

# Serum pepsinogen level as a biomarker for atrophy, reflux esophagitis, and gastric cancer screening in Indonesia

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**Background:** Chronic dyspepsia's symptoms are frequently seen in primary to tertiary healthcare in Indonesia. This study aimed to describe the potential usability of pepsinogen (PG) values in determining gastric mucosal conditions, including superficial gastritis and atrophic gastritis. **Materials and Methods:** We recruited 646 adult dyspeptic patients and then analyzed PG values (including PGI, PGII, and PGI/II ratio) with endoscopic findings, gastric mucosal damages, and *Helicobacter pylori* infection. The gastric mucosal damage and *H. pylori* infection were evaluated using histological examination based on the updated Sydney system. **Results:** Among 646 enrolled patients, 308 (47.2%), 212 (32.8%), 91 (14.1%), 34 (5.2%), and 1 (0.2%) patient were diagnosed with normal mucosa, gastritis, reflux esophagitis, peptic ulcer disease, and gastric cancer, respectively. Significant differences in PGI, PGII, and PGI/II ratio values were observed among ethnic groups (all  $P < 0.01$ ). The PGI and PGII levels were significantly higher and PGI/II was significantly lower in *H. pylori*-infected patients than in uninfected ones (all  $P < 0.001$ ). The optimal cutoff value for PGII and PGI/II was 12.45 ng/mL with an area under the curve (AUC) value of 0.755 (0.702–0.811), sensitivity 59.3%, and specificity 77.1%; and 4.75 with AUC value of 0.821 (0.763–0.855), sensitivity 81.5%, and specificity 78.7%, respectively, to determine moderate–severe atrophy. **Conclusion:** Serum PG levels, a useful biomarker, represent the endoscopic findings, especially for reflux esophagitis. In addition,

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the benefits of PG values detecting atrophic gastritis were limited to moderate–severe atrophic gastritis. This usefulness requires careful attention for several ethnic groups in Indonesia.

**Key words:** Atrophic gastritis, human and disease, *Helicobacter pylori*, Indonesia, neoplasms, pepsinogens, reflux esophagitis

## INTRODUCTION

Chronic dyspepsia's symptoms are frequently seen in primary to tertiary healthcare in Indonesia,<sup>[1]</sup> with underlying pathologies such as atrophic gastritis, intestinal metaplasia, and peptic ulcers which may act as the cause of the symptoms.<sup>[2-4]</sup> Those pathologies are influenced by *Helicobacter pylori* infection, which is known as the carcinogenic bacteria and is suffered by approximately half of the world population.<sup>[5]</sup> Therefore, the diagnosis of *H. pylori* and gastric mucosa status are still concerning for clinicians. The accuracy of diagnosis and appropriate therapy need to be performed as early as possible to overcome chronic gastritis and prevent more severe clinical manifestations, such as gastric adenocarcinoma, which was reported to have only 25.1% of 5-year survival rate.<sup>[6]</sup>

The diagnostic methods determining the gastric mucosa condition and *H. pylori* status include invasive techniques, through endoscopic and biopsy sampling.<sup>[7]</sup> However, this method is less comfortable, relatively risky for patients, and considered an expensive examination in some places, especially in Indonesia. Serological tests including anti-*H. pylori* antibodies detection are more convenient and the result can be obtained faster. Pepsinogen (PG) I is mainly produced by the chief cells and mucus neck cells in the gastric fundus, while PGII is produced in the stomach with the addition of the proximal duodenum, and these can be examined from the serum specimen.<sup>[8]</sup> PGI and PGII are proposed to have good diagnostic values in predicting gastric mucosal status such as atrophic gastritis. Alongside serological tests for *H. pylori* infection, they may have a benefit as the gastric cancer screening method.<sup>[9]</sup> The combination of PGs and antibody *H. pylori* examination was proposed to improve diagnostic accuracy.<sup>[10]</sup> The ABC method, which was initially introduced by Miki *et al.*, is a classification method to stratify gastric cancer risk based on the serum PG and *H. pylori* infection status. The ABC method consists of *H. pylori*-negative/PG-negative (group A), *H. pylori*-positive/PG-negative (group B), *H. pylori*-positive/PG-positive (group C), and *H. pylori*-negative/PG-positive (group D).<sup>[11]</sup> The use of ABC method is proven useful in countries with a high risk of *H. pylori* infection and gastric cancer, including China, Japan, Mongolia, and Bhutan.<sup>[12-15]</sup> However, the reliability of PGs and ABC method are still questionable when applied to different populations and regions.

