Plasma ghrelin concentration and pepsinogen I/II ratio as non-invasive markers for upper gastrointestinal malignancy

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Abstract

Malignancy in the upper gastrointestinal tract is an important health problem worldwide. It is often detected late because there aren't typical early symptoms. If the cancer is caught soon enough with identifying useful diagnostic and prognostic markers, patients can have a high survival rate through endoscopy-assisted treatment and surgical therapy.

This cross sectional study was done on 308 referred patients to an endoscopy department of a University hospital affiliated in Iran. Demographic data (age, gender), body weight, stature and any history of systemic disorder were recorded. The blood samples were collected to measure C-reactive protein, fasting blood sugar, Albumin, complete blood count, serum pepsinogen I and II and ghrelin plasma levels after 12 hours of fasting, before upper gastrointestinal endoscopy was performed. Enzyme-linked immunosorbent assay was used to measure serum pepsinogen I and II and ghrelin plasma levels.

The patients were divided into three groups: patients with cancer (14.6%), patients with benign lesions (27.6%), and others with normal endoscopy. Patients with malignancy showed significant lower levels of PGI (median, 73 ng/ml), ratios of PGI/PGII (median, 4.29) and ghrelin (4.94 pg/ml). The average of ghrelin level and PGI/PGII ratio in the women was significantly higher than in the men. The mean of ghrelin level was significantly lower in the stomach lesions than in the esophagus and antrum lesions.

Inverse associations between ghrelin in addition to the PGI/II ratio and some gastrointestinal cancers suggest a potential role for serum ghrelin and in addition to the PGI/II ratio, as two biomarkers of upper gastrointestinal cancers.

Key words: upper gastrointestinal diseases, gastric cancer, ghrelin, pepsinogen I and pepsinogen II, non-invasive marker, endoscopy.
PG: Pepsinogen
Pg/mL: Pico gram/milliliter
Ng/ml: Nano gram/milliliter

Please cite this article as:
Reza Eghdam Zamiri et al. Plasma ghrelin concentration and pepsinogen I/II ratio as non-invasive markers for upper gastrointestinal malignancy. 2018;16(2):223-227. World Family Medicine.
DOI: 10.5742/MEWFM.2018.93266

Introduction

Malignancy in the upper gastrointestinal tract is an important health problem worldwide (1). Upper gastrointestinal cancer is the fourth-most-common cancer in the world and also the second-highest cause of cancer death. There has been a dramatic decrease in the incidence of this cancer and the death rate during the past numerous years. It is often detected late because there aren't typically early symptoms, so the survival rate drops significantly. The overall prognosis is poor due to high incidence and late diagnosis, nonetheless, the appropriate treatments and early diagnosis improve the prognosis. If the cancer is caught soon enough with identifying useful diagnostic and prognostic markers, patients can have a high survival rate through endoscopy-assisted resection, particularly with submucosal dissection, even without surgical therapy (2).

Barium meal studies, particular double-contrast barium meal, are alternative options to mass screening of gastrointestinal tract in some countries such as Japan, but they are not as sensitive (2). At this time, there are no serum analysis screening tests yet (2). Stomach cancer is the most common cancer with high mortality rate in both sexes in Zanjan of Iran (3), so any study to help resolve this issue is definitely valuable.

Ghrelin is a 28-amino acid peptide hormone that is produced by ghrelinergic cells in the gastrointestinal tract and in other organs and tissues particularly in the stomach. It is the endogenous ligand for the growth hormone secretagogue receptor which present on pituitary cells secreting growth hormone. It is suggesting effects of stimulation of growth hormone in the pituitary and in the regulation of gastrointestinal function (4, 5). Ghrelin exhibits numerous physiological functions, such as stimulation of growth hormone release, anti-inflammatory effects and regulation of energy expenditure. It also exhibits some protective effects (6). The gastric fundus secretes 10 to 20 times more ghrelin than the duodenum; also concentrations of ghrelin in the jejunum and ileum is lesser. In other words, concentrations of ghrelin are generally diminished per gram of tissue with increasing distance from the stomach. Decrease in ghrelin levels following gastrectomy or small-bowel resection inculcate that approximately two thirds of circulating human ghrelin secretes from the stomach and one third from the small intestine (7). Serum ghrelin levels in different gastrointestinal diseases have not yet been determined (8).

