Prevalence of *H. pylori* Infection and Atrophic Gastritis Among Symptomatic and Dyspeptic Adults in Kazakhstan. A Hospital-based Screening Study Using a Panel of Serum Biomarkers

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Abstract. *Background: Health authorities of Kazakhstan are seeking for effective measures to interrupt the untoward trend, projected to increase the current number of gastric cancer (GC) cases (n=3,316) by 50% until the year 2030. Objective: Use of a non-invasive blood test with four stomach-specific biomarkers [Pepsinogen-I (PG-I) and -II (PG-II), amidated gastrin-17 (G-17), and Helicobacter pylori (HP) IgG antibodies], to assess for the prevalence of stomach conditions: Helicobacter pylori (HP) infection and atrophic gastritis (AG), both known to increase GC risk of in Kazakhstan. Materials and Methods: A cohort of 835 (symptomatic and asymptomatic) cases (473 women and 362 men)(median age 46.8 years; range 13.6-74.8) was examined with a panel of biomarkers. Results were assigned in five categories: 1) Healthy stomach, 2) HP infection, 3) atrophic gastritis (AG) of the antrum, 4) AG of the corpus, and 5) AG of both antrum and corpus (pangastritis). Results: The distribution in these five categories was identical in both sexes (p=0.259). Healthy stomach was detected only in 196 (23.5%) subjects, whereas the vast majority, 62.3% (n=519) had HP infection (with no AG). In 118 (14.1%) subjects, results were consistent with AG; in antrum (n=72), corpus (n=42) or pangastritis (n=4). Prevalence of AG increased with patient's age in both sexes. There was no age-related pattern in biomarker levels, and only slight differences between the genders. Conclusion: While capable of detecting the subjects at risk for GC (HP or AG), GP seems to be a cost-effective means to intervene the current ominous trend in GC incidence in Kazakhstan.*

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Dyspeptic symptoms are among the most common gastric complaints (1), experienced by 25-40% of the people during their lifetime (1, 2). When managed in primary health care, most of these subjects receive treatment (*e.g.* proton pump inhibitors, PPI) without diagnosis confirmation (3-5). The majority of these complaints are due to functional dyspepsia or gastro-esophageal reflux disease (GERD), while a small minority are classified as organic in origin (4, 6). In the latter, the two most important clinical conditions are *Helicobacter pylori* (HP) infection and atrophic gastritis (AG); two conditions that are closely interrelated (7, 8).

HP is the causal factor for several clinically important diseases in gastric and duodenal mucosa (7-11), and, in 1994, the IARC expert group classified HP infection as a group-I carcinogen for humans (12). This bacterial infection (usually acquired in childhood) initially affects only the antral mucosa causing superficial gastritis. If not eradicated, HP-infection remains chronic and progresses to corpus-predominant gastritis or pangastritis, with mucosal atrophy as the end result (13, 14). The exact mechanism by which HP-infection causes gastric cancer (GC) remains to be elucidated, but there is little doubt that HP-associated AG plays a key role in this development (14, 15).

AG is the single most powerful independent risk factor for distal (non-cardia) GC (8, 16-18). It is estimated that 50% of all GC cases develop through the "Correa cascade" (16, 19-21), leading from HP-associated gastritis to mucosal atrophy, intestinal metaplasia, dysplasia, and to invasive adenocarcinoma. There are some implications that early eradication of HP infection can slow-down or even revert this cascade (7, 13). Because this process takes several decades, there should be good prospects for early detection of pre-cancerous lesions (22), but the major problem is lack of a suitable test for GC screening (23). Furthermore, most of the patients report only a short period of symptoms before the diagnosis of GC, and up to 40% report no dyspeptic symptoms at all (24).

Several diagnostic tests are available for dyspeptic symptoms, including endoscopy, radiography and HP-infection testing (25). In many clinics, endoscopy with biopsies remains the golden-standard diagnostic tool, disclosing HP-infection, mucosal atrophy, intestinal metaplasia or dysplasia, and their topography (8, 14). However, this invasive method is uncomfortable, distressing and quite costly, emphasizing the need for rapid, reliable and inexpensive non-invasive tests for screening and monitoring the patients with dyspeptic symptoms (23-25).

