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EXPRESSION OF GASTRIN-17, PEPSINOGEN I AND II AND *HELICOBACTER PYLORI* IgG IN A SRI LANKAN DYSPEPTIC PATIENT POPULATION USING GASTROPANEL

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ABSTRACT

Objective: This study was aimed to assess the usefulness of Gastropanel assay, an ELISA-based non-invasive diagnostic technique, in diagnosis of *H. pylori* infection and disease severity in a Sri Lankan population. **Methods:** Blood specimens were collected from dyspeptic patients attending routine upper-gastrointestinal endoscopy at a tertiary care hospital. Serum Gastrin-17, Pepsinogen I and II were measured using the Gastropanel assay (Biohit Oyj, Finland). *H. pylori* infection was diagnosed using Gastropanel *H. pylori*-IgG (Biohit Oyj, Finland), histology and *H. pylori*-IgG (Bioactiva Diagnostica, Germany). Antral biopsies were histologically graded to determine the disease severity. **Results:** Among the dyspeptic population, *H. pylori* infection was diagnosed in 22 patients by histology and in another 15 by *H. pylori*-IgG assays. The expression of G-17, PG I and II did not show a significant difference between histologically confirmed *H. pylori*-positive patients and *H. pylori*-negative patients. The PG I/II ratio were normal in the

study population. Gastropanel assay identified 1 patient of the 22 histology positive patients as having atrophic gastritis of the antrum who was histologically diagnosed as mild antral gastritis while the other 21/22 were categorized as active *H. pylori* infection. Of the 44 *H. pylori*-negative dyspeptic patients, 15 were categorized as having healthy mucosa. However, 14/15 were diagnosed with mild antral gastritis while 1 patient had moderate to severe antral gastritis by histology. **Conclusions:** The results of histology and Gastropanel assay were not comparable in this population from Sri Lanka. Larger study for validation of cut off values for the local population is needed.

KEYWORDS: *Helicobacter pylori*, Gastropanel, ELISA, Sri Lanka.

INTRODUCTION

Helicobacter pylori infection causes dyspepsia which can develop into gastritis, gastric ulcers and gastric cancer.[1] Currently in Sri Lanka diagnosis of upper gastrointestinal diseases are usually carried out by endoscopy and histological investigations on gastric biopsies which is invasive and time consuming. Gastropanel kit is an ELISA (Enzyme linked immunosorbant assay) based technique which has recently been introduced as an alternative non-invasive diagnostic test to determine the stomach mucosal health.[2] Further this test has potential as a screening method to identify patients who are at high risk of developing gastric cancer thereby justifying endoscopic investigations for those at risk.[3]

This kit is based on the determination of three serum biomarkers; Gastrin-17 (G-17), Pepsinogen I (PG I) and Pepsinogen II (PG II) together with *Helicobacter pylori* IgG which serve as indicators for mucosal status of the antrum and the corpus of the stomach. Serum hormone G-17 is a well-known biomarker for antral atrophic gastritis while PG I and PG I/II ratio are biomarkers for atrophic gastritis in the corpus.[4,5] Gastrin-17 is produced by the G cells in the antrum and is known to regulate gastric acid secretion by parietal cells and promotes growth of the gastric epithelia.[6,7] Atrophy in the gastric antrum results in reduced expression of G-17.[8] Pepsinogen I is exclusively secreted by the chief and mucous neck cells of the corpus and the fundus.[9] The expression of this hormone reflects the histological status of the corpus. Pepsinogen II is produced by the chief cells and mucous neck cells in corpus along with pylori glands in antrum and Brunner's glands in proximal duodenum.[10] The serum level of PG II reflects the structure and function of the overall stomach. The PG I/II ratio together with the PG I expression is of value in diagnosis of the atrophy of the gastric body mucosa.

MATERIALS AND METHODS

Ethical approval

Ethical approval was obtained from the Ethics Review Committee of University of Sri Jayewardenepura (Ref no. 14/15) and Colombo South Teaching Hospital (Application no. 450).

Patient selection

Dyspeptic patients attending routine upper gastrointestinal endoscopy at a tertiary care hospital in Sri Lanka were enrolled in the study after obtaining written informed consent.

