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ORIGINAL ARTICLE

Evaluation of serum markers for gastric cancer and its precursor diseases among high incidence and mortality rate of gastric cancer area

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Abstract

Background Mongolia has the highest mortality rate of gastric cancer. The early detection of cancer and down-staging screening for high risk patients are essential. Therefore, we aimed to validate serum markers for stratifying patients for further management.

Methods Endoscopy and histological examination were performed to determine high risk and gastric cancer patients. Rapid urease test, culture and histological tests were performed to diagnose *Helicobacter pylori* infection. Serum pepsinogen (PG) I and II and anti-*H. pylori* IgG were measured by ELISA. Receiver Operating Characteristic analysis was used to extract the best cut-off point.

Results Totally 752 non-cancer and 50 consecutive gastric cancer patients were involved. The corpus chronic gastritis (72%: 36/50 vs. 56.4%: 427/752), corpus atrophy (42.0%: 21/50 vs. 18.2%: 137/752) and intestinal metaplasia (IM) (64.0%: 32/50 vs. 21.5%: 162/752) were significantly higher in gastric cancer than non-cancer patients, respectively. Therefore, corpus chronic gastritis, corpus atrophy and IM were considered as high risk disease. The best serum marker to predict the high risk status was PGI/II < 3.1 (sensitivity 67.2%, specificity 61%) and PGI/II further reduced to < 2.2 (sensitivity 66%, specificity 65.1%) together with PGI < 28 ng/mL (sensitivity 70%, specificity 70%) were the best prediction for gastric cancer. The best cut-off point to diagnose *H. pylori* infection was anti-*H. pylori* IgG > 8 U/mL. Multivariate analysis showed that anti-*H. pylori* IgG > 8 U/mL and PGI/II < 3.1 increased risk for high risk status and PGI/II < 3.1 remained to increase risk for gastric cancer.

Conclusion The serum diagnosis using PGI/II < 3.1 cut-off value is valuable marker to predict high risk patients for population based massive screening.

Keywords Serum markers · Gastric cancer · High risk disease · *Helicobacter pylori* · Pepsinogen · Mongolia

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Introduction

Mongolia has the highest mortality rate of gastric cancer (GC); the age standardized rate per 100,000 (ASR) of GC death for both sexes was 25.3 in 2012 [1]. As for the incidence of GC, top three countries in 2012 were Korea, followed by Mongolia and Japan; ASRs of the incidence for both sexes were 41.8, 32.5 and 29.9, respectively [1]. GC is considered as a high mortality cancer due to its delayed diagnoses because there is no specific clinical symptom in the early stage [2–5]. Therefore, the nationwide GC screening program had been already provided including endoscopic examination in high incidence GC countries such as Japan and Korea [6–8]. Early detection of GC could reduce GC mortality rate by 30–65% [9, 10]. Endoscopy follow-up with taking proper biopsy specimens remains the standard for early detection of GC and its related premalignant lesions [11].

However, since the histological evaluation is invasive and expensive, serum markers such as anti-*Helicobacter pylori* IgG and pepsinogens (PGs) have been used as non-invasive serological surrogate markers for detecting high risk patients to develop GC [12, 13]. PGs are consisting of two types: PGI, which is mainly secreted by the fundic mucosa, and PGII secreted by chief cells but also by the pyloric glands and the proximal duodenal mucosa. Both PGI and PGII decreased by the development of atrophy and loss of specialized cells. PGI usually shows a more marked decrease than PGII, thus a low PGI level, a low PGI/II ratio, or both, are good indicators of atrophic changes in the gastric mucosa [14]. It is well known that the majority of GC is caused by *H. pylori* infection [15]. Therefore, Japanese researchers (Miki et al.) had developed ABC (D) screening program which is combination of PGs (PGI < 70 ng/mL and PGI/II < 3.0 as positive PGs) for atrophic marker, and anti-*H. pylori* IgG for etiological marker to stratify high risk patients for further follow-up [13]. It is widely used in Japan and its modified version is used in Korea [16]. Meta-analysis study showed the best cut-off values to predict atrophy were varied depending on the countries [17]. The long term longitudinal cohort studies based on Miki's criteria showed GC was developed from not only atrophic gastritis patients but also it could be arisen from non-atrophic patients which is likely to be progressed diffuse type GC [18, 19]. Uemura et al. followed up the high risk patients to progress GC. Their results showed that GC was developed from atrophy and pan- or corpus-predominant gastritis patients. Interestingly patients with pan- (relative risk [RR] 15.6) or corpus-predominant gastritis (RR 34.6) more increased relative risk than those with moderate (RR 1.7), severe atrophy (RR 4.9) and intestinal metaplasia (IM) (RR 6.4) [20]. These results suggest that considering only atrophic gastritis are not sufficient criteria to determine high risk disease for GC development. Therefore, we aimed to validate serum markers based on not only atrophy, but also chronic corpus gastritis as a high risk disease for GC targeting Mongolian population as the highest GC mortality rate country in the world.

