

Original Research Article

## Prevalence of *H. pylori* Infection and Atrophic Gastritis among dyspeptic subjects in Cameroon using a Panel of Serum Biomarkers (PGI, PGII, G-17, HpIgG)

Ebule I.A.,<sup>1,2</sup> Djune, Fokou, A. K.,<sup>1,2</sup> Sitedjeya, Moko, I. L.<sup>2</sup>, Tanni B.,<sup>3</sup> Heugueu C.<sup>2</sup>, Longdoh A.N.,<sup>4</sup> Noah Noah D.<sup>5</sup>, Okomo Assoumou M.C.<sup>6</sup>, Paloheimo L.<sup>7</sup>, Njoya O.<sup>8</sup>, Syrjanen K.<sup>7</sup>

<sup>1</sup>Faculty of Science, University of Buea, Cameroon

<sup>2</sup>School of laboratory Technologies, Yaounde, Cameroon

<sup>3</sup>Protestant University of Central Africa

<sup>4</sup>Faculty of Health Sciences, University of Buea, Cameroon

<sup>5</sup>Yaounde Central Hospital, Faculty of Medicine and Pharmaceuticals Sciences, University of Douala, Cameroon

<sup>6</sup>National Public Health laboratory, Faculty of Medicine and Biomedical Sciences, University of Yaounde, Cameroon

<sup>7</sup>Biohit HealthCare Oyj, Helsinki, Finland

<sup>8</sup>University Teaching Hospital, Faculty of Medicine and Biomedical Sciences, University of Yaounde, Cameroon

### \*Corresponding author

Alonge Ivo Ebule

Email: [alolevo@yahoo.com/alonge.ivo@ubuea.cm](mailto:alolevo@yahoo.com/alonge.ivo@ubuea.cm)

**Abstract:** *H. pylori* infection induces chronic active gastritis that develops with time in a proportion of infected people to atrophic gastritis (AG) and acid free or hypochlorhydric stomach. Objective: Use of a non-invasive serum biomarker test [Pepsinogen-I (PG-I) and -II (PG-II), amidated gastrin-17 (G-17), and *Helicobacter pylori* (*H. pylori*) IgG antibodies], to determine the prevalence of *Helicobacter pylori* infection and atrophic gastritis (AG), both known to increase the risk of gastric cancer (GC). Subjects consulting for various dyspeptic complaints were prospectively recruited on voluntary basis at the Tombel District Hospital, Yaounde Central Hospital and University Teaching Hospital, from 2008 to 2016. 287 were enrolled 18-84 years (mean±SD 40.08±15.37), including 80 males aged 22-84 years (mean±SD 45.15±15.72) and 207 females aged 18-83 years (mean±SD 46.43±15.24) were examined with a panel of biomarkers. The results were assigned in five categories according to the test panel: 1) Healthy stomach, 2) superficial or non atrophic gastritis associated *H. pylori* infection, 3) atrophic gastritis (AG) of the corpus, 4) AG of the antrum, and 5) AG of both antrum and corpus (pangastritis). Results: 51 (17.8%) of the subjects were interpreted as normal. 226 (78.7%) were positive for *H. pylori* (all cases included), 209 (72.8%) had *H. pylori*-infection (with superficial or no atrophic gastritis). 27 subjects (9.4%) presented with the Gastro Panel results consistent with mucosal atrophy, including atrophic gastritis of the antrum (n=9, 3.1%), corpus, (n=10, 3.5%) or both, pangastritis (n=8, 3.1%).

**Keywords:** Prevalence, *H. pylori* Infection, Atrophic Gastritis, dyspeptic, Serum Biomarkers

### INTRODUCTION

Dyspeptic symptoms are among the most common gastric complaints, experienced by 25-40% of the people during their lifetime. The most common cause of dyspepsia is gastritis. Infection with spiral rod shaped bacteria *Helicobacter pylori* is now identified as the causal factor for several clinically important diseases in gastric and duodenal mucosa [1]. In 1994, the IARC expert group classified *H. pylori* infection as a group-I carcinogen for humans [2]. This bacterial infection (usually acquired in childhood) initially affects only the antral mucosa causing superficial gastritis. If not eradicated, *H. pylori*-infection remains

chronic and progresses to corpus-predominant gastritis or pangastritis, with mucosal atrophy as the end result [1]. *H. pylori* infection induces chronic active gastritis that develops with time in a proportion of infected people to atrophic gastritis (AG) and acid free or hypochlorhydric stomach [3]. Chronic gastritis is one of the most common life-long, serious and insidious illnesses in human beings and it has been estimate that more than half of the world population has this disease in some degree and extent, indicating that even many hundreds of millions of people worldwide may have chronic gastritis in a form or other [4]. On average, half of the case with *H. pylori* infection will develop AG of