Specimen collection and processing

During endoscopy, 2 gastric biopsies from the antrum and a whole blood specimen was collected from each patient. The specimens were transported to the Department of Pathology and Department of Microbiology at a state university in Sri Lanka. The biopsy specimens were dehydrated using an alcohol gradient, embedded in paraffin wax and sectioned in to 4 micron sections which were placed on clean glass slides. The sections were stained with Hematoxylin and Eosin stain and Giemsa stain before being graded according to the updated Sydney system by a consultant pathologist. The blood specimen was centrifuged and serum was separated into aliquots which were stored in -80^0C until the Gastropanel assay was performed.

Gastropanel assay

The specimens were first thawed in a room temperature water bath and placed in crushed ice. All standard and microplates were allowed to reach room temperature. The serum specimens were diluted according to the manufacturers' instructions. Blank solution, calibrators, negative and positive controls and specimens were pipetted into the wells. Each specimen and standard was added in duplicate. The plate was covered with incubation cover and incubated according to the instructions given in the assay. After incubation, the wells were washed with diluted washing buffer 3 times. Conjugate solution was added and the plate was incubated after covering with incubation cover. The plate was again washed with diluted washing buffer and the substrate solution was added. The plate was covered with aluminum foil and allowed to incubate. Stop solution was added before measuring the absorbance at 450 nm.

RESULTS

The expression of G-17, PG I, PG II and PG I/II ratio was compared in the 22 histologically confirmed *H. pylori*-positive patients and 44 patients *H. pylori*-negative by histology.

Association of G-17 expression with *H. pylori* infection and severity of antral gastritis

Mucosal G-17 levels are associated with the antral structure and function. Based on the expression levels, validated by the Gastropanel assay, the G-17 expression was categorized into 3 groups; Low (<1 pmol/L), normal (1-7 pmol/L) and high (>7 pmol/L). The mean G-17 concentrations in the *H. pylori*-positive and negative groups are described in Table 1. No significant association was seen for G-17 expression levels (low, normal and high) among *H. pylori*-positive and negative groups using the Mann Whitney U test.

Table 1: Expression of G-17 among *H. pylori*-positive and *H. pylori*-negative patients.

G-17 Expression (pmol/L)	*H. pylori*-positive patients			*H. pylori*-negative patients			P value
	N	Mean±SD	Range	N[1]	Mean±SD	Range	
<1	2	0.92±0.09	0.85-0.99	3	0.73±0.20	0.54-0.94	0.4000
1-7	12	2.66±1.34	1.08-4.72	26	3.37±1.73	1.12-6.89	0.2152
>7	8	17.04±12.38	7.17-44.31	15	20.97±12.33	7.17-38.21	0.5479

N=no. of patients *H. pylori*-positive by histology; N[1]=no. of patients *H. pylori*-negative by histology.

Among the *H. pylori*-positive dyspeptic patient population, only 2 patients had Low G-17 expression (0.92 pmol/L) and both were diagnosed as having mild antral gastritis while in the *H. pylori*-negative patient group 3 patients had mild antral gastritis (0.73 pmol/L). In patients with normal G-17 expression, 8 *H. pylori*-positive patients (2.97pmol/L) were diagnosed with mild antral gastritis and 4 patients (2.09pmol/L) were diagnosed with moderate to severe antral gastritis. In the *H. pylori*-negative dyspeptic patient population having normal levels of G-17 expression, 25 patients had mild antral gastritis (3.4pmol/L) while only one patient had moderate to severe antral gastritis. In the high G-17 expression group 3 *H. pylori*-positive patients were diagnosed with mild antral gastritis (12.39pmol/L) while 5 patients had moderate to severe antral gastritis (19.83pmol/L). In comparison, among *H. pylori*-negative patients 15 had mild antral gastritis (20.97 pmol/L) while none had moderate to severe antral gastritis (Table 2). Association of G-17 expression did not have a significant association with the severity of antral gastritis in *H. pylori*-positive patients.

Table 2: Association of G-17 with severity of antral gastritis in the study population.

G-17 expression	*H. pylori*-positive patients				P value	*H. pylori*-negative patients				P value
	N^2	Mild antral gastritis	N^3	Moderate to severe antral gastritis		N^4	Mild antral gastritis	N^5	Moderate to severe antral gastritis	
<1 pmol/L	2	0.92±0.09	-	-	-	3	0.73±0.20	-	-	-
1-7 pmol/L	8	2.94±1.52	4	2.09+0.82	0.3899	25	3.40±1.76	1	-	-
>7 pmol/L	3	12.39±8.39	5	19.83+14.38	0.3929	15	20.97±12.33	-	-	-

N^2=*H. pylori*-positive patients with mild antral gastritis; N3=*H. pylori*-positive patients with moderate to severe antral gastritis; N^4=*H. pylori*-negative patients with mild antral gastritis; N^5=*H. pylori*-negative patients with moderate to severe antral gastritis.

