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ORIGINAL RESEARCH

Reference Ranges and Comparison of Pepsinogen by Chemiluminescence Immunoassay and Enzyme-Linked Immunosorbent Assay in Chinese Population

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Objective: Serum pepsinogen (PG) is a good indicator of atrophic changes in the gastric mucosa. Gastric mucosal atrophy is a high-risk factor for gastric cancer. Serological testing for PG combined with endoscopy can help to improve gastric cancer screening. In this study, we established the reference ranges of serum PG-I, PG-II, and the PG-I/II ratio (PGR) in the Chinese population by chemiluminescence immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA). Besides, in the real world, doctors are often confused by the results of different testing platforms. Thus, a comparison of methods CLIA and ELISA was performed.

Methods: 2904 individuals were enrolled from six regions in China as part of the Chinese Adult Digestive Diseases Surveillance (2016) program. The individuals completed questionnaires and volunteered to undergo examinations, including gastroscopy, urea breath test, abdominal ultrasound examination and routine serologic tests. Serum was collected to measure PGs (including PG-I, PG-II and PGR) by CLIA and ELISA. Participants who were found obvious abnormalities or absent from the examinations were excluded. Ultimately, 747 healthy individuals were enrolled in this study. The Kolmogorov–Smirnov test was used to assess the distribution of variables. The Kruskal–Wallis *H* or Mann–Whitney *U*-tests were used to compare different sex, age, and geographical groups. The 95% reference ranges of PGs obtained by the two methods were established according to document CLSI-EP28-A3, with covariates of sex, age, and region. Spearman correlation analysis, linear regression analysis and allowable total error (ATE) zone analysis were utilized for comparing the two methods.

Results: On overall, the 95% reference ranges of PG-I, PG-II, and PGR measured by CLIA were 23.00–110.64 ng/mL, 2.50–19.13 ng/mL, and 3.87–13.30, respectively. Meanwhile, the reference ranges of PG-I, PG-II, and PGR measured by ELISA were 36.93–205.06 ng/mL, 1.65–17.96 ng/mL, and 7.50–33.60, respectively. Both PG-I and PG-II levels measured by the two platforms were found to be influenced by sex and age. PGR measured by CLIA was influenced by age but not by sex, while PGR measured by ELISA was not affected by either age or sex. Regional factors did not significantly impact the PG results, except for PG-I detected by ELISA. Ultimately, reference ranges for PGs were established based on age and sex stratification. Additionally, the Spearman correlation analysis revealed that the correlation coefficients for PG-I, PG-II, and PGR detected by the two methods were 0.899, 0.887, and 0.777, respectively, indicating a strong correlation between the two methods. The regression equation for the PG levels detected by two methods was obtained through linear regression analysis. The ATE analysis provided a visual depiction of the consistency between the two methods, clearly indicating the poor agreement between them.

Conclusion: This study established the reference ranges of PGs by strict and intact enrollment standard. In addition, the results indicated a strong linear relationship between the two methods, yet with a clear bias, which was valuable for laboratory interpretation.

Keywords: pepsinogen, reference range, gastric cancer

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Introduction

Gastric cancer (GC) ranked fifth in incidence and fourth in cancer-related mortality worldwide in 2020.[1] The prognosis of GC is closely related to the clinical stage. Early diagnosis and treatment are crucial.[2,3] Gastroscopy is the primary method for GC screening; however, patient intolerance to the procedure and the high cost make it unsuitable for routine screening. Several studies have shown that serum pepsinogen I (PG-I), PG-II, and the PG-I/II ratio (PGR) are closely related to the state of the gastric mucosa.[4,5] Abnormal serum PGs (including PG-I, PG-II and PGR) results indicate potential presence of atrophic gastritis, which is considered a significant risk factor for GC.[5,6] Studies have shown that combining serum concentrations of PG-I, PG-II and PGR with *Helicobacter pylori* (*H. pylori*) antibody titers can stratify populations into low-risk and high-risk populations for GC. Individuals who test positive for atrophic gastritis by serum pepsinogen need to undergo endoscopic examination to detect the presence of GC.[7,8] The key advantages of pepsinogen in GC screening are its non-invasive nature, making it a convenient and cost-effective tool for population-based screening programs. Thus, establishing appropriate reference ranges of PGs is of substantial significance for GC screening.