Such non-invasive methods providing information on the structure and function of gastric mucosa were introduced in the early 1980's, when Miki *et al*. (26) and Samloff *et al*. (27) developed assays measuring pepsinogen (PG) concentrations in the blood. The latest development in this field represents a panel which combines serum pepsinogen-I (PGI) and -II (PGII), gastrin-17 (G-17) and HP IgG antibodies (IgG-HP) using an ELISA technique for detection (GastroPanel® test, Biohit Oyj), proposed as the first-line diagnostic test for dyspeptic symptoms (28-30). According to a recent meta-analysis, serum PGs are not suitable for GC screening, but they proved useful for detecting the patients at risk for GC (31). Consequently, these stomach-specific biomarkers were recommended by an international group of experts for diagnosis and screening of AG (32).

In the present study, we evaluated the feasibility of the marker panel in diagnosing the gastric conditions (HP and AG) associated with an increased risk of GC, in a hospital-based cohort in Astana, Kazakhstan. In this country, GC represents a major cancer burden, with an annual incidence rate of 20,6/100,000 and mortality rate of 17,6/100,000, with 3,316 new cases and 2,831 cancer deaths each year (33). No data are available on the population prevalence of HP infection and the different phenotypes of HP-associated gastritis in Kazakhstan.

Materials and Methods

Study design. The present study is a hospital-based trial using the Biohit HealthCare's GastroPanel® test in order to i) Examine patients with dyspeptic symptoms and those participating in an annual health control and ii) to clarify the population prevalence for the different stomach conditions in the Republic of Kazakhstan. The target group consists of adult subjects who attended hospitals for annual health control, or due to different referral indications (gastrointestinal symptoms).

Patients. This trial was conducted by the Medical Center of the President's Affairs Administration of the Republic of Kazakhstan (MCPAA), Astana City. Between September and November 2012, a total of 835 subjects were enrolled in the study at MCPAA, 62% of whom participated in an annual health control and 38% attending the hospital because of upper gastrointestinal symptoms. In the symptomatic patients, 15% reported epigastric pain, 20% had sensation of discomfort in the epigastrium, 5% felt rapid satiety after meal, 6% complained of abdominal bloating, 10% had abdominal distention, 5% reported nausea, 2% had frequent vomiting, 30% suffered from reflux symptoms, and 7% suffered from regurgitation. The final cohort included 473 women and 362 men, with a mean age of 46.9 years [standard deviation (SD)=11.4 years, median: 46.8; range: 13.6-74.8]. Women were slightly younger, mean age 45.3 (SD=10.9) years than men (47.6±11.8 years) (p=0.003), the age range of 22-71 years and 13-74 years, respectively.

Patient enrollment took place in two steps. In brief, the potentially eligible patients were identified among the outpatient clinic attendants by the members of the research team. At this stage, every patient was asked to consent for the study and sign a form to participate. Eligible patients are all adult females and males (over 45 years of age), irrespective whether symptomatic or asymptomatic. However, the following patients were considered non-eligible: 1) Patients who were referred to hospital for gastroscopy, 2) patients whose treatment required surgery, or immediate follow-up treatment for major symptoms and 3) those who refused to participate.

Rational use of GastroPanel® examination necessitates some preparatory measures of the patients (34, 35). The patients were not expected to drink, eat or smoke for at least 4 h before blood sampling. The patients were allowed to receive medication, except for PPI inhibitors, H2-blockers or medication neutralizing gastric acid secretion (34, 35).

Collection of samples. The GastroPanel® tests is a combination of four biomarkers analyzed in a single blood sample using an ELISA technique: 1) Pepsinogen-I (PG-I), 2) pepsinogen-II (PG-II), 3) Gastrin-17 (G-17) and 4) *H. pylori* antibody (HpAb) (34, 35). A minimum of 2 ml plasma in EDTA from a fasting individual was obtained. Use of Gastrin-17 stabilizer 100 μl/2 ml plasma (Biohit Cat. No. 601 050 or 601 051) allowed for sample transfer at room temperature (20-25˚C), and permitted for ELISA tests within 4 days from sample collection.

