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Association of Helicobacter Pylori Infection with Cardiovascular Diseases Subjects from Cameroon, Using GastroPanel® Serological Biomarker Panel (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, GE, NKT, CKMF and KNNR 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 infection is associated with gastrointestinal diseases including atrophic gastritis and gastric cancer. Epidemiologic studies have demonstrated associations between H. pylori infection andextra gastrointestinal organ involvements including coronary artery disease and peripherical artery disease, dyslipidemia, insulin resistance and hematologic disorders. We sought to evaluate the prevalence of H. pylori infection in patients suffering cardiovascular diseases attending the Yaounde Central Hospital.

Materials and Methods: Patients suffering from various cardiovascular complications were recruited at the Yaounde Central Hospital, between January and May 2019. The clinical and socio-demographic information of the patients was recorded. Five ml of blood were collected aseptically for Pepsinogen I and II enzymes, gastrin17 hormone and IgG anti H. pylori anti-body. The test parameters were analyzed using a GastroSoft software application. The data was analyzed using Epi Info 7.0. All statistics were 95% CI. Ethical clearance was also obtained from the National Ethics Committee. The study was accepted by the authorities of the Yaounde CentralHospital. All patients signed an informed consent form.

Results: A total of 62 subjects were recruited aged 30-75 years, (mean±SD 52.03 ± 12,78 years); 34(54,84%) females aged 30 to 75years (mean±SD 54,47 ± 13,47 years) and 28 (45,16%) males aged 30 to 65,(mean±SD 49,07± 11,44years). Female/male ratio was 1:2. H. pylori seropisitivity occurred in 58 (93,55%) of the subjects (IgG ≥30 EIU). H. pylori seropositivity was significantly associated with high blood pressure (RR=2.2 95%C.I 1.67 -2.96, p=0, 024). Significantly low Triglyceride concentrations were observed in H. pyloripositive(0,51±0,03g/l) compared to negative subjects (1,04 ± 0,50g/l**),** (p=0.03). A significant inverse correlation was observed between IgG levels and blood glucose levels (r= 0,4 p=0, 004).

Conclussion: The study indicates that H. pylori infection is highly associated with various cardiovascular complications and disease risk factors.

Keywords: Helicobacter pylori infection; cardiovascular diseases; association to; gastropanel; serological biomarkers; Pepsinogen I; Pepsinogen II; Gastrin-17.

1. INTRODUCTION

Endemic non infectious diseases including cardiovascular diseases are among the most deadly diseases of the 21st century [1]. Various risk factors including hypertension, increased lipid level, obesity, diet with highfat, physical inactivity, diabetes, and stress conditions are generally associated with cardiovascular diseases (CVD) [2]. Some microorganisms have been reported to contribute in the development of cardiovascular diseases and are considered as risk factors, amongst them the spiral rod-shaped gram-negative bacterium, *Helicobacter pylori* (*H. pylori*) [3,4]. *Helicobacterpylori* is associated withmany gastrointestinal diseases including gastritis, peptic ulcer disease, gastroesophageal reflux disease, chronic atrophic gastritis (CAG) and gastric cancer [5,6]. *Helicobacter pylori* infection remains high on a global scale with an estimatedof 4.4 billion people infected but differs significantly among populations in various geographical regions [7]. Chronic infection with *H. pylori* strongly increases the risk of CAG which causes vitamin B12 deficiency that was found responsible for CVD. Because deficiency of vitamin B12 is one of the causes of hyper-homocysteinemia [3,8]. Data on the association of *H. pylori* and cardiovascular diseases are scarcein Cameroon. We therefore sought to evaluate the prevalence of *H. pylori* infection in patientswith cardiovascular diseases and also find out it there exist any significant correlation between *H .pylori* IgG antibodies and biological markers in patients attending the Yaounde Central Hospital.

2. METHODS

2.1 Patient Information

Patients consulting for various cardiovascular complications and consented to participate were prospectively used for this study between February and May 2019 at the cardiology unit of the Yaounde Central Hospital.

2.2 Blood Samples

Basal blood was aseptically collected after at least 4hours of fasting by venipuncture into

EDTA tubes and immediately centrifuged at 2000G for 15 minutes. The plasma samples were then distributed into cryo tubes and stored at -20 °C until analyzed. Plasma concentrations of PGI, PGII, G- 17 and *H. pylori* IgG determined by the Gastro Panel (Biohit plc Helsinki, Finland) (9) 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 GastroPanel® examination were evaluated using the Gastro Soft® interpretation software [9].

2.3 Assay Analysis

Based on the clinically-validated cut-off valuesfor 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, respectively. *H. pylori* IgG antibody levels above 30 EIU (enzyme-immunoassay units) were considered as indicator of *H. pylori* infection.

2.4 Statistical Analysis

Statistical analysis was performed using the EPI Info 7.0 software package. Data were expressed as mean±SD. Thedifferences 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 χ2 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 committee of the Yaounde Central Hospital. All patients signed an informed consent form.

