

ORIGINAL ARTICLE

A comparative study on changes in intestinal flora, pepsinogen and gastrin in patients with gastric cancer and atrophic gastritis

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Summary

Purpose: To explore the changes in the intestinal flora, pepsinogen (PG) and gastrin in patients with gastric cancer (GC) and atrophic gastritis (AG), hoping to provide references for the diagnostic screening of AG and early diagnosis of GC.

Methods: A total of 630 patients with gastrointestinal diseases in our hospital were selected and divided into healthy group (n=260), GC group (n=125) and AG group (n=245) according to the histopathological examination results. The changes in the serum PGI, PGI/PGII ratio (PGR) and gastrin-17 (G-17) were compared among the three groups, and the helicobacter pylori immunoglobulin G (HP-IgG) antibody in subjects was qualitatively analyzed.

Results: The changes in levels of serum PGI, PGII, PGR and G-17 in AG patients had certain associations with the at-

rophy site in the stomach. In GC group and AG group, the levels of serum PGI, PGII, PGR and G-17 in positive patients were significantly higher than those in negative patients ($p < 0.05$). According to the receiver operating characteristic (ROC) curve analysis, the area under the ROC curve of serum PGI was 0.691 and 0.894, respectively, in the diagnosis of AG and GC.

Conclusion: The decline in the levels of serum G-17, PGR and PGI is the biomarker for the incidence of AG, and they can be used to screen AG and GC to improve the quality of life and the diagnostic rate of GC.

Key words: gastric cancer, atrophic gastritis, intestinal flora, pepsinogen, gastrin

Introduction

The morbidity and mortality rates of digestive tract tumors rank first in the world, among which gastric cancer (GC) has the highest mortality rate, accounting for about 1/4 of the total deaths of malignant tumors, and the relatively low early diagnosis rate is an important factor causing the higher mortality rate [1]. Studies have demonstrated that the major causes of GC are precancerous lesions and precancerous diseases, and the most important of the latter is chronic atrophic gastritis (AG) [2]. Kim et al [2] believed that chronic AG can gradually

develop into GC, and its risk is mainly determined by the atrophy site, atrophy severity and complication of atypical hyperplasia or not, which also has a certain correlation with genetic susceptibility and genotype of patients. Cui et al [3] argued that compared with that in normal people, the risk of development of AG into GC in patients with antral atrophy is increased by about 18-fold, and it will be increased by 90-fold in the case of mucosal atrophy in the gastric body and antrum. Therefore, the early diagnosis of AG and GC appears to be particularly

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important. At present, AG and GC are still clinically diagnosed using the complex invasive gastroscopic biopsy [4]. In recent years, early diagnosis has been gradually made for AG and GC using such serological indexes as gastrin and pepsinogen (PG) in China and beyond, achieving certain results [5]. *Helicobacter pylori* (HP) is a kind of gastric parasite closely related to the occurrence and development of gastric diseases, and serum gastrin and PG will change to some extent in the case of lesions or HP infection of gastric mucosa.

In this paper, therefore, the changes in the intestinal flora, PG and gastrin in AG and GC patients were explored, hoping to provide references for the diagnostic screening of AG and early diagnosis of GC.

Methods

General data

A total of 630 patients diagnosed with gastroduodenal diseases via gastroscopy in our hospital from June 2015 to March 2019 were selected as subjects of study, and they were divided into healthy group (n=260), GC group (n=125) and AG group (n=245) according to the histopathological examination results. In healthy group, there were 190 males and 70 females aged 35-75 years with an average of 54.36±10.29 years, and they had mild superficial gastritis or normal gastric mucosa. In AG group, there were 176 males and 69 females aged 33-72 years with an average of 55.19±10.84 years. In terms of the atrophy site, multifocal atrophy occurred in 62 cases, antral atrophy in 131 cases, and gastric body atrophy in 52 cases. According to different atrophy sites, they were further divided into multifocal atrophy group, antral atrophy group and gastric body atrophy group. In

GC group, there were 79 males and 46 females aged 34-76 years with an average of 54.59±9.98 years. In terms of the lesion site, 15 cases had gastric cardia cancer, 69 cases had gastric body cancer, and 41 cases had gastric antral cancer. As for infiltration depth of tumor into the gastric wall, there were 96 cases of advanced GC and 29 cases of early GC. This study was approved by the Ethics Committee of the no.1 Hospital of Shijiazhuang. Signed written informed consents were obtained from all participants before the study.