Method

Sampling and endoscopy

We recruited patients who met our inclusion criteria that over 16 years old, patients with dyspeptic symptoms and suspected GC patients. Exclusion criteria included a history of partial or total gastrectomy, endoscopic mucosal dissection, treatment with bismuth-containing compounds, H2-receptor blockers, or proton pumps inhibitors within 2 weeks prior to the start of the study and a history of previous *H. pylori* eradication therapy.

The experienced endoscopists performed endoscopy and collected samples from dyspeptic patients in Ulaanbaatar City (November 18–22, 2014), western (Uvs Province; July 14–21, 2015), northern (Khuvsgul Province; July 19–25, 2015), southern (Umnugovi Province; August 4–8, 2016) and eastern (Khentii Province; August 9–12, 2016) parts of Mongolia. The consecutive suspected GC patients were collected from National Cancer Center Hospital (Ulaanbaatar City; October 2015–August 2016).

Blood samples from all participants were collected on the same day. Written informed consent was obtained from all participants, and the ethical permission was approved by the Mongolian Ministry of Health Mongolian National University of Medical Sciences, and Oita University Faculty of Medicine (Yufu, Japan). The questionnaire was filled out by clinicians before endoscopic examination. Our study had the limitation that the local ethical committee allowed us to take maximum five biopsy specimens in cases of the absence of suspected GC. Therefore, we followed the guideline by American Society for Gastrointestinal Endoscopy for gastric mucosal sampling for histological diagnosis [21]. During endoscopic examination three biopsy specimens were taken from the antrum (approximately 2 cm from the pyloric ring in the greater curvature). One was taken for rapid urease test, one for *H. pylori* culture and remaining one was for histological examination. One more biopsy specimen was taken from the greater curvature of the corpus (8–10 cm from the esophagogastric junction) for histological examination. Additionally, if suspected GC exists at least one more specimens were taken for histological diagnosis. In cases only small tissues or poor histological preparation were

detected for histological diagnosis, we excluded the cases for further analysis (Supplementary Figure 1).

Serum markers

Blood samples were centrifuged within 3 h of collection. Serum was kept at 2–8 °C for transfer to a − 80 °C freezer in Ulaanbaatar. After thawing, sera were used for serological identification of anti-*H. pylori* IgG and PGI and PGII serum levels were measured by commercially available ELISA kits (Eiken Co., Ltd., Tokyo, Japan). After evaluated the suitable cut-off value using Receiver operating curve (ROC) analyses we determined the positive status of each value.

H. pylori infection status

In our criteria; one test positive for culture or histology confirmed by immunohistochemistry (IHC) was considered as the positive for *H. pylori* infection. IHC was performed to confirm *H. pylori* infection as previously described [22]. This criterion was used to validate serum anti-*H. pylori* IgG test. Samples with bacterial loads ≥ grade 1 by the updated Sydney system [23] were considered as the positive for current *H. pylori* infection by histology.