Indonesia is the fourth largest populated country worldwide. It occupies a very wide area over numerous islands. In addition, it is inhabited by various ethnic groups with different hygiene and food habits. Although in the national survey, overall Indonesia had a low prevalence of *H. pylori* (10.4%),<sup>[16]</sup> there was a difference in ethnic prevalence; a lower prevalence was found in Javanese ethnic who mostly lived in the urban area with endoscopic facilities. However, the prevalence of *H. pylori* was high in several places, such as Jayapura (Papua Island), Makassar (Sulawesi Island), and Medan (Sumatra Island).<sup>[17]</sup> These places are considered to have remote areas that have limited access to endoscopic equipment. Therefore, a noninvasive reliable diagnostic method that can detect not only *H. pylori* infection but also gastric mucosal status, using PGs and/or ABC method, is necessary. In our previous study, the validation of PGs and *H. pylori* serology has been carried out only in three cities in Indonesia, and we found that PG can be beneficial.<sup>[18]</sup> However, it may not represent all of the Indonesian population. Therefore, a new survey involving populations in areas with higher *H. pylori* prevalence should be performed to examine the reliability of PGs use in a nationwide approach. In this study, we aimed to examine the reliability of serum PGs as a biomarker for gastroesophageal disease detection in Indonesia. We also described the diagnostic accuracy of ABC method in Indonesia. In addition, we analyzed the distribution of serum PGs secretion in various determinant factors (*H. pylori* infection, sex, age, and ethnicity).

## MATERIALS AND METHODS

### Study population

This cross-sectional study was conducted between October 2014 and March 2017, enrolling adult dyspeptic patients from 13 cities in Indonesia, including Aceh, Bangli, Cimacan, Gunung Sitoli, Kolaka, Kupang Manado, Merauke, Padang, Palembang, Palu, Surabaya, and Ternate. This study population also included 158 samples from our previous study.<sup>[18]</sup> The inclusion criteria for this study were adult dyspeptic patients. Exclusion factors were as follows: History of *H. pylori* eradication therapy, partial or total gastrectomy, contraindication to endoscopy, and non-fasting subjects. An experienced endoscopist acquired two gastric biopsy specimens during each endoscopic procedure and made a diagnosis of peptic ulcer, identifying the presence of a mucosal break due to reflux esophagitis. One specimen was obtained from the lesser curvature of

the antrum, approximately 3 cm from the pyloric ring, and the other from the greater curvature of the corpus. Both specimens were histologically examined. Furthermore, on the endoscopy's day, fasting serum was collected and stored at  $-20^{\circ}\text{C}$ . Furthermore, subjects were interviewed to obtain socio-demographic data: body mass index, smoking and drinking habits, and use of nonsteroidal anti-inflammatory drugs (NSAIDs). All participants signed written informed consent. The study protocol was approved by the ethics committees of Dr. Cipto Mangunkusumo Teaching Hospital (Jakarta, Indonesia), Dr. Soetomo Teaching Hospital (Surabaya, Indonesia), Dr. Wahidin Sudirohusodo Teaching Hospital (Makassar, Indonesia), and Oita University Faculty of Medicine (Yufu, Japan).

### Histology and immunohistochemistry

Collected biopsy material was stored in 10% buffered formalin and then embedded in paraffin. Hematoxylin–eosin and May–Giemsa staining were performed on serial sections. The degree of inflammation, atrophy, and bacterial density was classified into four grades according to the updated Sydney System: 0, normal; 1, mild; 2, moderate; and 3, marked.<sup>[19]</sup> Samples with bacterial loads  $\geq$  grade 1 were considered positive for *H. pylori*. In addition, we also performed immunohistochemistry analysis to increase the diagnostic accuracy, as previously described.<sup>[20]</sup> We incubated the histology specimens using anti- $\alpha$ -*H. pylori* antibody (DAKO, Glostrup, Denmark) after inactivation of endogenous peroxidase activity. We then incubated the 2<sup>nd</sup> antibody using biotinylated goat anti-rabbit IgG (Nichirei Co., Tokyo, Japan), followed by avidin-conjugated horseradish peroxidase solution (Vectastain Elite ABC Kit; Vector Laboratories Inc., Burlingame, CA, USA) for attaching the peroxidase. Detection of peroxidase activity on the specimens was performed by  $\text{H}_2\text{O}_2$ /diaminobenzidine substrate solution. The experienced pathologist was examining our current specimen, who also examines our other works in Myanmar, Vietnam, Bhutan, Dominican Republic, and Indonesia.<sup>[21–26]</sup>

### Determination of *Helicobacter pylori* serology and pepsinogen levels

Using separated sera, we measured both the *H. pylori* antibody titers with an enzyme linked immunosorbent assay (ELISA) kit (Eiken, Co. Ltd., Tokyo, Japan) and the PGI and PGII levels by using PG ELISA (Eiken), as per the manufacturer's instruction. *H. pylori* was considered positive if its antibody titers were  $\geq 10\text{U/mL}$ . The PGI and PGI/II ratio were interpreted as PG positive if the PGI levels  $\leq 70\text{ ng/mL}$  and PGI/II ratio  $\leq 3.0$ , according to the Miki criteria, which is commonly used in Japan.<sup>[11]</sup> In addition, we performed the ABC method evaluation for gastric cancer screening. Subjects were categorized into four groups: *H. pylori*-negative/PG-negative (group A),

*pylori*-positive/PG-negative (group B), *H. pylori*-positive/PG-positive (group C), and *H. pylori*-negative/PG-positive (group D).<sup>[11]</sup>

### Determination of disease

Experienced endoscopists observed the mucosal condition of the upper gastroduodenal tract from esophagus to duodenum. Reflux esophagitis was identified based on the observation of mucosal break on the gastroesophageal junction. The ulcerations in the stomach and duodenum were also identified based on endoscopic examination. Gastric cancer was determined based on endoscopic examination, confirmed by histopathology. The subjects without reflux esophagitis, ulcerations, and gastric cancer, including normal-looking mucosa, were further analyzed based on histological examination. The gastritis individuals were participants with the presence of neutrophil infiltration, monocyte infiltration, atrophy, or intestinal metaplasia. When the subjects did not have any histological gastric mucosal damage, we concluded these as a normal group.