Association of PG I expression with *H. pylori* infection

PG I secretion is reduced in the presence of mucosal damage resulting in loss of chief cells. Thus the severity of antral gastritis does not have a direct effect on PG I expression and the association of gastric severity with PG I was not analyzed in this study as a corpus biopsy was not obtained. The Gastropanel kit has grouped the PG I expression levels into three reference categories; low (<30 µg/L), normal (30-160 µg/L) and high (>160 µg/L).

In patients with normal PG I expression (30-160 µg/L), the mean PG I level was 79.24 µg/L in both *H. pylori*-positive and negative patient groups. In the patients with high PG I expression (>160 µg/L), *H. pylori*-positive patients had a mean PG I expression of 216 µg/L compared to *H. pylori*-negative patients 208.6 µg/L (Table 3). No significant association was observed between *H. pylori*-positive and negative patients with normal or high PG I expression. None of the patients had low PG I expression in this study, indicating absence of corpus atrophy.

Table 3: Expression of PG I among *H. pylori*-positive and *H. pylori*-negative patients.

PGI Expression (µg/L)	*H. pylori*-positive patients			*H. pylori*-negative patients			P value
	N	Mean+SD	Range	N^1	Mean+SD	Range	
30-160	16	79.24±24.43	39.95-142.3	33	79.24±29.26	44.69-122.7	0.8084
>160	6	216±52.08	162.1-273.3	11	208.6±43.23	160.8-261.9	0.5908

N=no. of patients *H. pylori*-positive by histology; N^1=no. of patients *H. pylori*-negative by histology.

Association of PG II expression with *H. pylori* infection and disease severity

Pepsinogen II (PG II) is secreted by the chief cells and mucous neck cells in corpus along with pylori glands in antrum and Brunner's glands in proximal duodenum.[10] Damage to the

mucosa of the stomach or duodenum can affect the PG II expression in an individual. The Gastropanel assay classifies PG II expression into three groups as low (<3 µg/L), normal (3-15 µg/L) and high (>15 µg/L).

None of the individuals had low PG II levels. Among *H. pylori*-positive patients with normal PG II expression (3-15 µg/L), individuals diagnosed with mild antral gastritis (n=9) had a mean PG II concentration of 9.45±3.44 µg/L and patients with moderate to severe antral gastritis (n=7) had a mean concentration of 9.36±3.69 µg/L. No significant difference in PG II expression was found between these two groups (p>0.9999). Among *H. pylori*-negative patients with normal PG II levels a mean expression of 8.29±2.62 µg/L was observed and 34 patients were diagnosed as having mild antral gastritis while only one patient was diagnosed as having moderate to severe antral gastritis. In patients with high PG II expression (>15 µg/L), the mean expression of PG II among *H. pylori*-positive patients was 33.84 µg/L while the mean expression was 22.79 µg/L among *H. pylori*-negative patients (Table 4). Among the 6 *H. pylori*-positive patients with high PG II levels, 4 had mild antral gastritis (23.71±5.93 µg/L) while 2 had moderate to severe antral gastritis (54.12±17.23 µg/L). In comparison, all 9 patients in the *H. pylori*-negative patient population had mild antral gastritis (22.79±6.21 µg/L). Although statistical analysis could not be carried out due to the small sample size *H. pylori*-positive patients with moderate to severe antral gastritis had more than 2 fold expression of PG II as *H. pylori*-positive patients having mild antral gastritis. Mann Whitney U test was used to determine the association of PG II expression with *H. pylori* infection. No significant difference was found between *H. pylori*-positive and *H. pylori*-negative patients with normal (p=0.3307) or high (p=0.1447) PG II expression. Since PG II secreting cells are located in corpus, antrum and duodenum, the expression of PG II levels cannot be used to directly reflect the disease severity observed in the antrum.

Table 4: Expression of PG II among *H. pylori*-positive and *H. pylori*-negative patients.