Sample processing. All samples were processed according to the instructions of the manufacturer in the laboratory of MCPAA, following a hands-on training by the manufacturer's staff. The details of sample processing and analysis by ELISA, as already described (34, 35). Both PG-I and PG-II ELISA is based on a sandwich enzyme immunoassay technique with PG-I- and PG-II-specific capture antibody, adsorbed on a microplate and the detection antibody labeled with horseradish peroxidase (HRP). The G-17 ELISA method in the GastroPanel® is specific to "amidated" G-17 molecule. This peptide is the biologically most active gastrin peptide, stimulating gastric acid secretion with 6-times higher potency than the biologically next most active gastrin, G-34. The G-17 ELISA in GastroPanel® assay does not react with G-34 or other

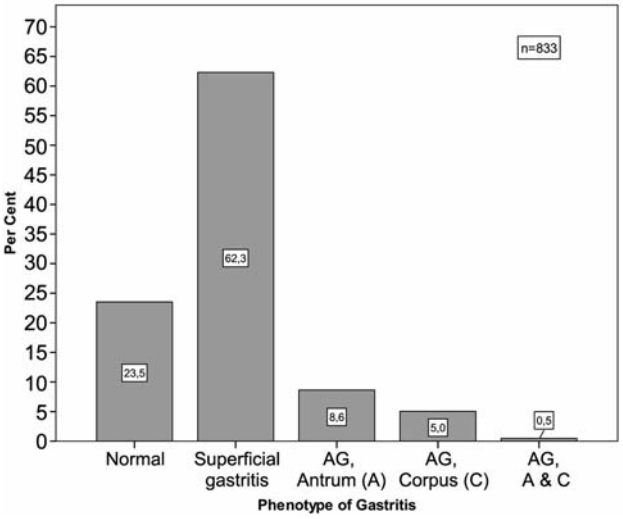

Figure 1. *Distribution of GastroPanel results in different categories.*

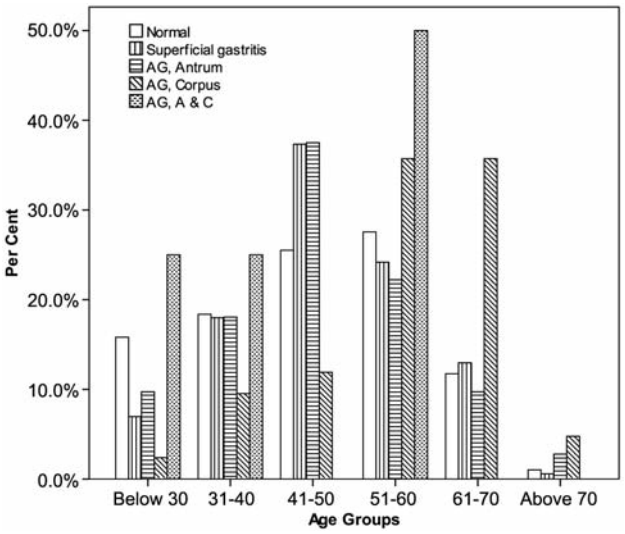

Figure 2. *GastroPanel results stratified by age.*

gastrin fragments (34, 35). If the sample could not be analyzed within 4 days, it was stored at –20˚C. In cases where the test report from the fasting sample implicated AG in the antrum, the G-17 test was repeated in a postprandial blood sample, following a stimulation by a protein drink [Biohit Cat. No. 601038] (34,35).

Evaluation of the marker panel results. The results of the GastroPanel® examination were evaluated using the GastroSoft® interpretation software (34, 35). The principles used by the software are based on the Updated Sydney System (USS) for classification of gastritis (36). Based on the clinically-validated cut-off values for each biomarker , the software classifies the test results into one of the five categories: 1) Normal result, 2) HP infection (without atrophy) 3) atrophic gastritis in the corpus, 4) atrophic gastritis in the antrum, and 5) atrophic pangastritis (8, 30, 34-36). The latest version of GastroSoft® is based on a stochastic algorithm (not probabilistic as the previous versions), giving an electronic (and printed) report, including the test results, cut-off values and verbal interpretation, classifying the GastroPanel® result into one of these five categories (34-36).