Several studies have reported the reference ranges of PGs.[9,10] However, in these studies the enrolled individuals were absent from prior gastroscopy evaluation. Thus, the "healthy people" were apparent healthy people. Besides, these studies used only one method to assess PGs, but there are two main commercial methods for detecting PGs: chemiluminescence immunoassay (CLIA) and enzyme-linked immunosorbent assay (ELISA). However, no studies have compared these two methods. How to interpret the results obtained from the two methods is of educational significance to physicians and laboratory technicians.

Therefore, in this study, we detected serum PG-I and PG-II levels by CLIA and ELISA in healthy volunteers excluding abnormal gastroscopy and urea breath test (UBT) results. Our study aimed to (1) establish the reference ranges of PG-I, PG-II, and the PGR using two different methods and (2) compare the results of PGs on two platforms.

Participants and Methods

Participants

The Participants were randomly selected from the Chinese Adult Digestive Diseases Surveillance program in 2016, a nationally representative cross-sectional survey. The survey was conducted by random cluster sampling, and village/resident groups were randomly selected by the national working group according to probability proportionate to size sampling guidelines. Six representative regions, Beijing (1140), Guiyang (862), Harbin (294), Nanchang (288), Yichun (288), and Beihai (32), were selected, covering northern (Beijing, Harbin) to southern (Guiyang, Nanchang, Yichun, and Beihai) China. 2904 participants were recruited in strict accordance with the principle of random stratified sampling. Trained medical personnel collected data via questionnaires, anthropometric measurements, gastroscopy, UBT, abdominal ultrasound, and serologic tests. The samples were also used to measure PGs levels for this study.

The exclusion criteria were as follows: (1) Obvious abnormalities in gastroscopy: GC, gastric ulcer, and chronic atrophic gastritis. (2) Obvious abnormalities in UBT: *H. pylori* infection. (3) Obvious abnormalities in abdominal ultrasound: abdominal tumors. (4) Obvious abnormalities in serologic tests: alanine aminotransferase (ALT)>100 U/L (2-fold of upper normal limit), total cholesterol (TC)>11.4 mmol/L (2-fold of upper normal limit), triglycerides (TG)>6.8 mmol/L (4-fold of upper normal limit). (5) Absence of these examinations. The flowchart illustrating participants' inclusion is shown in Figure 1.

Collection of Blood Samples

Nine milliliters of venous fasting blood were collected from each participant. The samples were centrifuged at 1500 ×*g* for 10 min to obtain serum samples. All serum samples were frozen at −80°C immediately and then sent to the central laboratory in Beijing via cold-chain transportation for subsequent detection.

