Pepsinogen I and II, Gastrin and Cag A Serum Levels in Shiraz

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ABSTRACT

BACKGROUND

Despite the similar rate of HP infection, the rate of gastric cancer (GC) differs in different regions of the country. There are conflicting reports for using a panel of serologic tests such as pepsinogens I, II (PG I and PG II), and gastrin for population screening. We designed this study to assess healthy appearing adults in Shiraz, southern Iran in order to evaluate the correlation of these serological tests with demographics and lifestyle in a region with a low rate of gastric malignancy.

METHODS

In a population-based study, 846 out of 1978 subjects who were selected by cluster random sampling based on postal code division in Shiraz agreed to participate in the present study. A questionnaire that included age, gender, weight and height, lifestyle such as physical activity, smoking and the use of nonsteroidal anti inflammatory drugs (NSAIDs) was completed. A blood sample was taken after overnight fasting for measurements of PG I, PG II and Cag A status by enzyme-linked immunosorbant assay (ELISA). Gastrin level was measured by radioimmunoassay (RIA).

RESULTS

The study included 305 men and 541 women. Their mean age was 50.53+11.4 (range: 35-99 years). The level of PG I was significantly more in males than females (116.6±57.1 vs. 103.1±55.8, $p < 0.001$), lower in older age groups ($p = 0.01$), and rural compared with urban residents (110.3+55.7 vs. 100.2+58.1, $p = 0.02$). The serum level of PG II was less in obese subjects ($p = 0.5$). There was no significant correlation between PG I, PG II, smoking, NSAID use and activity. Gastrin level were not correlated with any of the demographic characteristics. The level of Cag A was significantly different between males and females (30.5±37 vs. 37.7±41.7, $p < 0.001$), more in older subjects ($p = 0.007$) and non smokers ($p = 0.001$). The serum levels of PG I and PG I/PG II ratio decreased significantly in subjects with positive Cag A serology ($p < 0.05$). The ratio of PG I/PG II was lower than 3 in 35 (4.1%) subjects.

CONCLUSION

In this area, the PG I/PG II ratio is less than 3 in 4% of subjects of which most are positive for Cag A serology and older than 50. We recommend comparison of these findings with high GC mortality regions.

KEYWORDS

Pepsinogen I, II; Cag A; Population study; Southern Iran

INTRODUCTION

Gastric cancer (GC) is the second cause of cancer death worldwide.[1] Early diagnosis of GC is essential to decrease mortality. The most common type of GC, the intestinal type, is usually preceded by chronic atrophic gastritis.[2] Human pepsinogens (PG) are inactive proenzymes of pepsin originating in the gastric mucosa and are classified biochemically and immunochemically into two groups: PG I and PG II. Serum PG levels reflect the morphologic and functional status of gastric mucosa. The combined assay of PG I and the PG I/PG II ratio has been used to identify participants at high risk of GC and considered as a serum biopsy for gastric disease.[3, 4] Gastrin, produced exclusively in the antrum and secreted directly in the blood, is the specific marker of gastric G cell function.[5] Low serum gastrin and PG I levels have been reported to predict the presence of atrophic gastritis in *Helicobacter pylori* (HP) infected persons.[6, 7]

HP is known to be the causative agent for several gastro-duodenal diseases.[8] This infection, particularly cytotoxin associated gene A (Cag A) positive types, may cause peptic ulcer disease and malignant gastric tumors or specifically gastric adenocarcinoma. Recently, the determination of serum PG I, PG II, and gastrin levels has been proposed as a series of non-invasive markers for the assessment of both the morphological and functional status of gastric mucosa in subjects with dyspeptic symptoms.[9-11] Controversy exists for using serum PG levels, and its cutoff point as a screening test for gastric atrophy. Some reports have proposed that a panel of serologic tests such as PG I, PG II, the PG I/PG II ratio, gastrin and HP may be helpful for indicating those in need of a gastroscopy and biopsy.[12-15]

Our area is an endemic region for HP infection, where up to 80% of the general population has a positive serology for this infection.[16] Despite the similar rate of HP infection, the rate of GC differs in different regions. In comparison to Ardabil, a city in northern Iran, Fars Province has a very low rate of gastric cancer (GC) occurrence.[17] We designed this study in healthy appearing adults in Shiraz, southern Iran to evaluate the correlation of these serological tests with demographics and lifestyles in this region that has a low rate of gastric malignant disease.