As for atrophic gastritis, we simplified the classification of degree of atrophic gastritis due to a small number of moderate and marked. This classification based on the highest value of atrophic gastritis score in the histological evaluation. Mild atrophic gastritis is defined as score of atrophic gastritis equal to 1 in either antrum or corpus; when we observed score  $>1$  of atrophic gastritis, we defined as moderate–severe. In addition, we also classified atrophic gastritis based on the topographical distribution. An individual was categorized as having antral-predominant gastritis if the atrophic scores in the antrum were greater than those in the corpus, whereas if the atrophic scores in the corpus were greater than those in the antrum, these were categorized as corpus-predominant gastritis. The pan-gastritis was determined if the atrophic gastritis scores both in the antrum and corpus were equal.<sup>[27–29]</sup>

### Data analyses

Relationship between discrete variables was tested with a Chi-square test. Association of continuous variables was tested with the Mann–Whitney *U*- and Kruskal–Wallis tests. Normal distribution test for continuous variables was Shapiro–Wilk test. Discrete variables were presented as *n* number and percentage, while the continuous variables were presented as mean with standard deviation. The Spearman rank coefficients (*r*) were determined to evaluate the association between PG levels and gastric-mucosal inflammation and atrophy. A  $P < 0.05$  was considered as statistically significant. Receiver-operating characteristic curves were used to calculate the best cutoff. These included the area under the curve (AUC) and predictive values for discriminating chronic and atrophic gastritis. Utilizing

the cut-off value obtained from the ROC analysis, we then calculate the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. The SPSS statistical software package version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

## RESULTS

### Baseline characteristics

A total of 646 subjects which consisted of 383 males and 263 females were included with an average age of  $44.93 \pm 12.98$  years (range, 14–83 years). Those patients were enrolled from Aceh ( $n = 38$ ), Padang ( $n = 33$ ), and Palembang ( $n = 38$ ) in Sumatra island; Gunungsitoli ( $n = 32$ ) in Nias island; Cimacan ( $n = 21$ ) and Surabaya ( $n = 144$ ) in Java island; Bangli ( $n = 59$ ) in Bali island; Kolaka ( $n = 50$ ), Manado ( $n = 57$ ), and Palu ( $n = 55$ ) in Sulawesi island; Kupang ( $n = 33$ ) in Timor island; Merauke ( $n = 42$ ) in Papua island; and Ternate ( $n = 44$ ) in Ternate island [Figure 1]. The distribution of ethnicity in the city of endoscopy was described in Supplementary Table 1.

We performed Shapiro–Wilk test and observed that the PGI, PGII, and PGI/II ratio were not normally distributed. Male patients had significantly lower PGI level and PGI/II ratio median values than female patients (42.9 vs. 48.6,  $P = 0.003$  and 5.5 vs. 5.8,  $P = 0.024$ , respectively) [Table 1]. In addition, we also observed significant positive correlation between PGI value and PGII value with age ( $r = 0.377$  and  $r = 0.359$ , respectively, both  $P < 0.001$ ). Ethnic group influenced the PGI value and PGI/II ratio (both  $P < 0.001$ ) with the lowest ethnic group being Tolaki for PGI and Bataknese for PGI/II ratio, whereas the highest was Chinese for both PGI and PGI/II ratio. PGII value was also influenced by the ethnic

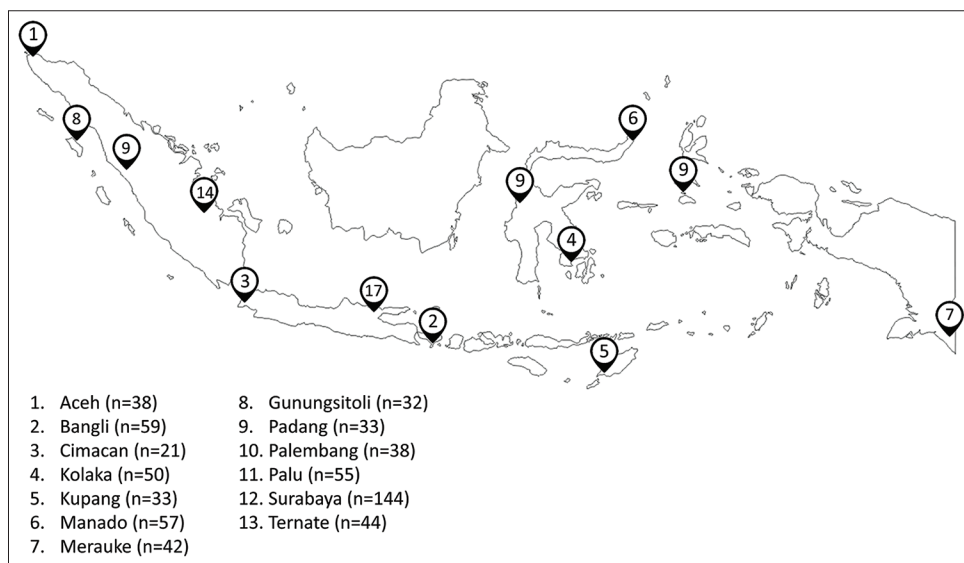
group ( $P = 0.015$ ) with the lowest being Tolaki and the highest being Chinese [Table 1].