Histological diagnosis and determination of high risk mucosal background

All biopsy materials were fixed in 10% buffered formalin and embedded in paraffin. Serial sections were stained with haematoxylin eosin and with May–Giemsa stain. The stained slides were examined by an experienced single pathologist (TU). The degree of acute inflammation (neutrophils infiltration), chronic inflammation (mononuclear cells infiltration), atrophy, IM and bacterial density scores were evaluated in three biopsy sites: antrum, angulus and corpus among gastritis and GC patients. The scores were classified into four grades: 0 "normal", 1 "mild", 2 "moderate", and 3 "marked" based on the updated Sydney system [23]. These scores ≥ grade 1 were considered as positive status. Previous study reported GC was developed from not only atrophy but also corpus predominant gastritis [20]. Atrophy-based GC progression usually develop intestinal type GC [24], whereas pan or corpus predominant chronic inflammation based gastric damage develop diffuse type GC [25, 26]. Therefore, corpus chronic gastritis, atrophy and IM status were examined for high risk mucosal background.

Statistical analysis

Continuous variables were tested by Kruskal–Wallis test, Mann–Whitney *U* test and *t* test for serum markers level. ROC curves were constructed to extract the corresponding cut-off values for serum markers. The area under curve and 95% confidence interval (CI) were calculated. The discriminatory ability of each biomarker was evaluated as follows: no discrimination (less than 0.7); acceptable (equal or more than 0.7, but less than 0.8); excellent (equal or more than 0.8, but less than 0.9); and outstanding discrimination (equal or more than 0.9). Statistical significance of the qualitative differences was calculated using the Chi-square test. All statistical analyses were performed using the SPSS 22 software (SPSS Inc., Chicago, IL, USA).

Result

Patient demographics, histology background and *H. pylori* infection status

Totally 815 consecutive non-cancer patients from the capital city (Ulaanbaatar, $n = 209$; November 18–22, 2014), western (Uvs Province, $n = 127$; July 14–21, 2015), northern (Khuvsgul Province, $n = 190$; July 19–25, 2015), southern (Umnugovi Province, $n = 144$; August 4–8, 2016), and eastern (Khentii Province, $n = 135$; August 9–12, 2016) parts of Mongolia. In addition, 51 consecutive GC patients (National cancer center hospital, Ulaanbaatar, Mongolia) were enrolled. Fifty-four patients were excluded for further analysis due to our exclusion criteria (Supplementary Figure 1). Among non-cancer patients 68.6% (516/752) was female and 31.4% (236/752) was male with the mean age ± SD was 44 ± 13.8 years old; ranged 16–87 years old. For GC patients 16% (8/50) was female and 84% (42/50) was male with 53.8 ± 12.1 years old and ranged 27–78 years old.

Histological background diseases according to non-cancer and GC group based on updated Sydney system score is shown in Fig. 1. All parameters in the corpus except for neutrophil infiltration scores were significantly higher in GC than in non-cancer group (mean [median]; 0.82 [1] vs. 0.71 [1]; $p < 0.03$ for mononuclear cell infiltration, 0.51 [0] vs. 0.22 [0]; $p < 0.0001$ for atrophy and 0.67 [0] vs. 0.06 [0]; $p < 0.0001$ for corpus IM). In addition IM scores in the antrum was significantly higher in GC than non-cancer group (0.7 [0] vs. 0.1 [0]; $p < 0.0001$). In contrast, neutrophil infiltration scores were significantly higher in non-cancer than in GC group (0.7 [1] vs. 0.3 [0]; $p < 0.0001$ in the antrum and 0.69 [1] vs. 0.44 [0]; $p < 0.009$ in the corpus).

Supplementary Figure 2 focused on the presence/absence of the corpus mononuclear cell infiltration, corpus atrophy and antrum/corpus IM in non-cancer and GC group taking care of *H. pylori* infection. The prevalence of *H. pylori* infection by gold standard method was 77% (579/752) in non-cancer group and was 54% (27/50) in GC group ($p < 0.0001$).

