RESEARCH ARTICLE

Serum Gastrin and the Pepsinogen I/II Ratio as Markers for Diagnosis of Premalignant Gastric Lesions

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Abstract

Background: Iran is a country with very high incidences of stomach cancer, especially in Northern parts. Here we assessed prognostic value of serum screening biomarkers among people >50 years old for early detection of precancerous lesions in a hot spot for gastric carcinoma in Guilan Province, North Iran. Methods: A cross-sectional population-based survey was conducted on 1,390 residents of Lashtenasha city with the mean age (SD) of 61.8 (9.02) years old (50.8% females) to assess the association of gastrin and the pepsinogen (PG) I/II ratio with premalignant gastric lesions. Blood samples were taken for CBC, blood group, and serologic exams (PGI, PGII, and gastrin 17) from each subject. Expert gastroenterologists performed upper GI endoscopy and ROC curves were generated to determine appropriate cutoff points. Results: Mean values of PGI, PGII, PGI/PGII and gastrin were significantly different between patients with and without atrophy or metaplasia (P<0.05). To diagnose atrophy and intestinal metaplasia, a significantly higher AUC was observed for the PGI/PGII ratio (70 and 72%, respectively) compared to the PGI (56, 55%), PGII (63, 64%) and gastrin (59, 61%) (all p<0.001). Conclusions: Biomarker tests such as the PGI/II ratio can be used in the screening and diagnosis of subjects at high gastric cancer risk in our region.

Keywords: Gastrin - pepsinogen I - pepsinogen II - preneoplastic lesion- gastric cancer - Northern Iran

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Introduction

Gastric cancer (GC), despite a recent gradual decline in incidence, is still one of the most common malignancies worldwide and remains the second most common leading cause of cancer death causing more than 700,000 deaths annually worldwide (Parkin et al., 2000; Axon, 2002; Parkin, 2004; Yanaoka et al., 2008).

In 2002, an estimated one million new cases of gastric cancer were diagnosed, with almost two-thirds occurring in developing countries. High-risk areas include Japan, China, South America, Eastern Europe, and Middle East (Parkin et al., 2000; Parkin et al., 2002; Maconi et al., 2008; Kwon et al., 2009; Miki, 2011).

Economically developing countries are facing an increasing burden of cancer as a result of population aging and growth as well as, increasingly, an adoption of cancer associated lifestyle choices including smoking, sedentary life, and ''westernized'' diets (Jemal et al., 2011).

Iran is a region with a high incidence of stomach cancer especially in Northern parts (Sadjadi et al., 2005; Mansour-Ghanaei et al., 2012). A strong spatial clustering of gastric cancer in both men and women has been described in Mazandaran and Golestan; two provinces of the Caspian Sea shore line (Malekzadeh et al., 2009). Data reported the highest incidence rate of gastric cancer of Iran in Ardebil- a north western province (49.1 per 100,000 in men and 25.4 per 100,000 in women) (Sadjadi et al., 2003).

As the early gastric cancer is asymptomatic or has non-specific symptoms, its diagnosis is usually made in the advance stages with a reported 5-year survival rate of less than 30% in most series (Maconi et al., 2008; Mansour-Ghanaei et al., 2012).Undoubtedly primary and secondary preventive activities decrease the burden of cancer patients to the hospital and minimize human suffering (Puri et al.,

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2010).

So it is important to introduce an efficient and cost-effective practical mass screening method for early detection of gastric cancer (Miki et al., 2011). At least for the intestinal type of gastric adenocarcinoma, a cascade of histopathologic lesions has been defined: chronic gastritis, atrophic chronic gastritis (ACG), intestinal metaplasia, and dysplasia (Carneiro et al., 2001; Dinis-Ribeiro et al., 2004). The identification of such lesions depends on invasive tests such as upper gastrointestinal endoscopy, and it represents a challenge because they are scattered and multifocal (Dinis-Ribeiro et al., 2004).