MATERIALS AND METHODS

In a population-based study, 3600 households selected by the cluster random sampling method based on postal code divisions of Shiraz, southern Iran were invited to participate in a gastroenterology health survey program. A total of 1978 subject enrolled in that study (response rate 54.9%). Of these, 846 subjects agreed to participate in the present study. The project was approved by the Ethics Committee of Shiraz University of Medical Sciences and written consents were obtained from each subject. The study was undertaken from April to September, 2004.[18, 19]

A questionnaire that included age, gender, weight and height, lifestyle such as physical activity (at least 30 min/week or sufficient to produce adequate sweating), smoking and the use of non steroidal anti-inflammatory drugs (NSAIDs) was completed.[18] Body mass index (BMI) was divided into five categories of thin (< 18 kg/m^2), normal (18-24.9 kg/m^2), overweight (25-29.9 kg/m^2), obese (30-40 kg/m^2) and very obese (> 40 kg/m^2). Participants were classified in four age groups; 29-44 years, 45-54 years, 55-64 years, and over the age of 65.

Determination of PG I, PG II, gastrin and Cag A

Following completion of the interviews, patients' basal blood samples were taken after overnight fasting for measurements of PG I, PG II and Cag A. The blood was centrifuged and serum aliquots stored immediately at -20^{oC}. Measurements of serum PG I and PG II were

obtained by ELISA (Biohit, Finland). Cag A was also measured by ELISA (Diapro, Italy) and gastrin by radioimmunoassay (RIA) (Biohit, Finland). All samples were analyzed at the Gastroenterohepatology Research Center Laboratory. Fasting serum gastrin levels over 32 pmol/l and Cag A more than 5 EIU/ml were considered abnormal.

Statistical analysis

Statistical analysis was performed using SPSS version 11.5. Descriptive variables such as mean, median and standard deviations were used. One-way analysis of variance (ANOVA) was performed for determining significance differences among the means of PG I, PG II, PG I/PG II ratio, gastrin, Cag A and age. Independent sample t-test was performed to elucidate the differences among the means of PG I, PG II, PG I/PG II ratio, gastrin, Cag A and BMI. Chi square was used for evaluating associations among lifestyle, demographic data and PG I, PG II, PG I/PG II, gastrin and Cag A values. P values less than 0.05 were considered significant.

RESULTS

The study included 305 men and 541 women with a mean age of 50.53+11.4 years (range: 35-99 years). There was no significant difference in PG II levels in the different age groups. The PG I level in the 29-44 age group was significantly different from other age groups ($p < 0.05$) and more in males than females. The level of gastrin did not correlate with any of the demographic characteristics. In subjects ≥ 65 years, the level of Cag A was significantly different compared to the 29-44 and 45-54 age groups ($p < 0.05$). There was a significant correlation between gender and smoking, which was significantly more in females and non-smokers (Table 1). BMI showed no significant correlation with serum PG I, gastrin and Cag A levels, but was significant with PG II. The serum level of PGI and PG I/PG II ratio decreased significantly in subjects with positive Cag A serology ($p < 0.05$). The results also indicated different values of PG I in participants who resided in urban regions ($p < 0.02$).

A total of 35 (4.1%, mean age: 57.5 years) subjects had a decreased ratio of PG I to PG II (< 3). The correlation of Cag A status and fasting gastrin levels in subjects with PG I/PG II ratios less than 3 are presented in Table 2.

DISCUSSION

Although serum PG levels could be related to age, however some disagreements have been reported in previous studies. In a Chinese study, Zhang et al. have reported significantly lower serum levels of PG I in subjects in the age ranges of 30-40 and above 70 years. The serum PG II level was significantly lower in the 50-60 year age group in comparison to other age groups. The PG I/PG II ratio showed a declining trend in those aged over 60.[20] In a Japanese study, Miki et al. have shown that the PG I level did not generally change with age.[21] In a Chinese study by Sun et al., there were higher serum PG I and II levels in males compared to females. In older adults, the PG I level was significantly lower. There was negative correlation between HP infection and PG level.[22]

Regarding the serum PG level, the general consensus is that PG II levels increase with age and remain stable until 50-60 years of age, while the PG I/PG II ratio decreases with aging due to a decreased PG I level, remaining stable until 60 years of age. In our study, PG I decreased significantly with age but no change was seen in PG II levels. Thus, we can assume that the PG I/PG II ratio should decrease accordingly. Derakhshan et al. have found PG I to be the most useful predictor of disturbances in acid secreting mucosa in patients with gastric atrophy. This finding may suggest disturbances in acid secretion in older subjects in our study.[23]

Table 1: Correlation between PG I, PG II, gastrin and Cag A serum levels with demographic and lifestyle characteristics (n=846).

