Serum Pepsinogen’s Potential Use as a Biomarker for Atrophy, Reflux Esophagitis, and Gastric Cancer Screening in Indonesia

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ABSTRACT

Background: Studies describing the benefit of pepsinogen (PG) values had been reported only in three Indonesian cities. However, to apply PG's benefits in Indonesia, a nation-wide approach is necessary. This study aimed to describe the potential usability of PG values determining gastric mucosal conditions, including superficial gastritis and anthropic gastritis.

Results: Among 646 enrolled patients, 308 (47.2%), 212 (32.8%), 91 (14.1%), 34 (5.2%) and 1 (0.2%) patients 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 $P < 0.01$). Additionally, a positive correlation was found between age and PGI ($r = 0.377$) and PGII ($r = 0.359$). PGI of gastritis and reflux esophagitis patients were significantly higher than that of normal patients ($P = 0.01$ and $P = 0.0015$, respectively). The PGI and PGII levels were significantly higher and PGI/II was significantly lower in *H. pylori* infected patients than uninfected ones (all $P < 0.001$). The optimal cutoff value for PGII and PGI/II were 12.45 ng/mL and 4.75, respectively to determine moderate-severe atrophy.

Conclusion: Serum PG levels represents a useful biomarker represents the endoscopy findings, especially for reflux esophagitis. Additionally, the benefits of PG values detecting atrophic gastritis were limited to moderate-severe atrophic gastritis. This usefulness requires careful attention for elderly patients and several ethnic groups in Indonesia.

Keywords: Pepsinogen, atrophic gastritis, reflux esophagitis, *H. pylori*, Indonesia, cancer

Background

Chronic dyspepsia's symptoms are frequently seen in primary to tertiary healthcare in Indonesia [1], with underlying pathologies such as atrophic gastritis, intestinal metaplasia, and peptic ulcers may act as the cause of the symptoms [2, 3]. 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 [4]. 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-years survival rate [5].

The diagnostic methods determining the gastric mucosa condition and *H. pylori* status include invasive techniques, through endoscopic and biopsy sampling [6]. However, this method is less comfortable, relatively risky for patients and considered as 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 and II are proposed to have good diagnostic values in predicting gastric mucosal status such as atrophic gastritis. Alongside with serological test for *H. pylori* infection, they may have a benefit as the gastric cancer screening method [7]. The combination of pepsinogens and antibody *H. pylori* examination were proposed to improve diagnostic accuracy [8]. 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 is consisting 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) [9]. 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 [10-

13]. However, reliability of PGs and ABC method are still questionable when applied in different populations and regions.

Indonesia is the fourth largest populated country worldwide. It occupies a very wide area over numerous islands. Additionally, 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%) [14], there was a difference among ethnics prevalence; a lower prevalence found in Javanese ethnic who mostly living in the urban area with endoscopic facilities. However, prevalence of *H. pylori* was high in several places, such as Jayapura (Papua island), Makassar (Sulawesi island) and Medan (Sumatra Island) [15]. These places are considered as have remote areas where have limited access to endoscopic equipment. Therefore, a non-invasive 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 [16]. However, it may not represent all of 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 nation-wide approach. In this study, we aimed to examine the reliability of serum Pepsinogen as biomarker for gastroesophageal diseases 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.

Results

Baseline Characteristic

A total of 646 subjects which consisted of 383 males and 263 females were included with the average age of 44.93±12.98 years old (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 on the **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 was 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 was Tolaki and the highest was Chinese (**Table 1**).

Table 1. PG I, PG II secretion and the determinant factor

Factors	N	Pepsinogen levels Mean [Median]		
		PGI	PGII	PGI/II
H. pylori Infection				
Positive	59	77.5 [54.4]*	19.4 [13.7]*	4.1 [4.0]*
Negative	587	61.6 [44.1]	10.2 [7.9]	6.0 [5.8]