Statistical analysis. All statistical analyses were performed using the SPSS 21.0.0 for Windows (IBM, NY, USA). Frequency tables were analyzed using the Chi-square test, with likelihood ratio (LR) or Fischer's exact test being used to assess the significance levels between the categorical variables. Odds Ratios (OR) and their 95% Confidence Intervals (95%CI) were calculated where appropriate, using the exact method. Differences in the means of continuous variables were analyzed using the non-parametric tests (Mann-Whitney, Kruskal-Wallis), with the mean (95%CI) values being derived from ANOVA (analysis of variance). In all tests, values with $p<0.05$ were regarded statistically significant.

Results

GastroPanel results. Figure 1 summarizes the distribution of the GP results in the cohort. Out of the 833 subjects with complete data, only 196 (23.5%) were interpreted as completely normal, whereas the vast majority, 62.3% (n=519) had HP-infection (with no AG). Altogether, 118 subjects (14.1%) presented with the GP results consistent with mucosal atrophy, including AG of the antrum (n=72), corpus (n=42) or both (pangastritis) (n=4). There was no difference in the distribution of these diagnostic categories by gender ($p=0.259$) (data not shown).

Distribution of the gastric conditions by age is summarized in Figure 2. There is a distinct drop of normal GP results after the age of 60. HP-associated non-atrophic gastritis peaks at the age of 41-50 years, with a decline thereafter. This parallels the distribution of AG of the antrum, also peaking at the same age group, with progressive disappearance among older subjects. AG of the corpus clearly takes longer time to develop, peaking in subjects 10 years older than those with antral AG, and remains equally prevalent in patients ageing between 61-70 years. Atrophic pangastritis was rare, only 4 cases were detected, with no distinct age pattern.

Biomarker levels by diagnostic categories. Figure 3 illustrates the serum biomarker levels stratified by the 5 diagnostic categories assigned by GastroSoft®. By definition, the levels of the four biomarkers were significantly different across the diagnostic categories ($p=0.0001$) (Figure 3). The levels of PG-I are within normal cut-off values (30-160 μg/l) in cases classified as normal, HP-gastritis and antral AG (Figure 3A), but there is a dramatic decline in cases classified as AG of the corpus or pangastritis, with mean 95%CI values of 17.9 (14.3-21.6) and 16.5 (0.3-34.5), respectively. As to PG-II (Figure 3B), the highest values (15.3, 95%CI=14.4-16.1) are detected in HP-associated non-AG, being the only condition where the reference levels (3-15 μg/l) are exceeded. PG-II levels are the lowest in subjects with atrophic pangastritis; 3.6