some degree during their lifetime, and in around 10% of the infected subjects, the AG will finally be moderate or severe. In the latter category, 2.5–5% may get a cancer. Nonatrophic *H. pylori* gastritis raises the risk of gastric cancer fourfold on average, and the risk may rise to 15-fold in patients with AG. In subjects with severe panatrophy (AG in both antrum and corpus, i.e., severe multifocal atrophic gastritis), irrespective of the presence or absence of ongoing *H. pylori* infection, the cancer risk may even be up to 90-fold compared with the risk in subjects with a healthy stomach mucosa [3, 5].

Atrophic gastritis is the single most powerful independent risk factor for distal (non-cardia) gastric cancer. It is estimated that 50% of all gastric cancer cases develop through the “Correa cascade”, leading from *H. pylori*-associated gastritis to mucosal atrophy, intestinal metaplasia, dysplasia, and to invasive adenocarcinoma [1]. Gastric cancer (GC) still remains a major public health problem worldwide and is fifth most frequent malignancy worldwide, with close to one million new cases annually [6] and the third most cause of cancer related deaths after lung and liver cancers [7]. The future of prevention of gastric cancer deaths now relies on the on timely and early diagnosis and surveillance of patients with precancerous lesions as well as early detection of the cancer, making higher survival rates and lower healthcare costs per patient achievable [8, 9]. Endoscopy with sampling of gastric biopsies is documented as the best and most effective option for screening for upper GI malignancies but however endoscopic screening for stomach cancer can only be cost-effective in moderate to high-risk populations and therefore limiting its applicability in low risk regions. Further, 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 [10, 1]. Now however, recent experience suggests that a systematic screening of the risk groups by stomach-specific biomarkers provides provide a potential solution. Plasma biomarker test are now be used for the screening of patients with a “sick stomach mucosa” and for those with AG The biomarker screening helps, in addition, in the identification of the patients with a “healthy” stomach mucosa, in whom the cancer risk is low, and in whom the endoscopy may not be the first important diagnostic procedure [6, 3]. The latest development in the field of biomarkers represents a panel which combines serum pepsinogen- I (PGI) and - II (PGII), gastrin-17 (G-17) and *H. Pylori* IgG antibodies (IgG-HP) using an ELISA technique for

detection (Gastro Panel® test, Biohit Oyj), proposed as the first-line diagnostic test for dyspeptic symptoms. 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. Consequently, these stomach-specific biomarkers were recommended by an international group of experts for diagnosis and screening of AG [6, 1, 11].

## **METHODS**

### **Patient information:**

Patients presenting with dyspeptic symptoms and consented to participate were prospectively collected between March 2016 and February 2017 at the Tombel District Hospital, the Yaounde Central Hospital, the University Teaching Hospital and Espoir Laboratory of Yaounde. Patients fasted 10 hours prior sample collections and authorized to take prescribed regular medications except those that had effects on gastric secretion such as Aluminium hydroxide containing drugs or gel, bismuth, sodium alginate, sodium bicarbonate, magnesium hydroxide gel, calcium carbonate.

### **Blood samples:**

Basal blood was aseptically collected by venipuncture into EDTA tubes and immediately centrifuged at 2000G for 15 minutes. The plasma samples were then distributed into cry and stored at -20 until analyzed. Plasma concentrations of PGI, PGII, G-17 and *H. pylori* IgG determined by the Gastro Panel (Biohit plc Helsinki, Finland) using the enzyme linked immunosorbent assay (ELISA), according to the manufacturer’s instructions for the measurement of absorbance after a peroxidation reaction at 450nm. The results of the Gastro Panel® examination were evaluated using the Gastro Soft® interpretation software ([www.biohithealthcare.com](http://www.biohithealthcare.com)).

### **Assay analysis:**

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) superficial gastritis (non atrophic gastritis), *H. pylori* infection without atrophy 3) atrophic gastritis in the corpus, 4) atrophic gastritis in the antrum, and 5) atrophic pangastritis. The recommended cut-off values were used for all 4 biomarkers as follows: pepsinogen I (PGI) <30 µg/l, the PGI/PGII ratio <2.5, and fasting G-17 <1 pmol/l (G-17b). Values below these cut-off levels implicate AG of the corpus (PGI, PGI/PGII) and AG of the antrum (G- 17b), respectively. Whereas low PGI/PGII ratio <2.5, or PGI <25 µg/l associated lo low G-17 <1 pmol/l was considered as atrophy of both

antrum and corpus. *H. pylori* IgG antibody levels above 30 EIU (enzyme-immunoassay units) were considered an indicator of *H. pylori* infection (ongoing or recent exposure).

