C respectively. Enzyme activity of HRP was assessed by addition of o-phenylene-diamine and H<sub>2</sub>O<sub>2</sub>. After 15-30 min the reaction was stopped by addition of 50 µL of 1 mol/L H<sub>2</sub>SO<sub>4</sub> per well. OD was measured at 450 nm using a microtiter plate reader (Multiskan FC, Thermo Scientific).

**Table 2.** The concentration of G-17, PG I, PG II, PRG in serum.

Groups	G-17 (pmol/l)	PG I (ug/l)	PG II (ug/l)	PG I/PG II (PRG)
Healthy (n=90)	1.89 (1.51~51.92) <sup>b,c</sup>	230.215 (45.42~766.32)	16.775 (0.36~66.74)	8.22 (1.04~172.44)
Non-chronic atrophic gastritis (n=39)	7.42 (0.81~63.33) <sup>a,b,c</sup>	159.91 (55.74~516.48) <sup>b,c</sup>	19.28 (3.01~52.98)	10.76 (2.49~49.31)
Chronic atrophic gastritis (n=49)	4.55 (0.97~47.58) <sup>a,b,c</sup>	235.377 (41.79~704.09) <sup>b,c</sup>	14.767 (2.10~76.85) <sup>a</sup>	10.99 (1.50~103.36) <sup>a</sup>
Gastric ulcer (n=41)	7.34	177.49	27.22	8.49

during 2010-2011. A total of 192 patients with gastric diseases were selected.

All patients were divided into five groups: early gastric cancer group; non-early gastric cancer group; gastric ulcer group; chronic atrophic gastritis group; non-chronic atrophic gastritis group, and 90 healthy people were selected as the control group (Table 1).

All patients signed consent forms before treatment.

### Statistical analysis

The results were entered into computer using SPSS 18 statistical software and analyzed by chi-square, t-test. Receiver operating curve (ROC) analysis was used to determine the cut-offs for PG I, PG I/II ratio and G17. A p value less than 0.05 is considered as significantly different. Sensitivity and specificity of these markers to distinguish the above mentioned groups (non-atrophic gastritis, GA, and GC) were calculated. Positive Predictive Value (PPV), Negative Predictive Value (NPV), Area under Curves (AUC) and the 95% Confidence Intervals (CI) were also calculated and presented.

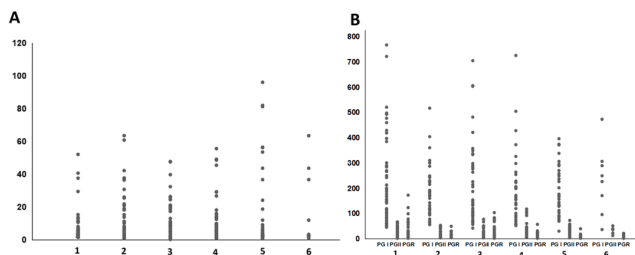
## Results

### Concentration of G-17, PG I, PG II, PRG in serum

The results show that the non-early gastric cancer in patients with G-17 and PG I expression are significantly different with the healthy group (P<0.05). PG II and PRG expression are not significant compared with healthy group (p>0.05). The expression of PG I in all patients had significant difference compared with healthy group, (p<0.05) (Figure 1 and Table 2).

	(0.92~55.45) <sup>a,b,c</sup>	(51.91~724.818) <sup>a,b,c</sup>	(4.54~117.65) <sup>b,c</sup>	(0.49~56.91)
Non-early gastric cancer (n=39)	3.67	138.11	20.21	7.51
	(0.45~95.90) <sup>a,c</sup>	(29.95~395.59) <sup>a</sup>	(0.32~72.71)	(0.32~39.46)
Early gastric cancer group (n=30)	2.86	156.74	22.09	8.46
	(0.78~63.54) <sup>a,b</sup>	(36.42~472.34) <sup>a</sup>	(11.73~41.33)	(1.51~21.56)

Note: <sup>a</sup>P<0.05, Compare to healthy group; <sup>b</sup>P<0.05, Compare to non-early gastric cancer group; <sup>c</sup>P<0.05, Compare to early gastric cancer group.