The second non-invasive option is the examination of gastric biomarkers from serum or plasma. Serum levels of pepsinogen (PG) have been used for decades to diagnose atrophic corpus gastritis non-invasively (Borch et al., 1989; Karnes et al., 1991; Iijima et al., 2009). In particular, in Japan, a country known to have a high prevalence of *H pylori* infection accompanied by gastric atrophy, the usefulness of the serum test to diagnose gastric atrophy has been extensively investigated, and there has been some success in screening subjects with a high risk of gastric cancer by determining the serum PG and PGI/II ratio (Borch et al., 1989).

Prevention unpublished works regarding cancer-related death in Guilan province during 1999-2002 in the Gastrointestinal and Liver Diseases Research Center (GLDRC) confirmed gastric cancer as the leading cause of cancer-related death which led to 31.4% of all cancer deaths over the province; These emerged the necessary of surveillance programs for GC especially among high risk population (Mansour-Ghanaei et al., 2010). This study is a part of a large-scaled study in hot point regions for GC designed to assess prognostic value of screening biomarkers like pepsinogen I/II and Gastrin among people >50 years as a cost-effective screening method for early detection of gastric carcinoma.

Materials and Methods

The pilot outcomes of Guilan Cancer Registry Study (GCRS) by management of GLDRC in Guilan University of Medical Sciences (GUMS) confirmed gastrointestinal cancers as the most common neoplasm in Guilan province (37%) and defined hot point regions for this cancer. The GCRS is a population-based cancer registry study which covers a population about 2.5 million and with a sample-size about 20,000 cases of various types of cancers. This study is designed and conducted as a mass screening program for GC between May 2010 to March 2011 in GLDRC to assess the association of serum level of PGI, II, its ratio and gastrin with precancerous lesions and early gastric cancer among people ≥50 years in a hot point regions for GC Lashtenesha. The study protocol was first approved by ethics committee of GLDRC.

One of the defined hot point regions for GC was Lashtenesha district which is located in the northeast of Rasht, the capital of Guilan Province. Information regarding population distribution of this region, and target population (>50 years) was collected through health-treatment centers. Lashtenesha has a population over 45000 persons (49.6% males, 50.4% females), among them 10500 persons have >50 years old. Most of the people settled in rural regions (70.0%) (Mansour-Ghanaei et al., 2010).

Study design

A cross-sectional population-based survey was conducted on 1390 residents of Lashtenesha city to assess the association of Gastrin and Pepsinogen I/II ratio with the premalignant gastric lesions. Two months prior the screening program, the target population were called mainly through two methods based on their locations. In rural regions, all people ≥50 years were defined with house-house direct refer by environmental health experts and Behvarzes (Auxiliary health personnel in health house network locally called the Behvarz) and with a close cooperation of health centers, sheriffdom and local governors.

The study was performed by Gastrointestinal and Liver Disease Research Center of Guilan University of Medical Sciences. The process was done by trained blinded Internists, General practitioners and pathologists. First of all the objectives of the survey were explained to the responders and they were asked if they would be prepared to help research by registering in it. Those who did not consent to answer the questions were excluded .A specific code was given to him/her to refer in a defined day to a specific health center for further evaluation. In urban area, residents were invited to register in the survey by posters, pamphlets, and public media.

Sample size

The sample was calculated 1390 people from the general population of Lashtenesha -Guilan province based on the prevalence among the pilot group (p=25% for premalignant gastric lesions) and considering the sensitivity of almost 70% and specificity of almost 90% and the type one error of 0.05. The sampling was done in a Randomized Systematic Clustering Method.

Data collection

Three trained general practitioners filled a detailed questionnaire including demographic characteristics, history of smoking, drug history and family history of GI cancers in the first-degree relatives for each subject and did the physical examination for each subject. The study goals and endoscopy procedure were again explained for each individual, a specific code was given to each participant and a time was set for endoscopy. An informed consent was obtained from each participant prior their enrollment. Participants who were consuming proton pomp inhibitors (PPIs) were ask to discontinue their drugs 4 weeks prior endoscopy. Persons who were under any antibiotics therapy were advised to complete their treatment and two weeks after finishing refer for endoscopy procedure.