3. RESULTS

A total of 62 patients were enrolled duringthestudy period, aged 30- 75 years (mean±SD 52.03 ± 12.78) years, including 34 (54.84%) females aged 30-75 years (mean±SD 54.47 ± 13.47) and 28 (45.16%) malesaged 30-65 years (mean±SD 49.07±11.44). Among the subjects with cardiovascular diseases, 14 (22.58%) presented with heart failure, 12 (19.35%) with ischemic stroke, 10 (16.13%) had obliterating arteriopathy of the lower limbs, 08 (12.90%) with ischemic heart disease, 04 (06,45%) hypertensive encephalopathies, 04 (06.45%) coronary heart disease, 02 (03.23%) hemorrhagic stroke,02 (03.23%) aortic dissections, 02 (03.23%) hypertrophy of the right ventricular, 02 (03.23%) cardiac tamponades and finally 02 (03.23%) with deep vein thrombosis. The results of the distribution of Gastropanel® test are summarized in Tables 1 and 2. Amongst the 62 subjects, 30 (48.39%) were interpreted as superficial gastritis (no atrophic gastritis), 32(51. 61%)were consistent with mucosal atrophy, including Atrophic Gastritis of the corpus, C (n= 14 (22.58%)(PG1< 30µg/l and/or PGI/PGII<3) and (n=18 , 29.39%) pangastritis), P(PG1< 30µg/l and/or PGI/PGII< 3 ; G-17< 1pmol/l). The prevalence of *H. pylori* in the study population was 58(93.55%). In subjects with superficial gastritis, *H. pylori* was responsible for 28(93.33%) and 02(6.67%) were due to either use of medication, alcohol, spices, bacterial or parasitic infection, viral infection or bile reflux as indicated by the GastroSoft. In those with atrophic gastritis,30 (93.75%) were due to *H. pylori* infection and 02 (6.25%) were due to autoimmune disease.

The *H. pylori* seropositivity of was higher in females 32/34 (94.12%) than in males 26/28 (92.86%). This difference was not significant (X2 = 0.04; p = 0.42). The positivity rate of *H. pylori* infection was higher (100%) for the 30-59 age group and lower in the 70-79 age group (66.67%). There was no statistically significant difference in the prevalence of *H. pylori* among different age groups (X2 = 1.45; P = 0.48).*H. pylori* positivity was significantly associated with patients with hypertension (RR=2.2 95% C.I 1.6 -2.96 ; p=0 ,024) ; diabetics (OR=1.16 95% C.I 1.04 -1.29 ; p=0.0001), nephropathy (OR=1.16 95%C.I 1.04-1.28, p=0.0001), hyperglycemia (OR=1.21 95% C.I 1.07 -1.36 ; p=0.0001) and non smokers (X2=5.23 ; p=0.04) (Table 1).

No significant differences in mean cholesterol, HDL and LDL levels were observed between *H. pylori* positive and negative patients (p=>0.05). A significant difference in mean Triglyceride levels in *H. pylori* positive and negative subjects was observed, (p=0.03) (Table 2).

Table 1. Association of *Helicobacter pylori* infection with potential risk factors

Variable	*H. pylori* positive (IgG≥30EIU)58(93.55%)	*H. pylori* negative (IgG<30EIU) 04(6.45%)	OR	95%CI	P-value
Sex			X2 =	/	0.42
Male	32(94.12%)	2(5.88%)	0.04		
Female	26(92.86%)	4(7.14%)			
Age group(years)			X2 =	/	
33-39	12(100%)	0(0%)	1.45		0.48
40-49	16(100%)	0(0%)			
50-59	14(100%)	0(0%)			
60-69	12(85.71%)	2(14.29%)			
70-79	4(66.67%)	2(33.33%)			
Smoker			X2 =	/	0.04
No	52(96.30%)	2(3.70%)	5.23		
Yes	6(75.00%)	2(25.00%)			
Diabetic					0.0001
No	50(92.59%)	4(7.41%)	Ref	95%C.I	
Yes	8(100%)	0(0%)	OR, 1.16	1.04-1.29	
Hyperglycemic					
No	48(92.31%)	4(7.69%)	Ref	95%C.I	0.0001
Yes	10(100%)	0(0%)	OR, 1.21	1.07-1.36	
Nephrotic syndrom					
No	50(92.59%	4(7.41%)	Ref	95%C.I	0.0001
Yes	8(100%)	0(0%)	OR, 1.16	1.04-1.28	
Hypertensive					
No	26(86.67%)	4(13.33%)	Ref	95%C.I	0.024
Yes	32(100%)	0(0%)	RR, 2.2	1.6-2.96	

Table 2. Mean CHO, TG, BS, HDL and LDL levels (±SD) according to *Helicobacter pylori* serostatus

Variable	*H. Pylori*			Atrophic class			
	Negative IgG<30EIU	Positive IgG≥30EIU	p-value	AGPan 18(29.39%)	AGC 14 (22.58%)	S 30(43.39%)	p-value
CHO (g/L)	01,84,±0,59	1,82,±0,31	0,93	01.89±0,65	1,91±0,67	1,77±0,49	0,68
TG (g/L)	1,04 ± 0,50	0,51 ± 0,03	0,03	0.88±0,43	1,06±0,42	1,05±0,56	0,47
HDL (g/L)	0,53 ± 0,21	0,59 ± 0,25	0,59	0,61±0,19	0,64±0,22	0,43±0,16	0,0008
LDL (g/L)	1,26 ± 0,45	1,18 ± 0,23	0,71	1,19±0,41	1,24±0,38	1,30±0,48	0,66