Sex				
Male	383	61.5 [42.9]*	11.0 [7.9]	5.7 [5.5]*
Female	263	65.2 [48.6]	11.0 [8.8]	6.0 [5.8]
Age (years)				
< 18	3	34.3 [35.7]‡	7.2 [9.0]	5.7 [4.6]
18 – 29	74	47.5 [38.5]	9.1 [7.0]‡	5.4 [5.4]
30 – 39	165	47.9 [37.0]	8.3 [7.1]	5.8 [5.7]
40 – 49	155	61.5 [42.0]	11.7 [7.5]	5.7 [5.6]
50 – 59	162	76.7 [54.3]	12.3 [9.9]	6.1 [5.8]
≥60	87	83.2 [71.6]¶	14.4 [12.4]¶	6.0 [5.8]
Ethnic				
Aceh	70	46.2 [38.3]	7.9 [7.5]	5.8 [5.5]
Balinese	61	64.8 [47.7]	12.1 [9.0]	5.6 [5.5]
Bataknese	2	41.4 [41.4]	10.1 [10.1]	4.1 [4.1]‡
Bugis	69	64.4 [39.9]	10.7 [7.6]	5.8 [5.4]
Chinese	40	85.3 [61.6]¶	13.3 [10.6]¶	6.7 [6.5]¶
Dayak	6	76.5 [49.6]	12.1 [9.2]	5.8 [6.1]
Javanese	118	77.1 [62.5]	12.4 [9.5]	6.4 [6.4]
Ternatese	46	59.2 [44.2]	9.6 [7.4]	6.0 [5.9]
Malay	36	73.0 [59.1]	11.8 [9.7]	6.4 [5.8]
Minahasanese	53	47.2 [44.3]	8.3 [8.0]	5.9 [5.8]
Nias	32	61.4 [44.0]	10.3 [8.4]	6.2 [5.6]
Kaili	12	49.8 [36.4]	9.2 [7.8]	5.5 [5.3]
Papuan	43	49.6 [39.0]	12.5 [9.1]	4.7 [4.4]
Timor	34	72.4 [46.2]	14.6 [9.2]	5.2 [4.7]

Tolaki	24	42.0 [33.4]‡	9.8 [7.1]‡	4.6 [4.6]

*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

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). Additionally, 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 PUD, normal and gastritis patients (P = 0.002, P <0.001 and P <0.001, respectively) (**Table 2**).

Table 2. Pepsinogen level and Disease

Pepsinogen Level	**Normal**	**Gastritis**	**Reflux Esophagitis**	**Peptic Ulcer Disease**	**Gastric Cancer**‡
n	308	212	91	34	1
PGI	55.0±43.0	63.4±53.8	87.4±75.4	65.4±55.6	123.0
PGII	9.4±6.6	12.1±10.1	12.7±10.6	12.6±10.9	93.1
PGI/II	5.9±1.6	5.6±1.9	6.7±1.8	5.4±1.4	1.3

‡There was only one patient with gastric cancer

PG 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 ≥1. The patients with atrophic gastritis had significantly higher PGII value and significantly lower PGI/II ratio than non-atrophic 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 any significant difference of PG levels among predominant locations.

Table 3. The Pepsinogen Levels between Atrophic Status, Predominant Location and Severity of Atrophic Gastritis

	n	PGI	PG II	PGI/PGII
Atrophy status				
Non-Atrophy	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*
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
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*

* The calculation showed statistically significant (Wilcoxon rank sum test, all P < 0.05)

We also classified the 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 ≥1) in the antrum and corpus, the PGI and PGII values were significantly higher in the inflammation group than non-inflammation one (both P <0.001). The PGI/II ratio were significantly lower in inflammation group than non-inflammation one (P value <0.001).

Value of Pepsinogen for Atrophic Gastritis

Based on the criteria by Miki et al [9], values: ≤70 ng/mL for PG I level and ≤3.0 for PG I/II ratio were considered as 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 ≥1 as the positive group, we found 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 ≥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 ≥1 as a good standard. When we considered atrophic score ≥2 as the standard determining positive group, we observed AUC for PGI, PGII and PGI/II ratio were 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 ≥12.45ng/mL, the sensitivity and specificity were 59.3% and 77.1%, respectively. When utilizing the PGI/II of ≤4.75 as the cutoff, we observed the sensitivity and specificity were 81.5% and 78.7%, respectively (**Table 4**). When we considered to use either PGII or PGI/II ratio to determine moderate-severe atrophic data, we found 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 sensitivity and specificity were 55.6% and 94.9%, respectively.

Table 4. The validation parameters for PG levels determining moderate-severe atrophy in Indonesia

Disease group	Parameters	Serum Pepsinogens (95%CI)		
		PGI	**PGII**	**PGI/II**
Moderate-severe atrophy	Cutoff value	-	≥ 12.45 ng/mL	≤ 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)

Validation for H. pylori IgG and ABC method of Indonesian Patients

Following manufacturer's standard for detecting *H. pylori* infection, we observed 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. Owning considerably low sensitivity value, we determined the new cutoff value. With the AUC 0.934 (95%CI = 0.890-0.976), the optimal cutoff value for serology test in Indonesia was ≥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 (≥6.7 U/mL) and the PGs value was same (PG1 < 70 ng/mL and PGI/II ratio ≤ 3). We observed 585 patients (90.6%) were classified as group A and 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 proportion in Papuan and Timor ethnic group (11.6% and 5.8%, respectively) (**Supplementary Table 2**).

