Iraqi Journal of Biotechnology, 2013,Vol.12, No.2 , 75-81

Determination of serum pepsinogen I and II level at high risk for stomach cancer

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Received: February 6,2013/ Accepted: June 16,2013

Abstract The levels of pepsinogen (PG) I and the PGI/II ratio are useful serologic markers for chronic atrophic gastritis. This study evaluated the performance and clinical implications of these markers in patients undergoing endoscopy. This study was carried at the gastrointestinal unit at AL-Yarmouk Teaching Hospital. Blood samples with antral biopsies were obtained from 127 patients who were complaining from gastroduodenal disease. Patients included 64 with chronic gastritis and 30 with gastric carcinoma. A further 33 healthy persons, uninfected with *H.pylori* were involved as a control group. Every gastric biopsy examined by histology and rapid urease test. In addition, patient's sera were screened for the presence of *H.pylori*IgG antibodies, serum level of PGI and PGII. According to the histological data it was demonstrated that the adenocarcinoma patients were more prevalent than other type of gastric cancer. There was a significant $p<0.05$ increased level of pepsinogen I and II in the serum of patients that infected with *H.pylori*, and the ratio SPGI/SPGII was reduced when compared with the subjects who were uninfected with *H.pylori*. The mean level of SPGI was significantly lower $p<0.05$ in patients with gastric carcinoma than patients with normal mucosa and chronic gastritis. However, the serum PGII increased significantly $p<0.05$ in gastric carcinoma, with lower ratio. In conclusion low serum PGI and lower ratio SPGI/SPGII might be used as a marker for predicting of gastric cancer.

Key words: Pepsinogen I and II, *H. pylori*, CagA, stomach cancer.

تحديد مستوى ارتفاع المصل مولد الببسين المستوى الأول والثاني في خطر الاصابة بسرطان المعدة

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الإستلام:شباط 6-2013/ القبول: حزيران 16-2013

الخلاصة:أجريت هذه الدراسة على المرضى الوافدين الى وحدة الناظور / قسم الباطنية التابع لمستشفى اليرموك التعليمي وشملت الدراسة 127 عينة دم وخزعه نسيجيه من أشخاص كانوا يعانون من امراض في المعدة. 64 من المرضى المصابين بالتهاب المعدة المزمن و30 مصابين بسرطان المعدة . كما ان هناك 33 شخصا" كانوا بحالة صحية سليمة وغير مصابين ببكتريا *Helicobacter pylori* واعتبروا كمجموعة سيطرة. اخضعت عينات الخزع النسيجية التي اخذت من الغار المعدي لكل شخص مريضا كان ام سليما الى تحليل اليوريز السريع والفحص النسيجي. وتم فحص المصول للكشف عن وجود اجسام مضادة نوع IgG لبكتريا *H.pylori* وحساب مدى ارتفاع

مستوى Pepsinogen I . II بواسطة مقايسة الممتز المناعي المرتبط بالانزيم (ELISA) كطريقة مصلية. وقد بينت الدراسة ان هناك ارتفاع معنوي $p<0.05$ ل pepsinogen I,II في مصل المرضى عند وجودالبكتريا وقلة النسبة PGI/PGII مقارنة بالاشخاص غير المصابين . كما وظهر انخفاض معنوي $p<0.01$ لمستوى SPGI في الاشخاص المصابين بسرطان المعدة مع ارتفاع معنوي $p<0.05$ لمستوى SPGII وقلة النسبة SPGI/SPGII بشكل ملحوظ مقارنة بالاشخاص المصابين بالتهاب المعدة المزمن والاشخاص الاصحاء الغير مصابين بالبكتريا .

Introduction

Gastric cancer remains one of the leading causes of cancer-related death worldwide(1). Pepsinogens are pro enzymes of the digestive enzyme pepsin that are organized in the gastric mucosa and are classified biochemically and immunochemically into two groups: pepsinogen I and pepsinogen II (2). Pepsinogen I is synthesized by the chief and mucus neck cells of the gastric corpus whereas pepsinogen II can be produced not only by the chief and mucous cell of the corpus, but also by the antrum pyloric glands and even by the Brunner's glands in the first part of the duodenum (3). Serum pepsinogens level reflects the morphologic and functional status of the stomach mucosa (2, 4). As gastritis progresses mild inflammation leads to elevated concentrations of PG I and PG II in the circulation (3). As the severity of atrophy advances, chief cells are replaced by pyloric glands and the concentration of PG II remains increased, while the concentration of PG I decrease. Consequently, the ratio between the concentrations of PG I and PG II is greatly reduced (5). Infections with *H.pylori* cause inflammation stimulating secretion by the glands and increasing the serum concentration of pepsinogen (6). Persistent *H. pylori* colonization promotes the induction of gastric atrophy, which is considered to be part of the causal pathway of intestinal-type gastric adenocarcinoma (7). Pepsinogens are aspartic proteinases that are predominantly produced and secreted into the gastric lumen by gastric epithelial cells. (8). Nevertheless, the spontaneous disappearance of *H.pylori* due to extended mucosal atrophy may lead to decreasing the level of serum concentration (3). Consequently, the ratio between the concentrations of PG I and PG II is greatly reduced. Thus serum PG concentration reflects the morphological and functional status of the gastric mucosa (9). Many gastric cancer develop in stomach mucosa affected by severe and extensive chronic atrophic gastritis. Therefore PG screening would enable the detection of subjects with extensive atrophic gastritis; such subjects have a high risk of developing gastric cancer (10).