Diagnostic and grouping criteria

All subjects were diagnosed and grouped based on the results of histopathological examination and gastroscopy: 1) GC diagnosis: The cancer tissues are confined to the submucosa or the mucosa, regardless of the presence or absence of lymph node metastasis and size, including high-grade intraepithelial neoplasia and severe dysplasia. The infiltration of cancer tissues into the submucosa indicates the advanced GC. 2) AG diagnosis [6]: It is based on the diagnostic criteria for gastric mucosal atrophy in the National Consensus Conference on Chronic Gastritis and Sydney System. 3) Healthy group: The patients had mild superficial gastritis or normal gastric mucosa.

Inclusion and exclusion criteria

Inclusion criteria: 1) subjects meeting the above corresponding diagnostic criteria, and confirmed by pathological examination and gastroscopy; 2) those receiving no special medication, such as H2 receptor antagonist and proton pump inhibitor, at 1 week before study; and 3) those who and whose families agreed and signed the informed consent. Exclusion criteria: 1) subjects complicated with severe hepatic or renal dysfunction; 2) those with a history of gastroduodenal surgery; 3) those with a history of radical cure of HP; 4) those using antibiotics recently; or 5) those who had poor compliance or quit halfway.

Table 1. Changes in levels of serum PGI, PGII, PGR and G-17 in each group

Group	PGI ($\mu\text{g/L}$)	PGII ($\mu\text{g/L}$)	PGR	G-17 (pg/mL)
Healthy group (n=260)	120.68±31.06	11.59±6.33	10.89±5.36	12.32±4.71
AG group (n=245)	86.58±15.31*	9.85±3.65*	8.59±2.19*	8.62±2.02*
GC group (n=125)	55.81±16.39**	18.69±3.75**	3.15±0.87**	18.63±3.54**
F	11.309	5.876	8.362	6.092
p	<0.001	<0.001	<0.001	<0.001

*p<0.05 vs. healthy group; **p<0.05 vs. AG group

Table 2. Changes in serum levels of PGI, PGII, PGR and G-17 in patients with AG at different atrophy sites

Group	PGI ($\mu\text{g/L}$)	PGII ($\mu\text{g/L}$)	PGR	G-17 (pg/mL)
Gastric body atrophy group (n=52)	42.51±10.25 ^a	7.80±1.42 ^a	5.12±0.57 ^a	15.19±4.67 ^a
Antral atrophy group (n=131)	87.36±11.39	13.69±3.57	6.80±0.69	5.62±1.97
Multifocal atrophy group (n=62)	36.74±6.80 ^a	7.89±1.36 ^a	4.55±0.61 ^a	6.69±3.36 ^a
F	16.874	6.325	4.521	9.681
p	<0.001	<0.001	<0.001	<0.001

^ap<0.05 vs. antral atrophy group

Methods

Before gastroscopy, 5 mL of fasting venous blood was drawn from all subjects in the morning and centrifuged at 3000 rpm for 15 min, and the serum separated was stored in a refrigerator at -75°C for later use. 1) Determination of serum PG and gastrin levels: The levels of serum PGI, PGII, PGI/PGII ratio (PGR) and gastrin-17 (G-17) were determined via enzyme-linked immunosorbent assay using the kits (Avioq) strictly according to the instructions. 2) Qualitative detection of HP-immunoglobulin G (IgG) antibody: The serum HP-IgG antibody was qualitatively detected through the colloidal gold method using the kits (Shanghai Kemin Biotechnology Co., Ltd., Shanghai, China) in strict accordance with the instructions.

Statistics

The above data were sorted and analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Measurement data were expressed as mean \pm standard deviation (SD). The Least Significant Difference (LSD) test was performed for the intergroup multiple comparisons, and analysis of variance for the comparison of means among groups. $P < 0.05$ suggested statistically significant difference of data. The optimal critical value of serum PGI, PGR and

G-17 in the diagnosis of GC and AG was analyzed using the receiver operating characteristic (ROC) curve.

Results

Changes in levels of serum PGI, PGII, PGR and G-17 in each group

The levels of serum PGI and PGR were significantly lower in the GC group and the AG group than those in healthy group, while they were significantly higher in AG group than those in the GC group ($p < 0.05$). The level of serum G-17 was the lowest in the AG group and the highest in the GC group, and there was a statistically significant difference ($p < 0.05$) (Table 1).

