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Analysis of the Value of *Helicobacter pylori* Test in Combination with the Determination of Plasma Propepsin and Gastrin 17 in Screening the Precancerous Status of Gastric Cancer

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ABSTRACT

To explore the value of the *Helicobacter pylori* test in combination with the determination of plasma pepsinogen (PG) and gastrin 17 in screening the precancerous status of gastric cancer and gastric cancer in the healthy population, between 2019 and 2022, we enrolled a total of 402 subjects who went to the physical examination in the Center of Health Management of Ganzhou people's Hospital and additionally underwent the urea (14C) breath test and determination of PGI, PGII and G-17. Anomalies in Hp, PG or G-17 ≥ 2, or a single anomaly in PG determination would be taken as positive, and the diagnosis should be further confirmed by the gastroscopy and pathological test. According to the results, subjects would be further divided into the gastric cancer group, precancerous lesion group, precancerous disease group and control group, aiming to clarify the relationship between Hp, PG and G-17 levels and the precancerous status and development of gastric cancer and the screening value. Results showed that Hp-positive infection was found in 341 subjects (84.82%). Hp infection rate in the control group was much lower than those in the precancerous disease group, precancerous lesion group and gastric cancer group ($P < 0.05$). The CagA positive rates in the gastric cancer group and precancerous lesion group were significantly higher than those in the precancerous disease group and control group, while the serum level of G-17 in the gastric cancer group was much higher than those in the precancerous lesion group, precancerous disease group and control group ($P < 0.05$), and the PG I/II ratio in the gastric cancer patients was also lower than those in the precancerous lesion group, precancerous disease group and control group ($P < 0.05$). As the disease progressed, the G-17 level also increased but PG I/II ratio decreased gradually ($P < 0.001$). Hp test in combination with PG and G-17 shows a high value in determining the precancerous status of gastric cancer and screening for gastric cancer in healthy subjects.

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Introduction

Gastric cancer (GC) remains highly pandemic in the world nowadays and is the second most common cause for cancer-related death (1). According to the *World Cancer Report 2018* issued by World Health Organization (WHO), there have been 1,033,071 new cases of GC worldwide (taking up 5.7% of all new cancer cases) and 782,685 death cases (taking up 8.2% of all cancer-related death cases) (2). The high death rate of GC has been found to be associated with delayed diagnosis, so early diagnosis and treatment should be pivotal to the reduction of GC-related death (3).

According to the *Guidelines for Screening of Gastric Cancer in China*, screening for GC is advised for all high-risk populations, defined as those who live in the GC prevalent region for more than 3 years, or those who have been infected by *H. pylori* or those with GC-positive familial history, or those with the risk factors of GC, including the high-salt diet, smoking or massive uptake of alcohol, at the age of 40 (4). There have been two choices, *i.e.* the endoscopic examination for the upper gastrointestinal tract and histological examination by endoscopic biopsy (5), for the diagnosis of atrophic gastritis and precancerous lesion as well as the identification of healthy and affected gastric mucosa. Nevertheless, screening for GC via gastroscope is inefficient and unrealistic, since only 1 to 3% of the population would be affected by GC. Additionally, about 300,000,000 people are at a high risk of GC, and it is impossible to provide a gastroscopic examination for them to screen for GC (6). Therefore, a method that could stratify the risk is required for this population as a pre-screening tool for the gastroscopic examination, which could further identify the individuals at a high risk of GC.

Serological parameters, including anti-PG I, PG II, G-17 and Hp antibodies, have been applied to detect the status of the gastric mucosa of people at different risks of GC (7). Pepsinogen in the serum is a kind of non-invasive marker indicating the status of the gastric mucosa that could generate two biochemically different PGs, PG I and PGII. PG I is mainly generated by fundus mucusa, while PG II is by fundus, sinuses ventriculi and duodenal mucosa. It has been suggested that a low level of PG I in serum or a low PG I/PG II ratio is associated with severe gastric atrophy and intestinal metaplasia, quite common in GC (8-11). Generally, PG I < 70 μg/L or PG I/PG II ratio < 3.0 has been used as a threshold for clarifying the high-risk population of GC (12). Gastrin (GAS) is also proved

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to be a functional marker of gastric mucosa (13). G-17 is secreted by G cells of sinuses ventriculi, and its level in serum could better reflect the atrophy of sinuses ventriculi than the total GAS in serum (14). *H. pylori* infection has been considered as the major cause of chronic gastritis, peptic ulcer, GC and mucosa-related lymphoma and other kinds of parenteral diseases (15,16). Thus, *H. pylori* test in combination with the determination of plasma PG and G-17 may serve as a tool for evaluating the serological risk of GC.

