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Plasma hPG80 (circulating progastrin) as a novel biomarker for detecting gastric cancer: a Japanese multicenter study

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Purpose: Early detection and treatment of cancer are important for prolonging life expectancy. hPG80 (circulating progastrin) is an 80-amino acid protein that could prove useful for detecting and following up cancer patients. However, no studies have clarified hPG80 levels in Japanese populations.

Patients and Methods: From 2018 to 2022, we prospectively measured hPG80 levels in 40 cancer patients and 18 healthy volunteers. A receiver operating characteristic (ROC) curve was used to assess the optimal cut-off for hPG80. According to this cut-off, we divided participants into a high-hPG80 group (n=30) and a low-hPG80 group (n=10) and compared clinical characteristics between groups.

Results: Levels of hPG80 were higher in cancer patients (5.9 pM) than in healthy volunteers (2.3 pM; p=0.036), especially for gastric cancer (7.2 pM). We identified an optimal cut-off for hPG80 at 3.42 pM. At this cut-off, the sensitivity was 93.3% and specificity 83.3% for gastric cancer. The proportion of gastric cancer patients (46.7% vs. 10.0%; p=0.040) was higher in the high-hPG80 group. Among gastric cancer patients, 7 of 8 patients (87.5%) with early-stage cancer showed high hPG80 levels.

Conclusion: Plasma hPG80 levels appear useful for early detection of cancer patients, especially gastric cancer.

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Key words: circulating progastrin; gastric cancer; hPG80; Japanese population

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Introduction

In 2020, close to 10 million people worldwide died from cancer[1]. Early detection and treatment of cancer are thus important for optimizing outcomes, and appropriate, timely treatments for recurrence and metastasis are likely to prove effective in maximizing both life expectancy and quality of life. Currently, various tumor markers are used in combination in clinical practice for cancer detection, diagnosis, and follow-up after treatment[2]. The search for new tumor markers with high accuracy is important to improve cancer treatments.

Physiologically, progastrin is the precursor of gastrin synthesized by antrum G cells and processed into gastrin[3]. As a consequence, progastrin is barely detectable in the blood of healthy subjects[4]. Interestingly, it was recently shown that hPG80 (circulating progastrin) can be detected at significantly higher concentrations in the blood of cancer patients than in healthy subjects including breast, endometrial, prostate, lung, stomach, pancreatic, and hepatocellular cancers and melanoma, as well as in colon cancer[5]. Furthermore, hPG80 is a good prognostic marker for multiple types of cancers, including breast cancer or liver cancer[6-8].

A previous report investigated specimens obtained from occidental patients, so whether plasma PG concentrations are also higher in Asian individuals with various cancer types is not yet clear[5]. Other reports showed that genetic, lifestyle, and environmental factors can contribute to differences in cancer incidence, histology, and prognosis between ethnic groups, and a test that is useful in one population will therefore not necessarily be useful in another[9-12].

The present study aimed to measure and compare plasma hPG80 concentrations in a Japanese population of healthy volunteers and patients with four different cancers. We also aimed to clarify the efficacy of hPG80 as a cancer detection marker.

Patients and Methods

This prospective investigation collected blood plasma from 40 adult Japanese patients diagnosed with four cancers (gastric cancer, colon cancer, pancreatic cancer, and gynecological cancer) and 18 healthy volunteers at Nagasaki University Hospital and Saga-ken Medical Centre KOSEIKAN between October 2018 and January 2022. Healthy volunteers and patients before receiving any treatment were assigned to the study group. Patients receiving treatment for other cancers at the same time were excluded. Healthy volunteers were adults who had no poorly controlled chronic diseases and no cancer and were non-current smokers. To match the age range of the patient group, healthy volunteers were defined as <40 years old. Levels of tumor markers CEA and carbohydrate antigen (CA)19-9 were also measured, and samples with elevated levels were excluded.