Changes in levels of serum PGI, PGII, PGR and G-17 in patients with AG at different atrophy sites

The level of serum G-17 was the highest in the gastric body atrophy group, and the levels of serum PGI and PGR were the highest in the antral atrophy

Table 3. HP-IgG antibody detected

Item	Healthy group (n=260) n (%)	AG group (n=245) n (%)	GC group (n=125) n (%)
+	148 (56.92)	210 (85.71)	107 (85.60)
-	112 (43.08)	35 (14.29)	18 (14.40)
Positive rate (%)	56.92	85.71	85.60

Table 4. Changes in levels of PGI, PGII, PGR and G-17 in patients with HP-related AG

Item	PGI ($\mu\text{g/L}$)	PGII ($\mu\text{g/L}$)	PGR	G-17 (pg/mL)
+	93.54 \pm 8.26	15.39 \pm 3.26	14.87 \pm 3.20	6.69 \pm 2.19
-	81.39 \pm 7.25	12.21 \pm 3.52	10.14 \pm 3.08	5.84 \pm 1.87
t	8.189	5.282	8.138	2.167
p	<0.001	<0.001	<0.001	0.016

Table 5. Optimal critical value of serum PGI, PGR and G-17 in the diagnosis of AG

Item	Sensitivity %	Specificity %	ROC	95%CI	Optimal critical value
PGI	73.15	50.00	0.691	0.652-0.876	91.45
PGR	72.70	53.31	0.650	0.612-0.856	9.00
G-17	65.98	63.89	0.613	0.556-0.813	6.21

Table 6. Optimal critical value of serum PGI, PGR and G-17 in the diagnosis of GC

Item	Sensitivity	Specificity	ROC	95%CI	Optimal critical value
PGI	85.67	75.11	0.894	0.712-0.923	74.03
PGR	89.11	62.45	0.766	0.687-0.845	4.01

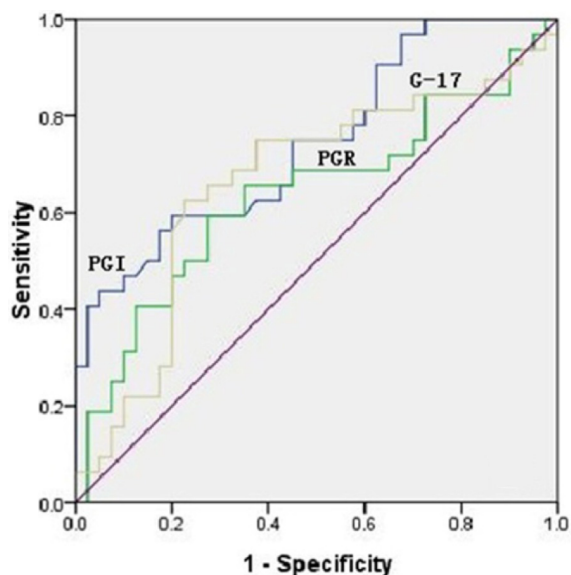


Figure 1. ROC curves of serum PGI, PGR and G-17 in the diagnosis of AG. The area under the ROC curve of serum PGI in the diagnosis of AG was 0.691 and its optimal critical value was 91.45 µg/L.

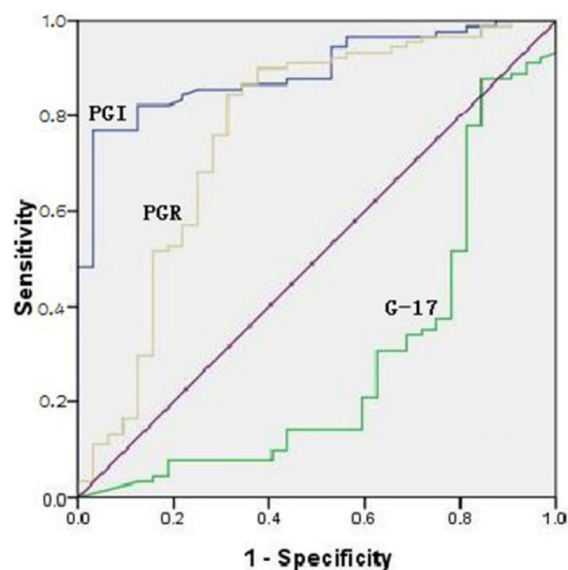


Figure 2. ROC curves of serum PGI, PGR and G-17 in the diagnosis of GC. The area under the ROC curve of serum PGI in the diagnosis of GC was 0.894 and its optimal critical value was 74.03 µg/L.

group, showing statistically significant differences ($p < 0.05$) (Table 2).

HP-IgG antibody detected

The positive rate of HP-IgG antibody was 56.92%, 85.71% and 85.60%, respectively, in the healthy group, AG group and GC group (Table 3).

Changes in levels of PGI, PGII, PGR and G-17 in patients with HP-related AG

The levels of serum PGI, PGII, PGR and G-17 in the positive AG patients were obviously higher than those in the negative patients, and there were statistically significant differences ($p < 0.05$) (Table 4).

Optimal critical value of serum PGI, PGR and G-17 in the diagnosis of AG

The area under the ROC curve of serum PGI in the diagnosis of AG was 0.691, and its optimal critical value was 91.45 µg/L (Table 5 & Figure 1).