All IM-positive cases in non-cancer group had gastric atrophy. Therefore, in addition to atrophy or IM we added

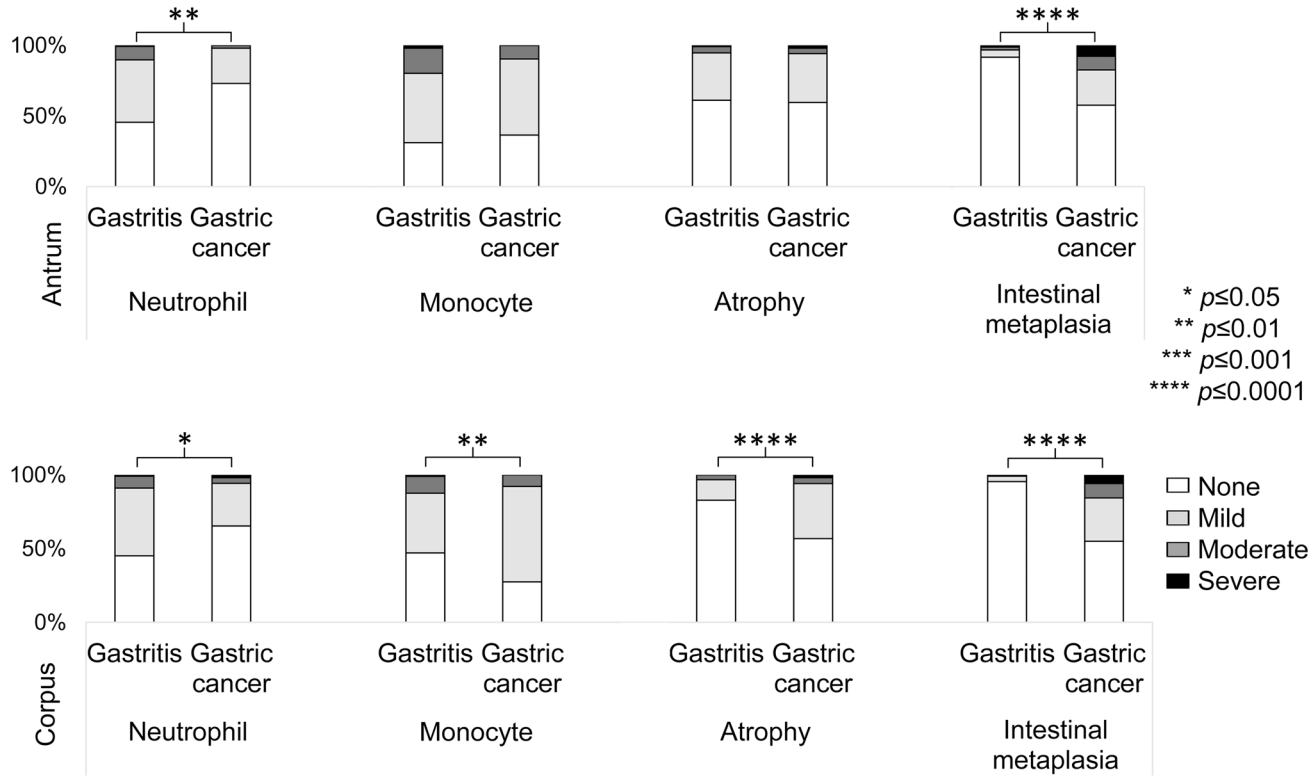

Fig. 1 Precursor disease for gastric cancer based on updated Sydney system scores

chronic gastritis (mononuclear cell infiltration) as the high risk status. We categorized the non-cancer patients into "low" and "high" risk group for developing GC; (1) low risk group ($n=302$): antrum limited chronic gastritis and/or atrophy, and (2) high risk group ($n=450$): corpus chronic gastritis and/or atrophy (Supplementary Figure 1). The definition of the antrum limited chronic gastritis and/or atrophy is that mononuclear cell infiltration and/or atrophy were limited in the antrum and there were no mononuclear cell infiltration and atrophy in the corpus, and the definition of corpus chronic gastritis and/or atrophy is that mononuclear cell infiltration and/or atrophy were observed in the corpus irrespective of the presence in the antrum.