This study was conducted with the approval of the Genetic and Medical Ethics Commission at Nagasaki University (approval no. 18082031-2) and Saga-ken Medical Centre KOSEIKAN (approval no. 18-12-01-09). All subjects provided informed consent before participating in the study.

The following patient data were collected to compare clinical characteristics between cancer patients and healthy volunteers: sex; age; smoking status; type of cancer; and levels of tumor markers CEA and CA19-9. TNM stage, T-factor, N-factor, and staging were classified according to the World Health Organization TNM staging 8th edition.

The primary objective was to evaluate the levels of hPG80 in patients diagnosed with each cancer. Plasma hPG80 level was measured by enzyme-linked immunosorbent assay (ELISA) and compared between patients and healthy volunteers using the DxPG80 lab kit (Biodena Care, France). All plasma and serum samples were stored in a freezer at -80℃ in each institution after blood collection. Samples were sent to and analyzed at Kyushu Pro Search LLP (Fukuoka, Japan) after collection of all samples was finished. The ELISA-based DxPG80 lab kit was used to measure hPG80 levels in all blood samples according to the instructions from the manufacturer[13].

A receiver operating characteristic (ROC) curve was used to assess the optimal cut-off for hPG80. We used *t*-tests to compare plasma hPG80 levels and chi-square tests to compare clinical parameters. Interaction P values were calculated with the likelihood ratio test. The statistical significance of differences among healthy volunteers and patients with the other four cancers was examined using Dunnett's test. Sensitivity represents the ability of a test to correctly classify a diseased individual as diseased, while specificity is the ability of a test to correctly classify a disease-free individual as disease-free. The threshold for significance was $p<0.05$. All statistical analyses were conducted using JMP version 16 software (SAS Institute Japan, Tokyo, Japan).

Results

Table 1 shows the comparison of clinical background factors between cancer patients (gastric cancer and other cancers, including colon cancer, pancreatic cancer, and gynecological cancer) and healthy volunteers. Age (gastric cancer vs. other cancers vs. healthy volunteer; 72 years vs. 67 years vs. 58

Table 1. Association between psychological distress and bone mass (univariate analysis)

	Cancer patients		Healthy volunteer	p-value	GC vs healthy volunteer	OC vs healthy volunteer
	GC	OC				
n	15	25	18			
Age	72 (59-84)	67 (43-85)	58 (40-79)	<0.001	<0.001	0.006
Sex				0.557	0.731	0.753
Male	8 (53.3)	9 (36.0)	8 (44.4)			
Female	7 (46.7)	16 (64.0)	10 (55.6)			
Smoking, yes	6 (40.0)	10 (40.0)	1 (5.5)	0.026	0.030	0.013
Cancer stage						
0	0 (0)	3 (12.0)	-			
1	5 (33.3)	1 (4.0)	-			
2	3 (20.0)	5 (20.0)	-			
3	4 (26.7)	4 (16.0)	-			
4	3 (20.0)	10 (40.0)	-			
unknown	0 (0)	2 (8.0)	-			
Level of hPG80(pM)	7.2 (3.4-94.2)	3.8 (1.4-21.8)	2.3 (1.4-7.5)	<0.001	0.012	0.850
		Colon cancer 4.3 (2.4-21.5)				
		Pancreatic cancer 4.6 (2.0-12.3)				
		gynecological cancer 3.1 (1.4-21.9)				

Data are presented as number of patients or median (range).
GC, Gastric cancer; *OC*, Other cancers (colon cancer, pancreatic cancer, and gynecological cancer); hPG80, circulating progastrin.

years; $p<0.001$) and frequency of being a smoker (40% vs. 40% vs. 0%; $p=0.030$) were greater in cancer patients. Median values by other carcinoma were 4.3 pM for colon cancer, 4.6 pM for pancreatic cancer, and 3.1 pM for gynecological cancer.