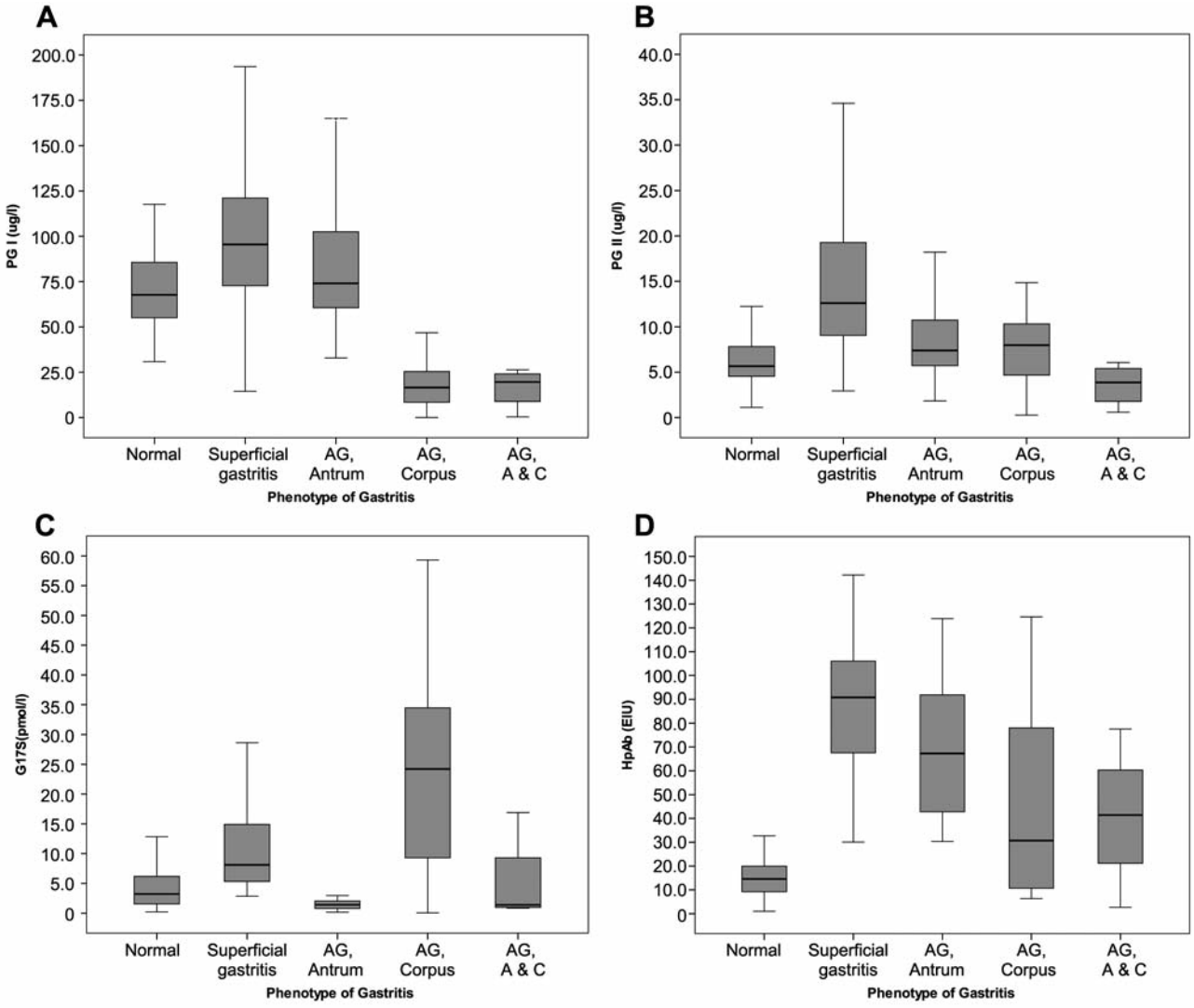

Figure 3. *Mean levels of the 4 biomarkers stratified in the five diagnostic categories. A: Levels of PG-1 by diagnostic categories, p=0.0001. B: Levels of PG-2 by diagnostic categories, p=0.0001. C: Levels of G-17s by diagnostic categories, p=0.0001. D: Levels of HP-Ab by diagnostic categories, p=0.0001.*

(95%CI=0.6-6.1). G-17 basal values below 7 pmol/l were detected in all other categories except for AG of the corpus, with mean basal G-17 levels of 27.6 (95%CI=20.5-34.8) (data not shown). After protein stimulation, G-17 levels were within normal range (3-30 pmol/l) in all other categories except for AG of the antrum, where also the stimulated G-17 values were very low; 1.4 (95%CI=0.2-2.9) (Figure 3C).

Among the subjects classified as having a healthy stomach (n=196), the mean HP-Ab titres were 15.4 (95%CI=14.4-16.3) (Figure 3D). In all other categories, HP-Ab titres were elevated, reaching the peak of 85.7 (95%CI=83.5-87.9) in HP-associated non-AG, followed by AG of the antrum 69.6 (95%CI=62.8-76.5).

Biomarker levels by age. Figure 4 illustrates the levels of the four biomarkers stratified by age groups. There is little variation in PG-I levels over the age, the mean values ranging between 86.4 and 97.6 (p=0.025, ANOVA, p=0.105 Kruskall-Wallis) (Figure 4A). There is more variation in PG-II levels across the age groups, reaching (p=0.001) significance (Figure 4B). Stimulated G-17 values do not significantly differ between the age groups (Figure 4C). HP-Ab titers are very similar across the age groups from 31 until 70 years, but much lower among younger individuals (56.0, 95%CI=46.7-65.2), and particularly among the subjects beyond 70 years of age (45.4, 95%CI=18.2-72.5), with p=0.026.