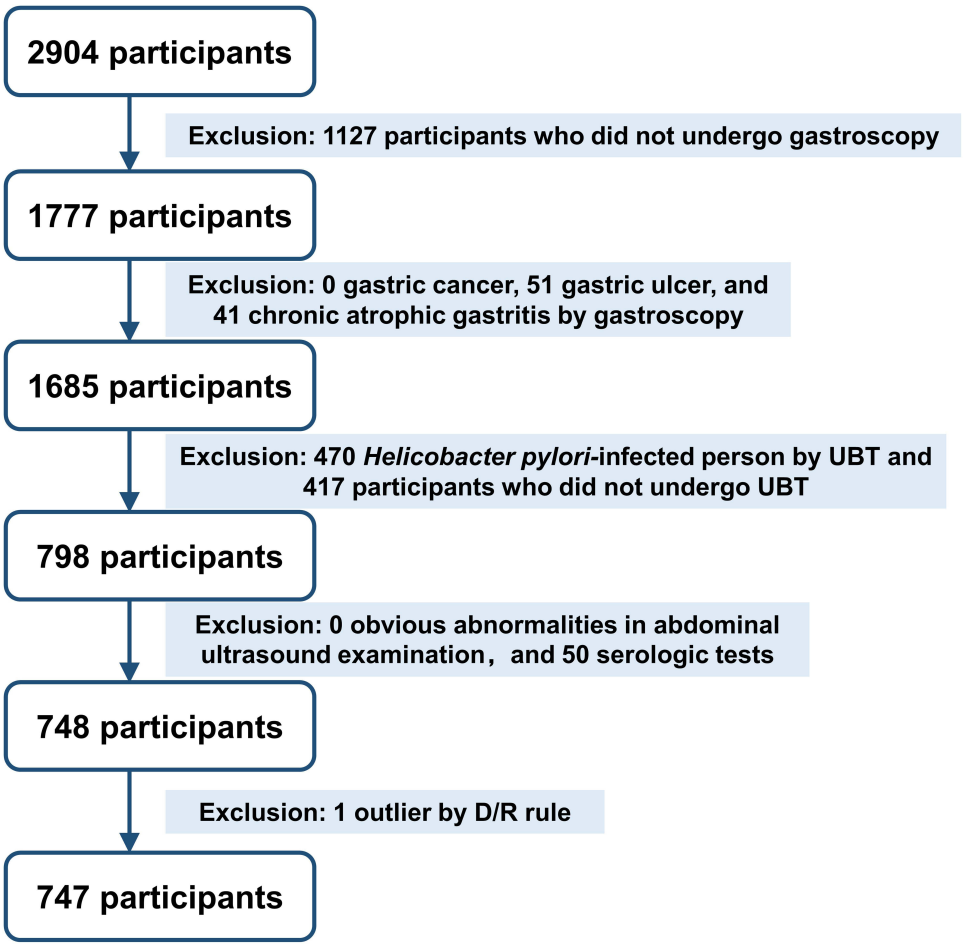

Figure 1 Flowchart for participants' inclusion.
Notes: The method of D/R rule to exclude outliers was adapted from Horowitz et al.[11]
Abbreviations: UBT, urea breath test.

Examination Methods

UBT

The UBT is a method for *H. pylori* detection that was established based on the ability of the *H. pylori* urease enzyme to break down an isotope-labeled urea solution ingested by the patient into carbon dioxide and ammonia.[12]

The exhaled gas was collected from the enrolled individuals on an empty stomach for the[13] C breath test. The breath was stored at room temperature and sent to the medical inspection institution designated by the national project team by cold-chain transportation for detection on the second day.

Abdominal Ultrasound Test

The centers for disease control of each district summoned the participants to the local hospital for abdominal ultrasound examination (bile duct, gallbladder, and liver) (15–20 persons per batch). All participants were examined on an empty stomach (no eating or drinking for 6 h; patients with high blood pressure could take their medication with a small amount of water) by qualified ultrasound doctors. The results were reported via the information collection and management system.

Gastroscopy

Gastroscopy was performed after the participants completed an abdominal ultrasound examination. All participants were examined on an empty stomach (no eating or drinking for 6 h; patients with high blood pressure could take their

medication with a small amount of water) by qualified gastroenterologists, and the results were reported via the information collection and management system.

Laboratory Analyses

Serum PG-I and PG-II levels were measured using an automated CLIA analyzer (Abbott ARCHITECT i2000) and commercial ELISA tests (Biohit, Finland) with the appropriate reagents, calibrators, and quality controls. All tests were performed according to the manufacturers' guidelines.

Carbohydrate antigen 72–4 was measured using Abbott ARCHITECT i2000. ALT, TC, and TG were measured using a Beckman AU5800 instrument with Beckman and Sekisui Medical reagents.

Statistics

95% reference ranges were determined according to the document CLSI-EP28-A3.[11] The outlying observations were deleted by D/R rule.[11] The 2.5th percentile ($P_{2.5}$) and the 97.5th percentile ($P_{97.5}$) of the PGs results were listed as bilateral 95% reference ranges. The 5th percentile (P_5) of the PGs results were also listed as unilateral 95% reference ranges. The Kolmogorov–Smirnov test was used to assess the distribution of variables. The Kruskal–Wallis *H* or Mann–Whitney *U*-tests were used to compare groups in non-Gaussian distributed data. Spearman correlation analysis was utilized to evaluate the correlation between two methods. The closer the absolute value of the correlation coefficient is to 1, the stronger the correlation between the two variables. Linear regression analysis was employed to derive regression equation. Allowable total error (ATE) zone analysis, based on a graphical technique and simple calculation, was applied to assess the consistency between two methods. 80% of the data fall in the ATE zone and cluster around the bisector, indicating that the two methods agree well. IBM SPSS Statistics 26.0 and GraphPad Prism 8.0 were used for the statistical analyses and data plotting. Two-sided *p*-values < 0.05 were considered to indicate significance. We divided the participants into three age groups according to the World Health Organization's age classification criteria: 18–44 years old, 45–59 years old, and ≥60 years old.

Results

The Baseline Characteristics of Participants

After applying the strict exclusion criteria, the total number of enrolled participants was 747 (age: 19–64 years; 298 male participants, 449 female participants). The baseline characteristics of participants were shown in Table 1.