Figure 1 shows the hPG80 concentration in healthy volunteers and patients with four types of cancer (i.e. gastric cancer, colon cancer, pancreatic cancer, and gynecological cancer). The median hPG80 level was 2.3 pM in healthy volunteers and was significantly lower than that of cancer patients (5.9 pM, $p=0.036$) (Fig. 1a). Levels of hPG80 were significantly higher in patients with gastric cancer (7.2 pM) than in healthy volunteers (2.3 pM) ($p=0.012$), but no significant differences were apparent between other cancers (Fig. 1b).

Figure. 2 shows the ROC curve comparing the series of samples from healthy volunteers and gastric cancer patients.

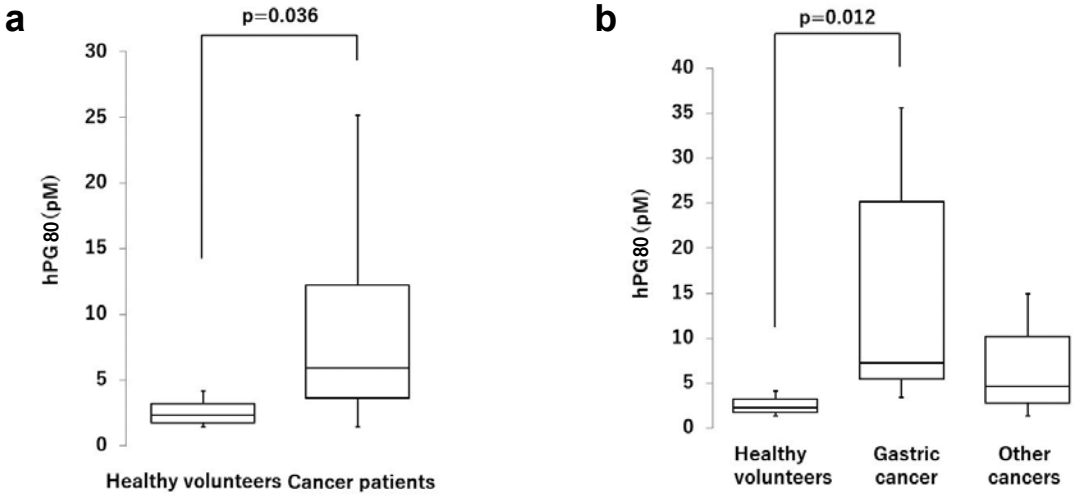

Figure 1. Concentrations of hPG80 in healthy volunteers and patients with each type of cancer.
The median hPG80 level was 2.3 pM in healthy volunteers, and significantly lower than that of cancer patients (5.9 pM, $p=0.036$) (a). Levels of hPG80 were significantly higher in patients with gastric cancer (7.2 pM) than in healthy volunteers (2.3 pM) ($p=0.012$), but no significant differences were apparent between other cancers (b). hPG80, circulating progastrin

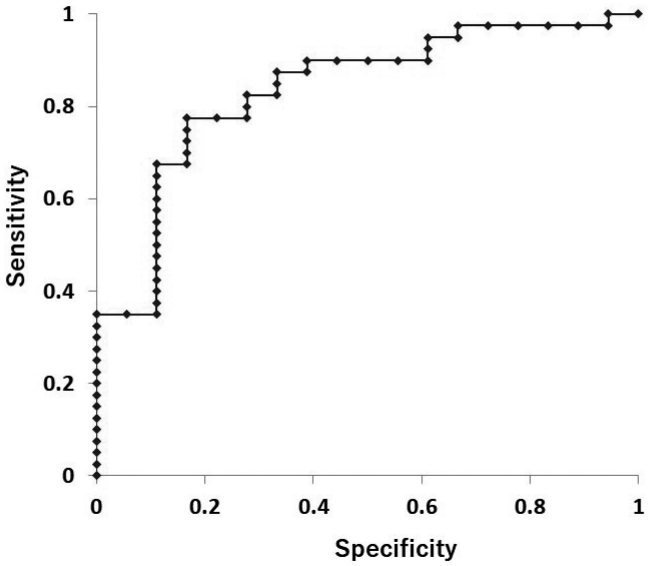

Figure 2. The receiver operating characteristic curve used to assess the optimal cut-off for hPG80.

