Association between the immune responses against Helicobacter pylori infections and the concentration of atrophic gastric biomarkers in Cameroon

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Abstract

Gastric colonization by *Helicobacter pylori* causes recruitment of inflammatory cells by the adherence of bacteria to the epithelium and the release of virulence factors. Gastritis induces disruption of acid secretion depending on the predominant location in the stomach, antrum or corpus. The purpose of this study was to examine the impact of the presence of *H. pylori* on acid secretion and to establish relationship between the level of immune responses against *H. pylori* and gastric biomarkers in patient with dyspeptic disorders.

Methods: From march to July 2011, all patients with dyspeptic underwent upper gastro-intestinal tract endoscopy with biopsy at the Yaounde Central Hospital and

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University Teaching Hospital. Basal blood was collected and tested for gastric biomarkers- pepsinogen (PG) I, pepsinogen (PG) II, gastrin (G)-17 levels and correlated with immunoglobulin (Ig)-G antibody concentration to *H. Pylori*.

Results: Depressed levels of G-17(P=0.016) and a negative association with *H. Pylori* IgG levels (r=-0.171, p=0.04) was noticed in *Helicobacter pylori* infected than non-infected individuals. Raised levels of PGI (p=0.002) and a positive correlation with *H. Pylori* IgG (r=0.241, p=0.047) was seen among *H. pylori* infected subjects. Significantly reduced levels of PGI (p=0.003) and G-17 (p= 0.037) with worsening of atrophy was observed.

Conclusion: The findings suggest that gastric inflammation with *H. Pylori* has a considerable impact on the gastric morphology and acid secretion and in turn influences the trend of gastric biomarkers (PGI, PGII, and G-17).

Keywords: *Helicobacter pylori*, atrophic gastritis, immunoglobulin, pepsinogen, gastrin

1 Introduction

Helicobacter (H.) pylori infection is the most important cause for chronic and atrophic gastritis. Other causes include autoimmune disease, drugs or bile reflux [1]. The gastroduodenal response to chronic *H. pylori* infection is characterized by infiltration of plasma cells, lymphocytes, neutrophils, and monocytes in to gastric mucosa. The gastric epithelium plays an active role in mucosal defense. Neutrophil activation and the production of reactive oxygen metabolites are induced directly by bacterial factors and indirectly via host derived cytokines and products of complement activation. As well as stimulating specific T and B cell responses and systemic immunoglobulin (Ig) G and A antibody production, *H. pylori* infection also induces a local proinflammatory cytokine response and the

development of gastric lymphoid follicles which are important in immune cells infiltration [2].

Among the well known parameters of atrophic gastritis are the levels of serum pepsinogens and gastrin. Gastrin-17 (G-17) and pepsinogen I (PGI) levels respectively reflect distal and proximal stomach, while pepsinogen II (PGII) level, reflects the status of the entire stomach and particularly inflammation [1,3]. PGI is produced by chief cells and mucous neck cells in the corpus area; G-17 is produced mainly by the G cells in the antrum. The more severe the atrophic gastritis in the mucosa of the corpus of the stomach, the lower are the measured concentrations of PGI and PGI/ II ratio in the blood sample. Correspondingly, the more severe the atrophic gastritis in the mucosa of the antrum of the stomach, the lower is the measured G-17 concentration. The measured concentrations of pepsinogens (I and II) and G-17 represent quantitatively the overall condition of the mucosa of the entire stomach, its functional status and the severity of atrophy [4]. According to the Maastricht III consensus conference, the gold standard for the diagnosis of *H. pylori* infection is firstly carbon-13 (C-13) urea breath test (C-13UBT), and secondly *H. pylori* stool antigen test (HpSA). However, in the test and treat strategy, neither test gave any information on the morphologic or gastric secretory status. Furthermore, C-13UBT, and HpSA give 40-50% false negative result in atrophic gastritis, MALT lymphoma, or upper bleeding, or if the patient has currently received antibiotics and protein pump inhibitors treatment [5-8]. In these cases the reliable identification and treatment of *H. pylori* is important. These limitations paved the way for serology and especially IgA and IgG because greater than 90% of *H. pylori* infected individuals exhibit specific IgG antibodies, with 70% of whom also exhibit IgA antibodies [9, 10]. The anti body levels remain elevated during the course of the infection and fall gradually within six months, during disappearance of the bacteria, and can be used to evaluate the success of treatment. In view of the role cells of the immune system play in patients with *H. pylori* and also the lack of information on immunoglobulin

