

## Accuracy of pepsinogens for early diagnosis of atrophic gastritis and gastric cancer in Iranian population

Abbas Zoalfaghari<sup>1</sup>, Najmeh Aletaha<sup>2</sup>, Nader Roushan<sup>3</sup>, Reza Taslimi<sup>4</sup>  
Hossein Foroutan<sup>5</sup>, Bita Faridnia<sup>6</sup>

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### Abstract

**Background:** Currently, non-invasive methods for screening atrophic gastritis and gastric cancer are lacking. The purpose of this study was to evaluate the value of serological parameters including serum pepsinogen I (PGI), pepsinogen II (PGII) and pepsinogen I: II ratio for the screening atrophic gastritis and gastric cancer.

**Methods:** The study population consisted of 132 dyspeptic patients who had undergone upper endoscopy with biopsy. Blood samples for ELISA assays of serum PGI, PGII and IgG antibodies against *Helicobacter pylori* were drawn. Comparison between the two groups was done by Student's t- test, and Mann Whitney test. Cut-off points were calculated using receiver operating curves (ROC).

**Results:** Mean ( $\pm$ SD) age of the study population was 51.4 ( $\pm$ 15.5) years. Values of PGI and PG ratio decreased significantly in the atrophic gastritis as compared with the control group ( $p < 0.05$ ). Values of PG and PG ratio didn't show any significant difference between the gastric cancer and control group ( $p > 0.05$ ). For patients with atrophic gastritis, the area under the ROC for PGI was 0.639 (95% CI: 0.538-0.741,  $p = 0.008$ ) in which the best cut-off value was 40  $\mu$ g/L (sensitivity 90%, specificity 67%, accuracy 69%, negative predictive value 92%, YI : 0.429). The area under the ROC for PG ratio was 0.711 (95% CI: 0.617-0.806,  $p = 0.0001$ ) and the best cut-off value was 8 (sensitivity 71%, specificity 71%, accuracy 71%, negative predictive value 86%, YI : 0.431).

**Conclusion:** It seems that PGI, PGI: PGII ratio is potential biomarkers for screening atrophic gastritis with high sensitivity, specificity, accuracy and negative predictive value. Serology could be used as a screening method for the detection of precancerous states due to its convenience, relative low cost and safety.

**Keywords:** Pepsinogens, Atrophic gastritis, Gastric cancer, Biomarkers.

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### Introduction

Incidence and mortality of gastric cancers (GC) have rapidly decreased in the past 50 years in many countries; however it remains a major cause of morbidity and mortality (1).

Mortality of GC is high in most parts of Asia, while it was reduced in Japan and Korea (2, 3). Atrophic gastritis (AG) is an important and prevalent condition that has

no symptom, leading to GC without any alarming sign (4,5).

Atrophic gastritis often desires to progress to premalignant lesions, and even to gastric cancer (5). Endoscopy with gastric biopsies was suggested as the best and most effective diagnostic method for screening of upper GI malignancies (3). Endoscopic screening is an invasive procedure, needing experienced endoscopist. In

1. (Corresponding author) Fellowship of Gastroenterology, Imam Khomeini hospital, Department of Internal Medicine, Division of Gastroenterology, Tehran University of Medical Sciences, Imam Khomeini Hospital, Tehran, Iran. abaszoalfaghari1354@yahoo.com

2. Assistant Professor, Department of Internal Medicine, Division of gastroenterology, Imam Khomeini hospital, Tehran University of Medical Sciences, Tehran, Iran. aletaha@ddrc.ac.ir

3. Assistant Professor, Department of Internal Medicine, Division of gastroenterology, Imam Khomeini hospital, Tehran University of Medical Sciences, Tehran, Iran. nroshan@tums.ac.ir.

4. Assistant Professor, Department of Internal Medicine, Division of gastroenterology, Tehran University of Medical Sciences, Tehran, Iran. rtaslimi@tums.ac.ir.

5. Professor of Gastroenterology, Department of Internal Medicine, Tehran University of Medical Sciences, Tehran, Iran. froutan@ams.ac.ir.