Pepsinogen is a substance which is secreted by the chief cells in the fundus of stomach wall before being converted into the proteolytic enzyme pepsin by gastric acid. It includes two major groups: Pepsinogen I and Pepsinogen II. Pepsinogen II is secreted from pyloric glands in the stomach antrum and Brunner's glands in the duodenum (9). Pepsinogen I is released into not only gastric lumen but also into the blood. The serum levels of the two types of pepsinogen represent the morphological and functional conditions of different parts of the gastric and duodenal mucosa, because they are secreted from different sources (9, 10).

Serum pepsinogen has been exerted as biomarkers of gastric inflammation. Serum pepsinogen I and pepsinogen II levels increase in the presence of Helicobacter pylori-related nonatrophic chronic gastritis (9). The serum pepsinogen is measured for information on the presence of intestinal metaplasia in addition to atrophic gastritis. In atrophic gastritis, serum pepsinogen I declines while serum pepsinogen II does not change, therefore the pepsinogen I to pepsinogen II ratio elevates (10). Atrophic gastritis is one of the main precursor lesions of gastric cancer (11). Serum pepsinogen analyses are helpful to screen as a test for cancer. (10).

Early diagnosis of upper gastrointestinal cancer is very important to survival increase in patients. So, this study investigated the serum ghrelin levels and pepsinogen I to pepsinogen II ratio as well as association between these two variables in different places in upper gastrointestinal tract, especially stomach, diseases. Also these two variables were investigated in both benign and malignant gastric ulcers in addition to other upper gastrointestinal tract diseases.

Materials and Methods

This cross sectional study was done on 308 referred patients to the endoscopy department of Vali-Asr University hospital affiliated to the Zanjan University of Medical Sciences, in Iran within one year. The study protocol was approved by the institutional board of human studies at Zanjan University of Medical Sciences (Registration number: A-12-482-6). Furthermore, before the inclusion of cases to this study, the study details were explained to each of the patients and their consent recorded prior to entering the study.

Demographic data (age, gender), body weight, stature and any history of systemic disorder were recorded. The Eastern Cooperative Oncology Group (ECOG) Score was determined to guage ability of all patients to tolerate therapies in serious illness. Data collection device was researcher made check list. Exclusion criteria included: No consent to participate in the study, ECOG Performance Status grade three and more than three, alcoholism and drug abuse, diabetes mellitus, major surgery within recent six months, organ failure and any severe chronic disorder. The blood tests were done to measure C-reactive protein, fasting blood sugar, Albumin and complete blood count. In addition, approximately eight milliliters of blood was collected from each patient after at least 4-hours fasting to measure serum pepsinogen I and II and ghrelin levels. Samples to measure serum ghrelin levels were immediately collected in Ethylenediaminetetraacetic acid and p-hydroxymercuribenzoic acid-containing tubes to prevent acylated ghrelin degradation by protease. Then samples were centrifuged at 3000 rpm for 5 minutes then the serum aliquot were stored immediately at -20°C. Serum pepsinogen I and II samples were kept in the freezer at -70°C. We used Enzyme-linked immunosorbent assay with BioVendor and BioHit Research and Diagnostic kits for ghrelin and pepsinogens tests. Microscopic examination of the gastric biopsies of every abnormal lesion was

conducted after the upper endoscopic process. The patients were divided into three groups: patients with cancer, patients with benign lesions, and patients with normal endoscopy.

Statistical analysis

Data was analyzed by SPSS 20 (SPSS, Chicago, IL, USA). ANOVA, nonparametric tests and Chi-square Test were used. P- Value less than 0.05 was considered to be statistically significant. The results were presented as mean (SE) or number (%) where appropriate.