antibody levels against *H. pylori* infected individuals in our study area, this investigation focused on the relationship between gastric biomarkers (PGI, PGII, G-17) and IgG production in *Helicobacter pylori* patients (IgG*Hp*Ab). We also compared IgG*Hp*Ab, PGI, PGII, and G-17 levels in patients with or without stomach mucosa atrophy.

2 Methods

Patient information: The study was carried out from March to July 2011, at the Yaounde Central Hospital (YCH) and University Teaching Hospital (UTH). Patients were recruited prospectively on voluntary basis according to the following criteria: Adult patients (over 20 years) and undergo endoscopy with biopsy for dyspeptic symptoms. Patients: in whom endoscopy is carried out for other than diagnostic reasons, or as an "emergency examination" due to bleeding etc., was excluded from the study. Patients fasted for 10 hours before sample collection and were exempted from medication which had effects on gastric acid secretion such as aluminium hydroxide containing drugs or gel, bismuth, sodium alginate, sodium bicarbonate, magnesium hydroxide gel, magnesium trisilicate mixture, magnesium carbonate, and calcium carbonate were avoided.

Blood samples: Basal blood was drawn into EDTA tubes from each patient and was instantly centrifuged at 2000 g for 15 minutes for plasma collection. Plasma samples were then distributed into cryovials and stored at –20°C until they were tested. The commercial kit GastroPanel (Biohit plc Helsinki, Finland) served to determine serological levels of PGI, PGII, G-17 and *H. pylori* IgG antibodies. Specific enzyme immnuno assays (ELISA) were performed on micro well plates according to instructions of the manufacturer for the measurement of the absorbance after a peroxidation reaction at 450 nm. Linear graphs on standard concentrations were used to estimate unknown sample concentrations and the *H.*

pylori antibodies were expressed as enzyme immune units (EIU). Any blood sample with EIU ≥30 was considered as positive for *H. pylori* antibodies. For the diagnosis of gastritis, any sample with EIU < 30 (negative for *H. pylori* IgG) and ratio PGI/PGII > 2.5 (or PGI≥25 µg/l) and G17 ≥ 1pmol/l was considered as normal mucosa (no gastritis); any sample with EIU ≥30 (positive for *H. pylori* IgG) + ratio PGI/PGII > 2.5 (or PGI≥25 µg/l) if 0.1 pmol/l < G17 <5 pmol/l was considered as non atrophic (superficial) gastritis, and low levels of G17 (≤ 0.05 pmol/l) indicated atrophy at antrum, while low PGI/PGII ratio (< 2.5) or PGI level (<25 µg/l) suggested atrophy at corpus. Samples with low levels of PGI/PGII ratio (< 2.5) or PGI level (<25 µg/l) associated to low levels of G17 (≤ 0.05 pmol/l) and *Hp* IgG Abs ≥30 EIU were considered as atrophic at both antrum and corpus (figure1) [4].

Histology: Routine gastroscopic examinations were complemented by four standard biopsies each from the antrum and corpus and set into formalin tubes. The biopsies were processed into paraffin blocks and histological sections were obtained using ordinary techniques and stains (Haematoxillin and Eosin, modified Giemsa (for *H. pylori*) at the routine histopathology laboratory of the YCH. Experienced pathologist studied the slides by using the Updated Sydney System as criteria in evaluation and grading of mucosal alterations [11].

Statistical analysis: The statistics, significance, prevalence, and mean values were calculated by means of the computer program SPSS 12.0 version. All calculations were realized at 95% confidence interval. Assay analyses were done using the GastroSoft, which is based on an algorithm used to classify gastritis as antral atrophic gastritis, corpus atrophic gastritis, and superficial gastritis or normal stomach. The study was approved by both local ethics committees of the YCH and the UTH. A formal clearance was obtained from the national ethics committee under the authorization Number 065/CNE/SE/2011 of the 17th of March 2011. All information related to patient health and laboratory results were

handled confidentially. All patients signed the informed consent form and were informed about their results when the study was completed.