Patients with gastric cancer or those with previous gastric surgery, and those who were not able to discontinue their medications were excluded. After giving consent, the subjects were referred for further evaluation. Blood samples were taken for CBC, blood group, and serologic exams (PGI, PGII, and Gastrin 17). Expert

gastroenterologist rechecked history and physical examination to ensure fitting inclusion and exclusion criteria and performed Upper GI endoscopy for them in a one-month period.

Subsequent two local anesthesia using Lidocaine 10% (with 10 minute interval) upper GI endoscopy (video-endoscope, GIF-Q240Z; Olympus Co., Tokyo, Japan) was performed by six experienced endoscopists and cooperation of a trained staff. Five samples were taken from different parts of stomach (body, fundus, antrum, angularis and one sample from antrum for RUT to detect *H pylori* infection). The samples were fixed in formalin 10%, labeled by subjects' codes and sent to the blinded pathologist.

Data analysis

Data were analyzed using STATA software (version 11, StataCorp, College Station, TX, USA). Demographic data were presented as mean (standard deviation (SD)) or number (%), as appropriate. Student's t test or nonparametric Mann-Whitney U test were used to compare the means of screening biomarkers with respect to precancerous lesions. The Receiver Operating Characteristic (ROC) curves were constructed to assess the accuracy of PGI, PGII, PGI/PGII ratio and Gastrin to diagnose atrophy, metaplasia and dysplasia and the areas under the curves (AUCs) and their 95% confidence intervals (CIs) were calculated.

Corresponding area under the ROC curves (AUC) were compared using the standard error of the test statistics as derived from the asymptotic variance covariance. Youden's index (J) was calculated to choose the optimal cutoff value. P value less than 0.05 was considered as statistically significant

Results

Totally 1390 subjects were included in the study. Mean age (SD) of the participants was 61.76 (9.02) with a range from 50 to 87 years old and 706 (50.8%) of them were females (Table 1) .

H pylori infection based on the RUT was positive in 66.6% (920) of participants (68.2% in men, 65% in women). The overall and gender specific prevalence of Atrophy, Intestinal Metaplasia and Dysplasia are shown in Table 2. The prevalence of Atrophy and Intestinal Metaplasia was significantly different between males and females (p= 0.004 and 0.001, respectively). Furthermore, about 38.35% of the subjects were shown to have at least one of the lesions (atrophy, metaplasia or dysplasia).

The mean value of PGI, PGII, PGI/PGII and Gastrin level were significantly different between patients with and without of atrophy or metaplasia. However we observed no difference in mean values of PGI, PGII and Gastrin in presence or absence of dysplasia but mean PGI/PGII was different with respect to dysplasia (Table 3). Then we used only PGI/PGII ratio to diagnose dysplasia.

The AUC, sensitivity, specificity, PPV and NPV of cutoffs that obtained based on Youden's index for screening biomarkers were summarized in Table 4.

The cutoff values based on Youden's J index for PGI, showed low sensitivity. Accordingly we decided to report more sensitive cutoff values. So we used the cutoff value of PGI<88.7 ng/ml with the sensitivity and specificity of 64.4% and 43% respectively for atrophy, cutoff value of PGI<90.2 with the sensitivity and specificity of 64% and 40% respectively for intestinal metaplasia and the cutoff value of PGI<90.6 ng/ml with the sensitivity and specificity of 64.7% and 40% respectively for any of three lesions.

To diagnose the atrophy and intestinal metaplasia, a significantly higher AUC was observed for the PGI/PGII ratio (70, 72%, respectively) compared to the PGI (56, 55%, respectively), PGII (63, 64%, respectively) and gastrin (59, 61%, respectively) (all p<0.001).