**Figure 1.** Scatter diagram of the concentration of G-17, PG I, PG-2 and PRG in serum detected by ELISA. A: concentration of G-17 in serum; B: concentration of G I, PG-2 and PRG in serum. Note: (1) Healthy group; (2) Non-chronic atrophic gastritis group; (3) Chronic atrophic gastritis group; (4) Gastric ulcer group; (5) Non-early gastric cancer group; (6) Early gastric cancer group.

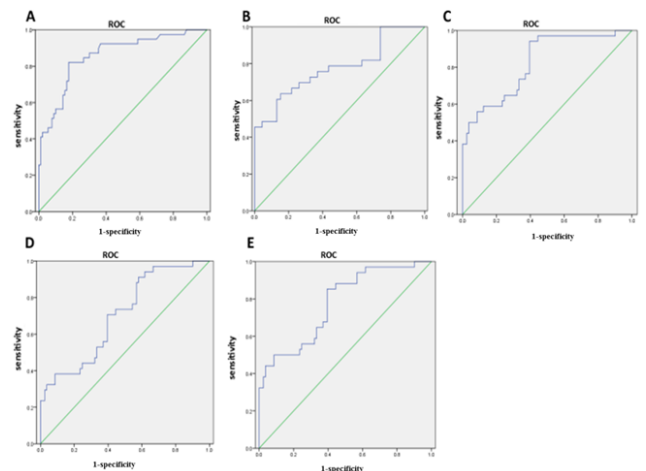
**The value of G-17, PG I, PG II and PRG in the diagnosis of gastric carcinoma**

The results show that when the concentration of G-17>3.119 pmol/l as cut-off points the sensitivity was 84.6%, false positive rate was 26.7%. When the concentration of G-17<2.053 pmol/l as cut-off points, the sensitivity was 76.7%, false positive rate was 23.1%, the area under the ROC curve (AUC) is 0.797, 95% curve of the confidence interval 0.721-0.873, p=0.000. When the concentration of PG I<88.15 ug/l, the sensitivity was 74.7%, false positive rate was 24.4%, AUC is 0.724, 95% curve of the confidence interval 0.741-0.861, p=0.000. When the concentration of PG II>18.68 ug/l as cut-off value, the sensitivity was 61.5%, false positive rate was 34.4%, the AUC is 0.783, 95% curve of the confidence interval 0.578~0.787, p=0.034. When the PRG<7.61 as cut-off value, the sensitivity was 76.8%, false positive rate was 25.8%, the AUC is 0.733, 95% curve of the confidence interval 0.538-0.728, p=0.005 (Figure 2 and Table 3).

**Table 3.** Indices of validity for serum levels of pepsinogen I (PG I), PG I/II Ratio and Gastrin-17 (G17) to distinguish gastric cancer, non-early gastric cancer and early gastric cancer.

Groups	Indices of validity	Gastric cancer (n=43)	Non-early gastric cancer (n=33)	Early gastric cancer (n=30)
G-17	Cut-off points (>pmol/l)	>3.119	>3.119	>3.119
	Specificity	0.733	0.726	0.834
	Sensitivity	0.846	0.787	0.9

	Cut-off points (<pmol/l)	<2.053	<2.053	<2.053
	Specificity	0.769	0.716	0.815
	Sensitivity	0.767	0.723	0.887
PG I	Cut-off points (ug/l)	88.15	88.15	88.15
	Specificity	0.756	0.643	0.651
	Sensitivity	0.747	0.727	0.7
PG II	Cut-off points (ug/l)	18.68	18.68	18.68
	Specificity	0.656	0.647	0.662
	Sensitivity	0.615	0.636	0.6
PRG	Cut-off points	7.61	7.61	7.61
	Specificity	0.742	0.709	0.791
	Sensitivity	0.768	0.727	0.8



**Figure 2.** The ROC curve of G-17, PG I, PG II and PRG in early and non-early gastric cancer group detected by ELISA. (A) The concentration of G-17>3.119pmol/l; (B) The concentration of G-17<2.053pmol/l; (C) The concentration of PG I<88.15 ug/l; (D) The concentration of PG II>18.68 ug/l; (E) PRG<7.61.