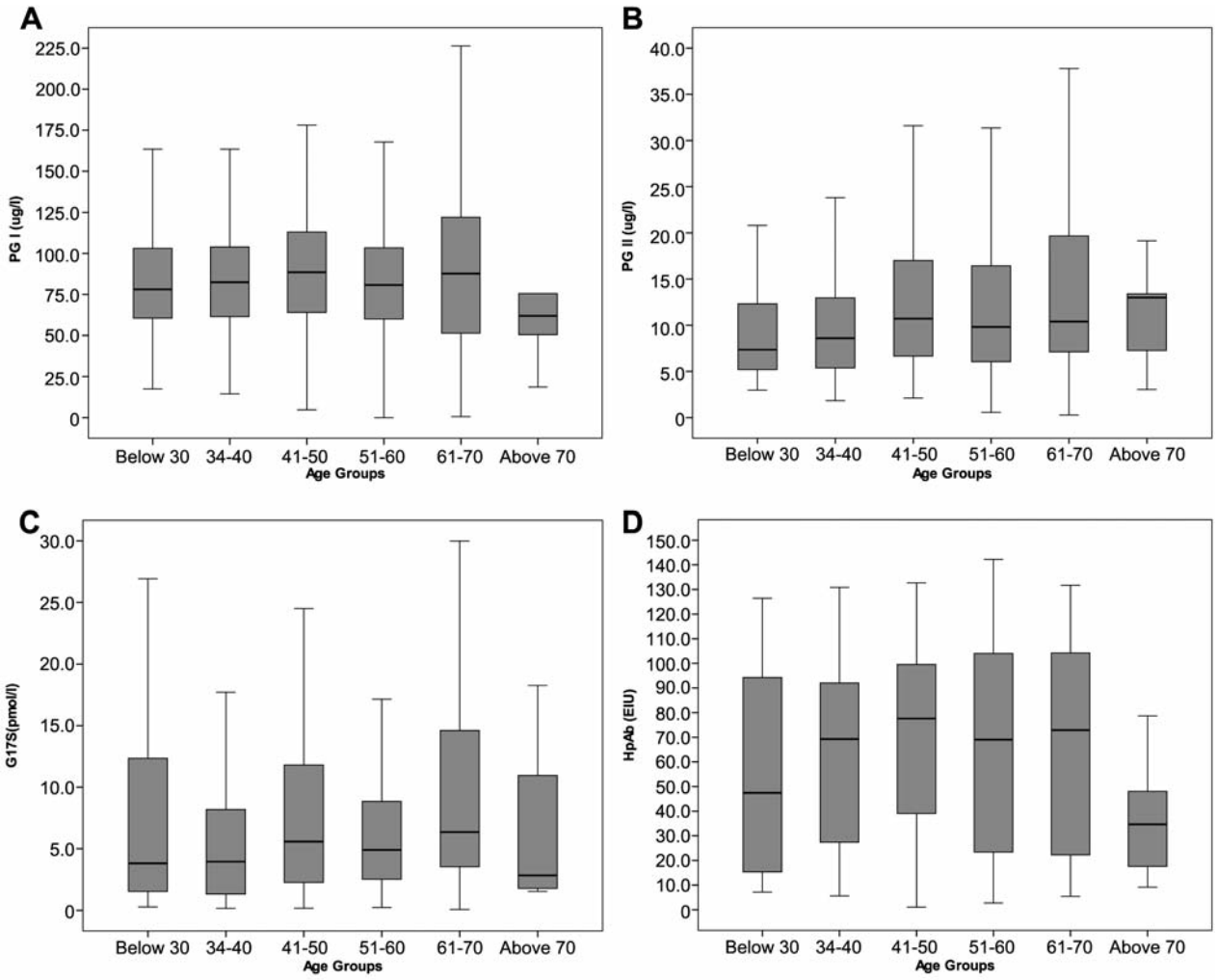

Figure 4. *Mean levels of the 4 biomarkers stratified by the age groups. A: Levels of PG-1 by age, p=0.025 (ANOVA); p=0.105 (Kruskal-Wallis. B: Levels of PG-2 by age, p=0.001 (ANOVA); p=0.001 (Kruskal-Wallis). C: Levels of G-17s by age, p=0.310 (ANOVA); p=0.094 (Kruskal-Wallis). D: Levels of HP-Ab by Age, p=0.026 (ANOVA); p=0.026 (Kruskal-Wallis).*

The serum levels of the 4 biomarkers were also stratified by sex (data not shown). PG-I levels were lower among women (86.0, 95%CI=82.2-89.8) than in men (95.7, 95%CI=90.0-101.4, p=0.011) (Kruskal-Wallis test). The reverse was true with G-17s levels that were higher among women (p=0.005). The levels of PG-II (p=0.197), PG-I/II ratio (p=0.351), G-17b (p=0.106), and HP-Ab (p=0.701) did not differ between the two genders.