Concentration of PGs in Healthy Individuals and Affecting Factors

The concentrations of PG-I measured by CLIA, PG-II measured by CLIA, PG-I measured by ELISA, and PG-II measured by ELISA were higher in males than in females ($p<0.05$), whereas PGR measured by CLIA and PGR measured by ELISA showed no significant differences between males and females ($p>0.05$) (Table 2).

Table 1 The Baseline Characteristics of Participants

Characteristics	Male	Female
No of individuals (n)	298	449
Age (years)[a]	42 (30, 51)	42 (32, 52)
Geographical location (Northern China/Southern China)	114/184	203/246
Body mass index (kg/m^2) [a]	24.67 (22.14, 27.30)	24.12 (21.52, 26.20)
Alanine aminotransferase (U/L) [a]	17.00 (11.00, 25.00)	12.00 (8.00, 16.00)
Total cholesterol (mmol/L) [a]	4.76 (4.05, 5.35)	4.56 (3.76, 5.32)
Triglycerides (mmol/L) [a]	1.31 (0.79, 1.96)	1.06 (0.69, 1.69)
Carbohydrate antigen 72–4 (U/mL) [a]	1.90 (1.00, 4.30)	2.20(1.10, 4.95)

Notes: [a]Values are expressed as median (first quartile, third quartile).

Table 2 Comparison of Serum PG-I Measured by CLIA, PG-II Measured by CLIA, PGR Measured by CLIA, PG-I Measured by ELISA, PG-II Measured by ELISA, and PGR Measured by ELISA by Sex, Age, and Geographical Location

Characteristic	Number	PG-I measured by CLIA (ng/mL)		PG-II measured by CLIA (ng/mL)		PGR measured by CLIA		PG-I measured by ELISA (ng/mL)		PG-II measured by ELISA (ng/mL)		PGR measured by ELISA	
		M (Q1, Q3)	*p*	M (Q1, Q3)	*p*	M (Q1, Q3)	*p*	M (Q1, Q3)	*p*	M (Q1, Q3)	*p*	M (Q1, Q3)	*p*
Sex			0.00		0.00		0.968		0.00		0.00		0.868
Male	298	50.50 (41.90, 64.83)		6.50 (4.90, 8.80)		8.05 (6.60, 9.40)		94.45 (76.67, 122.05)		4.81 (3.54, 6.91)		19.73 (15.50, 23.45)	
Female	449	42.10 (34.40, 54.45)		5.50 (4.10, 7.30)		8.00 (6.70, 9.45)		76.73 (61.22, 101.31)		3.82 (2.91, 5.60)		19.37 (16.03, 23.46)	
Age			0.00		0.00		0.00		0.00		0.00		0.056
18–44	428	43.55 (35.80, 55.78)		5.40 (4.10, 7.10)		8.30 (7.10, 9.70)		78.65 (62.13, 103,40)		3.95 (2.93, 5.64)		19.99 (16.29, 23.62)	
45–59	253	49.70 (40.25, 63.00)		6.40 (4.85, 8.85)		7.70 (6.50, 9.15)		90.16 (73.47, 118.57)		4.69 (3.37, 6.91)		18.96 (15.49, 23.62)	
≥60	66	47.65 (36.28, 65.40)		7.00 (5.20, 9.93)		7.30 (5.70, 8.70)		97.78 (70.42, 130.59)		5.14 (3.48, 8.16)		17.57 (14.66, 22.81)	
Geographical location			0.525		0.236		0.087		0.001		0.109		0.491
Northern China	317	46.50 (36.50, 60.40)		5.90 (4.35, 8.65)		7.90 (6.40, 9.30)		88.96 (69.40, 118.78)		4.39 (3.14, 6.61)		19.88 (15.55, 24.44)	
Southern China	430	45.65 (37.38, 57.60)		5.60 (4.38, 7.80)		8.15 (6.90, 9.50)		80.42 (64.60, 103.68)		4.11 (3.06, 5.84)		19.32 (16.03, 23.09)	

Abbreviations: CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; PG-I, pepsinogen I; PG-II, pepsinogen II; PGR, the PG-I/II ratio; M (Q1, Q3), median (first quartile, third quartile).