The area under the curve is 0.836. An hPG80 of 3.42 offers optimal sensitivity (93.3%) and specificity (83.3%) and was therefore used as the cut-off. hPG80, circulating progastrin

The area under the curve (AUC) was 0.836. An hPG80 level of 3.42 pM offered the optimal combination of sensitivity (93.3%) and specificity (83.3%) and was therefore used as the cut-off for further analyses.

We then divided cancer patients into a high-hPG80 group (hPG80 >3.42 pM, n=30) and a low-hPG80 group (hPG80 <3.42 pM, n=10). Table 2 shows a comparison of clinical background factors of cancer patients between high- and low-hPG80 groups. The frequencies of gastric cancer (p=0.040) were greater in the high-hPG80 group. Among gastric cancer patients, 7 of 8 patients (87.5%) with early-stage cancer showed high hPG80 levels. Other factors including age, sex, smoking, and the level of tumor marker were not significantly different between the groups.

Table 3 shows correlations between the presence of gastric cancer and laboratory markers hPG80, CEA, and CA19-9. The sensitivities of hPG80, CEA, and CA19-9 for detecting gastric cancer were 93.3%, 13.3%, and 13.3%, respectively, for specificities ranging from 83.3%, 100%, and 100%, respectively.

Discussion

In this study, plasma hPG80 levels were compared between Japanese healthy volunteers and patients with four types of cancer (gastric cancer, colon cancer, pancreatic cancer, and gynecological cancer). Plasma hPG80 levels were significantly higher in cancer patients, especially patients with gastric cancer, compared to healthy volunteers. With a cutoff value set at 3.42 pM, sensitivity and specificity for gastric cancer were 93.3% and 83.3%, respectively, showing high accuracy.

hPG80 has shown high blood levels in 80% of patients with colon cancer[14]. hPG80 is absent in healthy intestinal epithelium and has been reported to promote cancer cell

Table 2. Comparison of clinical background between high and low hPG80 levels of cancer patients

	High hPG80 (≥3.42)	Low hPG80 (<3.42)	p-value
n	30	10	
Age, years	69 (47-84)	66 (43-85)	0.300
Sex			0.274
Male	11 (36.7)	6 (60.0)	
Female	19 (63.3)	4 (40.0)	
Smoking, yes	10 (33.3)	4 (40.0)	0.717
Level of hPG80(pM)			
Gastric cancer	14 (46.7)	1 (10.0)	0.040
Stage			0.232
1	5 (35.7)	0 (0)	
2	2 (14.3)	1 (100)	
3	4 (28.6)	0 (0)	
4	3 (21.4)	0 (0)	
Elevated tumor markers			0.472
No	13 (43.3)	6 (60.0)	
Yes	17 (56.7)	4 (40.0)	

Data are presented as number of patients or median (range).
hPG80, circulating progastrin.

Table 3. Correlations between presence of gastric cancer and laboratory markers, including hPG80, CEA and CA19-9.

Table 3a		Gastric cancer	
		Present	Absent
hPG80	High	14	3
	Low	1	15

Sensitivity: 93.3%; specificity: 83.3%.

Table 3b		Gastric cancer	
		Present	Absent
CEA	High	2	0
	Low	13	18

Sensitivity: 13.3%; specificity: 100%.

