

Study on the Clinical Value of Serum Pepsinogen II in Gastritis Detection

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Gao *et al.*: Serum Pepsinogen II in Gastritis Detection

The present study aims to explore the clinical value of pepsinogen II and gastritis detection. Firstly, serum expressions of pepsinogen II in patients with *Helicobacter pylori*-caused gastritis, gastric ulcer and atrophic gastritis were detected using enzyme-linked immunosorbent assay, followed by statistical analysis. In comparison with the normal group, serum pepsinogen II levels were increased in the above groups ($p < 0.01$). Next, the positive cut-off value for pepsinogen II in detecting gastritis was obtained by the receiver operating characteristic curve. When the value of pepsinogen II was 10.55 $\mu\text{g/l}$, the specificity and sensitivity was 83.6 % and 74.4 % in the diagnosis of *Helicobacter pylori*-causing gastritis, 81.6 % and 80.0 % in gastric ulcer and 81.6 % and 53.1 % in atrophic gastritis. Thus, pepsinogen II at 10.55 $\mu\text{g/l}$ was recommended as the positive cut-off value in gastritis detection. Besides, we observed that the ribonucleic acid and protein expressions of pepsinogen II in different gastritis groups were up-regulated by Western blotting and real-time polymerase chain reaction. Moreover, the immunohistochemistry indicated that pepsinogen II was highly expressed in gastritis tissues compared with normal controls. In conclusion, pepsinogen II could be an independent indicator in gastritis detection.

Key words: Pepsinogen II, gastritis, *Helicobacter pylori*, serological screening, enzyme-linked immunosorbent assay

Gastritis is histologically defined as the inflammation of the gastric mucosa and it is a frequent, severe and occult disease in human life. In clinic, it generally can be divided into chronic and acute ones. Based on etiology, it contains *Helicobacter pylori* (*H. pylori*) related, atrophic and autoimmune gastritis^[1] and so on^[2]. There is a wealth of information that gastric atrophy is a substantial risk for the incidence of Gastric Cancer (GC)^[3]. Over 50 % people in the world are affected by this disease and millions of people pass away each year as a result of GC or ulcers caused by gastritis^[4,5]. These indicate that gastritis is ruining the health of hundreds of millions of people globally.

Currently, serological screening of stomach, especially for Pepsinogen (PG), gastrin-17 and *H. pylori* antibodies, is a non-invasive, simple and low-cost technique that has been widely used in screening for GC and precancerous lesions. In some countries

like Japan and China, serological screening has been listed in the national plan for GC prevention and control^[6,7]. PG is an inactive precursor of pepsin. Based on biochemistry and immune activity, human PG could be further classified into two subgroups, namely PG I and PG II. PG I is chiefly produced by main cells and mucus neck cells in gastric glands, whereas PG II by gastric glands, pyloric glands and Brunner's glands in proximal duodenum^[8,9]. Previously, only the ratio between PG II and PG I is applied in clinical tests and few studies have reported the clinical significance of PG II in gastric diseases^[10-14].

Herein, this study aims to explore the clinical value of PG II in different gastritis. We analyzed the differential expressions of PG II in patients with *H. pylori* caused gastritis, gastric ulcer, atrophic gastritis and set normal people as controls and the relative expressions of PG II were also detected in gastritis

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samples by functional experiments. This study will update the knowledge of gastritis pathogenesis and offer a scientific foundation for the exploration of new diagnostic biomarkers in gastritis.

MATERIALS AND METHODS

General information:

All the patients involved in this study received gastroscopy in Anhui province and Zhejiang province between 2014 and 2018, diagnosed by gastroscopy and pathological examination. No patient had a history of special medication in 2 w before the examination and patients with acute upper gastrointestinal hemorrhage could not participate in this study. These patients were classified into 3 groups; *H. pylori* caused gastritis (n=853), gastric ulcer (n=121) and atrophic gastritis (n=188), compared with healthy control (n=929). For serum PG II detection, 3 ml of fasting blood was collected and the serum was immediately isolated and preserved at -20°. The Enzyme-Linked Immunosorbent Assay (ELISA) kit from Biohit (Finland) was used for the measurement (Table 1). Then, all the data were analyzed by Receiver Operator Characteristic (ROC) curve analysis to calculate the sensitivity and specificity of the diagnosis indicators.