#### **STATISTICAL ANALYSIS:**

Statistical analysis was performed using the SPSS 16.0 software package (SPSS Inc. Chicago, IL, USA). Data were expressed as mean±SD. The differences between groups were analyzed by the Student's *t*-test, Mann-Whitney U-test and Significance of differences between means was estimated with ANOVA, and between proportions using  $\chi^2$  test. In all tests, values with  $p < 0.05$  were regarded statistically significant. Ethical clearance was obtained from the national ethics committee. The study was accepted by the ethics committees of the Tombel District Hospital, Yaounde Central Hospital, the University Teaching Hospital and the Espoir Laboratory. All patients signed an informed consent form.

#### **RESULTS**

In total, 287 subjects were enrolled during the study period aged 18-84 years (mean±SD 40.08±15.37), including 80 males aged 22-84 years (mean±SD 45.15±15.72) and 207 females aged 18-83 years (mean±SD 46.43±15.24). The results for the distribution of Gastro Panel are summarized in Figure 1. Amongst the 287 subjects 51 (17.8%) were interpreted as normal, whereas the vast majority, 209 (72.8%) had *H. pylori*-infection (with superficial or no atrophic gastritis). In total 27 subjects (9.4%) presented with the Gastro Panel results consistent with mucosal atrophy, including Atrophic Gastritis of the antrum, A (n=9, 3.1%), corpus, C (n=10, 3.5%) or both (pangastritis), P (n=8, 3.1%).

Distribution of the gastric phenotypes by age is shown in Figure 2. There was a drop of normal gastric mucosa after the age of 50. Non-atrophic or superficial gastritis associated *H. pylori* peaked at the age of 31-40

years, and declined thereafter. This paralleled the distribution of atrophic pangastritis, also peaking at the same age group, with progressive disappearance among older subjects. Atrophic gastritis of the antrum peaked equally at the age groups 31-40, 41-50, 51-60 and then falling gradually. Atrophic gastritis of the corpus peaked at the 41-50 age groups and the falling progressively.

The serum biomarker levels (PGI, PGII, PGI/PGII, G-17 and HpIgG) were stratified by the 5 phenotypes of gastritis assigned by Gastro Soft®. The mean PG-I levels decreased significantly with increasing grade of atrophic gastritis of corpus (57.82±38.99) and pangastritis (65.38 ±52.52) compared normal (99.75 ±71.57) and subjects with non-atrophic gastritis (129.83±67.32) ( $p=0.038$ ), (figure 3). Mean PG-II levels did not differ significantly ( $p=0.349$ , figure 4) among the different phenotypes of gastritis, atrophic antrum gastritis (37.98±18.86), atrophic corpus gastritis (15.92±8.54), atrophic pangastritis (18.00±6.94), Nonatrophic gastritis (25.46±17.01) and normal gastric mucosa (21.90±18.99). Raised levels of mean PGI/PGII were observed in normal mucosa (8.34±6.48) and nonatrophic gastritis (8.96±18.67) than in atrophic antrum gastritis (5.06±1.55), atrophic corpus gastritis (3.23±4.12) and atrophic pangastritis (1.92±0.78), ( $p=0.019$ , figure 5).

Mean G-17 levels were within normal range (1-10 pmol/l) in the categories of atrophic corpus gastritis (7.46±12.79), nonatrophic gastritis (1.89±3.60), normal stomach mucosa (6.28±16.75) except atrophic antrum gastritis (0.46±0.63) and atrophic pangastritis (0.61±0.63), ( $p=0.0021$ , figure 6). Raised HpIgG titers above 30 EIU were observed in all categories except in normal mucosa ( $p=0.118$ , figure 7), non-atrophic gastritis (240.57±227.72), atrophic antrum gastritis (200.51±152.39), atrophic corpus gastritis (40.43±85.52), atrophic pangastritis (150.86±135.08) and normal mucosa (19.29±20.28).