Results

In the present study; 308 cases (52.6% males and 47.4% females) were investigated by upper gastrointestinal endoscopy. Total of the patients were divided into three groups according to endoscopic Biopsy Specimens: patients with malignant lesions (14.6%) including adenocarcinomas and squamous cell carcinoma, patients with benign lesions (27.6%) including inflammation, erosion, ulcer, hyperplastic polyp, intestinal metaplasia and celiac, and others with normal endoscopy (without lesion). (Table 1) The malignant lesions were especially prominent in men (p-value=0.02). There was no significant difference between body weight index in three groups. (Table 1)

Table 1: Comparison of different characteristics of the study patients including; age, serum concentrations of PGI [ng/ml], PGII [ng/ml], PG I to PG II Ratio and serum concentrations of Ghrelin [pg/ml] among studied groups (n=308).

	Normal group	Benign LESION group	Malignant LESION group	P-VALUE
Age [Median; Mean (SE)]	51.5;53.8 (4.3)	52;56.6(4.9)	70;70.9 (6.1)	<0.0001
SEX				0.002
WOMEN [number(percent)]	95(53%)	37(43.5%)	14(31.1%)	
MEN [number(percent)]	37(46.6%)	48(56.5%)	31(68.9%)	
places of lesions				
Stomach [number(percent)]		23(57.5%)	17(42.5%)	
Esophagus [number (percent)]		15(41.7%)	21(58.3%)	
Antrum [number(percent)]		47(87%)	7(13%)	
total [number(percent)]		85(65.4%)	45(34.6%)	
body weight index	24.99;25.86(2.45)	26.23;27.8(2.5)	24.3;23.1(2.3)	0.051
PGI [ng/ml Median; Mean (SE)]	123.7;145.61(12.3)	117.2;154.44(11.6)	73;117.4(7.2)	0.002
PGII [ng/ml Median; Mean (SE)]	14.2;19.85(1.6)	15.8;23.09(1.7)	20.4;30.66(1.9)	0.069
PGI/PGII ratio	8;9.6(0.8)	6.39;9.03(0.6)	4.29;5.97(0.3)	<0.0001
Ghrelin [Pg/ml Median; Mean (SE)]	16.82;27.19(2)	15.06;19.42(1.3)	4.94;20.41(6.2)	<0.0001
albumin	405;4.54(0.43)	4.4;4.49(0.41)	3.9;4.26(0.39)	0.023
serum Creactive protein	4;21.4(1.2)	5;27.68(1.4)	15;37.3(1.6)	0.014
platelet count*1000	213;200(22)	198;195(23)	254;222(27)	0.021

The mean of age, Serum levels of PGI, PGII, ghrelin and PG I to PG II ratio in the three groups are shown in Table 1. The mean of age of cases in the malignant group was significantly higher than the benign and normal groups (median: 70 years) (p-value <0.0001). Patients with malignancy showed significant lower levels of PGI (median, 73 ng/ml), ratios of PGI/PGII (median: 4.29) and ghrelin (4.94 pg/ml) (Table 1). There was significant difference between Albumins, serum creative protein (CRP) and platelet count in the three groups. (Table 1)

In the cancerous group; the malignant lesions were prominent in 42.5% of the stomach (the fundus, body, and stomach small bend), 58.3% of the esophageal (the lower esophagus and cardia) and 13% of the antral (the antrum, pylorus, and duodenum). The average of ghrelin level and PGI/PGII ratio in the women was significantly higher than in the men (Table 2). There was no significant association between ghrelin levels and age, serum levels of PGI, PGII, and PGI/PGII ratio in total in the three groups. There was no significant association between ghrelin levels and grading of differentiation of adenocarcinoma. The mean of ghrelin level was significantly lower in the stomach lesions than in the esophagus and antrum lesions, but there was no significant difference in mean PG I to PG II among different places of the lesions (Table 2).