Table 5. Distribution of modified ABC Method classification among Indonesian Patients

	N	Group A (%)	Group B (%)	Group C (%)	Group D (%)
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)
Female	263	246 (93.5)	12 (4.6)	2 (0.8)	3 (1.1)
Age group					
<18	3	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)
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 (0.0)
≥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)
Reflux esophagitis	91	88 (96.7)	3 (3.3)	0 (0.0)	0 (0.0)
PUD	34	29 (85.3)	4 (11.8)	0 (0.0)	1 (2.9)
Cancer	1	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)

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Discussion

This study is the nation-wide approach study to validate the potential benefit of PG levels to determine if patients require further endoscopic examination. We observed the atrophy group has significantly lower PGI/II ratio in both *H. pylori*-positive and -negative in concordance with previous studies [16, 28]. The level of PGI in the atrophy group in this study was higher than the non-atrophy group which also reported in the previous study [29] but not in other studies in Korea and Japan [30-32]. This was probably due to the higher proportion of mild atrophy. Severe atrophy was related to the loss of glands which caused significant decrease of pepsinogen production [33]. This result was supported by remarkably lower PGI in severe atrophy compared to the mild atrophy. Furthermore, PGII value has the greater rise as the result of chronic inflammation [31, 33]. The presence of inflammation may also give the increase of PGI and PGII [30] as shown by a significant difference between inflammation and no inflammation either in the antrum or corpus. 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 [33, 34].

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 [33]. Different cutoff value was implemented to determine atrophy [35-37]. In this study, first we applied the cutoff based on the criteria by Miki et al [9]. However, the determined cutoff value from the original study had a low sensitivity and specificity as reported in several countries [38, 39], thus a validation is necessary. We calculated the new cutoff adjusted to Indonesian population. Our newly determined standard cutoff increased the ability to distinguish normal group 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. Inflammation in esophagitis can increase PGI secretion, where it mainly produced in the fundus might be related to this process [40]. Previous study reported the link between PG and endoscopy finding which showed higher PG production in the peptic ulcer and nodular gastritis and also higher PGI/II ratio in erosive esophagitis [41]. Other studies in Korean and Japanese population also showed increasing PGI/II ratio in reflux esophagitis [42, 43]. Reflux esophagitis is proposed as the causal pathology of dyspepsia in area with low prevalence of *H. pylori* infection as its possible protective mechanism to progression of *H. pylori* related disease [44]. This finding may promote the utility of PG for gastroesophageal reflux screening as suggested by prior study [45]. 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 [46]. However, our current study found there was no significant difference of 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 [47] 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 of either PGI, PGII or PGI/PGII ratio between the peptic ulcer and gastritis patients.

However, the measurement in elderly patient should be cautious. We found that PG level was affected by the age, it is in concordance with several studies [48, 49]. The level of PGI and PGII were increased as the inclining age may be due to the increasing prevalence of

superficial gastritis and *H. pylori* infection in the older age individuals [28]. In addition, the decreasing glomerular filtration rate in the elderly reduce the pepsinogen excretion [48]. 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 [50, 51]. Genetic factors plays role in determining the PG density [52]. We observed that Bataknese had lowest PGI/II ratio among all ethnic group we analyzed, suggesting that particular ethnic group more likely to have atrophic gastritis. In addition, our histologic examination analysis showed the Bataknese was grouped as intermediate risk group for gastric cancer [53]. 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 [54]. 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 [32, 33]. Several possible reasons are that *H. pylori* infection induces the somatostatin deficiency and inflammation by producing cytokines such as Leukotriene and TNFα thus increase the gastrin and gastric acid secretion. The increased gastrin secretion would later increases PG secretion [49, 55] . Infection process may also damage the mucous and chief cells which resulted in the leakage of zymogen cells and released the pepsinogen before converting to pepsin [54]. Therefore, the combination of PG with the *H. pylori* serological test is also proposed to improve gastric cancer screening, known as the ABC method [9, 56]. 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 [57]. 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 [11, 13], suggesting Indonesia is far less having gastric cancer risk than those countries. These findings were concordance with the age-standardized ratio of gastric cancer incidence from GLOBOCAN that showed 1.5/100,000 population (GLOBOCAN, 2018, available at: 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 [14].

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 resulting 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 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.

Conclusions

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 benefit represent the endoscopy finding, especially for reflux esophagitis. This usefulness needs to carefully take attention for older age.