Serum markers evaluation for *H. pylori* infection and stomach diseases

First we validated anti-*H. pylori* IgG as the etiological marker among non-GC patients ($n=752$) based on our gold standard tests for *H. pylori* infection (culture, histology confirmed by IHC). Supplementary Figure 3 showed ROC curve. Although the cut-off point recommended by the instruction of the test is 10.0 U/mL, the best cut-off point in this Mongolian population was anti-*H. pylori* IgG > 8.0 U/mL with area under curve of 0.84 (95% CI 0.8–0.9); the sensitivity was 82.1% and specificity was 75% ($p<0.0001$).

Figure 2 showed the comparison of mean ± standard error (SE) for serum PGs based on *H. pylori* infection status and following disease groups: low risk group, high risk group and GC group. PGI level was significantly decreased in GC group than low risk group regardless *H. pylori* infection. PGII level was significantly increased in high risk group than low risk and GC groups regardless *H. pylori* infection. The PGI/II was significantly decreased both in high risk and GC group than low risk group regardless *H. pylori* infection.

Figure 3 showed the best cut-off values to predict high risk group and GC patients based on ROC analysis. The best marker to predict GC was PGI and PGI/II. Then we focused on down-staging from GC to high risk group. The best serological marker to predict high risk disease was PGI/II among non-cancer patients. Table 1 showed detailed cut-off values for to predict high risk diseases and GC. Among low- and high-risk patients, the PGI/II < 3.1 (sensitivity 67.2%, specificity 61%, area under curve of 0.72) was the best cut-off point to predict high risk diseases. Among high risk and GC patients, the PGI/II < 2.2 (sensitivity 66%, specificity 65.1%, area under curve of 0.70) and PGI < 28 ng/mL (sensitivity 70%, specificity 70%, area under curve of 0.76) were the best cut-off point to predict GC patient. Among high risk patients sub-analysis was performed serum marker evaluation for predicting corpus chronic gastritis, atrophy and IM separately. The ROC analysis is shown in Supplementary

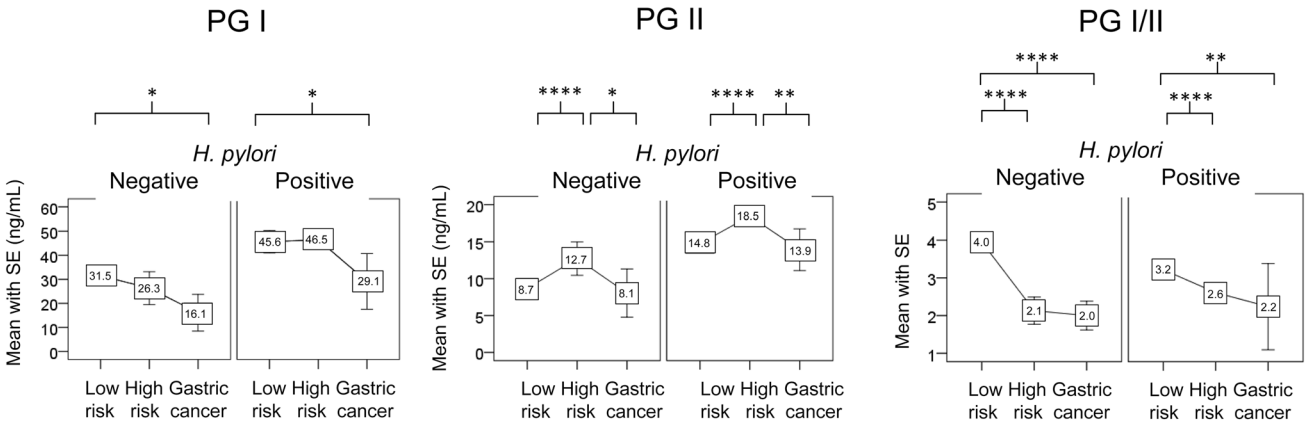

Fig. 2 Mean with standard error of pepsinogens based on severity of gastric diseases and *H. pylori* infection status