Also to diagnose the any of three lesions, the PGI/PGII ratio had higher AUC than the PGI, PGII and gastrin (all p<0.001) (Figure 1).

Table 1. Participant's Demographic and Clinical Data (n=1390)

Variable		Frequency (%)
Gender	Male	684 (49.2)
	Female	706 (50.8)
Mean age (year)		61.7±9
Age group	50-59	752 (54.1)
	60-69	335 (24.1)
	70-79	246 (17.7)
	≥80	57 (4.1)
Marital state	Married	1369 (98.5)
	Single	21 (1.5)
Smoking		154 (11.1)
Family history of cancer		171 (12.3)
Drug history	Aspirin	169 (12.2)
	Warfarin	6 (0.43)

Table 2. Overall and Gender Specific Prevalence of Atrophy, Intestinal Metaplasia and Dysplasia

	Prevalence (number)			P-value
	Female	Male	Total	
Atrophy	24.96(176)	31.96(218)	28.41(394)	0.004
Intestinal Metaplasia	26.91(190)	35.38(242)	31.08(432)	0.001
Dysplasia	2.42(17)	2.93(20)	2.67(37)	0.56
Any of three lesions	33.52(235)	43.32(295)	38.35(530)	<0.001

Table 3. Mean (SD) Value of PGI, PGII, PGI/PGII and Gastrin for Patients With and Without of Atrophy, Metaplasia and Dysplasia

		PGI	PGII	PGI/PGII	Gastrin
Total	Mean(SD)	89.33(43.33)	15.46(10.56)	7.30(3.91)	4.92(9.34)
Atrophy					
	Positive	83.25(40.82)	17.90 (9.94)	5.46(2.78)	6.31(10.73)
	Negative	91.77(44.12)	14.52(10.65)	8.02(4.06)	4.39(8.68)
	P-value	0.001	<0.001	<0.001	<0.001
Intestinal Metaplasia					
	Positive	84.94(43.82)	18.56(11.7)	5.50(2.99)	5.96(9.58)
	Negative	91.31(42.98)	14.06(9.68)	8.11(4.01)	4.45(9.19)
	P-value	0.011	<0.001	<0.001	0.005
Dysplasia					
	Positive	89.59(58.84)	17.30(11.04)	5.87(2.78)	5.96(6.76)
	Negative	89.26(42.91)	15.36(10.52)	7.35(3.93)	4.88(9.4)
	P-value	0.18	0.27	0.02	0.05

*PGI: pepsinogen I, PGII: pepsinogen II

Table 4. Screening Characteristics of PGI, PGII, PGI/PGII Ratio and Gastrin for Diagnosis of Atrophy, Metaplasia and Dysplasia

Cutoff Value		Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV	AUC (95% CI)
Atrophy	PGI<73.61	47.8 (42.8-52.9)	63.1 (60-66.1)	34	75.3	0.56(0.52-0.59)
	PGII>12.89	65.4 (60.5-70.1)	58.2 (55.1-61.3)	38.3	80.9	0.63(0.6-0.66)
	PGI/PGII<6.79	73.7 (69-78)	57.3 (54-60.3)	40.6	84.5	0.7 (0.67-0.73)
	Gastrin>2.49	57.3 (52.2-62.2)	59.9 (56.8-62.9)	36	78.1	0.59(0.56-0.62)
Intestinal metaplasia	PGI<59	27.8 (23.7-32.3)	83.2 (80.5-85.4)	42.6	71.8	0.55(0.52-0.58)
	PGII>14.9	56.1 (51.3-60.9)	68.1 (65-71)	44.2	77.5	0.64(0.61-0.67)
	PGI/PGII<6.69	73.8 (69.4-77.9)	61.0 (57.7-64)	45.9	83.8	0.72(0.69-0.74)
	Gastrin>1.39	78.6 (74.4-82.4)	40.9 (37.8-44.1)	37.4	81	0.61(0.58-0.64)
Dysplasia	PGI/PGII<8	86.5 (71.2-95.5)	39.3 (35.7-41)	3.73	99	0.61(0.53-0.7)
Any of three lesions	PGI<65.61	35.0 (30.9-39.2)	74.7 (71.5-77.5)	46.1	64.8	0.55(0.52-0.58)
	PGII>13	63.3 (59.1-67.4)	61.9 (58.5-65.2)	50.8	73.1	0.65(0.62-0.67)
	PGI/PGII<6.79	72.8 (68.8-76.6)	61.8 (58.4-65.1)	54.2	78.5	0.72(0.69-0.74)
	Gastrin>2.49	56.2 (51.8-60.5)	62.0 (58.7-65.3)	47.8	69.6	0.6 (0.58-0.64)