In this study, with the result of pathological biopsy as the gold criteria, we explored the value of the Hp test in combination with the determination of plasma PG and G-17 in detecting the precancerous status of GC and GC in a healthy population.

Materials and Methods

General data

This study was carried out in Ganzhou people's Hospital from January 2019 and June 2022, during which a total of 402 consecutive patients were enrolled. According to the histological pathology finding, subjects were divided into four groups: gastric cancer group, precancerous lesion group, precancerous disease group and control group.

Criteria for inclusion into the gastric cancer group: Patients with the diagnosis of GC confirmed by gastroscope and pathological findings, including the GC at an early stage and progressive stage. Criteria for inclusion into the precancerous lesion group: Patients with GC-related pathological changes, including moderate to severe intestinal metaplasia and low-grade intraepithelial neoplasia. Criteria for inclusion into the precancerous disease group: Patients with benign diseases that could increase the risk of GC, including chronic atrophic gastritis, gastric ulcer or gastric polyps. Criteria for inclusion into the control group: Subjects with no GC, precancerous lesion or precancerous diseases.

Criteria for exclusion: Patients with a history of chemotherapy, surgery in the stomach, eradication of *H. pylori*, or anticoagulation therapy, or a history of general diseases, including diabetes mellitus, liver cirrhosis or chronic renal failure.

Urea (^{13}C) breathe test

Subjects who attended the physical examination were required to keep their fasting status and sit quietly to collect the exhaled gas for 10 min. Then, a bottle of urea (^{13}C) was taken with 80 to 100 mL of cool water. After 30 min, exhaled gas was collected and subjected to the test. The final result was the difference value between the micrometer of the sample at 30 min and that at 0 min, and a final result ≥4.0±0.4 would be considered as Hp positive (17).

Serological test

For each subject, 10 mL fasting venous blood was collected into the tubes for centrifugation at 3000 r/min for 10 min to obtain the serum which was then preserved at -80°C. Enzyme-linked immunosorbent assay (ELISA) was applied to determine the levels of G-17, PG I, PG II and PG I/PG II ratio. For interpretation of results, PGI normal range is set between 60 and 240 μg/L, PG II between 0 and 27 μg/L, G-17 between 1 and 15 pmol/L, while results of PGI ≤ 70μg/L and PG I/PG II ratio ≤3.0 would be taken as an anomaly of pepsin, and the level of G-17 exceeding the normal range would be taken as abnormal (4).

Statistical analysis

SPSS 22.0 software was used to analyze the data. Continuous variables were described as mean ± standard deviation (SD), while the categorical variables as percentage or frequency. All data were subjected to the test of normal distribution by Kolmogorov- Smirnov, and the homogeneity of variance would be verified by the Levene test. Comparison of data between two independent samples in normal distribution would be verified by *t*-test, while the continuous data not in normal distribution would be described by using the median. Comparison of the medians among groups would be testified by the Kruskal-Wallis H non-parameter test. $P < 0.05$ would be taken as statistically significant. Then, the overall diagnostic efficiency of G-17, PG I, PG II and PG I/PG II ratio would be further calculated to clarify the optimal critical value, sensitivity and specificity.

Results

Characteristics of patient

A total of 402 subjects who attended to the physical examination in the health management center of Ganzhou people's Hospital between 2019 and 2022 were enrolled in this study. Among the subjects, the average age was (54.14 ± 11.79) years, and the male/female ratio was 0.6. No significant difference was found in the comparison of age and sex ($P > 0.05$; Table 1). This study was approved by the Ethical Committee of Ganzhou people's Hospital, and the patients signed the written informed consent prior to enrollment.

Results of gastroscopy

In this study, 5 subjects were grouped into the GC group, including 3 at the early stage and 2 at the progressive stage; 47 subjects were grouped into the precancerous lesion group, including 36 with moderate to severe intestinal metaplasia and 11 with low-grade intraepithelial neoplasia; 168 patients were grouped into the precancerous disease group, including 82 with chronic atrophic gastritis, 35 with gastric ulcer, 38 with gastric polyps and 13 with the history of stomach surgery; 182 subjects were grouped into the control group.