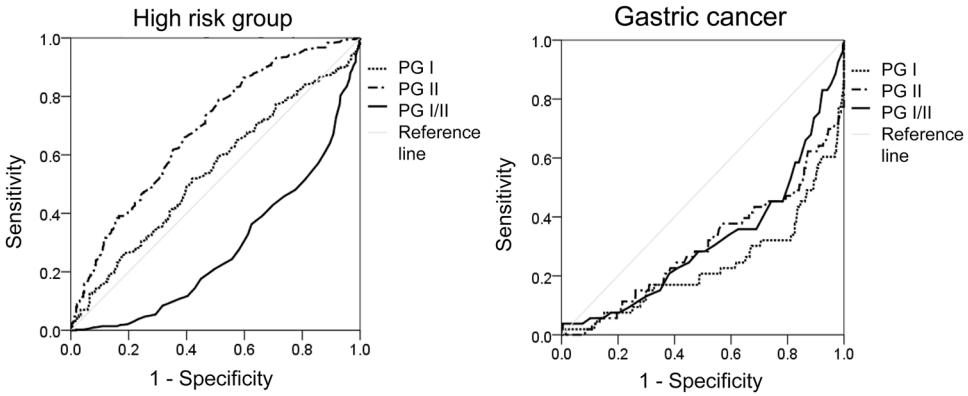

Fig. 3 Receiver operating curve analysis of pepsinogens for high risk and gastric cancer patients

Table 1 Best cut-off values of PGs for disease prediction

Disease group (total $n=802$)	Parameters	Serum pepsinogens		
		PGI	PGII	PGI/II
High risk ($n=450$)[a]	Cut-off value	–	–	3.1
	AUC ROC (95% CI); *P* value	–	–	0.72 (0.68–0.76); 0.0001
	Sensitivity	–	–	67.2%
	Specificity	–	–	61%
Gastric cancer ($n=50$)[b]	Cut-off value	28		2.2
	AUC ROC (95% CI); *P* value	0.76 (0.68–0.84); 0.0001	–	0.70 (0.62–0.77); 0.0001
	Sensitivity	70%	–	66%
	Specificity	70%	–	65.1%

AUC ROC area under the curve for receiver operating characteristics

[a]Low-risk group $n=302$ and high risk group $n=450$

[b]High-risk group $n=450$ and gastric cancer $n=50$

Figure 4 and its detailed cut-off values are summarized in Supplementary Table 1.

We considered PGI/II < 3.1 as the functional serological marker to predict high risk disease and anti-*H. pylori* IgG > 8 U/mL as the etiological marker for high risk disease. Further we tested using PGI/II < 3.1 for predicting diseases (low risk, high risk and GC) based on *H. pylori* status. Figure 4 showed using our criteria positive serum diagnosis was significantly higher in high risk disease and GC regardless serum *H. pylori* diagnosis that among *H.*