PG II expression (µg/L)	*H. pylori*-positive patients			*H. pylori*-negative patients			P value
	N	Mean+SD	Range	N^1	Mean+SD	Range	
3-15	16	9.41±3.43	4.40-14.79	35	8.29±2.59	3.01-13.7	0.3307
>15	6	33.84±18.09	18.41-66.3	9	22.79±6.21	16.1-30.8	0.1447

N=no. of patients *H. pylori*-positive by histology; N^1=no. of patients *H. pylori*-negative by histology.

PG I/II ratio among *H. pylori*-positive and *H. pylori*-negative dyspeptic patients

The PG I/II ratio are useful as a diagnostic marker. The reduction of PG I/II ratio is correlated with increasing grade of atrophic gastritis in the corpus.[11] Therefore patients with low PG I/II ratio may be at an increased risk of developing gastric cancer.[12] PG I/II ratio between 3-20 are observed among normal subjects.

In the present study all patients were found to be having a PG I/II ratio in the normal range. Among the *H. pylori*-positive patients the mean PG I/II ratio was 8.48±2.88 having a range between 3.92 - 15.05 while in *H. pylori*-negative patients the ratio was 10.2±2.21 having a range between 6.37 - 15.32. As PG I /II ratio is reported to reflect the gastric severity of the corpus(5), antral histological severity with the PG I/II ratio was not analyzed.

Diagnosis of the condition of the stomach mucosa using the Gastropanel assay

The Gastropanel assay was applied to 66 dyspeptic patients including 22 patients *H. pylori*-positive by histology and another 15 by serology for *H. pylori* IgG by one of the two ELISA assays. The diagnostic categories of Gastropanel assay is indicated in Table 5. Based on the Gastropanel interpretation criteria the study population was classified in to 8 different groups.

According to the diagnostic criteria of the 66 dyspeptic patients, 15 (22.7%) were diagnosed as having healthy mucosa with no atrophy and no *H. pylori* infection. High acid output was detected in three patients (4.54%) while 8 (12.1%) were identified as having low acid production due to PPI (Proton pump inhibitor) medication. Active *H. pylori* infection was diagnosed in a total of 35 patients (53.0%) (21 by histology of antral biopsy and another 14 by serum *H. pylori I*gG) One patient who presented with low G-17 concentration, normal PG I and II concentration and *H. pylori* infection was diagnosed as having antral atrophic gastritis (Category 6), indicating risk for developing gastric cancer. Four of the patients could not be included into the given categories which included 1 patient with *H. pylori*-positive by *H. pylori* IgG assay (Table 6).

Table 5: Categorization of patients according to the Gastropanel interpretation.

	Gastropanel Biomarkers						Interpretation
	Pepsinogen I (30-160 µg/L)	**Pepsinogen II (3-15 µg/L)**	**PG I/II ratio (3-20)**	**Gastrin-17b (1-7 pmol/L)**	**Gastrin-17s (3-30 pmol/L)**	***H. pylori* IgG Antibody level (<30 EIU)**	
1	N	N	N	N	N	N	Healthy mucosa (no atrophy, no *H. pylori* infection)
2	N	N	N	L*	N	N	Healthy mucosa. High acid output in the corpus
3	N or H^	N or H^	N	H**	N	N	Healthy mucosa. Low acid output due to, e.g., PPI medication
4a	N or H^	N or H^	N	N or H^	ND	H	Active *H. pylori* infection, not treated
4b	N	N	N	N	ND	N or H[†]	*H. pylori* infection successfully eradicated
4c	N	H	N	H	ND	H	*H. pylori* eradication failed
5	L	L	L	H	L	N^^ or H	Atrophic gastritis in the corpus
6	N	N	N	L	L	H	Atrophic gastritis in the antrum
7	L	L	L	L	ND	N^^ or H	Atrophic gastritis in the antrum and corpus (pan gastritis)
8	H	H	N	H		N	Short (4-10 day) break in PPI treatment

N=normal; L=low; H=high; *Test PPI medication for two weeks, G-17b should normalize; **Stop PPI medication, G-17b should normalize in two weeks; ND, no need for testing; ^PG I, PG II and G-17 can be elevated due to mucosal inflammation; ^^*H. pylori* antibodies can disappear in mucosal atrophy with prolonged course; [†]*H. pylori* antibody levels can remain elevated for months after successful eradication of *H. pylori*.