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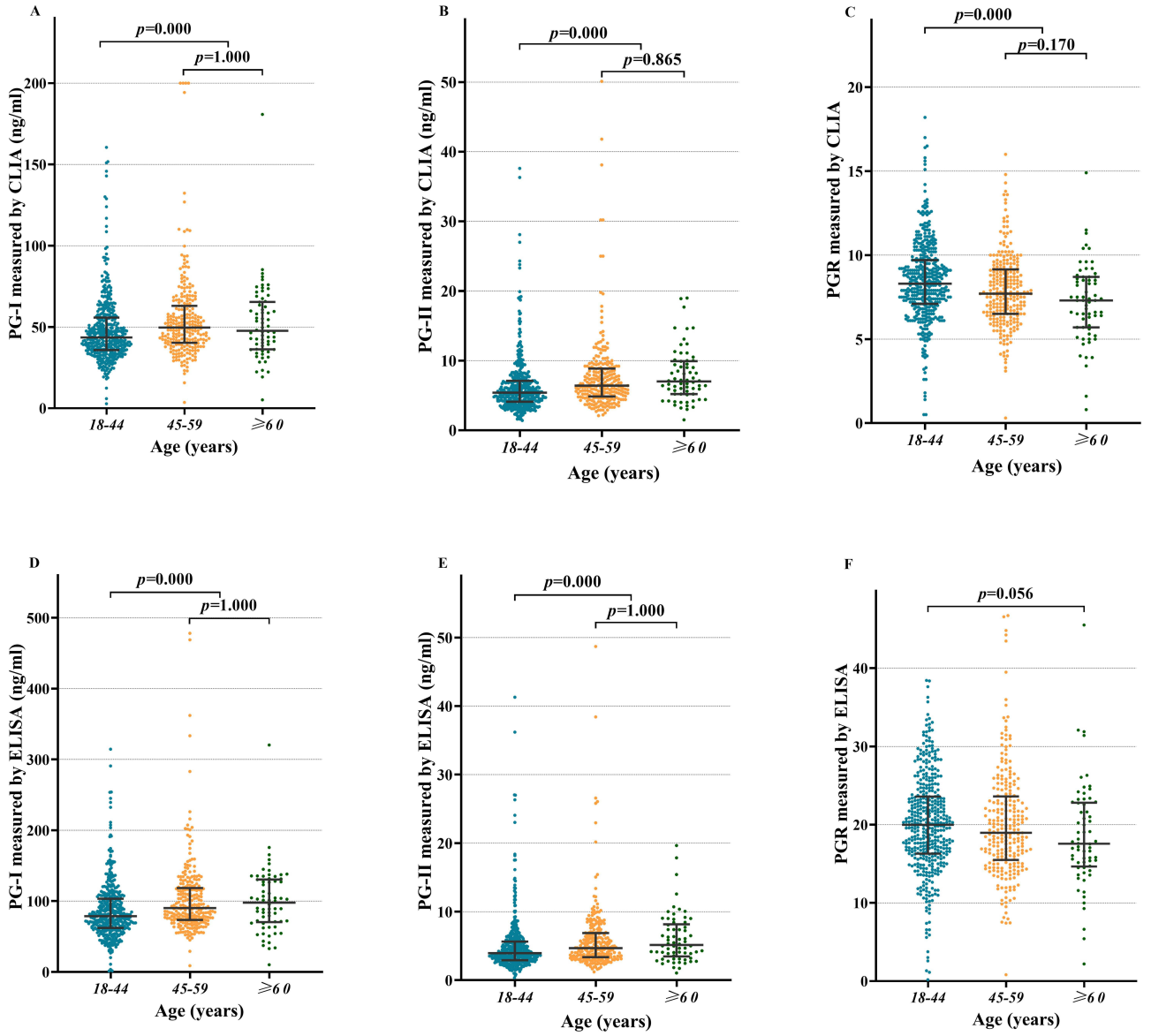

Figure 2 Comparison of serum PGs among different age groups. (**A**) Comparison of serum PG-I measured by CLIA among different age groups. (**B**) Comparison of serum PG-II measured by CLIA among different age groups. (**C**) Comparison of serum PGR measured by CLIA among different age groups. (**D**) Comparison of serum PG-I measured by ELISA among different age groups. (**E**) Comparison of serum PG-II measured by ELISA among different age groups. (**F**) Comparison of serum PGR measured by ELISA among different age groups.

Abbreviations: CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; PGs, pepsinogens (including PG-I, PG-II and PGR); PG-I, pepsinogen I; PG-II, pepsinogen II; PGR, the PG-I/II ratio.