6. Specialist of Internal Medicine, Tehran, Iran. B.faridnia@yahoo.com.

addition, it is relatively costly, uncomfortable for patients, and is not appropriate in low risk areas; therefore non-invasive screening modalities are required in this population. The inflamed gastric mucosa shifts specific factors to the blood, leading to the presence of diagnostic serologic biomarkers. In previous investigations, a variety of serologic markers have been explained, including, pepsinogen I (PGI), pepsinogen II (PGII) and IgG antibodies against *Helicobacter pylori* (4, 6). Due to the variance in ethnic, dietary, environmental and disease factors, the application of serum pepsinogen (PG) screening requires local validation in different geographic regions (7). As the prognosis is a major concern to most patients with gastric lesions, the importance of a suitable and cost-benefit screening program for early detection of the disease which could result in reduction of mortality in gastric cancer is highlighted (8) and a proper screening test which reduces the endoscopy loading, affirms a simple, safe and effective method in the first stage for diagnosis of AG is required (6). Currently, few data is available on serologic PG levels in Iranian patients with AG and gastritis cancer. Herein, the authors used serum PG level, as the first choice screening test for AG and gastric cancer and confirmed the diagnosis by endoscopy and biopsy. In this study, we have investigated the characteristics of PG screening and the convenient cut off points for the identification of AG and GC using receiver operator characteristics (ROC) curves. In addition, we compared PG screening with screening by endoscopy.

## Methods

### *Participants*

A total of 132 consecutive patients with upper gastrointestinal symptoms entered the study. The patients were referred to the endoscopy ward of Imam Khomeini hospital, Tehran, Iran during 2011-2013. They were excluded if they had *H. pylori* treatment before the test, any type of gastrointestinal malignancies other than gastric

cancer, or a drug history of anti-secretory medication. Patients with non-atrophic mild gastritis and without gastritis comprised the control group. All patients were informed about the study and signed a written informed consent. The project was approved by Ethical Committee of Tehran University of Medical Sciences.

### *Endoscopy and histology*

All patients underwent upper gastrointestinal endoscopy. At least four biopsy specimens were taken from the fundus of the stomach (two specimens from each site) and were sent to the Department of Pathology for histological examination by experienced pathologists. All specimens were reviewed as recommended in the last edition of Sydney System classification of gastritis (9).

Regards to Rugge M et al. study, we considered for Reliability, agreement between two observers with really having good Kappa =0.78 (10).

### *Blood samples*

Blood samples for the measurement of PGI, PGII and IgG antibodies for *H. pylori* were drawn after endoscopy, immediately put into an ice bottle for 30 minutes, and then centrifuged at 200 g for 10 min to separate the serum for the assays. The serum samples were stored at  $-70^{\circ}\text{C}$  until analysis.

### *Serological testing*

PGI, PGII and antibodies to *H. pylori* were determined using specific commercial ELISA kits11 (Pepsinogen I, Pepsinogen II, *H. pylori* ELISA Kit, Shanghai Huitai Medical Technology Company, China). To decrease the bias, all calibrators and samples were assayed as duplicates. For determination of PGI and PGII, standard curves were used to extrapolate the concentrations of unknown samples. According to the given cut-off value of the kit, IgG  $\geq 0.1$  enzyme immunoassay units (EIU) were considered *H. pylori* positive.

### Statistical Analysis

Data were analyzed using the SPSS 11.0 software. Serum concentrations of PGI, PGII and the ratio of PGI: PGII were compared between subjects with AG, non-AG, GC, non-GC and H. pylori positive, H. pylori negative were. We evaluated the difference between two groups with Student's t- test, and Mann Whitney test. Cut-off points of PGI and the PGI: PGII ratios were calculated using receiver operating curves (ROC).

### Results

The mean age ( $\pm$ SD) of the study patients was 51.4 ( $\pm$ 15.5) years, ranging from 12 to 84 years. Of the overall 132 patients, 83 were men (62.9%) and 49 women (37.1%). According to the histologic examination of the fundic mucosa, 51 (39.2%) patients had atrophic gastritis, and 22 (16.7%) had gastric cancer. Values of PGI and PG ratio decreased significantly in the atrophic gastri-