Characteristics	Categories	N (%)	PG I (30-165 µg/l)	*p*	PG II (3 - 15 µg/l)	*p*	Gastrin (1-10 p mol/l)	*p*	Cag A (0 - 30 EIU)	*p*
Gender	Male	305 (36.1)	116.6±57.1	0.001	17.8±11.1	.322	6±4.5	.573	30.5±37	0.001
	Female	541 (63.9)	103.1±55.8		17±11.7		5.8±4		37.7±41.7	
Age (years)	29-44	302 (35.7)	118±60.8	0.01	18.6±12.1		5.9±4.8	0.133	27.4±35	0.007
	45-54	268 (31.7)	112.7±59.3		19.4±12.2		5.4±2.2		32.6±39.6	
	55-64	155 (18.3)	108±57		18±11.7		6.3±4.6		37.9±41	
	>65	121 (14.3)	100.8±52.3		14.9±10.2		6.2±5.3		36.3±41	
BMI	Thin	10 (1.2)	103.5±52.3	0.2	18±7.6	0.05	5.8±3.5	0.8	37.2±39.1	0.8
	Normal	266 (31.5)	112.8±61		18.8±12.7		5.8±4.2		34±40.3	
	Overweight	381 (45)	107.5±55.1		16.6±10.8		6±4.5		35.9±40.1	
	Obese	189 (22.3)	102.5±53.1		16.2±11		5.6±3.6		35.4±40.5	
Smoking	Negative	656 (77.5)	106.9±56.2	0.3	17.1±11.4	0.48	5.9±4.2	0.6	37±41.2	0.001
	Positive	190 (22.5)	111.6±58		17.8±11.5		5.7±4.2		29.3±36.4	
Residence	Urban	618 (73)	110.3±55.7	0.02	17.3±11.6	0.9	5.8±4.2	0.9	34.6±40.2	0.4
	Rural	228 (27)	100.2±58.1		17.2±11.2		5.8±4.4		36.2±40.4	
NSAID use	Negative	617 (72.9)	108.9±57.1	0.4	17.4±12.1	0.4	5.9±4.2	0.6	34.4±40.1	0.2
	Positive	229 (27.1)	105.5±55.2		16.8±9.7		5.7±4.3		37±40.5	
Activity	Negative	495 (58.5)	106.3±57.7	0.3	17.8±11.9	0.08	6±4.5	0.2	34.8±39.8	0.7
	Positive	351 (41.5)	110.3±54.9		16.4±10.8		5.6±3.7		35.6±40.9	

Table 2: Correlation of Cag A status and fasting serum gastrin levels in subjects with a ratio of serum PG I/PG II <3 (n=35/846).

Helicobacter pylori-associated Cag A serology (ng/ml)		Fasting serum gastrin < 3 (age range)	PG I/PG II ratio
Positive	<32	19 (47±11.14)	
	>32	2 (70±11.31)	
Negative	<32	14 (70.0±11.31)	
	>32	-----	

In our study, the level of gastrin did not correlate with any of the demographic characteristics. Aly et al. and Sipponen et al. have reported that serum gastrin increases linearly with an increase in atrophy grade of body mucosa.[24, 25]

When atrophic gastritis develops, the loss of antral glands leads to a reduction in G cells and serum gastrin.[24] Thus it is supposed that G-17 is a good serum biomarker of gastric antrum cellular activity.[26] Cao et al. have found that the level of G-17 in atrophic antral gastritis was significantly lower than atrophic corpus gastritis and this decrease was in accordance with atrophic severity.[15]

Bolukbas et al. have demonstrated that the usage of PG II as a serum marker in predicting atrophic gastritis could be more reliable than PG I or the PG I/PG II ratio.[13]

The level of PG I and PG I /PG II ratio decreased significantly in Cag A seropositive subjects, which has indicated a possible role of the HP Cag A genotype in the predisposition of our subjects to gastric atrophy and precancerous lesions. Despite no significant differences of Cag A status between urban and rural living, however the level of PG I was different.