As shown in Table 2 and Figure 2, the levels of PG-I measured by CLIA increased with age but gradually decreased after age 60 (Figure 2A). PG-II measured by CLIA, PG-I measured by ELISA, and PG-II measured by ELISA consistently increased with age (Figure 2B, D, and E), while PGR measured by CLIA decreased with age (Figure 2C). There was no difference in PGR measured by ELISA among the different age groups (Figure 2F). Moreover, the Mann–Whitney *U*-test revealed no significant difference in all PGs Results between the 45–59 and >60 age groups ($p>0.05$). Therefore, the participants were divided into 18–44 and ≥ 45-year groups.

The six regions in this study (Beijing, Harbin, Guiyang, Nanchang, Yichun, and Beihai) were divided into two groups, southern China and northern China, according to the country's geographic dividing line. The Kruskal–Wallis *H*-test was used to assess the contribution of these regions to the PGs results. PG-I measured by ELISA was significantly higher in

northern China than in southern China ($p<0.05$), while other PGs results were not affected by geographical location ($p>0.05$) (Table 2).

Comparison of the Results of Serum PG-I, PG-II, and the PGR Between CLIA and ELISA

Linear regression analysis revealed that the linear regression equation for PG-I is PG-I measured by ELISA = 1.815 * PG-I measured by CLIA + 0.827. Spearman correlation analysis revealed that the correlation coefficient is 0.899 ($p<0.001$). The linear regression equation for PG-II is PG-II measured by ELISA = 0.968 * PG-II measured by CLIA − 1.249, and the correlation coefficient is 0.887 ($p<0.001$). The linear regression equation for PGR is PGR measured by ELISA = 2.213 * PGR measured by CLIA + 1.918, and the correlation coefficient is 0.777 ($p<0.001$).

To compare the consistency of CLIA and ELISA methods, we also refer to another graphical method—allowable total error and limits for erroneous results (ATE-LER) zones—as recommended by the United States Food and Drug Administration (FDA).[13] According to the criteria for external quality control established by the National Center for Clinical Laboratories in China, the allowable total errors of PG-I, PG-II, and PGR were target value±30%, and 80% of the data should be within the allowable total errors. Based on this standard, the ATE zone is the gray area between the lines y=−30%*x and y=30%*x in this study (Figure 3). However, only fewer PG-I (2.54%) (Figure 3A), PG-II (62.12%) (Figure 3B), and PGR (0.80%) (Figure 3C) data points fell in the ATE zones. Thus, CLIA and ELISA exhibit poor agreement. Due to the lack of an LER cut-off value, we did not set the LER zones.

Establishment of the Reference Ranges for PGs on Two Platforms

Bilateral reference ranges and unilateral reference ranges of serum PG-I, PG-II, and the PGR by ELISA and CLIA were established for the Chinese population. The reference ranges were stratified by sex and age group (Table 3).

Discussion

The gold standard for determining gastric mucosal lesions is histological examination. Considering the difficulty in obtaining gastric mucosal tissue samples, more convenient serum samples are a valuable alternative for atrophic gastritis and GC screening. PG-I and PG-II secreted by gastric mucosa cells are proenzymes for pepsin. PG-I is secreted by glands in the gastric fundus and body, and PG-II is secreted in the gastric antrum and duodenum.[14] Both PG-I and PG-II decrease in gastric atrophy. PG-I usually shows a more marked decrease than PG-II. Thus, a low PG-I level, a low PGR, or both are good indicators of atrophic changes in the gastric mucosa.[14,15] Moreover, atrophic gastritis are associated with elevated GC risk.[16,17] It is also well known that the majority of GC is caused by *H. pylori* infection.[18] Therefore, Japanese researchers developed the ABC (D) screening program that combines PGs (PG-I < 70 ng/mL and PGR < 3.0 as positive) atrophic markers and the anti-*H. Pylori* IgG etiological marker to stratify high-risk patients for further follow-up; however, the sensitivity and specificity of this approach need to be further improved.[19] A meta-analysis showed that