tis group as compared with the control group ( $86.4\pm 77.7$  vs.  $113.7\pm 75.7\mu\text{g/L}$ ,  $16.4\pm 42.1$  vs.  $17.4\pm 10.5$ ,  $p=0.008$ ,  $0.01$ , respectively). Values of PG and PG ratio didn't show any significant difference between the two groups in the gastric cancer and control ( $p> 0.05$ ). Values of PG and PG ratio decreased significantly in the atrophic gastritis group without H. pylori infection as compared with control group (all  $p<0.05$ ); in addition, PGI decreased significantly in those with atrophic gastritis with H. pylori infection as compared with control group ( $97.3\pm 85.2$  vs.  $126.5\pm 79.6\mu\text{g/L}$ ;  $p=0.025$ ,  $0.012$ , respectively). While values of PGI and PG ratio changed significantly in the atrophic gastritis with H. pylori infection as compared with the control group, however, values of PG and PG ratio didn't show any significant difference between two groups in the gastric cancer (Table 1).

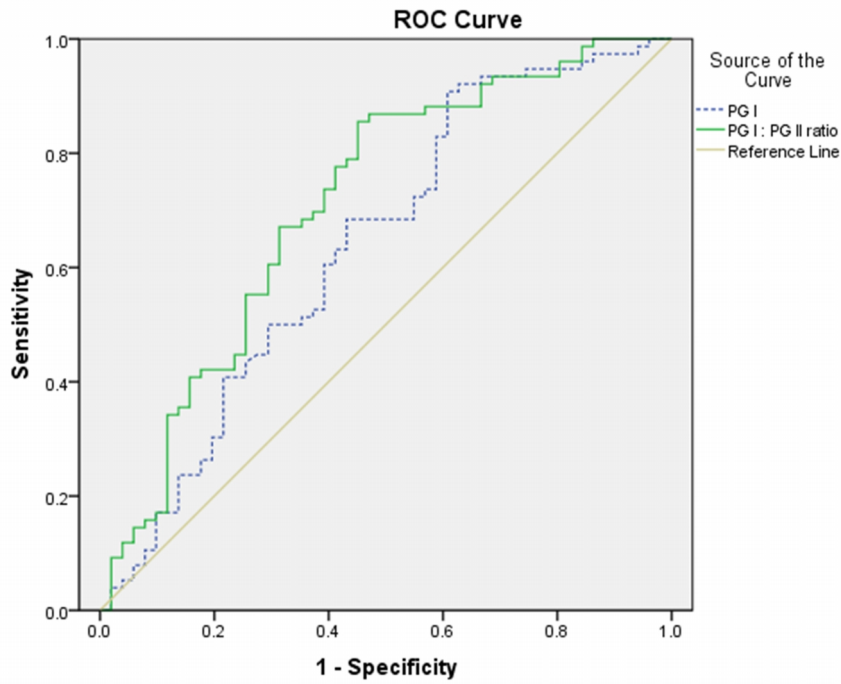
For patients with atrophic gastritis, the area under the ROC for PGI was 0.639

Table1. Comparison of the serum markers between different status of patients and controls.

Serum Marker	PGI ( $\mu\text{g/L}$ )	PGII ( $\mu\text{g/L}$ )	PGI:PGII Ratio
AG (Mean $\pm$ SD)	86.4 $\pm$ 77.7	12.3 $\pm$ 16.7	16.4 $\pm$ 42.1
Non AG (Mean $\pm$ SD)	113.7 $\pm$ 75.5	8.9 $\pm$ 9.2	17.4 $\pm$ 10.5
p value	0.008	0.360	0.010
GC (Mean $\pm$ SD)	100.3 $\pm$ 86.4	10.5 $\pm$ 10.7	25.7 $\pm$ 64.5
Non GC (Mean $\pm$ SD)	103.3 $\pm$ 75.8	10.2 $\pm$ 13.3	15.3 $\pm$ 10.5
p value	0.498	0.678	0.402
AG H.pylori Negative (Mean $\pm$ SD)	54.6 $\pm$ 36.07	15.8 $\pm$ 25.3	7.2 $\pm$ 5.9
Non AG H.pylori Negative (Mean $\pm$ SD)	75.5 $\pm$ 44.5	4.09 $\pm$ 2.6	21.9 $\pm$ 12.8
p value	0.015	0.005	<0.001
AG H.pylori Positive (Mean $\pm$ SD)	97.3 $\pm$ 85.2	11.05 $\pm$ 12.9	19.5 $\pm$ 48.4
Non AG H.pylori Positive (Mean $\pm$ SD)	126.5 $\pm$ 79.6	10.5 $\pm$ 10.06	15.9 $\pm$ 9.2
p value	0.025	0.559	0.012
GC H.pylori Negative (Mean $\pm$ SD)	33.5 $\pm$ 22.8	5 $\pm$ 4.07	9.5 $\pm$ 6.8
Non GC H.pylori Negative (Mean $\pm$ SD)	71.8 $\pm$ 42.2	9.4 $\pm$ 18	16.9 $\pm$ 13.3
p value	0.053	0.550	0.333
GC H.pylori Positive (Mean $\pm$ SD)	116 $\pm$ 88.6	11.8 $\pm$ 11.5	29.5 $\pm$ 71.5
Non GC H.pylori Positive (Mean $\pm$ SD)	114.5 $\pm$ 81.9	10.5 $\pm$ 11.2	14.7 $\pm$ 9.4
p value	0.930	0.541	0.067