TABLE 1: SERUM EXPRESSIONS OF PG II IN DIFFERENT GROUPS BY ELISA

Group	No.	PG II ($\mu\text{g/l}$)
Healthy control	929	7.48 \pm 4.89
<i>H. pylori</i> caused gastritis	853	16.24 \pm 8.60*
Gastric ulcer	121	17.79 \pm 9.28*
Atrophic gastritis	188	13.20 \pm 9.72*

Note: *p<0.01, when compared with the healthy control

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR):

qRT-PCR was conducted by SYBR Premix Extaq (Takara, United States of America (USA)). Endogenous Beta (β)-actin expression was the standardized control. The primers were listed below; PG II, 5'-TCACCAGCATTCCTCAAGGT-3' and 5'-CCAAGTGAAGCTCCCTCAGA-3'; β -actin, 5'-CCCTGGAGAAGAGCTACGAG-3' and 5'-GGAAGGAAGGCTGGAAGAGT-3'. The $2^{-\Delta\Delta Ct}$ method was adopted to calculate pertinent expression. qRT-PCR was conducted in *H. pylori* caused gastritis, gastric ulcer, atrophic gastritis and healthy control groups and each had 30 samples.

Western Blot (WB) analysis:

In Radioimmunoprecipitation Assay (RIPA) buffer, the entire protein was lysed and extracted. The proteins were separated using a 10 % Sodium Dodecyl Sulphate (SDS) polyacrylamide gel. The membranes were maintained overnight at 4° with antibodies to PG II (Affinity, DF6543, 1:1000) or β -actin (Zs-BIO, TA-09, 1:1000) after adding 5 % fat-free milk for 1 h. Phosphate Buffered Saline With Tween 20 (PBST) was applied to rinse blots and then they were cultured with the secondary antibody for 1 h. Each group with 5 samples, respectively.

Immunohistochemistry (IHC) analysis:

IHC was performed on paraffin-embedded tissue slices. After dewaxing with xylene, dehydration with gradient ethanol and antigen retrieval with sodium citrate buffer solution in a microwave, the peroxidase was blocked with a 3 % Hydrogen peroxide (H_2O_2) blocker. Sections were sealed in 5 % Bovine Serum Albumin (BSA) with the primary antibody (PG II, BIOHIT Healthcare, China, diluted at 1:500) and put in a humid incubator at 4° overnight. The next day, Phosphate-Buffered Saline (PBS) was adopted to wash the slices for 3 times and the prepared universal secondary antibody was cultured. Then, color development was achieved *via* Diaminobenzidine (DAB). The images were collected under a microscope.

Statistical analysis:

Statistical Package for Social Sciences (SPSS) 17.0 software was applied to analyze the statistics. The data were presented as mean \pm Standard Deviation (SD). Intergroup comparison was made by Analysis of Variance (ANOVA). p<0.05 indicated significance in statistics.

RESULTS AND DISCUSSION

According to the study, the level of serum PG II in *H. pylori* caused gastritis group (16.24 \pm 8.60 $\mu\text{g/l}$) was substantially higher compared with healthy control (7.48 \pm 4.89 $\mu\text{g/l}$). The other two groups, gastric ulcer (17.79 \pm 9.28 $\mu\text{g/l}$) and atrophic gastritis (13.20 \pm 9.72 $\mu\text{g/l}$), were also significantly higher (p<0.01, Table 1).

The ideal cut-off value was chosen as the point closest to the upper-left corner of the ROC curve, so the ROC curve's Area Under Curve (AUC) was determined. The above measurement data were then

analyzed by ROC curve. When the value of PG II was 10.55 $\mu\text{g/l}$, the specificity and sensitivity were 83.6 % and 74.4 % in *H. pylori* caused gastritis group (AUC=0.848, fig. 1A), 81.6 % and 80.0 % in gastric ulcer group (AUC=0.872, fig. 1B) and 81.6 % and 53.1 % in atrophic gastritis group (AUC=0.691, fig. 1C). Thus, 10.55 $\mu\text{g/l}$ PG II was consistent in detecting gastritis due to three different causes, it was recommended as the positive cut-off value in gastritis detection.