According to the *H. pylori* infection status by histology and immunohistochemistry, PGI level was significantly higher in *H. pylori*-positive than in *H. pylori*-negative patients ( $P = 0.002$ ). In addition, PGII level was significantly higher in *H. pylori*-positive than in *H. pylori*-negative patients [ $P < 0.001$ , Table 1], whereas the PGI/II ratios were significantly lower in *H. pylori*-positive than in *H. pylori*-negative patients ( $P < 0.001$ ).

PGI of gastritis and reflux esophagitis patients was significantly higher than normal patients ( $P = 0.010$  and  $P = 0.002$ , respectively) [Table 2]. Gastritis patients had significantly higher PGII than normal patients ( $P < 0.001$ ). Reflux esophagitis patients had significantly higher PGI/II than peptic ulcer disease (PUD), normal, and gastritis patients ( $P = 0.002$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively) [Table 2].

### Pepsinogen levels and atrophic gastritis

We observed 122 (20.8%) patients had atrophy either in the antrum or corpus based on histological examination of atrophy score  $\geq 1$ . The patients with atrophic gastritis had significantly higher PGII values and significantly lower PGI/II ratio than nonatrophic gastritis individuals (median = 9.6 vs. 7.9,  $P = 0.001$  and 5.4 vs. 5.7,  $P = 0.002$ , respectively), but no significant association was observed on the PGI level [Table 3]. In addition, among those patients with gastritis atrophy, we observed 154 patients (93.0%), 3 patients (1.8%), and 14 patients (8.2%) were classified as antral predominant, corporal predominant, and pan-gastritis, respectively. However, we could not find



**Figure 1:** Map of the enrolled patients in the current study. The map was drawn by the author showing the cities we visited on survey. We performed endoscopy examination to 646 patients from 13 cities in Indonesia

**Table 1: Pepsinogen I, pepsinogen II secretion, and the determinant factor**

Factors	n	Pepsinogen levels, mean±SD (median)		
		PG I	PG II	PG I/II
<i>Helicobacter pylori</i> infection				
Positive	59	77.5±77.6 (54.4)*	19.4±16.8 (13.7)*	4.1±1.2 (4.0)*
Negative	587	61.6±50.8 (44.1)	10.2±7.8 (7.9)	6.0±1.74 (5.8)
P		0.02	<0.001	<0.001
Sex				
Male	383	61.5±55.8 (42.9)*	11.0±10.1 (7.9)	5.7±1.79 (5.5)*
Female	263	65.2±51.0 (48.6)	11.0±8.28 (8.8)	6.0±1.75 (5.8)
P		0.003	0.074	0.023
Age (years)				
<18	3	34.3±8.19 (35.7)‡	7.2±3.23 (9.0)	5.7±2.3 (4.6)
18-29	74	47.5±44.3 (38.5)	9.1±7.68 (7.0)‡	5.4±1.4 (5.4)
30-39	165	47.9±42.1 (37.0)	8.3±6.53 (7.1)	5.8±1.5 (5.7)
40-49	155	61.5±48.7 (42.0)	11.7±11.6 (7.5)	5.7±1.5 (5.6)
50-59	162	76.7±67.8 (54.3)	12.3±9.46 (9.9)	6.1±2.2 (5.8)
≥60	87	83.2±49.6 (71.6)¶	14.4±9.35 (12.4)¶	6.0±1.9 (5.8)
P		<0.001	<0.001	0.257
Ethnic				
Aceh	70	46.2±28.7 (38.3)	7.9±3.7 (7.5)	5.8±1.6 (5.5)
Balinese	61	64.8±61.2 (47.7)	12.1±11.7 (9.0)	5.6±1.3 (5.5)
Bataknese	2	41.4±12.6 (41.4)	10.1±3.11 (10.1)	4.1±1.0 (4.1)‡
Bugis	69	64.4±59.5 (39.9)	10.7±7.3 (7.6)	5.8±2.1 (5.4)
Chinese	40	85.3±55.1 (61.6)¶	13.3±8.7 (10.6)¶	6.7±2.1 (6.5)¶
Dayak	6	76.5±66.5 (49.6)	12.1±8.1 (9.2)	5.8±1.4 (6.1)
Javanese	118	77.1±60.2 (62.5)	12.4±10.9 (9.5)	6.4±1.7 (6.4)
Ternatense	46	59.2±59.2 (44.2)	9.6±7.3 (7.4)	6.0±1.4 (5.9)
Malay	36	73.0±47.9 (59.1)	11.8±7.9 (9.7)	6.4±1.7 (5.8)
Minahasanese	53	47.2±23.3 (44.3)	8.3±3.7 (8.0)	5.9±1.3 (5.8)
Nias	32	61.4±42.8 (44.0)	10.3±6.6 (8.4)	6.2±2.1 (5.6)
Kaili	12	49.8±31.6 (36.4)	9.2±4.9 (7.8)	5.5±1.7 (5.3)
Papuan	43	49.6±35.5 (39.0)	12.5±14.0 (9.1)	4.7±1.5 (4.4)
Timor	34	72.4±90.6 (46.2)	14.6±14.9 (9.2)	5.2±1.8 (4.7)
Tolaki	24	42.0±30.5 (33.4)‡	9.8±9.0 (7.1)‡	4.6±0.9 (4.6)
P		<0.001	0.015	<0.001