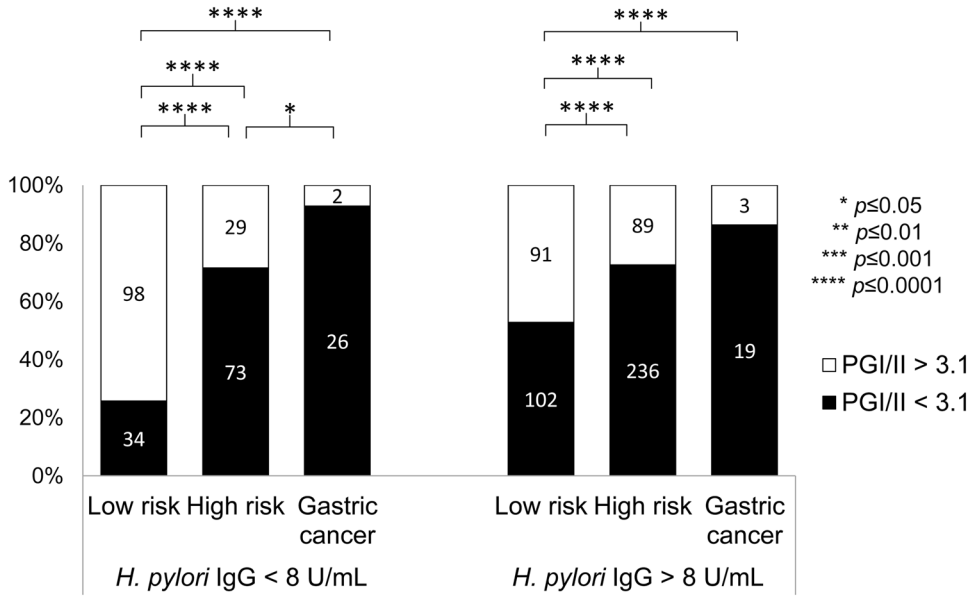

Fig. 4 Serum markers screening for high risk and gastric cancer patients

pylori negative group PG positive serum diagnosis was 25.8% (34/132) in low risk disease, 71.6% (73.102) in high risk disease and 92.9% (26/28) in GC. Among *H. pylori* positive group it was 52.8, 72.6 and 86.4%, respectively. Supplementary Figure 5 showed the distribution of ABC (D) stratification using evaluated serum markers (PGI/II < 3.1 and anti-*H. pylori* IgG > 8 U/mL) based on diseases (low risk, high risk and GC group). Group C and D were predominant in high risk group and GC patients.

Miki's criteria (PGI < 70 ng/mL and PGI/II < 3.0) is commonly accepted in Japan to predict atrophy [27]; therefore, we compared with our result (PGI/II < 3.1). Our modified PG criteria had higher detection rate and odds ratio (OR) than Miki's criteria (85% [119/140] and OR 5.6; 95% CI 3.4–9.1, $p < 0.0001$ vs. 72.9% [102/140] and 3.5; 95% CI 2.4–5.3, $p < 0.0001$, respectively). Furthermore, our modified criteria were remained to increase risk for atrophy by multivariate backward logistic regression analysis. Then we checked serum markers (PGI/II < 3.1 and anti-*H. pylori* IgG > 8 U/mL) by multivariate logistic regression analysis adjusted with age and gender. Detailed results are summarized in Table 2. Anti-*H. pylori* IgG > 8 U/mL and PGI/II < 3.1 were increased risk for high risk disease and PGI/II < 3.1 was remained to increase risk for GC.

Discussion

Our finding highlighted the application of PG is not only prognostic tool for gastric atrophy but also it can be used to predict corpus chronic gastritis (inflammation). Chronic inflammation is considered as high risk disease for GC due to its possibilities to induce point mutation and aberrant DNA methylation on gastric mucosal cells which further leads cancerization [28, 29]. It is conventionally described that the reduced PGI and PGI/II are markers for gastric atrophy [27, 30], further have a chance to develop GC [20] which is usually caused by *H. pylori* infection [15]. Our data showed in addition to atrophy, corpus extended gastritis also highly distributed in GC group than non-cancer group regardless *H. pylori* infection (Figs. 1, 2). It is reported that 20–30% of GC is developed from non-atrophic gastritis [25, 26]. Therefore, we examined serum markers and its best cut-off point to predict high risk diseases (corpus chronic gastritis, corpus atrophy and IM) and GC.

Table 2 Age and gender adjusted multivariate analysis for serum markers

Parameters	High risk group		Gastric cancer	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Gender (male)	1.1 (0.8–1.6)	NS	12.5 (5.3–29.6)	0.0001
Age group (over 40 years)	1.0 (0.7–1.4)	NS	3.4 (1.2–9.1)	0.02
Anti-*H. pylori* IgG > 8.0 U/mL (yes)	1.8 (1.3–2.5)	0.001	0.5 (0.2–1.0)	NS
PGI/II < 3.1 (yes)	3.3 (2.4–4.6)	0.0001	16.9 (6.1–46.9)	0.0001