Discussion

In contrast to most Western countries, where the incidence of GC has dramatically declined during the past decades, no such trend is witnessed in Kazakhstan (8, 12, 14, 16, 19, 20, 21). On the contrary, it is estimated that the current numbers (n=3,316) of GC cases will continue to increase, reaching 5,000 cases by 2030 (33). In countries where evidenced, the declining trends in GC incidence have been attributed to a marked reduction in the exposure to key risk factors, most notably HP-infection (7, 9-15), and AG (8, 18, 22). Whether this concept holds true for Kazakhstan remains to be elucidated, because no previous data are available on the population prevalence of HP-infection and AG until now. Needless to emphasize, interfering with this untoward development in GC epidemiology is among the top priorities of health policies in the country, and undertaking the present study is a step to this direction.

In the present cohort of 835 subjects, only 196 (23.5%) were interpreted as having a healthy stomach, whereas the

vast majority, (62.3%, n=519) had HP infection (with no signs of atrophic gastritis). These figures are in sharp contrast to those recently reported in two Nordic countries (Finland and Sweden), where the overall prevalence of HP-infection was only 19% and 28.7% and the stomach was classified as normal in 77% and 62.5%, respectively (34, 35). In those two studies, the prevalence of AG was only 3.5% (35) and 6.5% (34), as compared with 14.1% in the present cohort. Out of those 118 subjects, mucosal atrophy was confined to antrum in 72 cases, in the corpus in 42 cases, and additional 4 (0.5%) subjects had atrophy at both sites (Figure 1). Given that HP infection and AG are the two most important risk factors of GC, it is feasible to conclude that differences in the population prevalence of HP and AG closely concur with the observed differences in the time trends of GC incidence in these three countries. Indeed, the incidence of and mortality due to GC in these two Nordic countries have dropped-down to a small fraction of the rates observed in the early 1950's, ascribed to improvements in the living standards, also entailing a reduced exposure to HP-infection (8, 14, 32).

Compared with Finland (6,0/100,000) and Sweden (3,7/100,000), the incidence of GC in Kazakhstan (20,6/100,000) is over 3- and 4-fold, respectively (33). Since no preventive measures are known for GC, the only means to revert this ominous development would be a systematic population-based screening to disclose the subjects at increased risk for GC. Indeed, this is one of the main areas of application of the GP test, which offers a non-invasive tool for sorting-out the individuals for whom gastroscopy is mandatory (31, 32, 34, 35, 37). Even with the current prevalence of HP infection and AG in Kazakhstan, the cost savings of this approach should be substantial. According to current recommendations, gastroscopy is considered mandatory only for subjects with AG, and to a minor proportion of subjects with HP-infection, most notably those with remaining symptoms after successful eradication of HP (32). This reasoning is based on the fact that compared with the subjects who have a healthy stomach, the risk of GC among HP-infected subjects is increased by OR=4.2, whereas in patients with severe AG, this risk can be increased up to 90-fold (8, 12-16).

Translated to the current setting, this would imply that only 118 out of 835 subjects would need gastroscopy as a first-line triage tool, following GP test. The exact proportion of gastroscopies among those 519 individuals with HP-infection remains to be determined after HP-eradication, based on clinical judgment of each individual case. Additional information for an appropriate resource allocation can be obtained by stratifying the GP results by age (Figure 2). Accordingly, AG is a rare condition among individuals less than 40 years of age; less than 28% of antral AG and <12% of corpus AG occur in these age groups. The vast majority of all AG cases do occur among individuals past 50 years of age, particularly AG of the corpus, which clearly increases in parallel with age, as confirmed in previous studies (7, 8, 14-18, 22). Thus, focusing the population-based screening to the age groups above 45-50 years will most likely be a more cost-effective strategy than including the younger age groups in the program. This applies equally to both sexes, because there were no differences between the genders in i) distribution of the five diagnostic categories, or ii) their relationship with age.

