



***Helicobacter pylori* Infection and Association with Anaemia in Cameroon Patients, Using GastroPanel® Serological Biomarkers (Pepsinogen I; Pepsinogen II; Gastrin-17; *Helicobacter pylori* IgG)**

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Authors' contributions

This work was carried out in collaboration among all authors. Author AIE conceptualized and together with author VNN, designed the study. Authors AIE, VNN, NE and NKT carried out sample collection, analysis, interpreted the data and drafted the manuscript. Authors NND, MM and KS provided technical advice and corrected the manuscript. Author KS is the director of this work and responsible for the general supervision of the study. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: *Helicobacter pylori* (*H. pylori*) together with chronic atrophic gastritis have also been associated to extragastric manifestations including neurological, dermatological, ocular, cardiovascular, metabolic, allergic diseases and hematologic disorders. Several studies have

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associated *H. pylori* with anaemia, iron deficiency and iron deficiency anemia. Little or no data on the association of *H. pylori* infection and anaemia exist in Cameroon. We therefore evaluated the prevalence *H. pylori* infection among patients with anaemia.

Methodology: Blood samples were aseptically collected for the measurements *Helicobacter pylori* IgG antibodies, pepsinogen I et II levels, gastrin-17 in a total of 150 patients during the period February to July 2020. The blood samples required for the study were collected prospectively. Ethical clearance was obtained from the Centre Regional Ethics Committee for Human Sciences. An authorization of research was obtained from the authorities of General Hospital of Yaounde. All participants signed an informed consent form.

Results: The GastroPanel® results showed that the prevalence of *H. pylori* infection was 90(60.00%). We observed a strong association of *H. pylori* with anaemia (OR 2.19, 95% CI 1.08–4.46; p=0,02).

Conclusion: The prevalence of *H. pylori* infection is high in patients with anaemia and thus requires continuous monitoring of these patients.

Keywords: *Helicobacter pylori*; anaemia; gastropanel®; serological biomarkers.

1. INTRODUCTION

Infection to spiral rod shaped bacteria *Helicobacter pylori* (*H. pylori*) is associated to many gastrointestinal diseases including gastritis, peptic ulcer disease, gastroesophageal reflux disease, chronic atrophic gastritis (CAG) and gastric cancer (GC) [1,2]. Chronic infection with *H. pylori* together with CAG has also been associated to extragastric manifestations including neurological, dermatological, ocular, cardiovascular, metabolic, allergic diseases and haematologic disorders [3-7]. Several studies have reported that *H. pylori* can be a causative factor for anemia, iron deficiency and iron deficiency anemia (IDA) [8-10]. Further, it was stated that eradication of this pathogen not only led to increase in response to oral iron therapy and level of ferritin, but also could cure anemia completely in several cases with unexplained IDA [5]. Iron deficiency anaemia (IDA) is a well-recognized extragastric manifestation of *H. pylori* infection and has already been fully accepted and included in the current guidelines for these conditions [7]. Reported data have supported the effectiveness of *H. pylori* treatment in patients with moderate to severe anemia when compared to those with mild anemia [11]. Idiopathic thrombocytopenic purpura (ITP) is the other hematologic disease shown to be related to infection of *H. pylori* [5]. It has been proposed that *H. pylori*-induced gastritis could be a leading factor of vitamin B12 deficiency in patients [12]. Altogether, ITP is considered as an accepted hematologic manifestation of *H. pylori* infection. Besides, it seems that the infection can possibly decrease level of iron storage, resulting in IDA. Therefore, *H. pylori* treatment can be indicated in

patients with IDA, ITP and vitamin B12 deficiency [5]. Data on the effects of *H. pylori* infection on anemia are scarce in Cameroon. We sought to evaluate the prevalence of *Helicobacter pylori* infection among subjects presenting with anaemia

2. METHODOLOGY

2.1 Study Design and Study Population

We undertook a cross sectional study at the General Hospital of Yaounde Cameroon. Patients consulting haematological reasons, in whom anemia was diagnosed were recruited prospectively on voluntary bases from March to July 2020. Patients fasted 10 hours prior sample collections and authorized to take prescribed regular medications except those that had effects on gastric secretion such as Aluminum hydroxide containing drugs or gel, bismuth, sodium alginate, sodium bicarbonate, magnesium hydroxide gel, calcium carbonate.