Optimal critical value of serum PGI, PGR and G-17 in the diagnosis of GC

The area under the ROC curve of serum PGI in the diagnosis of GC was 0.894, and its optimal critical value was 74.03 µg/L (Table 6 & Figure 2).

Discussion

PG is the pepsin precursor without biological activity, which can be detected in the human urine, serum and gastric juice [7]. Under the action

of hydrochloric acid in the stomach, PG is transformed into pepsin with a certain biological activity, and PG *in vivo* can be synthesized and secreted upon the food stimulus [8]. PG is mainly stored at the top of chief cells, and about 1% of PG will be secreted into the blood circulation after the cells are stimulated, most of which enter the glandular cavity. Therefore, the level of serum PG can well reflect the functional structure of the gastric mucosa. In addition, PG can be divided into PGI and PGII according to its different immunocompetence. PGI secreted by mucous cells and chief cells of gastric fundus glands can be used as an indicative marker for the function of gastric oxyntic gland cells. When the gastric mucosa is damaged to some extent, the level of serum PGI shows a decreasing trend [9]. PGII is synthesized and secreted by the duodenal Brunner's glands, pyloric glands in gastric antrum and cardiac glands in gastric cardia. In this study, the levels of serum PGII, PGI and PGR in the AG group and GC group had changes. The main reason is that the release of inflammatory factors will promote the increase of serum PGII and PGI levels when the inflammatory response in a certain degree occurs in the gastric mucosa, and with the gradual development of disease into gastric mucosal atrophy, chief cells will be replaced with pseudopyloric gland cell, with increased synthesis of PGII and decreased synthesis of PGI, thereby lowering PGR [10]. The above findings suggest that the levels of serum PGII and PGI can reflect the number of gastric mucosal cells and glands, and also indirectly manifest the secretory function of

different sites of the gastric mucosa. Recent studies have shown that gastrin plays an important role in the occurrence and development of GC, and it has a certain impact on the deterioration and growth of cancer cells [11]. About 95% of gastrin in the human body has certain biological activity and exists in the form of α -amidated gastrin, among which G-17 synthesized and secreted by G cells in the gastric antrum holds the highest proportion [12]. When AG occurs, gastric antral glands will be lost, thereby reducing the number of G cells in the gastric antrum, and lowering the level of serum G-17, which can be used as a marker for judging the function of G cells [13]. In this paper, the detection results of G-17 demonstrate that when gastric mucosal atrophy occurs, the secretion of gastrin will be affected, the intrinsic glands will disappear or decline, and both G-17 secreted by gastric antrum and PGI secreted by gastric body are significantly decreased.

Studies have revealed that HP infection is closely related to the occurrence of various gastric diseases [14,15]. Currently, it is clinically recognized that HP causes GC mainly through the following two mechanisms. First, the persistence of pathogens will lead to inflammatory cell infiltration and excessive immune response, thereby producing pro-inflammatory factors to cause chronic inflammation. Second, the interaction between the host and the long-standing virulence factors will induce epithelial neoplasia [16]. In addition, HP can also cause gastric epithelial damage through production of cytotoxins, phospholipases and ammonia, thus resulting in persistent epithelial regeneration and destruction, and raising the risk of cancerization of gastric stem cells [17]. According to the changes in the related indexes of HP-related AG patients, HP infection has a certain correlation with the serum PG level, and the thorough eradi-

cation of HP can improve inflammatory cell infiltration and restore gastric secretory function to a certain extent, and affect the serum PG level [18]. In this paper, the optimal critical value of serum PGI and PGR in the diagnosis of AG was 91.45 $\mu\text{g/L}$ and 9.00 $\mu\text{g/L}$, respectively. The regular examination for patients with the above values can improve the diagnostic effect of GC and survival rate to a certain extent. Moreover, the optimal critical value of serum PGI in the diagnosis of GC was 74.03 $\mu\text{g/L}$ in this study, consistent with that in the study of Lv et al [19]. At present, there has been no clinical study proving that the detection of serum PG can directly reduce the mortality rate of GC. However, the aim of detection of serum G-17 and PG levels is not to directly diagnose GC and AG, but to screen the high-risk group, and increase the living quality of patients and the early diagnostic rate of disease. Besides, the serological detection is simple, economical and non-invasive, which can be used for large-scale physical examination for healthy people and screening of high-risk group [20].

Conclusions

In conclusion, the decline in levels of serum G-17, PGR and PGI is the biomarker for the occurrence of AG, and AG and GC can be screened according to the optimal critical value. In addition, there is an association between HP infection and serum PG level in patients, which can provide references for large-scale screening of AG and GC and the physical examination for healthy people, and also improves the quality of life and the diagnostic rate of GC.

Conflict of interests

The authors declare no conflict of interests.

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