Table 3c		Gastric cancer	
		Present	Absent
CA19-9	High	2	0
	Low	13	18

Sensitivity: 13.3%; specificity: 100%.

hPG80, circulating progastrin; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

growth and survival. The French company Biodena Care has developed a kit to measure hPG80 in plasma using anti-hPG80 antibodies that do not recognize the maturated by-products of progastrin maturation, among which gastrin[13]. You et al. used this kit to measure hPG80 levels in patients with 11 types of cancer (breast, uterine, ovarian, prostate, kidney, colon, pancreatic, esophageal/gastric, liver, skin melanoma, lung cancer) and healthy volunteers[5]. The results showed that hPG80 levels were significantly higher in cancer patients than in healthy volunteers (median hPG80: 4.88 pM vs. 1.05 pM; $p<0.0001$). Median ranges for each tumor were 6.92 pM for gastric cancer (including esophageal cancer), 4.36 pM for colon cancer, 6.47 pM for pancreatic cancer, and 2.92 pM for gynecologic cancer. In the present study, the median value for cancer patients was 5.9 pM, significantly higher than that in healthy volunteers (2.3 pM; $p=0.036$). Median values by carcinoma were 7.2 pM for gastric cancer, 4.3 pM for colon cancer, 4.6 pM for pancreatic cancer, and 3.1 pM for gynecological cancer, similar to previous reports in different populations.

Previous studies have shown statistical differences between healthy subjects and patients with different types of cancers, including gastrointestinal cancers, gynecological cancers, urological cancers, and neuro-endocrine tumors with occidental origin[5,15]. In the present study, we evaluated for the first time the Japanese population and only gastric cancer patients showed significantly higher levels of hPG80 than healthy volunteers. One explanation could be the small number of patients per cancer in the OC cohort. Gastric cancer has always had a high incidence in Asian countries and a low rate in Western countries[9,10]. In addition, a higher percentage of esophagogastric junction cancers are reported in Europe and the United States, whereas gastric body cancers are more common in Asia[11]. In fact, You et al. measured hPG80 in a combined cohort of gastric and esophageal cancer in Western patients. The present study is the first to measure hPG80 levels only for gastric body cancer, and the results suggest the utility of plasma hPG80 for diagnosing gastric body cancer.

Clinically applied tumor markers in gastric cancer patients currently include CEA and CA19-9[16]. Takahashi et al. reported the proportions of patients with high levels of CEA and CA19-9 were only 28.3% and 29.2% before gastric surgery and up to 65.8% and 55.0% at the time of recurrence, respectively[2]. CEA and CA19-9 levels tend to be higher for advanced or recurrent tumors[2]. In the present study, the positive rate for both CEA and CA19-9 was low, at only 13.3% (2/15) in gastric cancer patients. One possible reason is that in the present study, 53.3% (8/15) had early-stage cancer and 46.7% (7/15) had advanced cancer, possibly due to the high number of early-stage cancers. On the other hand, no reports have clarified optimal cutoff values for hPG80 in cancer diagnosis. In the present study, a cutoff of 3.45 pM was set from the ROC analysis, showing very high sensitivity and specificity. Among gastric cancer, most of the patients with early-stage cancer (87.5%) showed high hPG80 levels, suggesting that hPG80 may be useful for detecting early-stage patients.

Several limitations to this study must be kept in mind when interpreting the results. First, the number of patients was relatively small, and the cancer types were limited. Second, in this study, healthy subjects were determined only by self-report and screening for CEA and CA19-9, so we cannot exclude the possibility that "healthy subjects" may have included cancer-bearing patients. Third, hPG80 was only measured at a single time point in this study. Previous publications have reported that measuring hPG80 levels before and throughout treatment is useful, but the data in this study did not allow such tracking. Long-term results were also not tracked in this study. The utility of hPG80 as a prognostic marker has also been reported in several studies[7]. In the future, we hope to clarify the role of hPG80 in long-term observations and follow-up of a larger cohort.

Conclusions

Despite the limitations of this study, plasma hPG80 levels appear useful as a marker for the early detection of cancer, particularly gastric cancer.

Declarations

Ethics approval

Not applicable.

Consent for publication

Written, informed consent was obtained from the patient for publication of this case report.

Competing interests

None.

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