*PGI: pepsinogen I, PGII: pepsinogen II, PPV: positive predictive value, NPV: negative predictive value AUC: area under curve

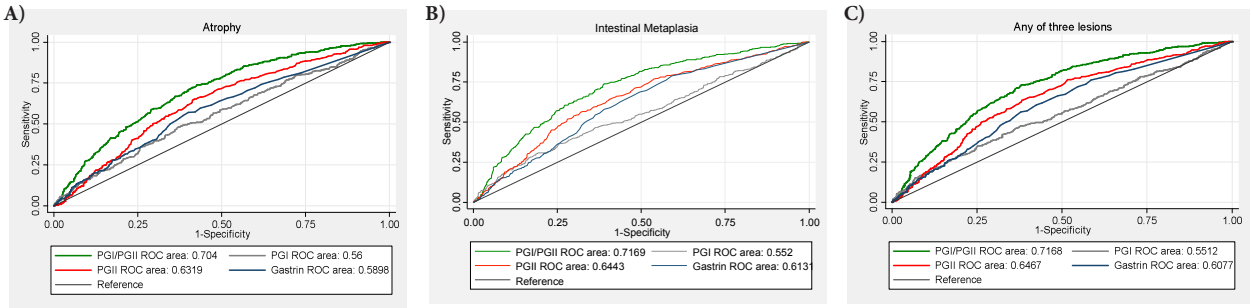

Figure 1. ROC Curves Based on Four Predictor Variables. A) Diagnosis of Atrophy, B) Diagnosis of Intestinal Metaplasia and C) Diagnosis of Subjects with any of three Lesions (Atrophy, Intestinal Metaplasia and Dysplasia)

Discussion

To improve the effectiveness of gastric cancer screening, serum pepsinogen (PG) assay has recently been introduced in Japan. As atrophic gastritis becomes more severe, normal gland function is lost and enzyme production is affected. PG, the precursor of pepsin, exists as two main types, I (PGI) and II (PGII), both of which are produced by the chief and mucus neck cells in the gastric fundus. PGII, but not PGI, is produced by the pyloric glands in the antrum and Brunner's glands in the proximal part of duodenum. As gastritis progresses, mild inflammation leads to elevated concentrations of PGI and PGII in the circulation. As the severity of atrophy advances, chief cells are replaced by pyloric glands and the concentration of PGII remains increased, while the concentration of PGI decreases. Consequently, the ratio between the concentrations of PGI and PGII is greatly reduced. Thus serum PG concentration reflects the morphological and functional status of the gastric mucosa. Many gastric cancers 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 (Miki, 1992; Kodoi et al., 1995; Kitahara et al., 1996; Kitahara et al., 1999).

In our survey, all of the parameters PGI, PGII and PGI/II were significantly different between the healthy people and those who had any of the premalignant histologic gastric lesions (metaplasia, atrophy and dysplasia). The ROC curves generated with serum PGI concentrations, PGII concentrations, or PGI/II ratios were examined.