2.2 Data Collection Questionnaire

A standardized questionnaire was completed by every participant, providing information on socio-demographic characteristics.

2.3 Blood Samples

For the GastroPanel test, an aliquot of 5 ml of blood was aseptically collected in EDTA anticoagulated tubes, which was kept undisturbed for 1 hour for plasma formation. The tubes were subsequently centrifuged at 1500rpm for 5 minutes. The plasma obtained was transferred into sterile cryotubes, for

GastroPanel® analysis, following the manufacturer's instructions. The biomarker profiles for PGI, PGII, G-17, and *H.pylori* IgG were interpreted using the GastroSoft software application [13]. All patients with *H. pylori* IgG ≥ 30 EIU were considered positive for *H. pylori* infection. All patients with PGI < 30 µg/l or PGI/PGII < 3.0 and those with G-17 < 1pmol/l were considered positive for AG of the corpus and AG of the antrum, respectively. Blood counts for the measurements of Hemoglobin and RBC indices were determined using an automated electronic counter (Sysmex ® XN3000, Sysmex Corporation, Kobe, Japan). The reference range for red cell indices were: mean corpuscular volume (MCV)= 80–100 fl, hemoglobin > 120.0 g/l, mean corpuscular haemoglobin (MCH)= 27–32pg and mean corpuscular haemoglobin concentration(MCHC)315–360g/l.

2.4 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 χ^2 test. In all tests, values with $p < 0.05$ were regarded statistically significant.

2.5 Ethical Consideration

The study was approved by the ethics committees of the General Hospital of Yaounde. Ethical clearances was obtained from the national ethics committee, written informed consent was obtained from each participant

3. RESULTS

3.1 Patient Information

A total of 150 patients aged 20 to 85years with a mean age 47.78 ± 17.06 including 81 females aged 20 to 85years mean (46.07 ± 17.45 years) and 69 males aged 21 to 80 years, mean (49.70 ± 16.52 years). The patient information and prevalence of *H. pylori* infection are presented in Table 1. Amongst the subjects 105(70%) were diagnosed as normo chromic anaemia (MCHC, MCV, and MCH normal) while 45(30%), presented with Microcytic hypochromic anaemia(MCHC, MCV, and MCH values lower than normal).The results of the distribution of

GastroPanel® test are summarized in Table 1. Amongst the 150 subjects, 42 (28.0) were interpreted as normal stomach mucosa (no inflammation, no atrophy), (n=21, 14.00%) superficial gastritis(no atrophic gastritis), 87(58.00%)were consistent with mucosal atrophy, including AG of the corpus (n=18, 12.00%)(PG1< 30µg/l and/or PGI/PGII<3), AG of the antrum (n=57, 38.00%) (G-17< 1pmol/l) and AG of corpus and antrum, pan-gastritis(n=12, 8.00%) (PG1< 30µg/l and/or PGI/PGII< 3 ; G-17< 1pmol/l). The prevalence of HP in the study population was 90(60.00%).

The HP-seropositivity of was more frequent amongmales 45/69 (65.22%) than in females 45/81 (55.56%)(OR 1.500 95% CI 0.7742-2.9065, $p=0.42$). The positivity rate of HP-infection increased with increasing age group 30-59 age group (50.00%), 34-47 age group(50.00%), 48-61 age group(58.33%), 62-75 age group(77.78%) and 76-99 age group (100.0%), but there was no statistically significant difference in the overall prevalence of *H. pylori* across all age groups ($X^2 = 1.45$; $P = 0.48$). *H. pylori* positivity was significantly associated anaemia (OR=2.1905 95% C.I 1.0760 -4.4594 ; $p=0.0162$).

4. DISCUSSION

Infection to *H. pylori* has been well established to be associated to chronic active gastritis, peptic ulcer disease, MALT lymphoma and gastric adenocarcinoma [1,14]. *H. pylori* infection consequence may be as result of several bacterial, host and environmental factors [15]. *H. pylori* infection has been reported to be associated as a causative factor for anemia, iron deficiency and iron deficiency anemia (IDA) [8-10].