3 Results

Patient information. The total number of enrolled patients was 86, including 64 women (74.4%) and 22 men (25.6%), and patient age ranged between 21 and 83 years old with a mean (± C.I.) of 46.4 (± 3.3) years old. The study sample comprised 37 (43%) patients from 21 to 39 years old, 32 (37.2%) from 40 to 59 years old and 17(19.8%) patients of 60 years old and above. In patient serum, the mean concentration (± C.I.) of serological markers in diagnosis of gastritis or *H. pylori* infection was 122.8 ± 14.1 µg/l for PGI (from 45 to 337 µg/l), 25.5 ± 3.2 µg/l for PGII (from 1.5 to 70 µg/l), 2.2 ± 1.6 pmol/l for G17 (from 0.04 to 70 pmol/l) and 217.9 ± 57.5 EIU for Hp IgG Ab (from 12 to 1584 EIU).

High levels of *H. pylori* IgG antibodies (≥30 EIU) referred as *H. pylori* infection were recorded among 73/86 (84.9%) of patients with serological test. This serological pattern of *H. pylori* infection was statistically similar to the histological pattern that showed 70/86 (81.4%) of *H. pylori* infection among patients (p>0.50) (table 1).

Association between gastric biomarkers and IgG*Hp*Ab. Table 1 shows the levels of gastric biomarkers in infected with *H. pylori* and non-infected individuals and association with IgG. Depressed levels of G-17 in *H. pylori* infected than non-infected subjects (p=0.016) and a negative correlation with IgG*Hp*Ab were observed (r=-0.171). Raised levels of PGI (p=0.002) and a positive correlation with IgG *HpAb* (r=0.241, p=0.047) were seen among *H. pylori* infected subjects. Similarly, raised levels of PG-II (p=0.047) and a positive correlation with IgG *HpAb* (r=0.248, p=0.021) were seen among *H. pylori* infected subjects. Also raised IgG *HpAb* level was observed in *H. pylori* positive than negative ones.

Trends of Pepsinogen (I and II), Gastrin-17 and IgG*Hp*Ab in atrophic gastristis. Table 2 shows the variation of gastric biomarkers and IgG*Hp*Ab with different stages of atrophic gastritis measured with the test panel. There was no significant difference between PGII, and IgG*Hp*Ab with progression to stomach atrophy (p >0.05). However, the PGI levels significantly reduced with worsening of corpus atrophy (p=0.003). Also G-17 levels significantly reduced with worsening of antrum atrophy (p=0.037). Additionally, in atrophic gastritis of both corpus and antrum the serum concentrations of PGI and G-17 were lower than their respective cut off values.

4 Discussion

The prevalence of *H. pylori* infection was 81.40%, and that for atrophic gastritis was 8.1%. This finding is consistent with the reports that atrophic gastritis prevalence in the world is about 10% [12, 13]. The serological pattern of *H. pylori* infection was statistically similar to the histological pattern (p>0.50) (see Table 1). Raised levels *H. pylori* IgG antibodies were observed in *H. pylori* infected than non infected cases. Infection with *H. pylori* and persistence stimulates specific T and B cell responses and systemic immunoglobulin (Ig) G and A antibody production. Levels of *H. pylori* IgG can however, remain elevated for up to six months even after eradication and thus not being able to distinguish between past and recent infection [1]. More studies are therefore needed to establish the immunopathology in *H. pylori* infections. There was no significant difference in sex and age in the levels of PGI, PGII, G-17 and *H. pylori* IgG antibodies (p>0.05).