AGPan, atrophic gastritis of antrum and corpus; AGC, atrophic gastritis of the corpus; CHO, total cholesterol ; TG, triglycerides ; HDL, high density lipoprotein cholesterol, LDL, low density lipoprotein cholesterol ; g/L, gram per litre

4. DISCUSSION

Several studies have reported a significant association between *H. pylori* infection and extragastric diseases such as coronary heart disease, dyslipidemia, hematological disorders and arteriosclerosis [2,10].

The results of GastroPanel® are presented in table 1 and 2. In this analysis of the biomarker profile of GastroPanel, atrophic gastritis appeared in 32 (56.61%) of the subjects (PGI<30 µg / l) and hypoclorhydria in 36 (58.06%) (G-17>10 pmol / 1) of the subjects. The results show that the prevalence of *H. pylori* infection was 58 (93.55%). This prevalence is in line with that reported in several studies on patients with cardiovascular disease e. g. [2] (80.2%), [11] (78.8%). This prevalence is also similar to those previously reported in dyspeptic patients [12] (81.40%), [13] (79.80%) and in diabetic subjects in Cameroon [14] (88.2%). However, our results contrast sharply with those reported by [15] (29.5%) using the PCR test, [15] (23.8%) using

antibodies against immunoglobulin A (IgA) and [15] (53.3%) using antibodies against immunoglobulin G (IgG). This may be as a result of diagnostic technique used. Several possible mechanisms and pathways have been described as to how *H. pylori* contribute to cardiovascular complications [10]. Repeated exposure to infection leads to failure of the inflammatory process and inability to control the progress of infectious agents that leads to a number of diseases such as heart disease and cancer [16,17]. Chronic stimulation due to bacterial infection in the gastric mucosa produces more induction of dyslipidemia, increases fibrinogen levels, stimulates the release of reactive protein and hyperhomocysteinemia leading to artery blockage and the development of heart problems [3]. Also, chronic infection *with H. pylori* is known to increase pH levels, increase gastric juice and decrease ascorbic acid levels, which will cause a reduction in folate absorption and thus increase the concentration of homocysteine that causes endothelial cell damage [18]. In addition, atrophic gastritis and hypochlorhydria observed in these subjects are a risk of enteric infections, low absorption of ATBs, vitamin B12 and some divalent micronutrients, including iron, calcium, magnesium and zinc, with an increased risk of clinically important sequelae, such as cognitive disorders, neurodegenerative and vascular disorders, encephalopathies, anemia and osteoporosis [19,20].

We found no association between *H. pylori* seropositivity with males (92.86%) and females (94.11%), (p=0,42). Similarly, no significant association (p=0.48) between *H. pylori* and age groups in patients with cardiovascular disease was observed, however, most diseases related to *H. pylori* are associated with males [21,22]. We observed a significant association between *H. pylori* seropositivity and hypertensive subjects (OR=2.2 95% C.I 1.6 -2 .96, p=0 ,024), diabetics (OR=1.16 95% C.I 1.04 -1.29, p=0.0001), nephropathy (OR=1.16 95%C.I 1.04-1.28, p=0.0001) and hyperglycemic subjects ((OR=1.21 95% C.I 1.07 -1.36, p=0.0001) (Table 1). It has been shown that *H. pylori* infection is involved in the regulation of 2 hormones leptin and ghrelin involved in energy hemostasis and their interaction affects obesity, insulin sensitivity and and glucose hemostasis [23]. We have previously demonstrated a strong association berween *H. pylori* infection and Diabetes mellitus type 2 subjects [14]. No significant differences in mean cholesterol, HDL and LDL levels were observed between *H. pylori* positive or negative patients (p>0.05). A significant difference in mean Triglyceride levels in *H. pylori* positive and negative subjects was observed, (p=0.03) (Table 2). [24] found significant differences between triglyceride and HDL values among *H. pylori* seropositive vs seronegative subjects. [25] did not observe any significant differences in plasma cholesterol, or triglyceride levels among seropositive and seronegative patients. The low triglyceride and cholesterol levels observed in our study disagreed with [2,26]. This difference may be due to the fact that our patients were followed nutritionally and therapeutically. *H. pylori* infection induces an increase in cholesterol and triglyceride levels with a decrease in HDL cholesterol contributing to the development of dyslipidemia, a well-known cardiovascular risk factor [26].

5. CONCLUSION

The study indicates that *H. pylori* infection is highly associated with various cardiovascular complications and disease risk factors. Thus, patients suffering from various cardiovascular diseases are at risk of gastric cancer and require continuous monitoring.

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

AVAILABILITY OF DATA AND MATERIALS

All data used during the current study are available from the corresponding author on reasonable request.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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