Several determinations of a suitable cutoff point for gastric cancer screening have previously been made based on the findings of photofluorography. Stemmermann et al used a PGI/II ratio of less than 2.0 as the cutoff point, to separate the subjects at high risk and those at low risk of developing gastric cancer (1987). Miki et al suggested using a serum PGI concentration of less than 70 ng/ml and a PGI/II ratio of less than 3.0 as the cutoff point (1993). Kodoi et al suggested a serum PGI concentration of less than 50 ng/ml and a PGI/II ratio of less than 3.0 as the cutoff point (1995). As all these reports are based on comparisons with photofluorography findings, their determinations of the false negative rate, sensitivity, specificity, and accuracy are not reliable. Therefore in this study, we determined a cutoff point using endoscopic diagnosis.

In a recent study , designed in our country , the best cutoff value in gastric atrophy assessment was calculated at PGI, 56 ng/ml (sensitivity: 61.9%, specificity: 94.8%) but we used the cutoff value of PGI <88.7 ng/ml (sensitivity and specificity of 64.4% and 43% respectively) for atrophy (Nasrollahzade et al., 2011). That work was an office based study and 309 persons were enrolled but present study was a population based survey on 1390 persons explaining the difference of cutoff between two studies.

Iijima's analysis showed that non-invasive serum PG assays accurately diagnosed Japanese patients with atrophic corpus gastritis. Similar findings were also obtained using both the conventional Japanese PG tests and the PG assays of the novel European GastroPanel examination in which, in addition to PG, the serum/

plasma levels of amidated gastrin-17 (G-17) and *H pylori* antibodies (IgG and IgA) were also measured. The diagnostic accuracy of both the Japanese test and the GastroPanel test was more than 80% when compared with endoscopic biopsy histology (2009). Also we found that PGI, PGII, and PGI/II could have the sensitivity of higher than 60% for detecting gastric premalignant lesions (especially PGI/II which had the sensitivity of more than 70%) but the specificity for these parameters is low. In a study by Kitahara et al, the most suitable cutoff point in screening for gastric cancer was found to be a PGI concentration of less than 70 ng/ml and a PGI/II ratio of less than 3.0. This cutoff point provided a sensitivity of 84.6%, a specificity of 73.5%, a positive predictive value of 81%, and a negative predictive value of 99.9% (1999).

In the study by Dinis-Ribeiro et al using a PGI/II ratio of ≤3 as the cutoff for dysplasia diagnosis, the sensitivity was 70% , the specificity was 65% (almost similar to the present survey) (2004).

Several authors from different parts of the world reported on the relationship between the serum levels of PGI and PGII as related to mucosal changes in the stomach, mostly in asymptomatic and/or unknown gastric mucosal lesions (Miki et al., 1989; Kitahara et al., 1999; Varis et al., 2000; Dinis-Ribeiro et al., 2004; Iijima et al., 2009), but another study in our country showed neither PGI nor PGI/PGII ratio were able to select those with precancerous conditions and corpus-predominant gastritis among the first-degree relatives of gastric cancer patients (Haj-Sheykholeslami et al., 2008). In the present study, the PGI, and PGI/II ratio tests were used in a set of patients with very high probability of a positive test in Iran.

We defined the same discriminative point (PGI/PGII ratio of ≤3) as the best cutoff, as in other studies with very similar results. Considering validity measurement estimates, we found sensitivity values that may not be cost-effective for screening purposes. However, if we consider negative predictive values even in a select high-risk sample as we did, we may argue that in a clinical background where no clear recommendations had been made until now, PGI and PGI/PGII serum level ratio may be useful tools (Dinis-Ribeiro et al., 2004).

Biomarker examinations from serum or plasma are free of the biases that affect biopsy histology or sampling. The biomarkers give an average view of the structure and function of the gastric mucosa.

One of the limitation of present study and similar ones is that Interpretation of the biopsy findings by pathologists may, therefore, easily fail, particularly in antral biopsies, in which the interobserver agreement, even between "expert" pathologists, is known to be imperfect and may require practice or even the application of morphometry (Iijima et al., 2009).