Materials and Methods

Study population

Endoscopy and assay of serum PG concentration were performed in 127 subjects who were screened for gastroduodenal diseases at AL-Yarmouk Teaching Hospital Gastroenterology and Hepatology Teaching Hospital. This study was carried out on 64 patients with chronic gastritis (CG) and 30 patients with gastric cancer (GC). The control group included 33 gastric normal tissue (N) and uninfected with *H. pylori.*

Diagnosis of *H. pylori* infection

Rapid urease test (RUT)

One biopsy was inoculated on urea agar slant, and then incubated at 37 C for 15 min. to1 hr. Slant was examined for color change from yellow to pink if the bacteria were present secret urease and hydrolysis the urea to ammonia and raises the pH of the medium which change the color.

Histological evaluation

One biopsy specimen from the antrum of the stomach was fixed in 10% formalin then processed paraffin wax embedded section was cut into 3-µthick section and stained with haematoxylin and eosin. Giemsa staining was performed to detect or absence of *H. pylori* were assessed by the pathologist, who were unaware of the clinical and endoscopic finding.

Blood samples

Blood samples were collected immediately after endoscopy, and then centrifuged for 15 min. at 1500 rpm; the sera were stored at -20^{o}C until further analyses were performed. Sera were screened for the presence of *H.pylori*IgG antibodies, level of SPGI and SPGII.

Serological methods

Enzyme linked immunosorbant assay was used for measurement of human IgG antibody to *H.pylori* (*H.pylori*IgG antibody ELISA Kit Cat. No. CS 001040 Biohit Plc, Helsinki, Finland), Pepsinogen I (ELISA ELISA Kit Cat. No. CS 001010 Biohit Plc, Helsinki, Finland) and Pepsinogen II (ELISA Kit Cat. No. CS 601 020.01Biohit Plc, Helsinki, Finland) in serum.All subjects were divided into three categories on the basis of histology and the results of the rapid urease test as those with: *H pylori* positive and *H pylori* negative and histological normal gastric mucosa. Patients were classified as positive for *H pylori* if two of three tests (RUT, Giemsa staining and human IgG antibody to *H.pylori*) were positive and as negative if both tests were negative.This pepsinogen I ELISA is based on sandwich enzyme immunoassay technique with PGI specific capture antibody adsorbed on microwell plate and detection antibody labeled with horseradish peroxidase (HRP). The assay proceeds according to the following reactions: a monoclonal antibody specific to human PGI on the polystyrene surface of the wells binds PGI, molecules present in the sample. Then wells are washed to remove the residual sample. An HRP- conjugated monoclonal detection antibody is added into the wells and it binds to the PGI molecules. The wells are washed and the substrate is added into them. The substrate is oxygenized by the enzyme and blue colored end product is produced. Lastly the enzyme reaction is terminated with stop solution. The solution in the microwells should turn yellow. The intensity of color developed is directly related to the PGI concentration of the sample.(PGI), pepsinogen II (PGII) and human IgG antibody to *H.pylori* in serum ELISA have the same principle. A low PGI result (PGI<25µg/l) indicates advanced (moderate and severe) atrophic gastritis of the corpus mucosa. A PGI/PGII ratio lowers than 2.5 indicate advanced corpus atrophy and increased risk of gastric cancer. The results of human IgG antibody to *H.pylori* were Negative < 34 EIU, Borderline 34-42 EIU (cut-off 38 EIU) and Positive > 42

EIU.These cut-off levels have been determined using the Biohit PGI, PGII and humanIgG antibody to *H.pylori* ELISA kit based on large clinical material.

Statistical analysis

The *t* test was used for the quantitative data.The chi-square used for the qualitative data.Means and standard errors (ES) were calculated. The lowest level of significance was when the probability ($p<0.05$) (11).

Results and Discussion

Bacteriological and histological study

Helicobacter pylori infection was detected in 94 (74.01%) from 127 patients, whereas the patients without *H.pylori* were 26.0% (Table -1). This result was very close with study that detected the *H.pylori* in 89 (74.78%) from 119 patients (12). In addition, the result was higher than that reported by other study who found the incidence of *.pylori* in 67 (67 %) from 100 patients (13).

