Diagnostic Role of Serum Pepsinogen Levels for Helicobacter Pylori Infection

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Abstract:
Background: Distributions of serum pepsinogen (PG) values were assessed in Helicobacter pylori-infected and non-infected Egyptian subjects. PG reflects gastric mucosal atrophy and inflammation caused by Helicobacter pylori (H. pylori) infection. Inflammation upregulates production of both PG I and PG II in gastric mucosal cells and increases the amount discharged to serum, where elevation of PG II is usually larger so that the PG I: II ratio declines. **Objectives:** evaluate PG serum levels as a reliable diagnostic marker for Helicobacter Pylori (HP) infection. **Subjects and Methods:** All individuals (100), controls (50) who were healthy and another HP infected patient group (50), were asked to provide serum samples, which were used to measure serum H. pylori antibodies using ELISA kits and PG values. The subjects, whose serum antibodies were positive, considered H. pylori infected. T-test used for comparison of two studied groups and Receiver operating characteristic (ROC) curve were used to evaluate the PGI, PGII serum levels for H. pylori infection. **Results:** Of the 100 subjects who provided blood samples, 50 were infected had serum antibody titer > 10 U/ml and 50 was considered negative <10 U/ml, the serum levels for PG I and PG II values and PG I to PG II ratio (PG I:II) were assessed , whereas in the infected subjects, these values were 46.618 ± 19.5031, 15.700 ± 8.2721 and 3.166 ± 0.5528 respectively in the other hand these values in the non-infected control were 45.220 ± 7.2834, 8.644 ± 0.7118 and 5.542 ±1.1305 with P value (= 0.684, < 0.001 and 0.001) respectively by using T-test for the statistical comparison also there was a significant difference between patients and control as regard Helicobacter antibody titer 36.16±19.736 and 5.82±1.804 P value < 0.001 Meanwhile, by using ROC curve there was insignificant difference for PGI with H pylori infection (Cut off value for PGI is 34.8 ng/ml with sensitivity of 82 % and specificity 75%) and there was high significant difference for PGII with H pylori infection (Cut off value for PGII is 8.7 ng/ml with sensitivity of 87.5 % and specificity 80%)
Conclusion: there was insignificant increase in PGI, high significant increase in PGII, significant decrease in PGI: PGII ratio. Statistical difference for H.pylori antibody titer in the serum levels between the estimated two groups.
Key words: Pepsinogen (PG) &Helicobacter pylori (HP)

Introduction

Pepsinogen is a precursor of pepsin, and human gastric mucosa cells produce two immune-chemically distinct forms of PG. (Samloff IM, 2017 and Kikuchi S, Wada O, Miki K, et al., 1994) PG I is secreted by the chief and mucus neck cells in the gastric fundic glands, and PG II is produced by these cells and by the cardiac, pyloric, and Brunner's glands in the gastric cardia and antrum and proximal duodenum. (Rotter JI, Wong FL, Samloff IM, et al. 1982) PG reflects gastric mucosal atrophy and inflammation, both of which Helicobacter pylori (H. pylori) infection provokes. Inflam-mation upregulates production of both PG I and PG II in gastric mucosal cells and increases the amount discharged to serum, where elevation of PG II is usually larger so that the PG I: II ratio declines. With the progression of atrophy, numbers of gastric mucosal cells producing PG II and I decease. As the decrease of cells producing PG I is more crucial, the PG I:II ratio declines with the progression of atrophy. (Miki K, Ichinose M, Shimizu A, et al., 1987 and Inoue K, Fujisawa T, Haruma K. 2010) In adults, PG values were used as a marker of gastric mucosal atrophy that is strongly related to gastric cancer risk. (Kikuchi S, Kato M, Mabe K, et al., 2017 and Kikuchi S, Wada O, Nakajima T, et al., 1995). Recently, criteria of PG values to distinguish subjects with and without H. pylori infection have been proposed because PG values differ depending on the infection among adult subjects. (Kikuchi S,