\*The calculation showed statistically significant (Wilcoxon rank sum test, all  $P < 0.05$ ). †The lowest group among determinant factor, ¶The highest group among determinant factor. PG=Pepsinogen; SD=Standard deviation

**Table 2: Pepsinogen level and disease**

PG level	Normal	Gastritis	Reflux esophagitis	PUD	Gastric cancer
n	308	212	91	34	1
PG I	55.0±43.0	63.4±53.8*	87.4±75.4*	65.4±55.6	123.0
PG II	9.4±6.6	12.1±10.1	12.7±10.6	12.6±10.9	93.1
PG I/II	5.9±1.6	5.6±1.9	6.7±1.8**	5.4±1.4	1.3

\*PG I level was significantly higher than normal individuals, \*\*PG I/II level was significantly higher compared to normal, gastritis and PUD groups. PUD=Peptic ulcer disease; PG=Pepsinogen

any significant difference of PG levels among predominant locations.

We also classified atrophic gastritis based on the severity observed by histological examination. Due to the low number of moderate and severe atrophic gastritis, we combined them into one group. Among all atrophic gastritis patients, we found 144 (84.2%) patients had mild atrophic lesion, while 27 (15.8%) patients had moderate–

severe atrophic lesion. The PGII value of moderate–severe atrophy patients was significantly higher than mild atrophy patients (median = 13.5 vs. 9.2,  $P = 0.001$ ), whereas the PGI/II ratio of moderate–severe atrophy patients was significantly lower than mild atrophy patients (4.1 vs. 5.6,  $P < 0.001$ ). In addition, we observed a significant positive correlation between PGII value and antral atrophic score based on Sydney System ( $r = 0.263$ ,  $P < 0.001$ ), but not in the corpus, and a significant negative correlation between

PGI/II and antral atrophic score ( $r = -0.316, P < 0.001$ ). When we analyzed the presence of inflammation (score of monocyte or neutrophil infiltration  $\geq 1$ ) in the antrum and corpus, the PGI and PGII values were significantly higher in the inflammation group than noninflammation one (both  $P < 0.001$ ). The PGI/II ratio was significantly lower in inflammation group than noninflammation one ( $P < 0.001$ ).

### Value of pepsinogen for atrophic gastritis

Based on the criteria by Miki et al.,<sup>[11]</sup> values  $\leq 70$  ng/mL for PG I level and  $\leq 3.0$  for PG I/II ratio were considered the PG-positive to detect gastric atrophy. By using the cutoff, we observed only 17 patients (2.6%) were considered as PG-positive group. By using histological examination of atrophic either in the antrum or corpus  $\geq 1$  as the positive group, we found that sensitivity and specificity were 7.6% (4.5–9.2) and 99.2% (98.2–99.8), respectively [Supplementary Table 2].

Considering a low value of sensitivity for the criteria, we recalculated the cutoff value of those measurements. By using atrophy score  $\geq 1$  as the standard determining positive group, we observed very low AUC value for PGI, PGII, and

PGI/II ratio (0.549, 0.589, and 0.581, respectively); thus, we may not consider atrophy score  $\geq 1$  as a good standard. When we considered atrophic score  $\geq 2$  as the standard determining positive group, we observed that AUC for PGI, PGII, and PGI/II ratio was 0.587, 0.755, and 0.821, respectively [Table 4]. As PGI had a considerably very low AUC value, we only determined the cutoff value for PGII and PGI/II ratio. The optimal cutoff value for PGII was 12.45 ng/mL and PGI/II ratio was 4.75 respectively [Table 4]. With the PGII cutoff of  $\geq 12.45$  ng/mL, the sensitivity and specificity were 59.3% and 77.1%, respectively. When utilizing the PGI/II of  $\leq 4.75$  as the cutoff, we observed that the sensitivity and specificity were 81.5% and 78.7%, respectively [Table 4]. When we considered using either PGII or PGI/II ratio to determine moderate–severe atrophic data, we found that the sensitivity and specificity were 85.2% and 60.8%, respectively. On the other hand, when using both PGII and PGI/II ratio, we found that the sensitivity and specificity were 55.6% and 94.9%, respectively.

