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ORIGINAL ARTICLE

Helicobacter pylori infection—A risk factor for lipid peroxidation and superoxide dismutase over-activity: A cross-sectional study among patients with dyspepsia in Cameroon

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Key words
antioxidant protective factors, *Helicobacter pylori* infection, lipid peroxidation, oxidative stress.

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Authors' contribution: Kouitcheu Mabeku Laure Brigitte conceived of the study, designed the experiments, and supervised the work. Ngatcha Ghislaine, Tagni Sartre Michele, and Talla Paul performed the physical examination, endoscopic examination, and collected the biopsy samples from participants. Faujo Nintewoue Ghislaine Florice and Tali Nguefak Lionel Danny performed *H. pylori* detection using rapid urease test. Menzy Moungo-Ndjole Carole Marlyse performed histological detection of *H. pylori*. Faujo Nintewoue Ghislaine Florice and Kouitcheu Mabeku Laure Brigitte detected oxidative stress biomarkers. Kouitcheu Mabeku Laure Brigitte drafted the manuscript. All authors read and approved the final manuscript.

Abstract

Background and Aim: There is an intimate relationship between oxidative stress and inflammation. *Helicobacter pylori (H. pylori)* infection leads to gastritis in almost all the hosts. So, we hypothesize that gastritis in *H. pylori* infection may be described as the accumulation of continuous oxidative damage.
Methods: The study was conducted from October 2020 to October 2021 at three reference health facilities in Cameroon. A total of 266 participants (131 males and 135 females) ranging from 15 to 88 years old with 48.28 ± 17.29 years as mean age were enrolled. Each participant gave a written informed consent and ethical committees approved the protocol. Biopsies samples were collected for *H. pylori* detection using histological examination and rapid urease test. Malondialdehyde (MDA) and glutathione (GSH) content, and catalase (CAT) and superoxide dismutase (SOD) activities were evaluated in serum as biomarkers of oxidative stress.
Results: *Helicobacter pylori* was detected in 71.80% of our sample population. Low income level was associated with higher GSH level ($P = 0.0249$) and having family history of gastric cancer to higher SOD activity ($P = 0.0156$). A significant higher MDA content ($P < 0.0001$) and SOD activity ($P = 0.0235$) was recorded among infected individuals compared with noninfected ones. A significantly higher MDA content and SOD activity was recorded among smokers ($P = 0.0461$) and participants older than 50 years old ($P = 0.0491$) with *H. pylori* positivity.
Conclusion: Our findings showed that *H. pylori* infection is associated with overproduction of reactive oxygen species and oxidative stress. The presence of this pathogen in elderly individuals or in smokers increased their risk for oxidative stress.
Helicobacter pylori infection induces the production of reactive oxygen species.

Introduction

Oxidative stress is frequently defined as an imbalance between pro-oxidants and antioxidants.[1] It arises when the production of reactive oxygen species (ROS) overwhelms the intrinsic antioxidants. Major ROS with physiological significance are superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($OH^{\bullet}$), hydroperoxyl radical ($H_2O^{\bullet}$), and hydrogen peroxide (H_2O_2).[2] Under physiological conditions, ROS function as cellular signaling agents in normal cellular metabolism.[3] Living cells are under constant oxidative attack from ROS, leading to oxidative damage, and the complex antioxidant defense system generally holds this attack in balance.[4] The balance is maintained either by enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase, peroxiredoxins, and paraoxonase, or by nonenzymatic antioxidants including glutathione (GSH), uric acid, cysteine, bilirubin, carotenoids, and vitamins A, E, and C.[5,6] Catalase (CAT) converts H_2O_2 to water and molecular oxygen.[7] SOD is a crucial part of the antioxidant defense against

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highly reactive superoxide radicals, partitioning them into H_2O_2 and O_2.[8] Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form glutathione (GSH).[8] Glutathione is a free radical scavengers, which neutralizes free radicals by donating electrons, thus preventing oxidative stress by maintaining proper cell function and GSSG/GSH ratio.[8] Minor disturbances of the balance between pro-oxidants and antioxidants lead to homeostatic adaptations, whereas significant perturbations may lead to irreparable damage and cell death.[4] In fact, a pathological shift in that balance leads to growing ROS concentrations, resulting in adverse modifications to cell components, such as lipids, proteins, and DNA.[2] For example, the oxidation of lipids known as lipid peroxidation leads to products such as malondialdehyde (MDA) or 4-hydroxynonenal, which can react with proteins present in the cell membranes, creating protein–lipid adducts.[8] They are able to diffuse through biological membranes and can cause a rupture in DNA strands.[8] They are cytotoxic, mutagenic, and carcinogenic.[8] Oxidative stress and oxidative damage are recognized to be important factors in the development of various diseases: Alzheimer's disease,[9] atherosclerosis,[10] and chronic inflammation.[11]