Hp and Cag A of subjects

In this study, 341 patients were Hp-positive (84.82%). In the control group, the infection rate of Hp was 69.23%, significantly lower than those of the precancerous disease group (97.02%), precancerous lesion group (100%) and GC group (100%) (all $P < 0.05$). Of 341 Hp positive subjects, 106 subjects were Cag A positive. Evaluation of the Cag A status was also carried out for those subjects, and the results indicated that the Cag A positive ratios in the GC group and precancerous lesion group were much higher than those in the precancerous disease group and the control group ($P < 0.05$). Comparison of the Cag A positive ratios either between the GC group and precancerous lesion group or between the precancerous disease group and control group showed no significant difference ($P > 0.05$; Table 2).

Table 1. Geographic characteristics of subjects.

	Total	Control group	Precancerous disease group	Precancerous lesion group	GC group	P value
n (%)	402 (100%)	182 (45.27%)	168 (41.79%)	47 (11.70%)	5 (1.24%)	
Age (mean ± SD)	55.14 ± 11.79	56.52 ± 11.15	53.18 ± 11.85	55.07 ± 11.91	53.92 ± 12.17	(0.553) * (0.693) ** (0.527) † (0.440) †† (0.748) ‡ (0.426) ‡‡
Sex (Female) (n, %)	252 (62.68%)	117 (64.28%)	104 (61.90%)	28 (59.57%)	3 (60%)	(0.792) * (0.602) ** (0.625) † (0.740) †† (0.348) ‡ (0.525) ‡‡

Intergroup comparison: * Control group *vs*. precancerous disease group; ** Control group *vs.* precancerous lesion group; † Control group *vs.* GC group; †† Precancerous disease group *vs.* precancerous lesion group; ‡ Precancerous disease group *vs.* GC group; ‡‡ Precancerous lesion group *vs*. GC group.

Table 2. Comparison of the Hp and Cag A status.

	Total	Control group	Precancerous disease group	Precancerous lesion group	GC Group	P value
n (%)	402 (100%)	182 (45.27%)	168 (41.79%)	47 (11.70%)	5 (1.24%)	
Hp positive	341/402 (84.82%)	126/182 (69.23%)	163/168 (97.02%)	47/47 (100%)	5/5 (100%)	
Cag A positive	106/341 (31.08%)	25/126 (19.8%)	34/163 (20.85%)	42/47(89.36%)	5/5 (100%)	(0.542) * (<0.001) ** (<0.001) † (<0.001)†† (<0.001) ‡ (0.134) ‡‡

Intergroup comparison: * Control group *vs*. precancerous disease group; ** Control group *vs.* precancerous lesion group; † Control group *vs.* GC group; †† Precancerous disease group *vs.* precancerous lesion group; ‡ Precancerous disease group *vs.* GC group; ‡‡ Precancerous lesion group *vs*. GC group.

Evaluation of the levels of PG I, PG II and G-17 in serum

Levels of PG I, PG II and G-17 in serum were shown in Table 3. Since levels of PG I, PG II and G-17 in serum and PG I/PG II ratios were not in a normal distribution, medians were utilized for statistical analysis. In the GC group, the level of G-17 in serum was significantly higher than that in the precancerous lesion group, precancerous disease group and control group ($P < 0.05$), while the PG I/II ratio was lower than those groups (all $P < 0.05$); differences of PG I levels among 4 groups showed no statistical significance ($P > 0.05$). Besides, we noted that the G-17 level would increase as the disease progressed, with a gradual decrease in the PG I/PG II ratio ($P < 0.001$).

Value of Hp in combination with the PG and G-17 in plasma in primary screening of precancerous status and GC

Levels of PG and G-17 in plasma have some value in primary screening of the precancerous status and GC. In this study, the critical value of the PG I/PG II ratio was 6.4, while that of G-17 was 16.1 pmol/L. Hp in combination with the plasma levels of PG and G-17 was found to be more valuable in the diagnosis of precancerous status and GC (AUC = 0.824, 95% CI: 0.813 – 0.846; Table 4).