We carried out qRT-PCR assays to measure its expressions in gastritis samples (3 different groups) and normal tissues. As fig. 2 indicated, PG II,

Ribonucleic Acid (RNA) expression was significantly up-regulated in the gastritis samples than that in normal tissues, especially in *H. pylori* caused gastritis group. In order to further explain the relationship between gastritis and PG II, WB was for measuring the protein expressions of PG II in 3 different groups of gastritis samples and normal tissues. The results showed that PG II protein expression levels were also significantly up-regulated in gastritis samples (fig. 3A and fig. 3B). Besides, the expression level of PG II was detected in different tissues by IHC. PG II was found to be highly expressed in tissue samples of 3 groups as shown in fig. 4A-fig. 4D.

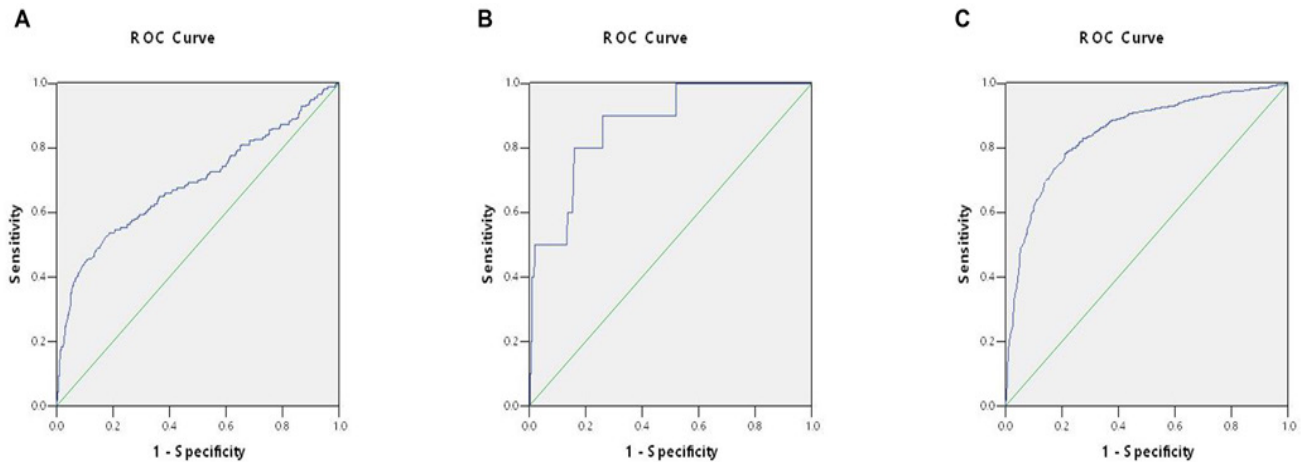


Fig. 1: The ROC curve analysis on PG II expressions in 3 different gastritis groups (A): *H. pylori* caused gastritis; (B): Gastric ulcer and (C): Atrophic gastritis