Nakajima T, Kobayashi O, et al., 2017). adults with H. pylori infection showed elevated PG I and PG II values and reduced PG I to PG II ratios. (Nakayama Y, Lin Y, Hongo M, Hidaka H, Kikuchi S , 2017). H. pylori infection causes lesions in most infected population, including nodular atrophic gastritis and duodenal erosion: ulcer, (Fukuda Y, Isomoto H, Ohnita K, et al., 2003) and a subset of infected subjects develop gastric cancer in the future. (Sipponen P, Härkönen M, Alanko A et al., 2002 and Mardh E, Mardh S, Mardh B et al 2002) In a previous study with 45 asymptomatic subjects aged 25-50 years. (Fukuda Y, Isomoto H, Ohnita K, et al., 2003) and another study analyzing sera from 120 asymptomatic individuals less than 45 years old, serum H. pylori antibody-positive subjects showed elevated PG I and PG II, and reduced PG I:II compared with the seronegative people. Thus, PG values can be used to diagnose H. pylori infection status in these intended patients. Nonetheless, it is still unclear whether distributions of PG values is variable and affected by age differences with reference to H. pylori infection status. The previous studies did not focused on these points. The inflamed gastric mucosa transmits specific factors to the blood, which allows the possibility of diagnosing gastritis by serologic analysis. A number of serologic markers have been described, such as pepsinogen I (PGI), pepsinogen II (PGII) and IgG antibodies against Helicobacter pylori. (Okuda M, Kamiya S, Booka M, et al., 2013).

Many studies have been done in Europe and Japan, (Mabe K, Kikuchi S, Okuda M, et al., 2017 and Kikuchi S. 2015) but because of potential ethnic, dietary, environmental and disease differences, the use of serum pepsinogen (PG) screening requires local validation. (Ueda J, Okuda M, Nishiyama T, et al., 2014).

There are no data available in Egypt on the serologic PG concentrations in patients with (HP), so we decided to use serum PG as the biomarker in the development of a novel noninvasive test, as the first option for a screening test for (HP) diagnosis &prevalence in Egyptian patients. The aim of this study was to assess the distributions of PG values in H. pylori infected and non-infected subjects in adult Egyptian population.

Subjects and methods:

The sample collection was conducted in Sayed Galal Hospital, Al-Azhar University. They were healthy subjects aged 25 –50 years and were asked to serum samples. The participants were informed of the study and gave the written consent. Blood samples were assayed using H. pylori IgG antibody kits. In addition, PG I and PG II levels were measured in the serum samples. For the serum antibody tests, serum H. pylori IgG antibody was quantified using a serum-HpELISA kit (E-plate EIKEN H. pylori, Eiken Chemical Co., Ltd. Japan). According to the manufacturer's instruction, serum antibody titers ≥10.0U/mL and titers <10.0U/mL were classified as positive and negative for H. pylori, respectively. The cutoff value gave 91.2% sensitivity and 97.4% specificity for adult subjects... (Kalach N, Legoedec J, Wann AR, Bergeret M, Dupont C, Raymond J. 2004) Levels of PG I and PG II and the ratio of PG I to PG II were evaluated between positive and negative (control) serum antibody tests.

Statistical analyses:

Data were analyzed using Statistical Program for Social Science version 18.0. Quantitative data were expressed as mean ± standard deviation. Qualitative data were expressed as frequency and percentage. The comparison between two assessed groups were statistically analyzed by T. test

Probability (P-value)

P-value <0.05 was considered significant. (Sig).

P-value <0.01 was considered as highly significant.

P-value>0.05 was considered non significant

Receiver operating characteristic (ROC) curves were used to evaluate the PGI, PGII serum levels for H. pylori infection with area under the curve was 0.437 and 0.816 with P value = 0.281 &< 0.001 respectively