Table 6: Categorization of the study population according to the Gastropanel kit interpretation.

	Interpretation	Patients	Percentage (%)
1	Healthy mucosa (no atrophy, no *H. pylori* infection)	15	22.7
2	Healthy mucosa. High acid output in the stomach	3	4.54
3	Healthy mucosa. Low acid output due to, e.g.., PPI medication	8	12.1
4a	Active *H. pylori* infection, not treated	35	53.0
4b	*H. pylori* infection successfully eradicated	-	-
4c	*H. pylori* eradication failed	-	-
5	Atrophic gastritis in the corpus	-	-
6	Atrophic gastritis in the antrum	1	1.5
7	Atrophic gastritis in the antrum and corpus (pan-gastritis)	-	-
8	Short (4-10 days) break in PPI treatment	-	-
	Not belonging to any of the above categories	4	6.06

DISCUSSION

The Gastropanel kit uses three serum biomarkers; Gastrin 17 (G-17), Pepsinogen I (PG I), Pepsinogen II (PG II) and Helicobacter *pylori* IgG to determine the mucosal status of the antrum and the corpus of the stomach.[13] Diagnosis of atrophic gastritis in patients may indicate a risk of developing Gastric cancer. The Gastropanel kit has been validated for diagnosis of atrophic gastritis in several populations in developed countries.[2,14] Its use in developing countries is not widely reported[15] and has never been validated in Sri Lanka. Based on the interpretation of the Gastropanel kit in this study, only one patient was diagnosed as having atrophic gastritis and thereby at risk of gastric cancer. This low proportion of atrophic gastritis appears to be a favorable finding in the local setting. However, atrophic gastritis was not identified in the study population when examined by histology. The updated Sydney system recommends at least 5 biopsies for histological investigations from five different sites.[16,17] However due to ethical constraints in this study, the limited number of biopsies used may have resulted in the inability to detect atrophic gastritis.

The sensitivity of the Gastropanel kit for detection of *H. pylori* in the study population was low as only two patients of the 22 *H. pylori*-positive group (by histology) were identified as positive for *H. pylori* Ig G by Gastropanel kit. Therefore to identify active *H. pylori* infection, histology and another *H. pylori* IgG assay (Bioactiva Diagnostica, GmBH) were used. The low sensitivity detected in the Gastropanel IgG kit could be due to the cutoff levels and the species variation which may differ in the local population.

The interpretation of the Gastropanel kit is based on eight diagnosis categories (Table 5). In this study population 15 patients were identified as having normal healthy mucosa. However, 14 of the 15 patients were identified as having mild antral gastritis while 1 patient had moderate antral gastritis by histology. The patient, who was diagnosed with atrophic gastritis of the antrum based on the interpretation of the Gastropanel kit, was identified as having mild antral gastritis by histology. None of the patients had atrophic gastritis based on histological evidence indicating that histology and Gastropanel assay are not consistent in this population from Sri Lanka. Further four patients could not be categorized by Gastropanel assay although histologically they were diagnosed as having mild antral gastritis.

Serum concentrations of Gastrin-17, Pepsinogen I and Pepsinogen II is dependent on several factors including sex, age, diet and medication.[18-21] Although the Gastropanel assay has been validated in several Caucasian countries such as Finland, Russia and Italy[2,14,22] limited studies have been conducted in Asia.[15] While some studies have recommended this assay as a diagnostic method with sensitivity and specificity of over 90% in Caucasian populations[3] a lower sensitivity (47%) has been reported by Koivusalo et al., in a group of children from Finland. This study suggested that the low sensitivity of Gastropanel assay made it too insensitive for *H. pylori* screening and to determine the state of the gastric mucosa and thus cannot replace endoscopy.[23]

Further it is important to consider if the cut off values indicated by the assay are appropriate for the population in Sri Lanka. It is important to carry out further studies using larger sample size to determine the cut off range for the tested serum biomarkers in the Sri Lankan population.