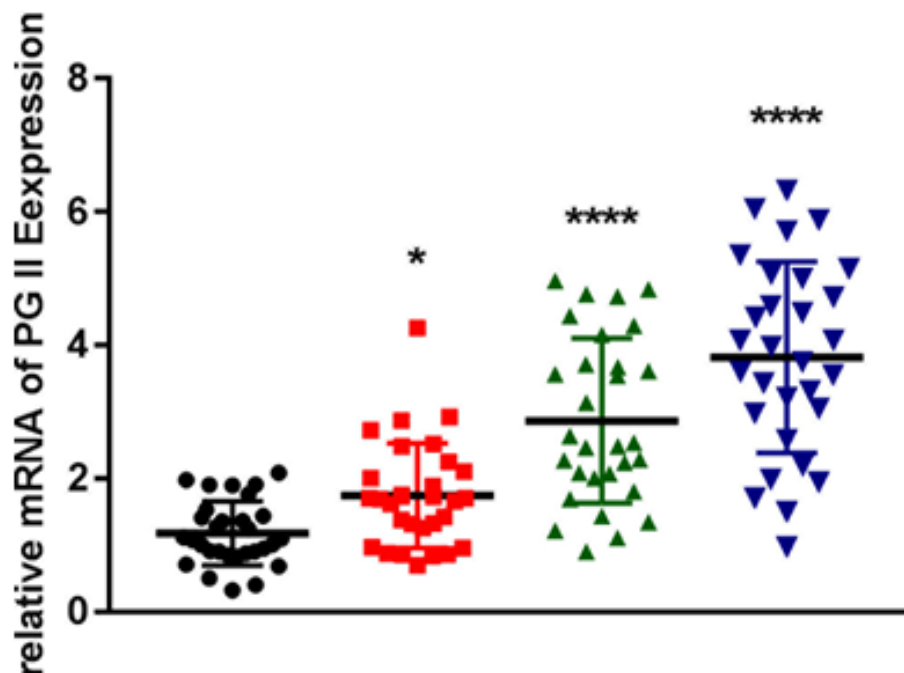


Fig. 2: The RNA expressions of PG II detected by RT-qPCR

Note: Each group had 30 samples, * $p < 0.05$ and **** $p < 0.0001$, (●): Normal control; (■): Gastric ulcer; (▲): Atrophic gastritis and (▼): *H. pylori* caused gastritis

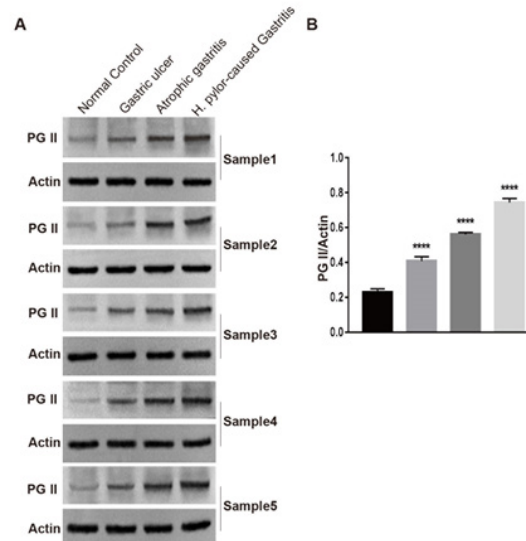


Fig. 3: (A): The images and (B): Relative protein expressions of PG II in 3 different gastritis groups detected by WB

Note: Each group had 5 samples, **** $p < 0.0001$, (■): Normal control; (■): Gastric ulcer; (■): Atrophic gastritis and (■): *H. pylori*-caused gastritis

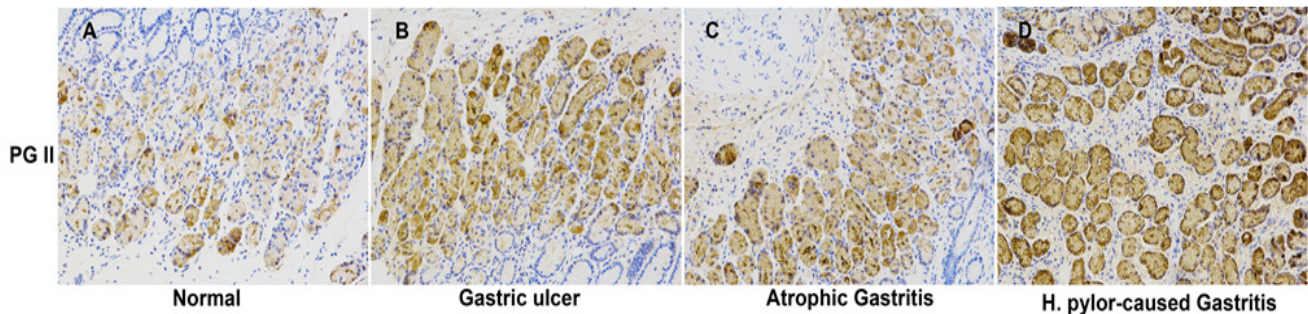


Fig. 4: IHC staining of PG II in (A): Normal control; (B): Gastric ulcer; (C): Atrophic gastritis and (D): *H. pylori* caused gastritis groups