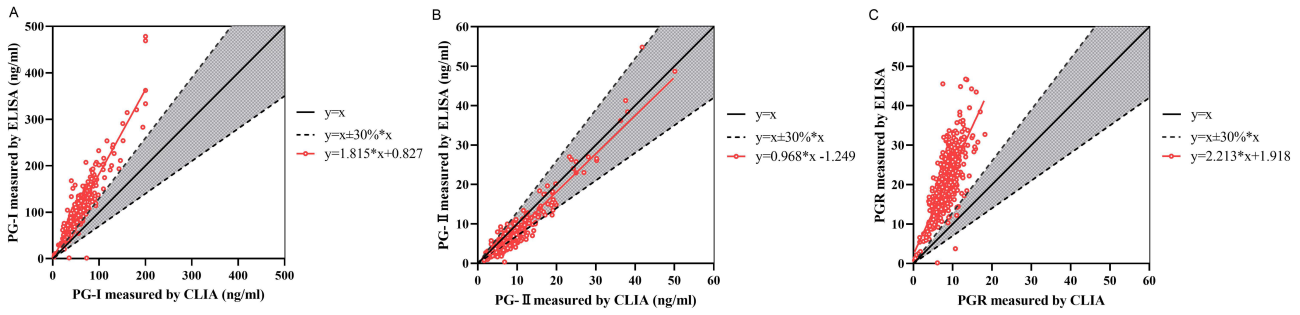

Figure 3 Consistencies between PGs measured by CLIA and ELISA using allowable total error zone analysis. (**A**) Consistencies between serum PG-I measured by CLIA and ELISA using allowable total error zone analysis. (**B**) Consistencies between serum PG-II measured by CLIA and ELISA using allowable total error zone analysis. (**C**) Consistencies between serum PGR measured by CLIA and ELISA using allowable total error zone analysis.

Abbreviations: CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; PGs, pepsinogens (including PG-I, PG-II and PGR); PG-I, pepsinogen I; PG-II, pepsinogen II; PGR, the PG-I/II ratio.

Table 3 Reference Ranges of PG-I Measured by CLIA, PG-II Measured by CLIA, PGR Measured by CLIA, PG-I Measured by ELISA, PG-II Measured by ELISA, and PGR Measured by ELISA by Sex and Age

Variables	Sex	Age group	N	Bilateral reference ranges ($P_{2.5}$–$P_{97.5}$)	Unilateral reference ranges (>P_5)
PG-I measured by CLIA (ng/mL)	M	18–44	175	29.84–127.66	>31.86
		≥45	123	33.31–193.23	>35.42
	F	18–44	253	19.51–89.96	>23.41
		≥45	196	21.15–111.36	>23.37
	Total		747	23.00–110.64	>26.28
PG-II measured by CLIA (ng/mL)	M	18–44	175	2.54–18.82	>2.98
		≥45	123	3.32–37.31	>3.72
	F	18–44	253	1.94–19.17	>2.50
		≥45	196	2.69–17.79	>3.10
	Total		747	2.50–19.13	>2.90
PGR measured by CLIA	M+ F	18–44	428	3.74–13.44	>5.00
	M+ F	≥45	319	3.80–13.30	>4.30
	Total		747	3.87–13.30	>4.73
PG-I measured by ELISA (ng/mL)	M	18–44	175	45.54–228.50	>52.90
		≥45	123	61.73–348.64	>64.84
	F	18–44	253	27.30–166.17	>35.75
		≥45	196	33.87–203.51	>48.01
	Total		747	36.93–205.06	>42.20
PG-II measured by ELISA (ng/mL)	M	18–44	175	1.71–17.54	>2.13
		≥45	123	2.47–37.18	>2.64
	F	18–44	253	1.42–17.47	>1.64
		≥45	196	1.71–15.41	>1.94
	Total		747	1.65–17.96	>1.93
PGR measured by ELISA	Total		747	7.50–33.60	>10.32

Abbreviations: CLIA, chemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; PG-I, pepsinogen I; PG-II, pepsinogen II; PGR, the PG-I/II ratio; M, male; F, female; N, number; $P_{2.5}$, 2.5th percentile; P_5, 5th percentile; $P_{97.5}$, 97.5th percentile.

the cut-off values to predict atrophy vary across countries and detection methods.[20] Therefore, it is important to establish appropriate PGs reference ranges according to different countries and detection methods.

In this study, we enrolled 747 healthy participants from a nationally representative population following strict exclusion criteria. Then, we set up the respective reference ranges of PG-I, PG-II, and the PGR stratified by age, sex, and method (Table 3). Several studies on the reference ranges of PGs have been reported.[9,10] The strengths of this study are as follows: (1) this study is part of the national Chinese Adult Digestive Diseases Surveillance program. Participants were recruited by random stratified sampling from six representative regions of China, covering the north to south regions. Thus, the conclusion could represent the whole Chinese population. Other reports were performed in a single region of China or other countries. (2) The exclusion criteria in this study were strict. Since all the 747 participants included in this study performed gastroscopy and UBT, those with gastric disease were ruled out accurately. The reference ranges from previous studies may be inappropriate for the enrollment of patients with gastric diseases. (3) The various methods for PGs level analysis may lead to differences in results. At least four PGs methods have been reported, namely CLIA, ELISA, time-resolved fluorescence analysis, and latex-enhanced immunoturbidimetry. However, no studies have compared PGs results between the two mainstream commercial Methods: CLIA and ELISA.