### Validation for *Helicobacter pylori* IgG and ABC method of Indonesian patients

Following manufacturer’s standard for detecting *H. pylori* infection, we observed that 46 patients (7.1%) were infected by *H. pylori*. Utilizing the manufacturer’s standard, the sensitivity, specificity, and overall accuracy were 69.5%, 99.2%, and 96.4%, respectively when histology results were used as gold standard. Owing considerably low sensitivity value, we determined the new cutoff value. With the AUC 0.934 (95% confidence interval [CI] = 0.890–0.976), the optimal cutoff value for serology test in Indonesia was  $\geq 6.7$  U/mL. By using new cutoff, the sensitivity and specificity were 83.5% (95% CI = 71.2–92.3) and 98.7% (97.3–99.4), respectively [Supplementary Table 2].

We classified each patient based on our modified ABC method, which we only changed the *H. pylori* infection cutoff point ( $\geq 6.7$  U/mL) and the PGs value was the same (PGI  $< 70$  ng/mL and PGI/II ratio  $\leq 3$ ). We observed that 585 patients (90.6%) were classified as group A followed by group B (44/646, 6.8%), group C (10/646, 1.5%), and group D (7/646, 1.1%) [Table 5]. When we evaluated ethnicity, we observed a considerably high group C

**Table 3: The pepsinogen levels between atrophic status, predominant location and severity of atrophic gastritis**

	n	PG I	PG II	PG I/PG II
Atrophy status				
Nonatrophy	475	62.0±60.9	10.6±9.4	6.0±1.7
Atrophy	171	65.9±59.8	12.3±9.2*	5.6±2.0*
P		0.058	<0.001	0.001
Predominant location				
Antral	154	65.9±60.9	12.0±8.9	5.7±2.0
Corporal	3	36.5±9.4	7.4±1.1	5.0±1.5
Pan-gastritis	14	72.6±53.4	17.2±12.0	4.6±1.7
P		0.135	0.098	0.587
Degree of atrophy				
Mild	144	67.7±64.3	11.9±9.6	5.8±1.9
Moderate-severe	27	56.3±22.5	14.4±6.3**	4.1±1.2**
P		0.543	0.001	<0.001

\*The calculation showed statistically significant compared to nonatrophic gastritis patients (Wilcoxon rank-sum test, all  $P < 0.05$ ). \*\*The calculation showed statistically significant compared to mild degree of atrophic gastritis patients (Wilcoxon rank-sum test, all  $P < 0.05$ ). PG=Pepsinogen

**Table 4: The validation parameters for pepsinogen levels determining moderate-severe atrophy in Indonesia**

Disease group	Parameters	Serum PG (95% CI)		
		PG I	PG II	PG I/II
Moderate-severe atrophy	Cutoff value	-	$\geq 12.45$ ng/mL	$\leq 4.75$
	AUC	0.587 (0.512-0.622)	0.755 (0.702-0.811)	0.821 (0.763-0.855)
	Sensitivity (%)	-	59.3 (38.8-77.6)	81.5 (61.9-93.7)
	Specificity (%)	-	77.1 (73.0-80.8)	78.7 (74.3-82.3)
	PPV (%)	-	12.8 (9.3-17.3)	17.9 (14.5-28.9)
	NPV (%)	-	97.1 (95.5-98.3)	98.7 (97.1-99.4)
	Overall accuracy (%)	-	76.1 (72.1-79.8)	78.9 (75.0-82.3)

AUC=Area under curve; PG=Pepsinogen; NPV=Negative prediction value; PPV=Positive prediction value; CI=Confidence interval

**Table 5: Distribution of modified ABC method classification among Indonesian patients**

	<i>n</i>	Group A (%)	Group B (%)	Group C (%)	Group D (%)	<i>P</i>
Overall	646	585 (90.6)	44 (6.8)	10 (1.5)	7 (1.1)	
Sex						
Male	383	339 (88.5)	32 (8.4)	8 (2.1)	4 (1.1)	0.004
Female	263	246 (93.5)	12 (4.6)	2 (0.8)	3 (1.1)	
Age group						
<18	3	3 (100)	0	0	0	0.010
18-29	74	69 (93.2)	3 (4.0)	1 (1.4)	1 (1.4)	
30-39	165	155 (93.9)	5 (3.0)	1 (0.6)	4 (2.4)	
40-49	155	138 (89.0)	13 (8.4)	3 (1.9)	1 (0.6)	
50-59	162	145 (89.5)	13 (8.0)	4 (2.5)	0	
≥60	87	75 (86.3)	10 (11.5)	1 (1.1)	1 (1.1)	
Disease						
Gastritis	212	160 (75.5)	36 (16.9)	10 (4.7)	6 (2.8)	0.001
Reflux esophagitis	91	88 (96.7)	3 (3.3)	0	0	
PUD	34	29 (85.3)	4 (11.8)	0	1 (2.9)	
Cancer	1	0	1 (100)	0	0	

All carried out statistical analysis were Fischer's exact test. *H. pylori*-negative/PG negative (group A); *H. pylori* positive/PG negative (group B); *H. pylori* positive/PG positive (group C); and *H. pylori*-negative/PG positive (group D). *H. pylori*=*Helicobacter pylori*; PG=Pepsinogen; PUD=Peptic ulcer disease

proportion in Papuan and Timor ethnic group (11.6% and 5.8%, respectively) [Supplementary Table 2].