Table 2.Comparison of Ghrelin level and PG I to PG II ratio between gender and different places of lesions of the study patients (n=308)

	PGI/PGII RATIO [MEAN (SE)]	GHRELIN [PG/ML, MEAN (SE)]
SEX		
WOMEN	8.78(0.4)	25.15(2.4)
MEN	7.36(0.4)	15.17(1.8)
P-VALUE	0.003	<0.0001
PLACES OF LESIONS		
STOMACH	6.90(0.67)	11.8(1.35)
ESOPHAGUS	6.53(0.53)	17.5(2.52)
ANTRUM	6.85(0.36)	17.17(1.35)
P-VALUE	0.39	0.025

Discussion

Nowadays, several studies have been conducted to find tumor markers for early diagnosis of different cancers (12-13). There was high prevalence of gastrointestinal tract (especially in upper segment) cancers in Asia, especially Japan, Iran and China, so most studies have been conducted in these regions (3, 14, and 15). The purpose of this study was to seek out an appropriate tumor marker for screening of upper gastrointestinal cancer.

According to the results obtained from this study, although the number of men was approximately proportional to the women, malignant lesions incidence in men was higher than in women. In addition, there were significant difference between men and women in ghrelin levels and PG I to PG II ratio, so gender may be a confounding factor in this study. The previous studies did not consider gender as a confounding factor, therefore there is a strength in the current study.

Miki et al. (1991-2005) in Japan pointed to elevation of the PG I to PG II ratio as identifying non-ulcerated differentiated asymptomatic cancer even limited to the mucosa that were well suited for endoscopic treatment (10). Xianghong Zhang et al suggested that the subjects with abnormal serum PG level in China were a high risk population for gastric carcinoma and development of gastric carcinoma (16). Watanabe et al. showed that combining these two serum tests (PGI/II ratio ≤ 3.0) and endoscopic examination for rugal hyperplastic gastritis were identification factors for more active gastritis and higher cancer risk. (17) Murphy et al. presented that low baseline concentrations of serum ghrelin were related with an increase in the risk of gastric non cardia adenocarcinoma and esophagogastric junction adenocarcinoma, as a potential role for gastric hormones in carcinogenesis (18).

In the current study; both the plasma levels of ghrelin and the PGI/II ratio decreased with increasing extent of malignant gastric lesion, but the plasma levels of ghrelin did not correlate with the PGI/II ratio. Suzuki et al. reported that the plasma levels of ghrelin correlated with the serum levels of PGI and also the PG I to PG II ratio in gastric mucosal atrophy as a non-invasive marker for chronic atrophic gastritis (19). They enrolled a small sample size (sixty-nine patients) to confirm their findings in atrophic gastritis. So the results of the two studies were partially different.

In this research, the serum ghrelin levels were associated with location of lesions (stomach, esophageal and antral). This is consistent with findings of Sadjadi et al. that serum ghrelin levels had an inverse relationship with the risk of non-cardia gastric cancer compared to cardia gastric cancer(20), because the main source of ghrelin is the gastric oxyntic epithelium, albeit ghrelin is produced by several different tissues. On the other hand, Huang et al. did not detect influence of the location of gastric cancer (proximal vs. distal) on ghrelin levels (21-26).

In the current study, the ghrelin level and PGI/II ratio were not significantly associated with the grades of tumor differentiation in the malignant lesions. This is contradictory with Isomoto et al's report that ghrelin levels of undifferentiated cases were higher than those of differentiated ones (8).

Conclusion

This study demonstrated that there is significant association between the serum ghrelin levels and PGI/II ratio with the malignant lesions particularly in stomach and esophagus, while there are nosignificant associations between the serums ghrelin levels with PGI/II ratio. Also gender is a

confounding factor, therefore it is recommended to conduct studies for men and women to arrive at more definitive conclusions so that the use of ghrelin and pepsinogen I and II, as tumor markers, to screen for stomach and esophageal cancers can be decided.

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