**Table 4.** Correlation analysis between G-17, PG I and PRG.

Detection index	G-17		PG I		PG- II	
	r	P	r	P	r	P
G-17						
PG I	0.36	0.26				

PG-II	0.75	0.17	0.18	0.27		
PRG	0.09	0.69	0.39	0.016	0.59	0.000

### **The combined detection of G-17 and PRG value in diagnosis of gastric cancer**

The diagnostic results of G-17 and PRG results were analyzed by using Spearman rank correlation method (Table 4). The results showed small correlation coefficient between G-17 and PRG ( $P>0.05$ ), a complementary diagnostic value for gastric cancer. All patients with gastric cancer, three indicators combined detection results are shown in Table 5, diagnostic sensitivity can be increased to 96.2% combined detection of G-17 and PRG.

**Table 5.** The combined detection of G-17 and PRG in the diagnosis of gastric cancer complementary diagnostic value.

	Sensitivity	Specificity	Accuracy
G-17 (+)	0.837	0.733	0.756
PRG (+)	0.768	0.742	0.767
G-17 (+)/PRG (+)	0.962	0.693	0.862

### **Discussion**

The aim of this study was to evaluate the accuracy of serum levels of PG and G17 as non-invasive screening methods for GC. Regarding the clinical importance of GA and GC, we mainly focused on assessing indices of validity of these markers to distinguish GA/GC patients. ELISA was used to detect the content of PG-I, PG-II and G-17 in gastric diseases patients and healthy people. The results showed that expression of G-17 and PG I all showed significant difference compared with the control group,  $P<0.05$ . Among the indexes, G-17 showed highest specification and sensitivity. However expression of PG II and the ratio of PG I/PG II (PRG) were not significantly different from the control. All G-17 and PG II showed good sensitivity and specificity in the gastric cancer group. However PRG and PG I showed lower sensitivity and specificity compared with other markers. In early gastric cancer group, G-17 demonstrated high sensibility and specificity which were significant higher than other indexes which was consistent with many studies [12]. As Edkins for the first time found gastrin, there were more and more scholars focusing on its activity [13]. Gastrin can stimulate gastric acid secretion; its expression can also be adjusted by acid feedback [14]. Among the many subtypes of gastrin, G-17 plays a significant role to regulate gastric acid secretion [15-17]. A large number of research results confirmed that hypergastrinemia is a risk factor for gastric cancer. Cao et al. an observational case-control study showed that serum G-17 was higher than the control group in gastric cancer patients [18]. Sun suggested the cut-off points of G-17 was 10.7 pmol/l, sensitivity and specificity were 50% and 83% out of a large sample of research data [19]. Japanese researchers found that the function of gastric mucosa can be reflected by the

expression of PG in serum [20]. The serum PG can be divided into two subtypes: PG-I and PG-II, which belongs to the aspartic protease precursor, consisting of 375 amino acids, 42 kDa molecular weight. Miki et al. suggested when PG I  $\leq 70$  ng/mL or PRG  $\leq 3$ , the diagnostic sensitivity was 77% and the false positive rate was 27%, the positive predictive value between 0.77% and 1.25%, the negative predictive value between 99.08% and 99.90% [21]. Finally we study the combined detection of G-17 and PRG value in diagnosis of gastric cancer. Spearman rank correlation method showed that the serum levels between G-17 and PRG is small ( $P>0.05$ ). All patients with gastric carcinoma, combined detection results of the three indexes showed that G-17 and PRG combined detection of diagnostic sensitivity can be increased to 96.2%. There are some reports on sign gesture recognition and we need to study further [22-26]. However, the results due to the smaller sample size and there are certain limitations, but the combined detection of G-17 and PRG still has the potential to be a reliable indicator of detection of gastric cancer.

### **Conclusion**

G-17 and PRG is a high potential for applications in the diagnosis of cancer. G-17 combined with PRG can play a complementary role in the diagnosis of gastric cancer, improving the diagnostic sensitivity of gastric cancer and accuracy.

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