OR odds ratio, *CI* confidence interval

The serum marker for predicting atrophy using PGs named as Miki's criteria was developed in Japan that the best cut-off values (PGI ≤ 70 ng/mL and a PGI/II ratio ≤ 3.0) are commonly described in East-Asian countries where exist high incidence of GC [31]. In these countries *H. pylori* infection played major role to increase GC risk because of severe atrophic damage of gastric mucosa [32, 33]. Comparing with Miki's criteria, our validated (PGI/II < 3.1) criteria remained to more increase risk for high risk disease by multivariate analysis. Same as Japan and Korea, Mongolia is Central-East Asian country and ranked second highest incidence of GC after Korea and before Japan [1]. Compared with Japanese patients overall gastric atrophy score of Mongolian patients were significantly lower [34]; however, our data showed in addition to atrophy or IM, corpus chronic gastritis was significantly higher in GC group than non-cancer group for both *H. pylori* negative and positive cases (Figs. 1, 2). Recent review summarized the pathogenesis of inflammation derived GC explained by hedgehog signaling pathways [35] and stem cell theory [36]. Since chronic gastritis is the fundamental status for atrophy, IM and GC progression (Fig. 1) we applied PG I/II < 3.1 as the best serological marker (Supplementary Table 1). We assumed that both PGI and PGII played important roles in addition to the declines of PGI/II. The serum PGII level was markedly increased in high risk disease group and both PGI and PGII markedly declined in GC group (Fig. 3). PGI/II was significantly decreased by the different groups (Supplementary Figure 3 and Supplementary Table 1). On the basis of physiological aspect, both PGI and PGII were important to reflect gastric mucosal status. Increasing serum PGII level is noted in the status of active inflammation [37–40]. Longstanding chronic active gastritis gastric mucosal glands are destroyed and turned to atrophic gastritis that initially lead serum PGI decline, by the time progression further PGII is gradually decreased [27, 41]. Our previous observation showed that Mongolian patients had higher proportion of diffuse type GC and characteristic location of GC was different from those among Japanese that the most of the GC among Mongolian patients located in the upper (46.5%) or middle (25.4%) region of the stomach [34]. Whereas only approximately 15% of GC in the Japanese registries located in the upper portion of the stomach and the remaining 85% located in the middle or lower parts [5]. For precursor disease Mongolian dyspeptic patients have less atrophic mucosal background comparing with Japanese patients [34], suggesting that chronic inflammation seems to play major role for GC rather than atrophy among Mongolian population.

From our result for etiological marker (anti-*H. pylori* IgG) evaluation, *H. pylori* infection is initially played main role for developing high risk disease (Table 2), so that applying anti-*H. pylori* IgG seemed as good etiological marker for high risk disease; however, in the final stage from high risk to GC it lost the screening value (Table 2). This result was also consistent with gold standard method (culture and/or histology confirmed by IHC) that the prevalence of *H. pylori* was significantly lower in GC than non-cancer group (54 vs 77%, $p < 0.0001$). Anti-*H. pylori* IgG might be good marker for planning eradication therapy for *H. pylori* related disease progression. In addition, recently *H. pylori*-negative gastritis was described and the prevalence was 18% [42]. Among *H. pylori*-negative gastritis, most cases were chronic non-active gastritis, and even gastric atrophy and IM were present in 13.0% of cases [42]. Except for *H. pylori* infection, other etiologies such as bile reflux, alcohol, salt and other bacterial or viral infections were still increased risk for high risk status and GC [43–49].

Based on our finding we concluded applying anti-*H. pylori* IgG > 8 U/mL as the etiological marker is not sufficient to screen high risk and GC patients. As for functional test, serum marker using PGI/II < 3.1 cut-off value is valuable and non-invasive tool to stratify high risk patients in population based massive screening.

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Author contributions BG, KO, DD, RS and YY conceived and designed the study; BG, KO, DB, YE, TT and YY contributed by collecting samples; BG and TU performed the experiments; BG and YY contributed to analysis and interpretation; BG and YY drafted the manuscript.

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Compliance with ethical standards

Conflict of interest The authors have no conflicts of interest to disclose.

Human rights statement and informed consent All procedures followed were in accordance with the ethical standard of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. Ethical approval was obtained from the Ethics Committees of Ministry of Health, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia and Oita University Faculty of Medicine, Japan. Informed consents were obtained from all patients for their inclusion in the study.

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