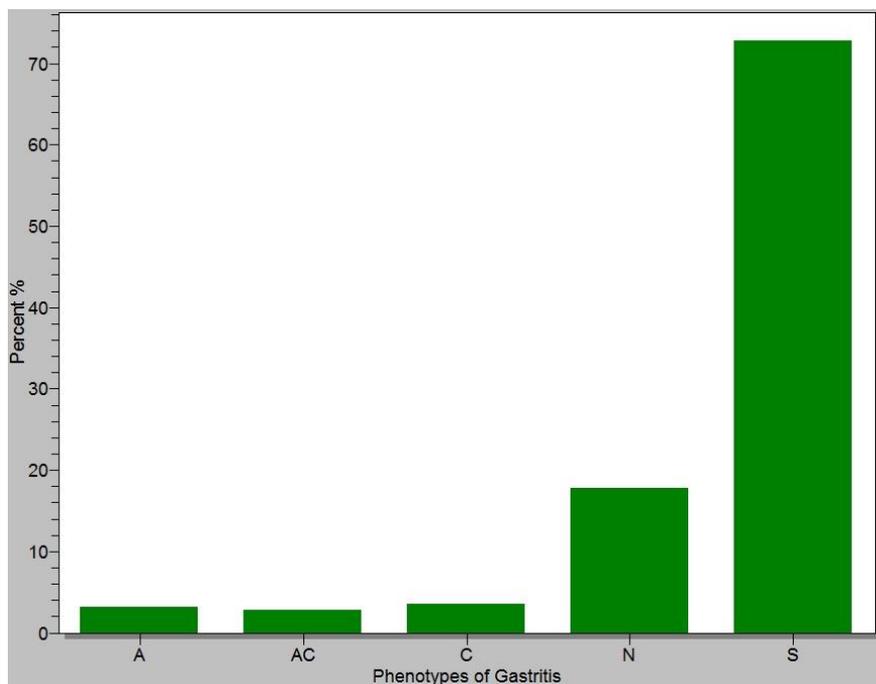


Fig 1: Summary distribution of Gastro Panel results

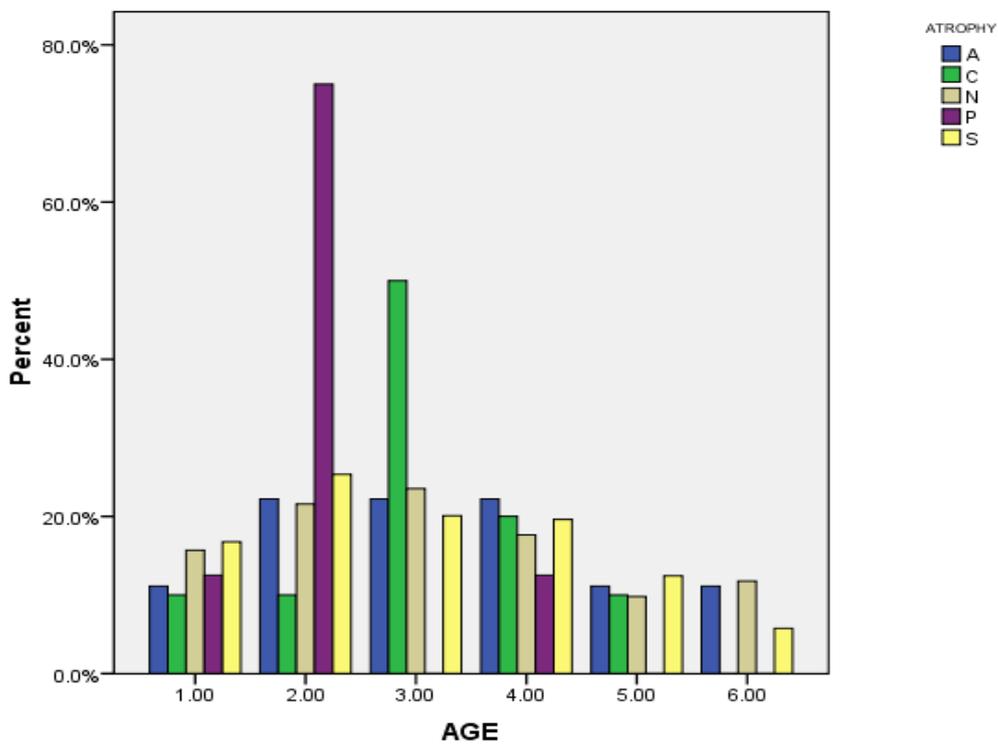


Fig 2: the distribution of the gastric conditions by age group: 1.00 = <30yrs; 2.00= 31-40 yrs; 3.00= 41-50 yrs; 4.00=51-60 yrs; 5.00=61-70 yrs 6.00 = >70 yrs. A= atrophic antrum gastritis; C= atrophic corpus gastritis; N= normal stomach mucosa; P= pangastritis, atrophic gastritis of both corpus and antrum; S= non atrophic or superficial gastritis associated with *H. pylori*.