Discussion

GC is a kind of disease caused by Hp infection that

Table 3. Evaluation of the levels of PG I, PG II and G-17 in serum.

	Control group	Precancerous disease group	Precancerous lesion group	GC Group	H value	P value
sPG I (μg/L)	221.43 (103.5-235.4)	192.21 (109.6-22.1)	154.5 (109.6-22.1)	145.9 (85.3-257.6)	2.163	>0.05
sPGII (μg/L)	12.7 (11.5-13.4)	13.1 (12.3-15.9)	13. 7 (12.4-16.3)	14.2 (10.3-17.7)	5.745	>0.05
sPGI/sPGII	12.4 (6.3-17.3)	11.5 (8.5-14.3)	10.6 (7.9-15.6)	8.4 (5.2-13.1)	30.436	<0.001
G-17 (pmol/L)	16.2 (0.8-25.1)	17.8 (1.3-29.4)	18.3 (0.9-28.3)	22.6 (13.2-36.7)	28.712	<0.001

Table 4. Value of Hp in combination with the PG and G-17 in plasma in primary screening of precancerous status and GC.

Item	Sensitivity	Specificity	Youden index	AUC	95%CI
Hp	0.652	0.384	0.247	0.574	0.531-0.652
PGI/PG2	0.847	0.463	0.359	0.723	0.647-0.758
G-17	0.523	0.865	0.525	0.738	0.702-0.795
Combined diagnosis	0.794	0.848	0.641	0.859	0.813-0.846

mainly induces oxidative mucosal atrophy. Atrophic gastritis leads to the reduction in the secretion of gastric acid, or inadequate gastric acid, or secondary hypergastrinemia (18). Gastroscopic screening is a kind of invasive operation with a high cost, which has limited its massive clinical application (19). Biomarkers in serum are simple, convenient, economic and non-invasive in measurement, so they may be used for the diagnosis of the population with precancerous lesion or GC at an early stage in epidemiology (20).

Hp causes the chronic inflammation of gastric mucosa which could progress into gastric adenocarcinoma through various precancerous stages, including atrophic gastritis, intestinal metaplasia and atypical hyperplasia (21). Subject carrying Cag A gene is associated with the increment in the risk of the precancerous lesion and GC (22,23). In our work, the frequency of anti-Cag A increased in the GC group and precancerous lesion group, coinciding with the findings of researchers (24) which showed that the existence of Cag PAI correlated with gastritis, digestive ulcer and histopathological finding of GC patients.

As reported, the average PG I level and PG I/PG II ratio of subjects with precancerous lesions were much lower (25). Our findings showed that the reduction of the PG I/PG II ratio was more common in the precancerous lesion group and GC group, which coincided with the report of the hotspot in Iran that evaluated the prognostic value of biomarkers, including PG I/PG II ratio and G-17 (26). In addition, Yanaoka *et al*. (27) conducted a 10-year follow-up for 5,209 asymptomatic subjects, suggesting that the risk of GC increased as the level of PG I or PG I/PG II ratio in serum decreased, and similar results were also obtained in our study.

Previous studies have shown that the increment of GAS in serum is the other marker indicative of the atrophy of the stomach in the advanced stage (28). In a study of over 834 GC patients at an early stage, G-17 was found to be able to predict the GC at an early stage with extensive atrophy (29). In addition, Lin *et al*. (30) reported that the level of G-17 in serum may be a promising biomarker of gastric atrophy or metaplasia. Results of this study suggested that the G-17 level in GC patients was much higher than that in the control group, and as the disease progressed, the G-17 level increased gradually, suggesting that GC patients were more susceptible to hypergastrinemia.

This study also indicated that a single application of PG I/PG II ratio or G-17 may be of some value for precancerous status and GC, while Hp in combination with PG I/PG II ratio and G-17 could be more efficient in the diagnosis of precancerous status and GC, which coincided with the findings of Wang *et al.* (17), suggesting that Hp-positive patients with anomalies in PG I/PG II ratio and G-17 should further take the gastroscopic examination to clarify the potential gastric diseases.

In summary, this study indicates that as the disease progresses, G-17 increases while PG I/PG II ratio decreases. As such, Hp in combination with plasma PG and G-17 may be a highly useful strategy in screening for a healthy population.

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Interest conflict
The authors declare that they have no conflict of interest.

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