## DISCUSSION

This study is the nationwide approach study to validate the potential benefit of PG levels to determine if patients require further endoscopic examination. We observed the atrophy group has a significantly lower PGI/II ratio in both *H. pylori*-positive and -negative in concordance with previous studies.<sup>[18,30]</sup> The level of PGI in the atrophy group in this study was higher than in the nonatrophy group, which also reported in the previous study,<sup>[31]</sup> but not in other studies in Korea and Japan.<sup>[32-34]</sup> This was probably due to the higher proportion of mild atrophy. Severe atrophy was related to the loss of glands which caused significant decrease in PG production.<sup>[35]</sup> This result was supported by remarkably lower PGI in severe atrophy compared to mild atrophy. Furthermore, PGII value has a greater rise as the result of chronic inflammation.<sup>[33,35]</sup> The presence of inflammation may also give an increase in PGI and PGII<sup>[32]</sup> as shown by a significant difference between inflammation and no inflammation either in the antrum or corpus, and by the presence of inflammation, it might respond to the high standard deviation values. However, we could not observe any significant difference of PG between the predominant locations of the atrophy. This phenomenon was also reported in several studies in Korea and Europe.<sup>[35,36]</sup>

PG's ability to distinguish between atrophy diverge according to the country which related to each population's risk of cancer and *H. pylori* infection rate.<sup>[35]</sup> Different cutoff value was implemented to determine atrophy.<sup>[37-39]</sup> In this study, first, we applied the cutoff based on the criteria by

Miki *et al.* However, the determined cutoff value from the original study had low sensitivity and specificity as reported in several countries;<sup>[40,41]</sup> thus, validation is necessary. We calculated the new cutoff adjusted to the Indonesian population. Our newly determined standard cutoff increased the ability to distinguish normal group and moderate–severe atrophy group with improved sensitivity and specificity. This finding suggests that the PG ability to distinguish atrophy group was limited to moderate–severe atrophy only in Indonesia. In addition, the diagnostic benefit of PG to diagnose moderate–severe atrophy in Indonesia was more likely only using PGI/II ratio value alone rather than combination with other values. Indeed, it has lower sensitivity value than the combination of PGII or PGI/II. However, the latter had considerably lower specificity than PGI/II alone. Therefore, PGI/II ratio had better balance between sensitivity and specificity.

We observed that PGI of reflux esophagitis and gastritis patients was significantly higher than patients with normal mucosa. PGI is mainly secreted by the fundus, which might responsible to the development of the esophagitis.<sup>[42]</sup> Previous study reported the link between PG and endoscopic finding which showed higher PG production in the peptic ulcer and nodular gastritis and also higher PGI/II ratio in erosive esophagitis.<sup>[43,44]</sup> Other studies in Korean and Japanese population also showed an increasing PGI/II ratio in reflux esophagitis.<sup>[44,45]</sup> Reflux esophagitis is proposed as the causal pathology of dyspepsia in the area with low prevalence of *H. pylori* infection as its possible protective mechanism for progression of *H. pylori*-related disease.<sup>[46]</sup> This finding may promote the utility of PG for gastroesophageal reflux screening as suggested by prior study.<sup>[47]</sup> Meanwhile, inflammation

occurred in PUD might affect PGII production in the antrum and duodenum resulting a decrease in PGI/II ratio value as reported before.<sup>[48]</sup> However, our current study found that there was no significant difference in PGI, PGII, or PGI/II ratio between PUD and gastritis. The increase in PGII which lowering the PGI/II ratio was closely associated with *H. pylori* infection,<sup>[44]</sup> thus inducing more severe inflammation. In our current study, the rate of *H. pylori* infection in PUD patients was quite low. Therefore, it may explain the insignificant differences in either PGI, PGII, or PGI/PGII ratio between peptic ulcer and gastritis patients.

However, the measurement of elderly patients should be cautious. We found that PG level was affected by age, it is in concordance with several studies.<sup>[49,50]</sup> The level of PGI and PGII was increased as the inclining age may be due to the increasing prevalence of superficial gastritis and *H. pylori* infection in older age individuals.<sup>[30]</sup> In addition, the decreasing glomerular filtration rate in the elderly reduces PG excretion.<sup>[49]</sup> In this study, we included 15 ethnics in Indonesia and found a significant association between PGI, PGII, and PGI/PGII ratio with the ethnic. This factor was also mentioned in the previous studies.<sup>[51,52]</sup> Genetic factors plays role in determining the PG density.<sup>[53]</sup> We observed that Batakese had the lowest PGI/II ratio among all ethnic groups we analyzed, suggesting that particular ethnic group more likely to have atrophic gastritis. In addition, our histologic examination analysis showed the Batakese was grouped as intermediate risk group for gastric cancer.<sup>[54]</sup> However, the sample number on that particular ethnic group was only 2, a caution that is important to be considered. Therefore, it is necessary to conduct further study with bigger sample size for each ethnic group to see bigger picture of gastric cancer in Indonesia.