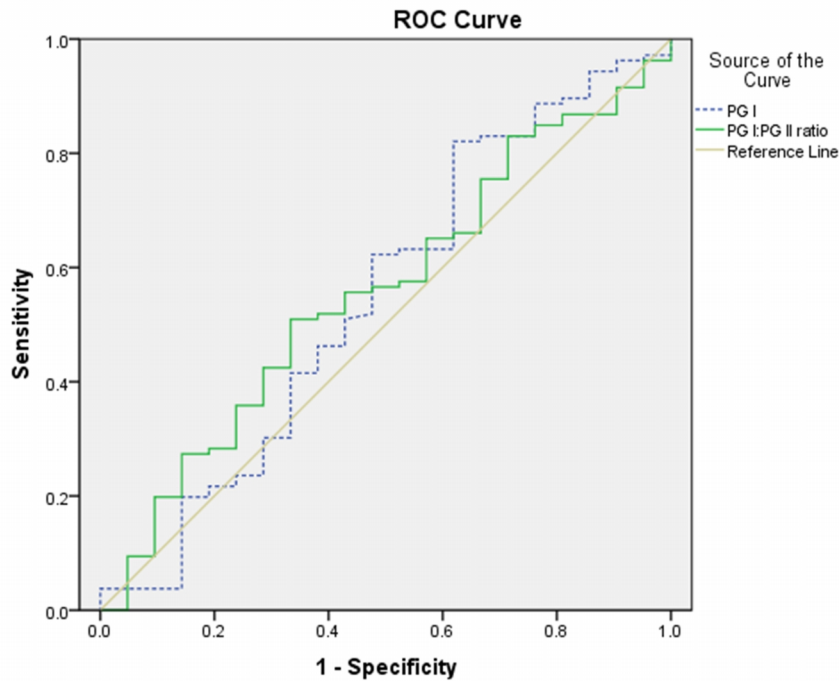
Table 2. Summary of the receiver operating characteristic (ROC) curve analysis for atrophic gastritis detection by the serum biomarkers

Serum marker	PGI ( $\mu\text{g/L}$ )	PGI:PGII ratio
AUC% (95% CI)	0.639	0.711
P value	0.008	0.0001
Cut-off	40	8
Sensitivity (95% CI)	90% (95% CI=76%-95%)	71% (95% CI=53%-85%)
Specificity (95% CI)	67% (95% CI=58%-76%)	71% (95% CI=61%-80%)
Accuracy (%)	69%	71%
PPV	35% (95% CI=22%-49%)	49% (95% CI=34%-63%)
NPV	92% (95% CI=83%-97%)	86% (95% CI=77%-93%)
Youden index	0.429 (95% CI=0.234-0.624)	0.431 (95% CI=0.256-0.607)



Diagonal segments are produced by ties.

Fig. 1. Receiver operating curve of pepsinogen I (PGI) (dashed line), PGI: PGII ratio (line) for the diagnosis of atrophic gastritis



Diagonal segments are produced by ties.

Fig. 2. Receiver operating curve of pepsinogen I (PGI) (dashed line), PGI: PGII ratio (line) for the diagnosis of gastric cancer

(95% CI: 0.538-0.741,  $p=0.008$ ). The best cut-off value was  $40\mu\text{g/L}$  (sensitivity 90%, specificity 67%, and accuracy 69%)

(Fig.1).