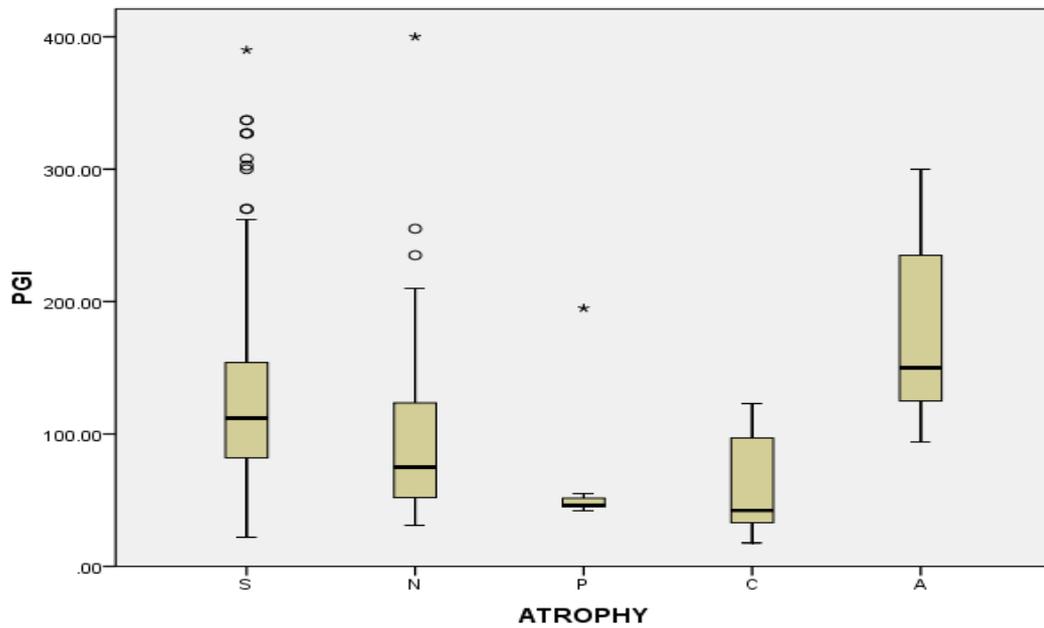


Fig 3: the variation of mean PGI levels with different grades of gastric phenotypes

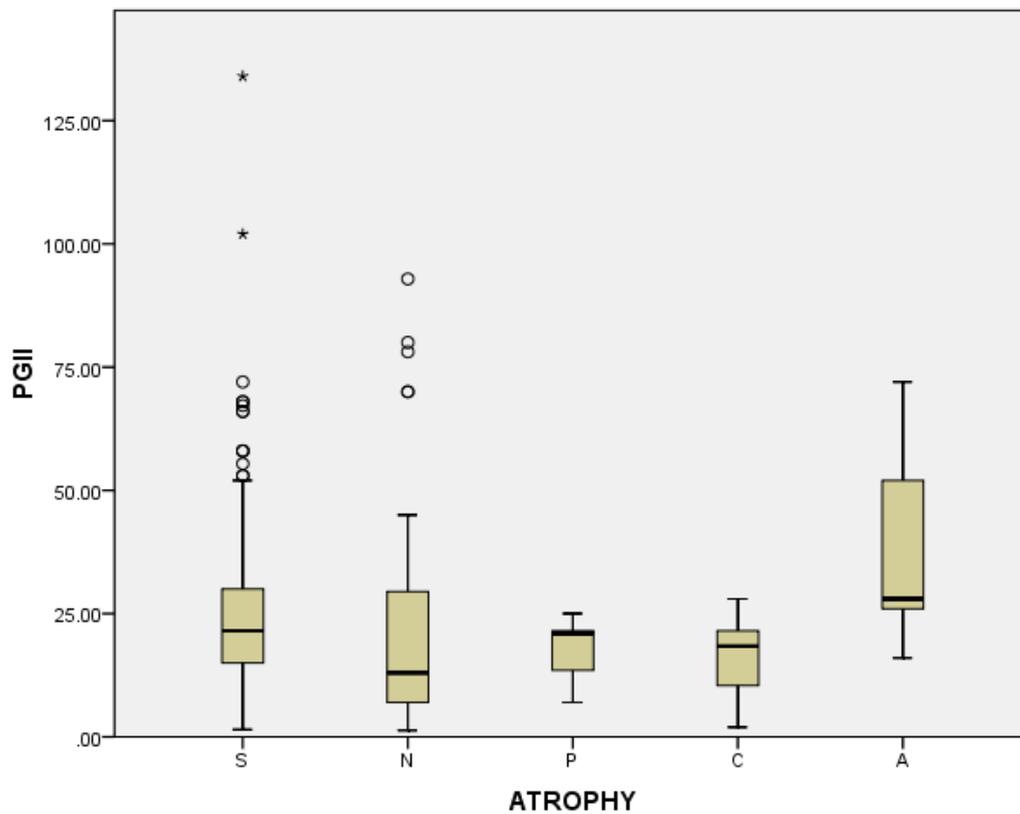


Fig 4: the variation of mean PGII levels with different grades of gastric phenotypes