The *H. pylori* prevalence obtained in this study (60.0%) is slightly lower than that reported previously in Cameroon among dyspeptic subjects by [16] (81.4%), [17] (79.8%), [18] (78.7%), and equally amongst diabetic subjects in Cameroon by [19] (80.5%). This slightly lower prevalence of *H. pylori* can be as a result of the high prevalence of atrophic gastritis observed [2,20].

We observed an association of *H. pylori* seropositivity with males 45/69 (65.22%) than females 45/81 (55.56%)(OR 1.500 95% CI 0.7742-2.9065). This difference was however,

Table 1. Patient information

Parameters	<i>Helicobacter Pylori</i>		p-value
	Positive 90(60, 0%)	Negative 60(40, 0%)	
Sex			OR=1.500
Females	45(55,56%)	36(44,44%)	p=0.1174
Males	45(65,22%)	24(34,78%)	
Age(years)			
20-33	21(50,00%)	21(50,00%)	p=0.48
34-47	18(50,00%)	18(50,00%)	
48-61	21(58,33%)	15(41,67%)	
62-75	21(77,78%)	6(22,22%)	
76-89	6(100,00%)	0(0,00%)	
Type of anemia			
Microcytic hypochromic	21(46,67%)	24(53,33%)	RR=1.7143
Normocytic normochromic	69(65,71%)	36(34,29%)	p=0.02

non significant ($p=0.42$). According to [21], most *H. pylori* diseases are associated males. The positivity rate of HP-infection increased with increasing age group, 30-59 age group (50.00%), 34-47 age group(50.00%), 48-61 age group(58.33%), 62-75 age group(77.78%) and 76-99 age group (100.0%), but there was no statistically significant difference in the overall prevalence of HP across all age groups ($X^2 = 1.45$; $P = 0.48$). The prevalence of *H. pylori* infection has been reported to increase with age [21], with about 50% of the population infected at ages above 60, and around 10% between 18 and 30 [7]. Since *H. pylori* infections are usually acquired in early childhood, it is reasonable to assume that chronic *H. pylori* infections in the elderly can cause anemia due to predisposition to gastrointestinal mucosal lesions [7].

We observed a strong association between *H. pylori* infection and anemia (OR 2.19, 95% CI 1.08–4.46). The prevalence of anemia in the positive group was higher than that in the negative group($p=0,02$). Similar results have been reported in several studies including [7] and [22] who respectively observed that *H. pylori* positive when compared to *H. pylori* negative groups, the OR of the group were (2.01 (95% CI: 0.92–4.40) and (OR 2.53, 95% CI 1.05–6.09). This observation is consistent with the view that anemia is considered as a complication of *H. pylori* infection [23–25]. *H. pylori* is suggested to cause anaemia and IDA by several mechanisms, including iron loss due to active hemorrhage secondary to gastritis, peptic ulcer disease or gastric cancer [26]; reduced iron absorption caused by pan gastritis and iron utilization for protein synthesis by the bacterium for colonization in the host environment

[27–28]. In addition, *H. pylori* strains possessing virulence factors CagA and VacA have also been reported to participate in iron acquisition and colonization without damaging host tissue, being responsible for chronicity as well as the immune response to *H. pylori*, considering that both host's innate and adaptive immune system play a crucial role in the initiation and progression of this infection [22]. Further, it has been stated that, the eradication of *H. pylori* can lead to an increase in response to oral iron therapy and complete treatment of anemia in several cases with unexplained iron deficiency anaemia [29].

5. CONCLUSION

Considering the biomarker panel results of PGI, PGI, G-17 and HplgG in this study, we have observed a high prevalence of *H. pylori* infection(60.00%) among the subjects and a strong association of *H. pylori* positivity with anaemia. Given that this infection represents an important risk factors of gastric cancer, and atrophic gastritis with risk of blood loss and poor absorption of iron and vitamin B12 and it would be paramount to monitor *H. pylori* in anaemia patients.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Ethical clearance was obtained from Center Regional Committee for Research on Human Health (CRERSH). An authorization was obtained from the authorities of the Jamot Hospital of Yaounde. All patients signed an informed consent form.

DECLARATION

Availability of data and materials: All data used during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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