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. These study population was also including 158 samples from our previous study [16]. 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 3cm from the *pyloric* ring, the other from the greater curvature of the corpus. Both specimens were histologically examined. Additionally, on the endoscopy's day, fasting serum was collected and stored at -20°C. Furthermore, subjects were interviewed to obtain the socio-demographic data: body mass index, smoking and drinking habits, and use of nonsteroidal anti-inflammatory drugs (NSAID). All participants signed a 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 were classified into four grades according to the updated Sydney System: 0, normal; 1, mild; 2, moderate; and 3, marked [17]. Samples with bacterial loads ≥ grade 1 were considered positive for *H. pylori*. In addition, we

To increase the accuracy of *H. pylori* detection, immunohistochemical confirmation was performed, as previously described [18]. We incubated the histology specimens using anti-α-*H. pylori* antibody (DAKO, Glostrup, Denmark) after inactivation of endogenous peroxidase activity. We then incubated for the 2nd 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 H_2O_2/diaminobenzidine substrate solution. The experienced pathologist was examining our current specimen, who also examine our other works in Myanmar, Vietnam, Bhutan, Dominican Republic, and Indonesia [19-24].

Determination of H. pylori Serology and PG Levels

Using separated sera, we measured both the *H. pylori* antibody titers with an ELISA kit (Eiken, Co. Ltd., Tokyo, Japan) and the PGI and PGII levels by using PG ELISA (Eiken), as per manufacturer's instruction. *H. pylori* was considered positive if its antibody titers were ≥ 10U/mL. The PGI and PGI/II ratio were interpreted as PG positive if the PGI levels ≤ 70ng/mL and PGI/II ratio ≤ 3.0, according to the Miki criteria, which commonly used in Japan [9]. Additionally, we performed the ABC method evaluation for gastric cancer

screening. Subjects were categorized into four groups: *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) [9].

Determination of Disease

Experienced endoscopists observed the mucosal condition of upper gastro-duodenal tract from esophagus to duodenum. Reflux esophagitis was identified based on the observation of mucosal break on the gastro-esophageal junction. The ulcerations in the stomach and duodenum were also identified based on endoscopic examination. The 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 damages, we concluded these as normal group.

As for atrophic gastritis, we simplified the classification of degree of atrophic gastritis due to small number of moderate and marked. This classification based on the highest value of atrophic gastritis score in the histological evaluation. Mild atrophic gastritis 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 the atrophic gastritis based on the topographical distribution. Individual was categorized having antral-predominant gastritis if the atrophic scores in the antrum was greater than those in the corpus, whereas if the atrophic scores in the corpus was 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 [25-27].

Data analyses

Discrete variables were tested with a chi-square test. Continuous variables were tested with the Mann–Whitney *U* and Kruskal–Wallis tests. The Spearman rank coefficients (r) were determined to evaluate the association between PG levels and gastric-mucosal inflammation and atrophy. All determinants with $P<0.10$ were jointly entered in the full model of logistic regression. The model was reduced by excluding variables with $P>0.10$. The OR and 95% confidence interval (CI) were used to estimate the risk. A P-value <0.05 was considered as statistically significant. Receiver-operating characteristic curves were used to calculate the best cutoff. These included the area under curve (AUC) and predictive values for discriminating chronic and atrophic gastritis. The SPSS statistical software package version 18.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Abbreviations

AUC: Area under curve; CI: confidence interval; ELISA: Enzyme linked Immunosorbent assay; NPV: Negative Prediction Value; NSAID: Nonsteroidal Anti-Inflammatory Drugs; PG: Serum Pepsinogen; PPV: Positive Prediction Value; PUD: Peptic Ulcer Disease; TNF-α: Tumor Necrosis Factor α.

Declarations

Ethics approval and consent to participate

All participants signed a written informed consent. The study protocol was approved by the ethics committees of Dr. Soetomo Teaching Hospital (Surabaya, Indonesia) which covers patients obtained in Surabaya (221/Panke.KKE/IX/2012), Dr. Wahidin Sudirohusodo Teaching Hospital (Makassar, Indonesia) which covers patients obtained in Makasar (0208/H4.8.4.5.31/PP36-KOMETIK/2015), Dr. Cipto Mangunkusumo Teaching Hospital (Jakarta, Indonesia) which a national ethics committee covers the rest of the obtained patients (206/112/P1/ETIK/2014), and Oita University Faculty of Medicine (Yufu, Japan) (P-12-10).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no conflicts of interest.

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Authors' contributions

MM, AFS and YY designed the study. LAW, MM, and TU performed the experiments. LAW, MM and YY analyzed the data. IAN, IDNW, YAAR, KAF, GS, FA, JBW, AMJS, AA, HM, MR, AR, YSN, ES, PA, UM, PBS, DD, TS and PSR contributed in reagents and data collection. LAW, AD, MM, TS and YY wrote the manuscript. All authors had read and approved the manuscript

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635 **Figure Legends**

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

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Serum Pepsinogen's Potential Use as a Biomarker for Atrophy, Reflux Esophagitis, and Gastric Cancer Screening in Indonesia

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