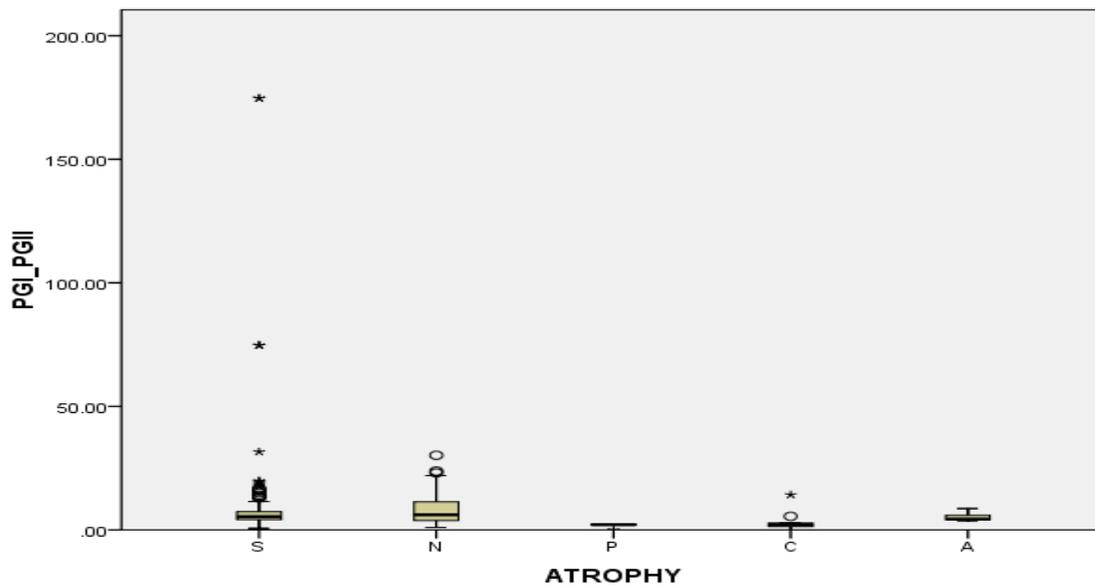


Fig 5: the variation of mean PGI/PGII ratio with different grades of gastric phenotypes

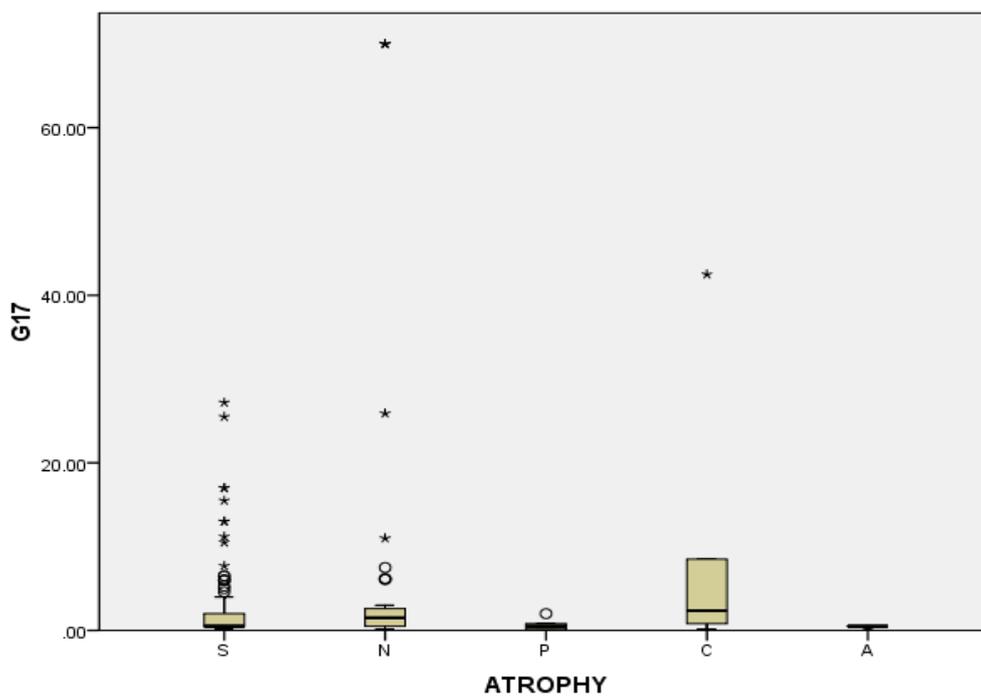


Fig 6: the variation of mean G-17 levels with different grades of gastric phenotypes