Determination of serum PG is not routine in clinical practice. PG levels have been checked in basic science research and population-based research studies for the diagnosis of gastric atrophy. There is a 2-10 fold increased risk of cancer in subjects with low PG I or low PG I/PG II ratios, but the sensitivity of the test is

inadequate for population screening.[27]

In the study by Cao et al. the levels of PG I, PG I/PG II ratio, gastrin-17 and HP serology were determined in subjects with atrophic gastritis, gastric ulcer, duodenal ulcer, GC and a control group. PG I and PG I/II ratio were significantly lower in gastric atrophy and GC subjects. The levels of PG I and PG I/PG II ratio decreased whereas the level of gastrin-17 increased significantly in subjects with atrophic gastritis. The researchers stated that GC can be screened by use of higher serum gastrin-17, a lower serum PG I level and PG I/PG II ratio.[15]

Tako and colleagues from Japan evaluated the correlation of positive pepsinogen test (PG I / II less than 3) and gastric mucosal atrophy in 319 patients with dyspepsia. They found significant correlations between the level of serum pepsinogen, endoscopic findings and histological changes in the stomach.[28]

Watabe et al. performed a GC screening program in 6983 subjects who were classified into four groups of: normal PG and negative HP serology, normal PG and positive HP serology, atrophic pepsinogen and positive HP serology, and atrophic pepsinogen and negative HP serology. These cases were followed for 4.7 years, the annual incidence of GC was 0.04%, 0.06%, 0.35% and 0.60%, respectively. They have concluded that serum PG and anti-HP serology is useful for GC screening. Their research has shown the annual risk of GC may increase up to 2% per year in males over the age of 60 years, with severe atrophic gastritis according to PG level and loss of antibody to HP.[29]

Some authors suggested HP eradication for low risk populations (HP infection with normal PG level) as well as HP eradication and screening programs for high risk groups (HP infection and low PG level).[30] In a long term follow up study from Japan, which they used endoscopic evaluation in cases with positive PG test (PGI $\leq$ 70 ng/ml and PGI / PGII $\leq$ 3), from 13 789 subjects who underwent endoscopies, 125 cases of GC were detected. Most of them were in the early stage.[31]

The use of serum PG and HP antibody have been suggested for detection of patients at high risk of gastric atrophy and cancer. However the feasibility and cost effectiveness of this method is not clear and needs further studies, particularly for sensitivity and specificity of these tests.[32] In a recent study from Japan the risks and benefits of four methods for population screening of GC, photofluorography, endoscopy, serum PG and HP testing were evaluated according to published articles from this country. Due to insufficient data, only photofluorography was recommended for this purpose.[33] In another study from Japan, GC screening was performed with the combination of serum PG and barium digital radiography (DR) in 17 647 middle-aged males. Forty-nine cases of GC were detected. The efficacy of cancer detection was equal for both methods. They suggested this screening method for high risk populations.[34]

For lowering the mortality of GC in high incidence areas such as Japan, screening asymptomatic subjects has been proposed. However, in low incidence areas, screening of the general population probably is not cost effective, therefore only screening of high risk groups is advised. However the value of serum PG, gastrin and HP serology is not clear and needs more evaluation before recommending this strategy for GC screening in these areas.[35]

The limitations of our study should be mentioned; our study subjects were from a low incidence area for GC so the findings are not applicable for other areas. In our study it was necessary to do endoscopic investigations of subjects with low PG I/PG II ratios. This procedure could help for better evaluation of the rate of atrophic gastritis or precancerous lesions in this population.

There is no general consensus for using the PG test as screening for GC. However, as we are supposed to use the PG panel for GC screening in our area, in the present study we have detected 35 (4%) subjects with ratios of PG I/PG II less than 3.

If we take into account that all GC subjects would have low PG I/PG II ratios and considering the rate of GC occurrence in Fars province (ASR=5.42), it is estimated that we should screen about 20 000 adults preferably over the age of 50 years with the serum PG test and perform endoscopies in 800 subjects in order to detect 1 GC case. Thus, the serum PG test, especially as a cross sectional checking lab test, is not feasible for GC screening in our general population. We suggest comparison of these results with a high prevalent region in our country.

The serum levels of PG I and PG I/PG II ratio decreased significantly in subjects with positive Cag A serology, and in older subjects. A panel of PG I, II, and CagA for those over 50 years may help to decrease the numbers of cases that need screening endoscopy for detection of gastric precancerous lesions. However, this panel is not feasible and cost effective for use in areas with low GC occurrence. Similar studies in areas with high GC prevalence such as Northwestern Iran are recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this work.

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