The study population presented with dyspepsia from 2 months to 1 year and were on PPI medication (Proton Pump Inhibitors). *H. pylori* normally infects the antrum in patients with normal acid production and may also infect the corpus when acid production decreases (due to PPI use).[20,24] Studies have reported that PPI treatment alone can change the pattern of *H. pylori* induced gastritis by a shift of the bacterium from the antrum to the corpus.[20] Corpus predominant gastritis in *H. pylori* infection is a significant risk factor for gastric cancer.[25] Although the Gastropanel assay determines the status of the stomach as a whole, a limitation of the current study was that corpus biopsy specimens were not collected and therefore the status of the corpus could not be determined. In this study histological diagnosis was based on the antrum biopsy alone. Thus the corpus atrophy and inflammation could not be

compared with results of the Gastropanel kit. Another limitation was the difficulty in obtaining several biopsy specimens for histology as recommended by updated Sydney system.[16,17]

Gastrin 17 is considered as a useful biomarker for atrophic antral gastritis.[8] *H. pylori* in the gastric mucosa produce urease which breaks down urea and produces ammonia, resulting in an increase in the local pH of the gastric mucosa. This results in a favorable environment optimal for colonization of *H. pylori*.[26] At higher pH, G-17 secretion in the stomach increases due to the inhibition of the negative feedback loop of somatostatin on G cells. G-17 is reduced in patients with atrophy in the mucosa of the gastric antrum, due to the destruction of the G cells responsible for G-17 secretion. There was no significant difference in G-17 expression between *H. pylori*-positive patients and *H. pylori*-negative patients in this study population. Similarly to the findings of the this study, Germana et al., did not find a significant difference in G-17 expression among *H. pylori*-positive and *H. pylori*-negative patients in a study done in Italy.[27] Further Fiocca et al., also found no significant difference in gastrin expression among long term PPI users (with high pH in the gastric mucosa) in the presence or absence of *H. pylori* infection.[28]

The PG I/II ratio together with the PG I expression is of value in diagnosis of the atrophy of the gastric body mucosa. Although the PG I/II ratio were within the normal range in the study population, *H. pylori* positive group had a lower PGI/II ratio than the *H. pylori* negative groups suggesting that a lower PG I/II ratio may be associated with *H. pylori* infection. Patients with lower PG I/II ratio are at increased risk of progressing to atrophic gastritis in the mucosa. Sun et al., reported that the PG I/II ratio are seen to decrease linearly with increasing disease severity.[10] Further a higher proportion of patients positive for *H. pylori* 40.9% (9/22) had moderate to severe gastritis compared to *H. pylori* negative patients 2.2% (1/44) in this study group.

CONCLUSIONS

The results of histology and Gastropanel assay were not comparable among the dyspeptic study population. A larger study need to be carried out for validation of cut off values for the Sri Lankan population.

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REFERENCES

1. Atherton JC. The pathogenesis of *Helicobacter pylori*–induced gastro-duodenal diseases. Annu Rev Pathol Mech Dis [Internet], 2006; 1(1): 63–96.
2. Noah DN, Claire M, Assoumou O, et al. Assessing GastroPanel serum markers as a non-invasive method for the diagnosis of atrophic gastritis and *Helicobacter pylori* infection. Open J Gastroenterol, 2012; 2012(8): 113–8.
3. Kurilovich S, Belkovets A, Reshetnikov O, et al. Stomach-specific biomarkers (GastroPanel) can predict the development of gastric cancer in a caucasian population : A longitudinal nested case-control study in Siberia. Anticancer Res., 2016; 254(36): 247–54.
4. Sipponen P, Valle J, Varis K, et al. Fasting levels of serum gastrin in different functional and morphologic states of the antrofundal mucosa. An analysis of 860 subjects. Scand J Gastroenterol, 1990; 25: 513–9.
5. Song HJ, Jang SJ, Yun SC, et al. Low levels of pepsinogen I and pepsinogen I/II ratio are valuable serologic markers for predicting extensive gastric corpus atrophy in patients undergoing endoscopic mucosectomy. Gut Liver, 2010; 4(4): 475–80.
6. In: Barrett KE, Barman SM, Boitano S, Brooks HL, editors.Ganong's Review of Medical Physiology. New York: McGraw-Hill Education, 2016.
7. Helander HF, Li H. Cell proliferation in the gastric epithelium of the ulcer rat. Scand J Gastroenterol, 2005; 40: 1386–93.
8. Zhang Z, Sun L, Gong YH, et al. Factors affecting the serum gastrin 17 level : an evidence-based analysis of 3906 serum samples among Chinese. J Dig Dis, 2007; 8: 72–6.
9. Pasechnikov VD, Chukov SZ, Kotelevets SM, et al. Invasive and non-invasive diagnosis

of *Helicobacter pylori* -associated atrophic gastritis: A comparative study. Scand J Gastroenterol, 2005; 40(8): 297–301.