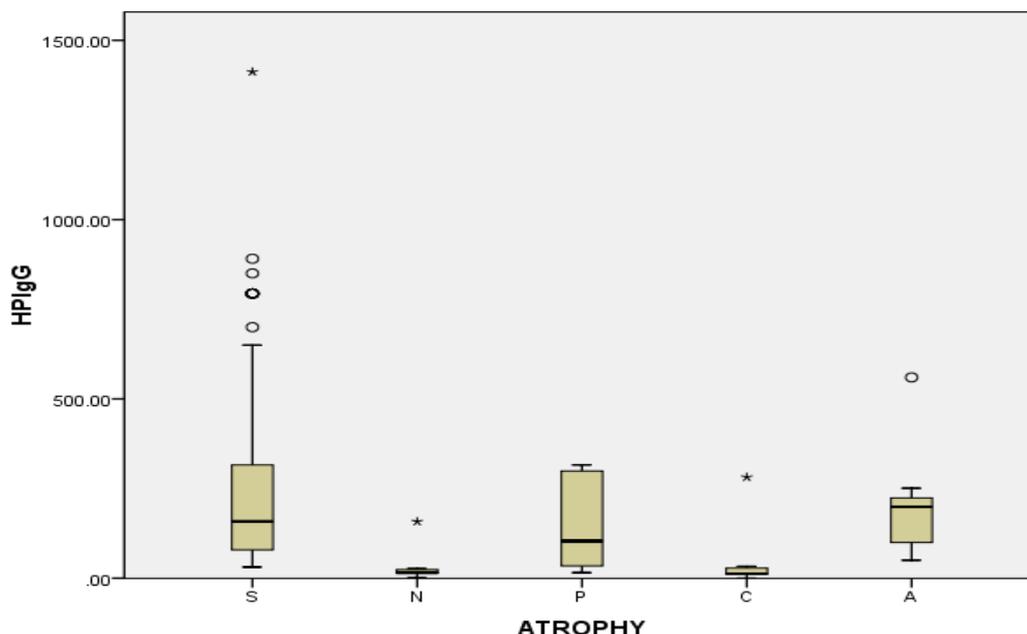


Fig 7: the variation of mean *H. pylori* IgG titers with different grades of gastric phenotypes

## DISCUSSIONS

Infections with *H. pylori* and atrophic gastritis have been identified as the highest risks conditions for gastric cancer. According to the recommendations of the Maastricht IV consensus statement, the gold standard for the diagnosis of atrophic gastritis is biopsy examination during gastroscopy [12]. However, gastroscopy is considered mandatory only for high risk subjects that is, those with AG, and to a minor proportions of subjects with HP-infection, most notably those with symptoms after successful eradication of HP. 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 4 fold, whereas in patients with severe AG, this risk can be increased up to 90-fold [1]. Since preventive measures for GC are scarce, a systematic screening to identify the subjects at increased risk for GC has been achieved easily with biomarker examination [3]. The biomarker panel is based on a combination analysis of PG-I, PG-II, amidated G-17 and HP-IgG 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. 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 [13, 6, 14].

The results for the distribution of Gastro Panel are represented in Figure 1. Amongst the 287 subjects, 226 were positive for *H. pylori* with a prevalence of 78.7% (95% C.I, 73.6-83.3%). 209 had superficial (non atrophic) gastritis associated *H. pylori*-infection with a prevalence of 72.8% (95% C.I, 67.3-77.9%). 51 (17.8%, 95% CI 13.5-22.7%) were interpreted as normal. 27 (9.4%) subjects (95% CI, 4.3- 17.6%) presented with the Gastro Panel results consistent with mucosal atrophy, including 9 with Atrophic Gastritis of the antrum, A (3.1%, 95% CI 1.4-5.9%), 10 with atrophic gastritis of the corpus, C (3.5%, 95% CI 1.7 – 6.3%) and 8 with both atrophic pangastritis, P ( 3.1%, 95% CI 1.2 – 5.4%). The results for the distribution of the Gastro Panel are similar with that of Benberin *et al.*; in 2013 [1] who in a cohort in Kazakhstan obtained 76.5% prevalence of *H. pylori* infection and 14.1% prevalence of atrophic gastritis with 23.5% prevalence of healthy stomach, 62.3%, for non-atrophic gastritis associated *H. pylori* infection. These figures are however in sharp contrast to those reported in by Storskrubb *et al.*; in 2008 [15] in Sweden and Telarata *et al.*; in 2010 [16] in Finland where the overall prevalence of *H. pylori* infection was respectively 19% and 28.7% and the prevalence of atrophic gastritis was only 6.5% and 3.5% respectively and the stomach was