In the present study, the biomarker tests were compared with endoscopic biopsy histology. Endoscopic biopsy histology is, however, not a reliable gold standard. Biopsy results are commonly biased by several factors, including such confounders as biopsy sampling, number of biopsies available from each gastric compartment, laboratory processing of the specimens, and interpretation of the biopsy by pathologists. In the similar studies such as Iijima's investigation in Japan, the biopsy analysis was based on only one biopsy from both the antrum and corpus, and so the study protocol did not strictly follow the guidelines of the Sydney System (the guidelines indicate at least two biopsies from each compartment) . But the advantage of our study is that we performed 5 biopsies from different parts of stomach.

Our results is based on a larger number of subjects, over a wider age range in contrary to other surveys such as the cutoff point suggested by Kikuchi et al. (2011), Iijima et al. (2009) and Miki et al. (1993). Therefore our results may be more reliable.

In conclusion, biomarker tests such as PGI, PGI/II and Gastrin levels can be used in the screening and diagnosis of subjects with a high cancer risk; i.e. subjects with atrophic gastritis in which a careful diagnostic endoscopy (gastroscopy) is mandatory to find possible neoplastic or precancerous lesions at an early and curable stage.

Serum PGI, PGI/II ratio and Gastrin-17 screening can identify non-ulcerated asymptomatic premalignant lesions irrespective of the size and location of the lesion. The PG method has many advantages in our region.

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References

Axon A (2002). Gastric cancer and Helicobacter pylori. *Aliment Pharmacol Ther*, **16**, 83-8.

Borch K, Axelsson CK, Halgreen H, et al (1989). The ratio of pepsinogen A to pepsinogen C: a sensitive test for atrophic gastritis. *Scand J Gastroenterol*, **24**, 870-6

Carneiro F, Machado JC, David L, (2001). Current thoughts on the histopathogenesis of gastric cancer. *Eur J Cancer Prev*, **10**, 101-2.

Dinis-Ribeiro M, da Costa-Pereira A, Lopes C, et al (2004). Validity of serum Pepsinogen I/II ratio for the diagnosis of gastric epithelial dysplasia and intestinal metaplasia during the follow-up of patients at risk for intestinal-type gastric adenocarcinoma. *Neoplasia*, **6**, 449-56.

Haj-Sheykholeslami A, Rakhshani N, Amirzargar A, et al (2008). Serum pepsinogen I, pepsinogen II, and gastrin 17 in relatives of gastric cancer patients: comparative study with type and severity of gastritis. *Clin Gastroenterol Hepatol*, **6**, 174-9.

Iijima K, Abe Y, Kikuchi R, et al (2009). Serum biomarker tests are useful in delineating between patients with gastric atrophy and normal, healthy stomach. *World J Gastroenterol*, **15**, 853-9.

Jemal A, Bray F, Center MM, et al (2011). Global cancer statistics. *CA Cancer J Clin*, **61**, 69-90.

Karnes WE Jr, Samloff IM, Siurala M, et al (1991). Positive serum antibody and negative tissue staining for Helicobacter pylori

in subjects with atrophic body gastritis. *Gastroenterology* , **101**, 167-74

Kikuchi R, Abe Y, Iijima K, et al (2011). Low serum levels of pepsinogen and gastrin 17 are predictive of extensive gastric atrophy with high-risk of early gastric cancer. *Tohoku J Exp Med*, **223**, 35-44.

Kitahara F, Kashiwagi A, Kanai T, et al (1996). A role of pepsinogen assay as the first cancer screening before screening by gastroscopy. *J Gastroenterological Mass Survey*, **116**, 42-8.

Kitahara F, Kobayashi K, Sato T, et al (1999). Accuracy of screening for gastric cancer using serum pepsinogen concentrations. *Gut*, **44**, 693-7.

Kodoi A, Yoshihara M, Sumii K, et al (1995). Serum pepsinogen in screening for gastric cancer. *J Gastroenterol*, **30**, 452-60.