The eradication of *H. pylori* significantly decrease the level of PGI in the peptic ulcer patients.<sup>[55]</sup> In our current study, it was also significantly increase the PGI but greater rise in PGII, thus decrease PGI/II ratio as stated in many studies.<sup>[34,35]</sup> Several possible reasons are that *H. pylori* infection induces somatostatin deficiency, and inflammation by producing cytokines such as leukotriene and TNF $\alpha$  thus increases the gastrin and gastric acid secretion.<sup>[50,56]</sup> The increased gastrin secretion would later increase PG secretion. Infection process may also damage the mucous and chief cells which resulted in the leakage of zymogen cells and released the PG before converting to pepsin.<sup>[55]</sup> Therefore, the combination of PG with the *H. pylori* serological test is also proposed to improve gastric cancer screening, known as the ABC method.<sup>[10,11]</sup> Group A has the lowest risk so may not require further endoscopy examination and the higher risk was group B, C, and D and require endoscopy examination every 3, 2, and 1 years, respectively.<sup>[57]</sup> Our overall observation showed an enormous number of group A and very low group C and D,

suggesting the atrophic condition in Indonesia, even with the presence of *H. pylori* in Indonesia is very low. As comparison to countries with higher gastric cancer incidence, including Japan and Bhutan, the group showed those countries had considerably higher number of group C and group D than Indonesia,<sup>[13,15]</sup> suggesting that Indonesia is far less having gastric cancer risk than those countries. These findings were in concordance with the age-standardized ratio of gastric cancer incidence from GLOBOCAN that showing 1.5/100,000 population (GLOBOCAN, 2018, available from: <https://gco.iarc.fr/>). In addition, we also observed that Papuan and Timor ethnic group had a considerably proportion of group C, which in concordance with our previous study which reported a high prevalence of *H. pylori* infection among those ethnicity.<sup>[16]</sup>

There were several limitations in this study. First, the patients included in this study was only dyspeptic patients without the addition of healthy subjects combined as a whole population. Therefore, it might decrease the predictive value of serum PGs analyzed in this study; therefore, it would be cautions in application for general population including asymptomatic subjects. Second, the low sample number was also a limitation in this current study, which resulted in a low sample number when our analysis deemed us to divide our samples into several ethnic groups. Therefore, our current works might only be regarded as preliminary generating study. Further study with larger sample number and including a healthy individual is necessary. In addition, even though histology and culture result appeared to be normal, it does not exclude possibility of the atrophy and inflammation due to sampling bias during endoscopy.

## CONCLUSION

Validation of indirect methods is essential before their application. We showed that serum PG levels are useful biomarkers for atrophic gastritis. However, the beneficial of PG values determining atrophic gastritis only limited to moderate–severe atrophic gastritis in Indonesia. In addition to atrophic gastritis, serum PG levels also have benefits that represent the endoscopy finding, especially for reflux esophagitis. This usefulness needs to carefully take attention for older age.

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### Conflicts of Interest

There are no conflicts of interest.

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**Supplementary Table 1: The distribution of each ethnic group based on Island and City in our current study**

<b>Ethnic</b>	<b>Island</b>	<b>City</b>
Aceh	Sumatra	Banda Aceh and Padang
Balinese	Bali	Bangli
Bataknese	Sumatra	Palembang
Bugis	Sulawesi and Java	Kolaka, Palu and Surabaya
Chinese	Java and Ternate	Surabaya and Ternate
Dayak	Java	Surabaya
Javanese	Almost in all largest Island of Indonesia	Cimacan, Kupang, Manado, Palembang, Palu and Surabaya
Ternatense	Java and Ternate	Surabaya and Ternate
Malay	Sumatra	Palembang
Minahasanese	Sulawesi	Manado and Palu
Nias	Nias	Gunungsitoli
Kaili	Sulawesi	Palu
Papuan	Papua	Merauke
Timor	Timor	Kupang
Tolaki	Sulawesi	Kolaka

**Supplementary Table 2: The classification based on ABC method based on ethnic group**

<b>Ethnic</b>	<b>n</b>	<b>Group A (%)</b>	<b>Group B (%)</b>	<b>Group C (%)</b>	<b>Group D (%)</b>
Aceh	70	49 (70)	1 (1.4)	0	20 (28.6)
Balinese	61	44 (72.1)	3 (4.9)	6 (9.8)	8 (13.1)
Batak	2	0	0	0	2 (100)
Bugis	69	42 (60.9)	4 (5.8)	8 (11.6)	15 (21.7)
Chinese	40	35 (87.5)	0	2 (5)	3 (7.5)
Dayak	6	5 (83.3)	0	0	1 (16.7)
Javanese	118	103 (87.3)	0	1 (0.8)	14 (11.9)
Ternatense	46	39 (84.8)	1 (2.2)	2 (4.3)	4 (8.7)
Melayu	36	31 (86.1)	0	1 (2.8)	4 (11.1)
Minahasa	53	38 (71.7)	4 (7.5)	2 (3.8)	9 (16.9)
Nias	32	24 (75)	1 (3.1)	1 (3.1)	6 (18.8)
Kaili	12	8 (66.7)	1 (8.3)	1 (8.3)	2 (16.7)
Papuan	43	20 (46.5)	0	9 (20.9)	14 (32.6)
Timor	34	14 (41.2)	2 (5.9)	9 (26.5)	9 (26.5)
Tolaki	24	10 (41.7)	0	1 (4.2)	13 (54.2)