classified as normal in 77% and 62.5%, respectively. These results are also similar with what has been reported previously in Cameroon by Noah *et al.*; in 2012 [17] and Ebule *et al.*; 2013 [18] who obtained respectively 81.40% and 79.80% for *H. pylori* infection and 8.1% and 6.6% for atrophic gastritis. Given that *H. pylori* infection and atrophic gastritis are the two most important risk factors of gastric cancer, it is feasible to conclude that differences in the population prevalence of atrophic gastritis and *H. pylori* closely concur with the observed differences in the time trends of gastric cancer incidence in these countries. The non-invasive Gastro Panel test could be a cost-effective means capable of interrupting the current rising trend in GC, when applied to a systematic population-based screening setting [1].

Significantly decreased PGI mean concentrations and PGI/PGII ratio with increasing atrophic stages in the corpus and pangastritis was observed ( $p=0.038$ , figure 3), ( $p=0.019$ , figure 5) respectively. Similarly, reduced levels of Mean G-17 with worsening of atrophic antrum gastritis ( $p=0.0021$ , figure 6). Although raised HpIgG titers above 30 EIU were observed in all categories of atrophy and non-atrophic gastritis, these high levels were however, no significant ( $p=0.118$ , figure 7), *H. pylori*-IgG antibodies measures potentially two different conditions: 1) An ongoing *H. pylori* infection, or 2) a previous exposure to *H. pylori* [13]. Highest *H. pylori*-antibody titers have been reported in *H. pylori*-related gastritis with no atrophy, and are also in antral AG, consistent with an ongoing active infection at this site. On the other hand, *H. pylori* can disappear in cases AG in the corpus, which permits AG of the corpus to be of either HP+ or HP- phenotype [1].

The findings of this study are consistent with the report of Pasechnikov *et al.*; in 2005 [19] that serum pepsinogens and gastrin 17 decreases as atrophic gastritis in the corpus and antrum worsens due to loss of mucosal glands and cells. Bornschein *et al.*; in 2013 [10], confirmed the association between gastric atrophy and decreased PGI levels in the serum as well as a decreased PGI/II-ratio. The use of serum pepsinogen I levels as an assessment of gastric acid secretion was adopted as early as in 1985 [20, 21]. The clinical significance of pepsinogen A and pepsinogen C and serum gastrin levels [22] and the role of serum pepsinogen I and serum gastrin in the screening of severe atrophic corpus gastritis had earlier been reported [23] and in screening of atrophic pangastritis with high risk of cancer Varis *et al.*, 1991). PGI reflects the status of the mucosa of the corpus and fundus of the

stomach and is a well-known indicator of the corpus mucosa [13]. G-17 is a well-established biomarker of the G-cells in the gastric antrum, providing information *e.g.* on mucosal atrophy in that topographic location. Low levels of G-17 are not exclusively inherent to antral AG, but may also reflect high gastric acid output. On the other way round, G-17 is upregulated (through a negative feedback loop) by a low acid content of the corpus, caused by either (i) AG of the corpus, or (ii) more frequently, by a prolonged use of proton pump inhibitor (PPI) medication [13, 6]. Several studies from all over the world have reported the diagnostic efficacy of the four biomarker test including PGI, PGII, G-17 and HpIgG to predict normal gastric mucosa with high sensitivity and specificity for gastric atrophy and that the biomarker test is a suitable tool for non-invasive screening and diagnosis of atrophic gastritis [25-30]. At present, longitudinal matched case controlled studies with the biomarker testing with baseline values of PGI, PGII and PGI/PGII ratio have been used in predicting incidence gastric cancer in population-based GC screening programs [31, 32, 6].

#### CONCLUSION:

Considering the biomarker panel results of PGI, PGII, G-17 and HpIgG in this study, we have observed an extremely high prevalences of *H. pylori*-infection (78.7%) and atrophic gastritis (9.4%). Given that these two conditions represent the single most important risk factors of gastric cancer, these data are in perfect alignment with what has been reported previously in Cameroon and the world at large. The non-invasive Gastro Panel (PGI, PGII, G-17 and HpIgG) test could be a cost-effective means capable of identifying patients at risk of gastric cancer *i.e.* those with *H. pylori* infection and atrophic gastritis.

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