Results

In this study, 100 subjects were participated, of all blood sample provided, comparison between mean serum level between control and H. pylori infected patients, (Figure 1). Also the comparison between two groups as regard serum levels for PGI, PGII, antibody H pylori titer and for Age using T. test & P value (Table 1). For control Ab H. pylori titer for control considered if below 10U/ml but for infected patients above 10U/ml. there was non-significant increase for PGI P value=0.684 but for PGII PGI:PGII and in H. pylori Ab titer show significant increase P

value <0.001 also there was increase in the mean serum levels in infected patients if compared with normal control (Figure 2). In the other hand and by using ROC curve there was non- significant statistical difference in PGI for H. pylori infection with Cut off value for PGI is 34.8ng/ml with sensitivity of 82% and specificity 75% P value=0.281 (Figure 3) meanwhile for PGII Cut off value is 8.7ng/ml with sensitivity of 87.5% and specificity 80% P value<0.001 (Figure 4). The serum levels for PG I and PG II values and PG I to PG II ratio (PG I:II) were assessed, whereas in the infected subjects, these values were 46.618 ± 19.5031, 15.700± 8.2721 and 3.166±0.5528 respectively in the other hand these values in the non-infected control were 45.220 ± 7.2834, 8.644 ± 0.7118 and 5.542±1.1305 with P value (=0.684, <0.001 and 0.001) respectively by using T-test for the statistical comparison (Table 1), also there was a significant difference between patients and control as regard helicobacter antibody titer 36.16±19.736 and 5.82±1.804 P value < 0.001 .also by using ROC curve there was insignificant difference for PGI with H pylori infection (Cut off value for PGI is 34.8 ng/ml with sensitivity of 82% and specificity 75%) and there was high significant difference for PGII with H pylori infection (Cut off value for PGII is 8.7 ng/ml with sensitivity of 87.5% and specificity 80%.

Table (1): Comparison between Control and Patients in Age, PGI, PGII, and Serum Ab titer foe HP Helicobacter by using **(T- test).**There is statistically significant difference between two groups in PGI, PGII and Serum Ab titer for HP

	Group No/50 for each	Mean	SD	P Value
Pepsinogen I(PGI) ng/ml	Control	45.220	7.2834	0.684
	Patients	46.618	19.5031	
Pepsinogen II(PGI) ng/ml	Control	8.644	.7118	**0.001**
	Patients	15.700	8.2721	
PGI/PGII Ratio	Control	5.542	1.1305	**0.001**
	Patients	3.166	.5528	
Age /years	Control	45.40	9.920	0.725
	Patients	46.06	8.765	
Serum Ab titer for HP	Control	5.82	1.804	**0.001**
	Patients	36.16	19.736	

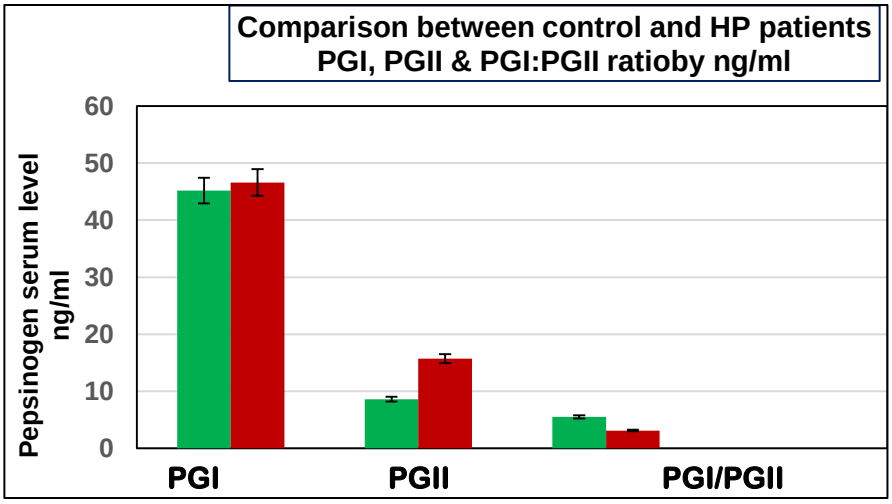

Figure (1): Comparison between mean of serum levels in Control and Patients: PGI, PGII, PGI/PGII ratio