Consistent with previous studies, this study showed that the PGs results were affected by sex and age.[21–23] As shown in Table 2, the serum PG-I and PG-II levels of males were significantly higher than those of females, while there was no difference in PGR between males and females. Iijima et al[24] demonstrated that the level of gastric acid secretion was higher in males than in females due to the inhibitory effect of estradiol on acid secretion.[25] Moreover, in *H. pylori*-negative participants, the serum pepsinogen concentrations correlated well with acid secretion, but there was no relationship between the PGR and acid secretion.[26] This finding may explain the PGs differences between the different sexes.

As shown in Table 2 and Figure 2, the levels of PG-I measured by CLIA increased with age but gradually decreased after age 60, similar to Sun's findings.[21] However, the levels of PG-I measured by ELISA consistently increased with age, similar to Hong's results.[23] Although serum pepsinogen concentrations were well correlated with acid secretion,[26] the effect of age on gastric acid secretion is controversial. Iijima[24] showed that aging is positively associated with gastric acid secretion in *H. pylori*-negative individuals, while K Haruma[27] found that advancing age does not influence gastric acid secretion in such individuals. In our study, PG-I measured by CLIA and PG-I measured by ELISA showed different trends with age, which requires further research. Besides, with both methods, PG-II levels gradually increased with age, and the PG-II levels were more significant than the PG-I levels, resulting in the gradual decline of the PGR with age.

The correlation coefficients of PG-I, PG-II, and the PGR between CLIA and ELISA were 0.899, 0.887, and 0.777, respectively, indicating a certain correlation between the two methods. Similar conclusions have been obtained in previous studies.[28,29] Chiang et al[28] compared the ELISA method and latex-enhanced turbidimetric immunoassay produced by GastroPanel (Helsinki, Finland) and LZ-Test (Tokyo, Japan) in the Taiwan population. The coefficient factor of PGR neared 0.7. Leja et al[29] studied a Latvian population using two ELISA assays and a latex agglutination assay produced by Biohit (Finland), Vector Best (Russia) and Eiken (Japan), respectively. The correlation of PGs showed a wide range of 0.79–0.90.

However, a high correlation coefficient can only indicate that the two methods are linearly related rather than in agreement with each other.[30] Considering the drawbacks of the correlation coefficient, the FDA suggested ATE-LER zones to obtain more accurate results.[29,30] This study refers to the approach. As shown in Figure 3A and C, most red points are above the ATE zone, meaning that the ELISA method for PG-I and the PGR deviates over 30% from the CLIA method. As shown in Figure 3B, although the systematic deviation is slight, only 62.12% of the red points are in the ATE zone, far below 80%. Overall, the two methods exhibit a strong linear relationship but less consistency. This finding can provide valuable insights for clinicians interpreting laboratory results. (1) Due to the poor consistency, it is advisable to establish distinct reference ranges for PGs detected by different methods. Clinicians can choose appropriate reference ranges based on the testing platform. (2) When PGs are used for continuous monitoring, it is recommended to consistently use the same testing platform. In cases where historical data includes results from both ELISA and CLIA, linear regression equations can be utilized by clinicians to convert results between the two methods, given their strong linear correlation.

Our study has several limitations. First, the reference range of PG-I measured by ELISA for southern China and northern China respectively could not be established due to the limited enrolled individuals. PG-I measured by ELISA was affected by geographical location, while the other PGs results were not. Second, Due to the lack of a gold standard for comparison, it is unknown which method is closer to the true value. Third, participants were recruited using a method of random stratified sampling, with the aim of reducing sampling bias. However, potential sampling bias may still exist. Fourth, due to limited funding support and personnel, as well as considerations for minimizing patients' safety, gastroscopic biopsy was not performed in this study.

Notwithstanding these limitations, this study provides a reliable reference range investigation and method comparison for PGs using two platforms. To the best of our knowledge, this is the first study to use gastroscopy to screen apparently healthy individuals to establish PGs reference ranges.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

This study was approved by the Ethical Committee of Peking Union Medical College Hospital (S-T272) and was conducted according to the principles of the Declaration of Helsinki. Informed consent was obtained from all subjects and/or their legal guardian(s).

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Disclosure

The authors declare that they have no competing interests.

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