10. Sun L, Gong Y, Wang L, et al. Serum pepsinogen levels and their influencing factors : A population-based study in 6990 Chinese from North China. World J Gastroenterol, 2007; 13(48): 6562–7.
11. Miki K. Gastric cancer screening by combined assay for serum anti- *Helicobacter pylori* IgG antibody and serum pepsinogen levels — " ABC method ." Proc Jpn Acad Ser B., 2011; 87(7): 405–14.
12. Agréus L, Kuipers EJ, Kupcinskas L, et al. Rationale in diagnosis and screening of atrophic gastritis with stomach-specific plasma biomarkers. Scand J Gastroenterol, 2012; 47(2): 136–47.
13. Syrjanen K. A Panel of Serum Biomarkers (GastroPanel®) in Non-invasive Diagnosis of Atrophic Gastritis. Systematic Review and Meta-analysis. Anticancer Res., 2016; 36: 5133–44.
14. Roman LD, Lukyanchuk R, Sablin OA, et al. Prevalence of *H. pylori* Infection and Atrophic Gastritis in a Population-based Screening with Serum Biomarker Panel (GastroPanel®) in St. Petersburg. Anticancer Res., 2016; 36: 4129–38.
15. Iijima K, Abe Y, Kikuchi R, et al. Serum biomarker tests are useful in delineating between patients with gastric atrophy and normal, healthy stomach. World J Gastroenterol, 2009; 15: 853–9.
16. Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis The updated Sydney system. Am J Surg Pathol, 1996; 20(10): 1161–81.
17. Price AB. The Sydney System: histological division. J Gastroenterol Hepatol., 1991; 6(10): 209–22.
18. Lou J-F, Chen X, Zhang J-X, et al. Discrepant impacts of age and gender factors on serum pepsinogens and gastrin-17 levels. J Lab Precis Med [Internet], 2017; 2: 42–42.
19. Gold schmiedt M, Barnett CC, Schwarz BE, et al. Effect of age on gastric acid secretion and serum gastrin concentrations in healthy men and women. Gastroenterology, 1991; 101: 977–90.
20. Kuipers E. Proton pump inhibitors and gastric neoplasia. Gut., 2006; 55: 1217–21.
21. Agreus L, Storskrubb T, Aro P, et al. Clinical use of proton-pump inhibitors but not H 2 -blockers or antacid / alginates raises the serum levels of amidated gastrin-17 , pepsinogen I and pepsinogen II in a random adult population. Scand J Gastroenterol, 2009; 44: 564–70.

22. Lombardo L, Leto R, Migliardi M, et al. Prevalence of atrophic gastritis in dyspeptic patients in Piedmont. A survey using the GastroPanel test. Clin Chem Lab Med, 2010; 48(9): 1327–32.
23. Koivusalo AI, Pakarinen MP, Kolho K. Is GastroPanel serum assay useful in the diagnosis of *Helicobacter pylori* infection and associated gastritis in children? Diagn Microbiol Infect Dis., 2007; 57: 35–8.
24. Kuipers EJ, Klinkenberg-knol EC, Meuwissen SGM. *Helicobacter pylori*, Proton Pump Inhibitors and Gastroesophageal Reflux Disease. Yale J Biol Med., 1999; 72: 211–8.
25. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of Gastric cancer. N Engl J Med., 2001; 345(11): 784–9.
26. Eaton KA, Krakowka S. Effect of gastric pH on urease-dependent colonization of gnotobiotic piglets by *Helicobacter pylori*. Infect Immun [Internet], 1994.
27. Germana B, Mario F Di, Cavallaro LG, et al. Clinical usefulness of serum pepsinogens I and II , gastrin-17 and anti- *Helicobacter pylori* antibodies in the management of dyspeptic patients in primary care. Dig Liver Dis., 2005; 37: 501–8.
28. Fiocca R, Mastracci L, Attwood SE, et al. Gastric exocrine and endocrine cell morphology under prolonged acid inhibition therapy: Results of a 5-year follow-up in the LOTUS trial. Aliment Pharmacol Ther., 2012; 36(10): 959–71.