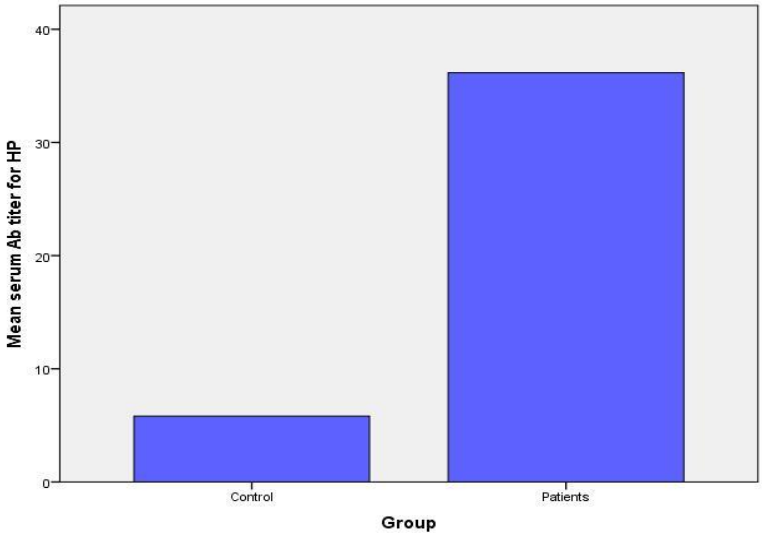

Figure (2): Mean Serum AB titer/U for HP between control and patients.

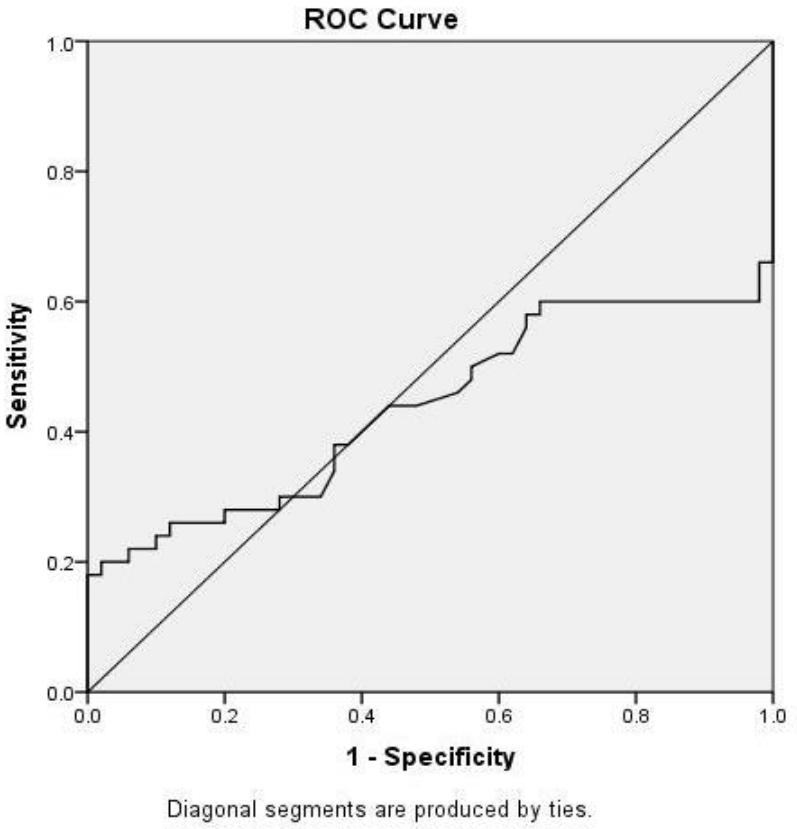

Figure (3): ROC curve of Serum PGI for H. Pylori infection.

Table (2): ROC curve of Serum PGI for H. Pylori infection.
Cut off value for PGI is 34.8 ng/ml with sensitivity of 82 % and specificity 75%

	Area under the curve	S. Error	95% CI	P value
Pepsinogen I(PGI) ng/ml	0.437	0.061	0.318-0.557	0.281