Decrease levels of G-17 were observed in *H. pylori* infected than non infected cases. According to Di Mario *et al.* [1], this decrease is due to the acid gastrin negative feedback mechanism. Decrease levels of G-17 with progression to antral

atrophy were observed. Serum G-17 concentrations is a valid marker of the morpho-functional condition of gastric antral mucosa. The G-17 is virtually solely synthesized and secreted from antral G-cells and disappears during the worsening of atrophic gastritis due to loss of glands. According to Di Mario *et al*. [1] and Väänänen *et al*. [16] high intragastric acid output and atrophic antrum gastritis are known to inhibit the release of G-17.

Raised levels of SPGI and SPGII in *H. pylori* infected than non infected cases (Table 2). These findings are consistent with the reports of Di Mario *et al*. [1], Pasechnikov *et al*. [3], Väänänen *et al*.[16], that patients with *H. pylori* related superficial chronic gastritis present with significant higher levels of both PGI and II than in *H. pylori* negative ones. This can further be supported by the strong positive correlation between: PGI and PGII, a weak correlation between PGI and *H. pylori* IgG and between PGII and *H. pylori* IgG (Table 2). The use of serum pepsinogen I levels as an assessment of gastric acid secretion was adopted as early as in 1985 [14, 15]. These findings were consistent with the report of Pasechnikov *et al.,* [3] that serum pepsinogens decrease as atrophic gastritis in the corpus worsens due to loss of mucosal glands and cells.

5 Conclusion

In conclusion, we have shown a negative association between G-17 and IgG levels in subjects with *H. pylori* infection. Also, subjects with superficial gastritis due to *H. pylori* demonstrated elevated levels of PGI and PGII levels compared to normal subjects. Further, we have also shown that gastric biomarkers (PGI, PGII, and G-17) decreased in subjects with atrophic gastritis than in the normal subjects. These findings suggest therefore that gastric inflammation with *H. Pylori*, has a considerable impact on the gastric morphology and acid secretion and in turn influences the trend of gastric biomarkers.

No potential conflict of interest relevant to this article was reported.

Table1:   Association between gastric biomarkers and IgG*Hp*Ab

	Atrophic gastritis PGI<25 µg/l or PGI/PGII<2.5	No atrophy PGII≥25µg/l or PGI/PGII≥2.5	All cases
Patients			
Women	12	52	64
Men	2	20	22
All samples	14	72	86
Mean age(±CI)	47.3(±10.2)	46.5(±3.5)	46.9(±3.3)
GastroPanel Markerss			
Pepsinogen I/ µg /l(±CI)	61.7(±14.3)	129.8 (±15.0)	122.5(±14.1)
Pepsinogen II/ µg /l(±CI)	30.3(±13.5)	24.6(±3.4)	25.2(±3.3)
Pepsinogen I/ II (±CI)	2.0 (±0.3)	8.8(±4.7)	8.1(±4.7)
Gastrin-17 pmol/l (±CI)	2.3(±2.0)	2.2(±1.8)	2.2(±1.7)
Hp IgG ELISA			
Prevalence (%)	14/15 (93.3)	59/71 (83.1)	73/86 (84.9)
Mean (±CI) positive samples (≥30EIU)	303.6(±189.6)	247.6(±69.1)	251.5(±64.5)
Mean (±CI) negative samples (<30EIU)	18.1(±3.5)	16.5(±2.2)	17.0(±2.1)
	5/7(71.4)	65/79(82.3)	70/86(81.4)
Histology			
H. pylori prevalence (%)			

Table 2: Concentrations of gastric biomarkers and association with IgG levels in
Helicobacter pylori infected and non infected subjects.

Gastric biomarker	Non infected N=13	Infected N=76	p-value	Correlation with IgG/EIU levels	p-value
PGI (µg/L)	80.3±15.4	130.32±14.9	0.002*	0.241*	0.047*
PGII (µg/L)	18.7±4.6	26.3±3.7	0.047*	0.248*	0.021*
PGI/PGII	5.5±2.6	8.5±4.4	0.637	-0.081	0.457
G-17(pmol/L)	6.8±2.2	1.3±0.9	0.016*	-0.171	0.488
*H.pylori*IgG (EIU)	22.1±2.1	220.5±55.4	0.0085*	/	/

* = Significant, PGI: pepsinogen I, PGII: pepsinogen II, G-17: gastrin-17

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