Kwon YM, Lim HT, Lee K, et al (2009). Factors associated with use of gastric cancer screening services in Korea. *World J Gastroenterol*, **15**, 3653-9.

Maconi G, Manes G, Porro GB (2008). Role of symptoms in diagnosis and outcome of gastric cancer. *World J Gastroenterol*, **14**, 1149-55.

Malekzadeh R, Derakhshan MH, Malekzadeh Z (2009).Gastric cancer in Iran: epidemiology and risk factors. *Arch Iran Med*, **12**, 576-83.

Mansour-Ghanaei F, Joukar F, Soati F, Mansour-Ghanaei A, Bakhshizadeh Naserani S (2012). Knowledge about gastric carcinoma in North of Iran, a high prevalent region for gastric carcinoma: a population-based telephone survey. *Asian Pac J Cancer Prev*, **13**, 3361-6.

Mansour-Ghanaei F, Sokhanvar H, Joukar F, et al (2010). Endoscopic findings in a mass screening program for cancer in a high risk region- Guilan province of Iran. *Asian Pac J Cancer Prev*, **13**, 1407-12.

Miki K (2011). Gastric cancer screening by combined assay for serum anti-Helicobacter Gastric cancer screening by combined assay for serum anti-Helicobacter. *Proc Jpn Acad Ser B Phys Biol Sci*, **87**, 405-14.

Miki K, Ichinose M, Ishikawa KB, et al (1993). Clinical application of serum pepsinogen I and II levels for mass screening to detect gastric cancer. *Jpn J Cancer Res*, **84**, 1086-90.

Miki K (1992). Mass screening of stomach neoplasms by serum pepsinogen analysis. *J Jpn Society of Internal Med*, **81**, 654-9.

Miki K, Ichinose M, Kawamura N, et al (1989). The significance of low serum pepsinogen levels to detect stomach cancer associated with extensive chronic a gastritis in Japanese subjects. *Jpn J Cancer Res*, **80**, 111-14.

Nasrollahzade D, Aghcheli K, Sotoudeh M, et al (2011). Accuracy and cut-off values of pepsinogens I,II, and Gastrin 17 for diagnosis of gastric fundic atrophy: Influence of gastritis. *PLoS one*, **6**, 26957.

Parkin DM, Bray F, Ferlay J, et al (2005). Global cancer statistics, 2002. *CA Cancer J Clin*, **55**, 74-108.

Parkin DM (2004). International variation. *Oncogene*, **23**, 6329-40.

Parkin DM, Bray FI, Devesa SS (2001). Cancer burden in the year 2000. The global picture. *Eur J Cancer*, **37**, 4-66 .

Puri S, Mangat C, Bhatia V, Kaur AP, Kohli DR (2010). Knowledge of cancer and its risk factors in Chandigarh, India. *Int J Epidemiol*, **8**, 1-7.

Sadjadi A, Malekzadeh R, Derakhshan MH, et al (2003). Cancer occurrence in Ardabil: results of a population-based cancer registry from Iran. *Int J Cancer*, **107**, 113-8.

Sadjadi A, Nouraie M, Mohagheghi MA, et al (2005). Cancer occurrence in Iran in 2002, an international perspective. *Asian Pac J Cancer Prev*, **6**, 359-63.

Stemmermann GN, Samloff IM, Nomura AM, et al (1987). Serum pepsinogens I and II and stomach cancer. *Clin Chim Acta*, **163**, 191-8.

Varis K, Sipponen P, Laxen F, et al (2000). Implications of serum pepsinogen I in early endoscopic diagnosis of gastric cancer and dysplasia. Helsinki Gastritis Study Group. *Scand J Gastroenterol*, **35**, 950-6.

Yanaoka K, Oka M, Mukoubayashi C, et al (2008). Cancer high-risk subjects identified by serum pepsinogen tests: outcomes after 10-year follow-up in asymptomatic middle-aged males. *Cancer Epidemiol Biomarkers Prev*, **17**, 838-45.