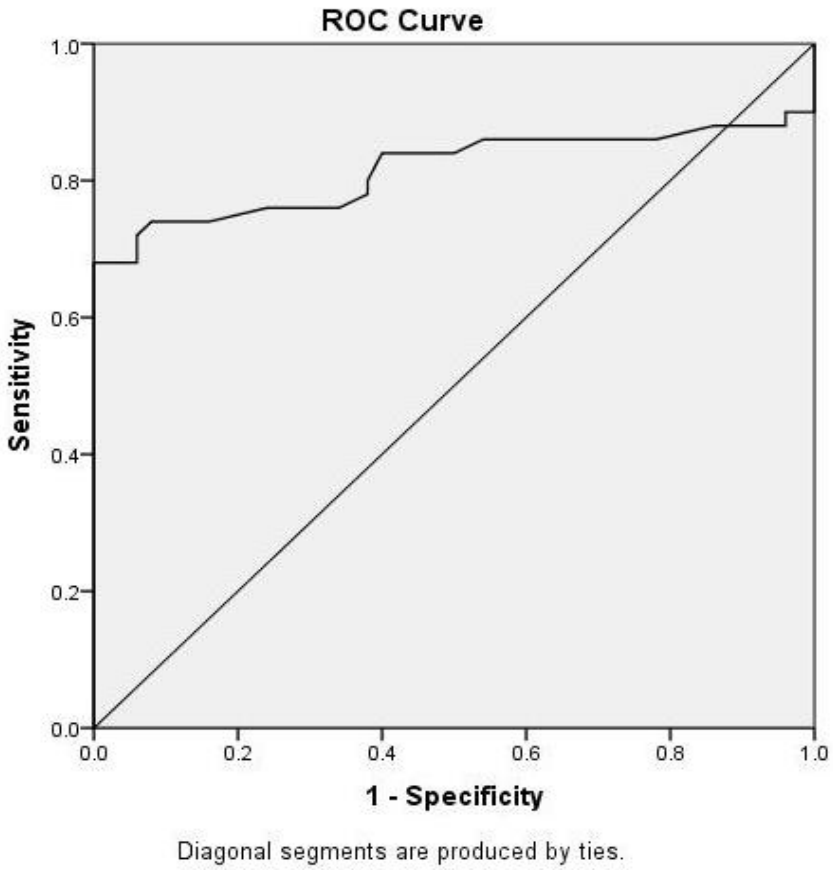

Figure (4): ROC curve of Serum PGII for H. Pylori infection.

Table (3): ROC curve of Serum PGII for H. Pylori infection.
Cut off value for PGII is 8.7 ng/ml with sensitivity of 87.5 % and specificity 80%

	Area under the curve	S. Error	95% CI	P value
Pepsinogen II(PGII) ng/ml	0.816	0.049	0.720-0.912	**0.001**

Discussion

50 out of 100 subjects gave positive results for serum antibody titer considered H. pylori infected > 10U/ml, and 50 control group with negative serum antibody titers of <10U/mL were considered no infected. In the H. pylori non-infected subjects, the means of PG I, PG II, and the PG I:II ratio were 45.22 ng/ml, 8.644 ng/ml, and 5.542 respectively, whereas in the infected subjects, these values were 46.618ng/mL, 15.700ng/mL, and 3.166, respectively (each P=0.684 and P <0.001; respectively (Table 1). In the previous studies with asymptomatic Japanese people the elevated PG I and PG II levels and the decreased PG I:II ratio in H. pylori infected subjects were consistent with the present study except for PGI serum level not significantly increased. In asymptomatic people aged 40-50 years in Chile, similar results were obtained, while the PG I:II ratio was elevated in the H. pylori seropositive population. (Koilvusalo AI, Pakarinen MP, Kolho KL 2007). In symptomatic patients, PG I and II were elevated and the PG I:II ratio was decreased in H. pylori seropositive patients, (de Angelis GL, Cavallaro LG, Maffini V, et al., 2007 and Kitamura Y, Yoshihara M, Ito M, et al., 2015) although a few exceptional results were reported, where PG I was not affected coinciding with our study or the PG I: II ratio was not affected. The elevated PG I and PG II and decreased PG I: II ratio in H. pylori infected patients seem to be consistent, although several exceptions exist. The change in PG values in the infected children were