Note: Magnification 200×

PG is an inactive precursor of pepsin in gastric juice, as a polypeptide chain consisting of 375 amino acids. PG is synthesized by ribosome, secreted by the Golgi body and activated by hydrochloric acid to form pepsin^[15]. Stomach is likely to be the only source of PG and the secretion volume varies at different stages. After synthesis, most PG would enter the gastric cavity and convert into pepsin after being activated by acidic gastric fluid. Moreover, a small amount of PG (about 1 %) would penetrate the mucosa and enter serum. When pathological changes occur in the gastric mucosa, the level of serum PG also changes. Thus, serum PG can reflect the secretion level of PG.

Based on biochemistry, immunogenicity, cellular origin and tissue distribution, there are two PG I (groups 1-5) and PG II (groups 6-7) subgroups^[16,17]. As mentioned above, the serum levels of PG I and PG II could reflect the functions of gastric mucosa at different locations. Serum PG I and PG I/PG II ratio are significantly associated with atrophic

gastritis and have been used for GC screening among high-risk populations in many countries^[18-22]. For example, Biasco *et al.*^[23] reported the decrease in the PG I:PG II ratio might be utilized to predict *H. pylori* infection and the Immunoglobulin G (IgG) antibody response to *H. pylori* in the blood. Su *et al.*^[24] found that a low PG I/II ratio was not just a sign of atrophic gastritis, but of nutritional and metabolic health. However, the clinical application of PG II as an independent indicator has been rarely reported.

In this study, we analyzed the PG II levels in gastritis patients and the results suggested that patients with *H. pylori* caused gastritis ($16.24 \pm 8.60 \mu\text{g/l}$), gastric ulcer ($17.79 \pm 9.28 \mu\text{g/l}$) and atrophic gastritis ($13.20 \pm 9.72 \mu\text{g/l}$) all had significantly higher serum PG II as compared to the control ($7.48 \pm 4.89 \mu\text{g/l}$). By plotting the ROC curve, we defined the cut-off value for PG II in gastritis diagnosis by calculating AUC. According to our results, when the value of PG II was $10.55 \mu\text{g/l}$; the specificity and sensitivity was 83.6 %

and 74.4 % with an AUC of 0.848 in diagnosing *H. pylori* caused gastritis, 81.6 % and 80.0 % with an AUC of 0.872, in diagnosing gastric ulcer and 81.6 % and 53.1 % with an AUC of 0.691 in diagnosing atrophic gastritis. For 3 different groups, the positive cut-off value of PG II was consistent in gastritis detection. Thus, 10.55 µg/l PG II is recommended as the positive cut-off value in gastritis diagnosis.

Besides, we found PG II had a high RNA and protein expressions in tissue samples of *H. pylori* caused gastritis, gastric ulcer and atrophic gastritis, especially *H. pylori* caused gastritis. *H. pylori* are a kind of spiral-shaped, micro-anaerobic bacteria only known microbial species surviving in stomach. The bacterial infection will cause chronic gastritis, which can lead to ulcers and atrophic stomach atrophy and even GC. Currently, it is believed that early detection and timely treatment of *H. pylori* infection is of importance for the prevention and controlling of GC. This study demonstrates that PG II expression is closely related to the pathogenesis of *H. pylori* caused gastritis, gastric ulcer and atrophic gastritis. The findings confirm that PG II could be a critical value standard for gastritis diagnosis and offer a scientific foundation for the application of PG II in clinical test as an independent biomarker.

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Ethics approval:

This study was approved by the Department of Gastroenterology, Sanmen County People's Hospital and Ethics Committee of Third Affiliated Hospital of Anhui Medical University (Hefei, China). Signed informed consents were obtained from all participants before the study.

Author's contributions:

Dongmei Gao and Jiaoe Chen have contributed equally to this work and Jun Zhao was considered as the co-corresponding author.

Conflict of interests:

The authors declared no conflict of interests.

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