The area under the ROC for PG ratio was 0.711 (95% CI: 0.617–0.806,  $p=0.0001$ )

and the best cut-off value was 8 (sensitivity 71%, specificity 71%, accuracy 71%) (Fig.1).

For patients with gastric cancer, the area under the ROC for PGI was 0.547 (95% CI: 0.400-0.694,  $p=0.498$ ), and for PGI: PGII the ratio was 0.558 (95% CI: 0.427-0.689,  $p=0.402$ ) which showed no value for the diagnosis of gastric cancer (Fig. 2). The results of ROC analysis and the corresponding diagnostic indices are summarized in Table 2.

### Discussion

Despite the decline in gastric cancer in western countries, it remains a leading cause of cancer-related deaths in Asian countries such as Japan and Iran (11, 12).

Definitive diagnosis of AG through endoscopy and histological examination is invasive. While histological examination is considered the gold standard diagnostic test, it may be subject to error due to the patchy nature of atrophy and the limitation in the number of biopsies. PGI and PGII can be used to indicate the function of the gastric mucosa (13-15). PGI and PGI: PGII ratios are decreased in atrophic chronic gastritis which is acknowledged as a major risk factor for the development of gastric cancer. Variable cut-off-values for PGI and the PG-ratio were applied in former studies (16), but levels below 70  $\mu\text{g}/\text{ml}$  and 3.0, respectively, were considered the most efficient resulting in a sensitivity of 66.7-84.6% and a specificity of 73.5-87.1% for the detection of atrophic gastritis (17-19).

In our study, the best cut off value for PGI was found to be 40  $\mu\text{g}/\text{L}$  with an accuracy of 69%, 90% sensitivity, 67% specificity and Youden index (YI) of 0.429. Additionally, the best cut off value for PGI: PGII ratio was 8, with 71% accuracy, 71% sensitivity, 71% specificity and YI 0.431, in discriminating atrophic from non-atrophic specimens. Other studies showed a broad range of cut off values for the diagnosis of gastritis atrophy, which can be attributed to the use of different methods for patients selection, atrophic in-

tensity, risk factors and different type of studies.

With respect to studies from Iran, Hosseini et al. reported that the PGI: PGII ratio is diagnostically significant in detecting gastric atrophy, while serum biomarkers of atrophic gastritis (PGI and PGII) are not useful screening tests due to their low sensitivity (50%) (20). Haj-Sheykholeslami et al. demonstrated that by setting a cutoff value of 7.5 microg/mL for PGII, all types of gastritis were diagnosed with 80% sensitivity and 80%. While PGII is a suitable marker for screening any gastritis from normal mucosa, PGI, PGI: PGII ratio or their combination failed to select those with precancerous conditions and corpus-predominant gastritis among first-degree relatives of gastric cancer patients (21).

In the study conducted by Nasrollahzadeh et al. for defining an efficient biomarker for gastric atrophy, the best cut off value for PGI was 56 mg/l (sensitivity: 61.9%, specificity: 94.8%), while a PGI: PGII ratio=5 presented as the most efficient cut off value (sensitivity: 75.0%, specificity: 91.0%) (22).

Irvani et al. described PGI: PGII ratio as an efficient biomarker with 96.1% sensitivity and 97.7% negative predictive value; PGI has the highest specificity (94.6%), and PGII also had a high negative predictive value (90.7%). Thus, Pepsinogen I/II ratio appears to be the most suitable single measurement for screening purposes in atrophic gastritis (23).

Storskrubb et al. reported a sensitivity and specificity of 71% and 98%, respectively. Serological biomarkers show a high degree of accuracy as a non-invasive method to diagnose corpus atrophy (24).

Most studies have indicated a superior ability of the combined PGI and PGI: PGII ratio (24) or the PGI: PGII ratio alone (25-27) in the diagnosis of atrophic gastritis, which was confirmed by our findings.

In the present study, serum PGI levels and PGI: PGII ratio was significantly decreased in patients with atrophic gastritis, whereas there was no significant associa-

tion between these biomarkers and gastric cancer.

### Conclusion

This study demonstrates that PGI and PGI: PGII ratios are potential serologic biomarkers for screening of atrophic gastritis in fundus region, possessing high sensitivity, specificity, accuracy and negative predictive value. Serologic biomarker could be used as a screening method for the detection of precancerous states due to its well-established advantages, such as its convenience, relative cheapness and safety.

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