similar to those of adult subjects without severe mucosal atrophy (Inoue K, Fujisawa T, Haruma K. 2010). PG I and PG II values can be affected by the kits that are used and the different criteria are proposed.(Kikuchi S, Nakajima T, Kobayashi O, et al., 2000 and Khanna B, Cutler A, Israel NR, et al., 1998) One study that used the same kit as the present study reported a 96.3% sensitivity and 82.8% specificity in adult subjects when a PG II value of ≥28 ng/mL or a PG I:II ratio of ≤8.0 were considered positive for the diagnosis of H. pylori infection. (Kikuchi S, Nakajima T, Kobayashi O, et al 2017).Nonetheless, 100% sensitivity and 81.9% specificity are obtained when PG II values of ≥31 ng/mL or PG I: II ratios of ≤5.3 are used to determine H. pylori infection status. ROC of PG II indicates 8.7 ng/ml with sensitivity of 87.5% and specificity 80% was the cutoff value available in this work. H. pylori infection in Egyptian sampled population and can be used with a serum antibody test as another marker of the infection to improve the diagnostic accuracy. The limitations in the present study were, First, the sample size of H. pylori positive subjects was relatively small and the small sample size might make the results unstable. The results of the present study regarding H. pylori positive subjects were consistent with other studies. (So JB-Y, Yeoh K-G, Moochala S et al. 2002 and Zagari RM, Nicolini G, Casanova S et al., 2002).

The effect of H. pylori infection on PG values may be typical, and the small sample size may not have a large effect... Some reports have suggested that the antibody test is inaccurate for the diagnosis of H. pylori infection in children, (Sunnerstam B, Kjerstadius T, Jansson L, et al., 1999) but the both serum and urine antibody kits showed a good diagnostic accuracy. (Koilvusalo AI, Pakarinen MP, Kolho KL 2007 and Lopes AI, Palha A, Lopes T, et al., 2006, and Okuda M, Osaki T, Lin Y, et al., 2015)

So, we recommend urine and serum samples to be assessed in future studies .We excluded 4 subjects with discrepant results and 2 subjects with negative high titers of serum antibody .

In conclusion, PG I values not affected and PG II values were higher and the PG I: II ratio was lower in H. pylori infected adult Egyptian patients compared to non-infected people. Therefore, PG serum levels considered significant markers to differentiate Pylori infected from healthy Egyptian individuals.

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الملخص العربي

الدور التشخيصي لقياس مستوى الببسونجين في دم المرضى المصابين بالجرثومة الحلزونية

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خلفية البحث: بقياس مستويات الببسنوجين في دم 100 من المصريين البالغين في المستوى العمري 25-50 عاما نصف هذا العدد اعتبر كمجموعة ضابطة والنصف الاخر من المرضى المصابين بالجرثومة الحلزونية حيث ان مستوي افراز الببسنوجين يعكس صحة جدار المعدة وعندما تلتهب تحت تأثير الإصابة يتم افراز الببسنوجين 1 وببسنوجين 2 بكميات كبيرة مع زيادة نسبية للببسنوجين 2 مما يسبب نقص معدل ناتج قسمتهما.
الغرض من البحث: تقييم مستوى الببسنوجين في الدم كدلالة تشخيصية للإصابة بالجرثومة الحلزونية.
الطرق المستخدمة في البحث: قد تم سحب عينات الدم من 100 شخص مصري منهم 50 كمجموعة ضابطة من الأصحاء والنصف الأخر كمجموعة من المرضى المصابين بالجرثومة الحلزونية وقد تم قياس مستوي الببسونوجين ELISA وكذلك قياس معدلات مضادات الجرثومة .وقد تم تقدير الدلالة الإحصائية عن طريق (ROC curve و T-test).
نتائج البحث: لوحظ أنه ليس هناك فروق ذات دلالة إحصائية بين المجموعتين الضابطة والمريضة بخصوص مستوي ببسنوجين 1 وهناك زيادة ذات دلالة إحصائية في مستوي ببسنوجين 2 وكذلك نقص ذو دلالة إحصائية في معدل ببسنوجين 1: ببسنوجين 2 وعلي الجانب الاخر هناك زيادة في معدل قياس مضادات الجرثومة في دم المرضى بالمقارنة بالمجموعة الضابطة.
الخلاصة: يمكن اعتبار قياس مستوى ببسنوجين 1 وببسنوجين 2 ومعدل ببسنوجين 1: ببسنوجين 2 له دلالة احصائية في تشخيص المرضى المصريين المصابين بالجرثومة الحلزونية.