Table -1: The prevalence of *H. pylorii*nfection in patients with gastroduodenal diseases

***H.pylori* status**	**Gastroduodenal disease (No. 127)**
***H.pylori*- positive**	94 (74.01%)
***H.pylori*- negative**	33 (26.0%)

***$p<0.05$**

Relationship between *H.pylori* infections, serum PGI, serum PGII

Table -2 reveals the relation between mean levels of serum pepsinogens (PGs) and *H.pylori*infection. The mean levels of SPGI and SPGII in patients infected with *H.pylori* were (104.2µg/l) and (17.8µg/l) respectively. While in patients uninfected with *H.pylori* the mean level of SPGI was (74.4µg/l), and the SPGII was (8.4µg/l) with significantly difference ($p<0.01$). The ratio SPG1/SPGII was significantly lower in patients with *H.pylori* (5.8) compared with that *H.pylori* negative patients (8.8) $p<0.05$. This result was very close to that obtained by other investigators (14, 15). The *H.pylori* infection ongoing in relation with gastritis activity and inflammation and stimulating the PGs to release (16). It has been suggested that PGII was a more sensitive indicator of *H.pylori* load than PGI. Furthermore, previous studies showed that PGII was sensitive marker of *H.pylori* eradication therapy than PGI in patients (14). PGI is restricted to the corpus mucosa, whereas PGII is present in both the corpus and antral mucosa (3). It has been suggested that *H.pylori*-associated gastritis was more severe in the antrum than in the corpus mucosa, possibly accounting for the greater relation between PGII levels and *H.pylori*-associated gastritis as

compared with PGI which was present to a lesser extent in the antrum (1).

Gastric cancer, SPGI, and SPGII

The mean level of SPGI was (41.4µg/l) in patients with gastric cancer (Table -3) which is significantly (p<0.01) lesser than that in patients with chronic gastritis (101.5µg/l) and individual with normal mucosa (71.6µg/l). The ratio of SPGI/SPGII was (1.8) in patients with gastric cancer which was significantly lower (p<0.01) than that normal mucosa (9.6 µg/l) and was (7.6 µg/l) in chronic gastritis. The mean level of SPGII was increased (23.2 µg/l) in patients with gastric cancer than in normal mucosa (7.5 µg/l), and in chronic gastritis patients (13.9 µg/l). The level of SPGI and the SPGI/SPGII ratio were significantly lower in patients with gastric cancer than patients with chronic gastritis or healthy group.

The presence of *H.pylori* infection lead to raise the SPGI in patients with chronic gastritis than those with gastric cancer patients (3,4). This possibly due to the opposite influence of gastric atrophy on SPGI in patients infected with *H.pylori*, which make SPGI level much lower than that of patients who were infected with *H.pylori* but had no gastric atrophy (2, 14). However, the number of glands, including chief cells reduced with replacement by intestinal metaplasia, which might cause decrease in the SPGI (18, 19). Therefore, it was a progressive decrease in the SPGI/SPGII, proportional to increase degree of infiltration of polynuclear cell and monocytes, atrophy, intestinal metaplasia, and the stage of precancerous lesion to gastric cancer (14, 18, 20).

Table -2: The effect of *H.pylori* infection on SPG I and SPG II

Variables	*H.pylori* status	No.	Mean ±SEm g/l	*P* Value
SPG I	Positive	94	104.2 ± 10.2	< 0.01
	Negative	33	74.4±4.6	
SPG II	Positive	94	17.8 ± 3.4	< 0.05
	Negative	33	8.4±0.4	
SPG I / SPG II	Positive	94	5.8 ± 0.7	< 0.05
	Negative	33	8.8±0.8	

The serum level of PGII does not decrease as the corpus mucosa progresses from normal mucosa to non-atrophic gastritis to atrophic gastritis to gastric cancer (4). The explanation for this elevated level of the SPGII in that it is synthesized not only by cells of the corpus, but also by gland of the antrum and the duodenum, and the infection with *H.pylori* stimulate secretion by the

glands in the antrum thus increases the concentration of SPGII (5, 21).

Table -3: Serum level of PGI and PGII and the ratio of PGI/PGII in subjects with normal mucosa, chronic gastritis and gastric cancer.

Variable	Groups	No.	Mean ± SE mg/l	Sig. between Groups
SPG I	NM	33	71.6±5.2	NM- GC**
	Gas	64	101.5 ± 7.1	Gas- GC**
	GC	30	41.4 ± 4.2	
SPG II	NM	33	7.5±1.6	NM- GC**
	Gas	64	13.9 ± 0.6	Gas- GC*
	GC	30	23.2 ± 5.2	
SPG I / SPG II	NM	33	9.6 ± 2.0	NM- GC**
	Gas	64	7.6 ± 0.4	Gas- GC**
	GC	30	1.8 ± 0.8	

*Significant at the 0.05 level,

** Significant at the 0.01 level.

NM**:** normal mucosa, Gas**:** chronic gastritis, GC**:** gastric cancer.

Conclusion

The pepsinogen test method can be used as a screening test for high-risk subjects with atrophic gastritis rather than as a tool for screening for cancer itself. Low serum PGI value with lowest ratio of serum PGI /PGII was the best indicator for the risk of developing malignancy. The variation in serum PGs levels with *H.pylori* status support the importance of this bacteria in development of gastric diseases. Therefore, *H.pylori* was the bacterial factor involved in the increased serum PGs secretion.

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