The biomarker panel is based on a combination analysis of PG-I, PG-II, amidated G-17 and HP-antibodies, designed to give information on both the structure and function of the stomach mucosa. Most importantly, this panel gives accurate estimates of the capacity of the corpus and antrum mucosa to secrete acid and G-17, respectively, as well as of important gastric pathologies like inflammation, grade and topography of AG (30, 38-40). Normal plasma levels of these biomarkers indicate that the stomach mucosa has normal structure and function, whereas the abnormal levels are signs of a non-healthy stomach, reflecting the disturbances in the feedback mechanisms between the acid output, PGs and G-17 (41).

The five diagnostic categories assigned by the GastroSoft software are characterized by significantly different biomarker profiles (Figure 3). Based on previous validation studies (with biopsy confirmation) (38, 39, 40, 41), levels of PG-I decrease in AG of the corpus (and in pangastritis), but remain within the normal range in all other conditions. The PG-II levels reflect mucosal inflammation, the highest values being detected among cases classified as HP-associated (non-atrophic) gastritis. The G-17s are highest in AG of the corpus, because of the missing negative feedback loop by the acid from an atrophic corpus mucosa, resulting in uninhibited secretion of G-17 by the normal antral mucosa after protein stimulation (38-41). By definition, when antral mucosa is atrophic and G cells are depleted, G-17 secretion remains very low after protein stimulation (41).

HP-IgG antibodies give significant added value to the three biomarkers (38-41). IgG serology for HP measures potentially two different conditions: 1) An ongoing HP infection, or 2) a previous exposure to HP (7- 15). In the GP test, there is no means to make distinction between these two, for which other tests are needed, *e.g.* the HPQT (*H. pylori* Quick Test, Biohit HealthCare), performed in gastric biopsies and when positive, demonstrates an ongoing HP infection.

By far the highest HP-antibody titers were encountered in cases classified as HP-related gastritis with no atrophy (8, 36). HP-Ab titers are high also in antral AG, consistent with an ongoing active infection at this site. Albeit the causal agent of AG in the corpus, HP itself can disappear in cases with prolonged course (7, 9-15). This is recognized by the GastroSoft algorithm, which permits AG of the corpus to be of either HP+ or HP– phenotype. According to current concepts, there is no AG of the antrum without HP-infection,

and because of this, GastroSoft assigns this diagnosis to HP+ cases only, if matching the other criteria (38-41).

When stratified by age, there was no consistent age-related pattern for any of the biomarker levels (Figure 4), implicating that their secretion is not related to age. This is in line with the view that age is not an independent risk factor of GC either (8, 21), but age is a surrogate of AG, which is the true risk factor. Similarly, the secretion of these 4 biomarkers is determined by the specific conditions of gastric mucosa, which these biomarkers are the indicators of. Thus, *e.g.* the fact that the levels of PG-I are the lowest among subjects beyond 70 years of age, is simply because AG of the corpus is more prevalent in this age group.

This is the first population-based study to use this non-invasive biomarker test for estimating the population prevalence of known risk factors for GC in a high-incidence GC country, the Republic of Kazakhstan. As such, the study provides valuable information, concerning intervention strategies to revert the rising trend of GC incidence in Kazakhstan. The weakness of this pilot study is failure to provide biopsy confirmation for the biomarker panel results, which precludes the possibility to calculate the performance indicators of the panel for the different study end-points.

Taken together, the biomarker panel results of this cohort are alarming, while disclosing i) an extremely high prevalence of HP-infection (76.5%, all cases included), and ii) a distressing prevalence of atrophic gastritis (14.1%). Given that these two conditions represent the single most important risk factors of GC, these data are in perfect alignment with the high incidence of GC in Kazakhstan. The non-invasive GastroPanel test could be a cost-effective means capable of interrupting the current rising trend in GC in Kazakhstan, when applied to a systematic population-based screening setting.

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