The critical role of *H. pylori* infection in chronic gastritis has been generally recognized.[12,13] *H. pylori* is a microaerophilic Gram-negative spiral shaped bacterium that colonizes both the mucosal layer and the epithelial lining of the stomach.[14,15] *Helicobacter pylori* chronically infects more than half of the population worldwide.[16,17] Several epidemiological studies carried out around the world reveal that the prevalence of this bacterial infection is greater in developing countries than in developed ones.[18] The long-term carriage of this bacterium leads to diverse gastrointestinal conditions, among which are gastritis in almost all the hosts and the more severe neoplastic diseases such as gastric adenocarcinoma and mucosa associated lymphoid tissue lymphoma (MALT lymphoma) in 1–3% and less than 0.1% of cases, respectively.[19] However, the pathogenesis from *H. pylori* infection to these physiopathology gastrointestinal conditions remains unclear. There is an intimate relationship between oxidative stress, inflammation, and functional impairment, resulting in various diseases affecting the entire human body. So, we hypothesize that chronic gastritis in *H. pylori* infection may be described as the accumulation of continuous oxidative damage.[20] Thus, analysis of ROS, antioxidants production, and level of peroxidation in relation to *H. pylori* infection could give a lot of promising data that potentially explain modification in gastric cell responses, which lead to these pathological gastrointestinal conditions. Therefore, in the current study, we strengthen the connection between *H. pylori* status and oxidative stress active compounds. For this purpose, we assess biomarkers of oxidative stress such as enzymatic and nonenzymatic antioxidants protective factors (SOD, catalase, and glutathione), products of lipid peroxidation (malondialdehyde) among *H. pylori*-infected individuals in comparison with noninfected individuals from health facilities in Cameroon.

Methods

Study design. This is a cross-sectional hospital-based study conducted at the Yaoundé General Hospital, the Centre Médicale la Cathédrale, and the Bafoussam Regional Hospital from October 2020 to October 2021 in Cameroon. The study was approved by the ethic committee of each selected health facility and the national ethical committee in Cameroon. The participants were enrolled at the gastroenterology unit of the mentioned health facilities.

Selection of participants. In this study, we enrolled 266 participants who complained of dyspepsia and who came for an upper endoscopy procedure at the gastroenterology unit of either selected health facilities. The recruitment of participants was done from October 2020 to October 2021. *Helicobacter pylori* status, risk factors for oxidative stress, and oxidative stress parameters were assessed. A consecutive sampling method was used for data collection from participants who fulfilled the eligible criteria for the study and provided a written informed consent to participate. We excluded patients with chronic diseases or medical conditions known to be associated with the health outcome. Patients who voluntarily refused to take part in this study were not included, as well as those for whom endoscopic examination was not possible. Patients under any gastrointestinal medication, such as antibiotics for *H. pylori* eradication or proton pump inhibitors, and those who frequently use nonsteroidal anti-inflammatory drugs (NSAIDs), were excluded from this study. Pregnant and breast feeding women and patients under 15 years old were also excluded.

For each participant, sociodemographic factors such as age, gender, and information on factors that could lead to the development of oxidative stress such as alcohol consumption, tobacco smoking, socioeconomic class or income level [low income (≤2500 $/month), middle income (2500–8500 $/month), and high income (≥8500 $/month)], and family history of gastric cancer were collected using a structured survey questionnaire.

Concerning sample collection, gastric biopsy samples were collected from the antrum and body of each participant during upper endoscopy procedure for *H. pylori* detection.

Fasting blood samples were also collected by peripheral venipuncture from the brachial region into dry tubes to assess biomarkers of oxidative stress.

Samples collection. All the selected patients underwent an upper gastrointestinal endoscopy during which gastric biopsy samples were taken. Endoscopy was carried by gastroenterologists. The endoscope was inserted through the patient's mouth, down the esophagus, and into the stomach. Once inside the stomach, gastric biopsy were collected in three topographical area of the stomach (fundus, angulus, and antrum) for *H pylori* detection.

Five milliliters of fasting blood samples were collected from each participant by peripheral venipuncture at the brachial region into dry tubes. The samples were centrifuged at 3000 rpm for 5 min and the serum samples isolated were frozen at −80°C for a maximum of 2 weeks before analysis.

Samples analysis

Helicobacter pylori *detection.* *H. pylori* detection was performed from the biopsy specimens using both histological examination and rapid urease test (RUT). The microorganism was said to be present when both tests were positive.

Helicobacter pylori *detection using RUT.* Rapid urease test was used for *Helicobacter pylori* detection from biopsies using AMA RUT 1 kit (AMA RUT 1, Association of Medicine and Analytics, Saint Petersburg, Russia). This test was carried out

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few minutes after sample collection. The gastric biopsy was carefully placed on the white reactive element and the protective cover resealed gently. The test slide was then inserted into the slot of the AMA RUT Reader and the button pressed until the display showed "RUN". The results were read 5 min later. The presence of a color spot on one side of the indicator disk was indicative of urease activity in the biopsy specimen or a positive result. No color indicated the absence of the bacteria in the analyzed specimen.

Helicobacter pylori detection using histological examination. The biopsy specimens collected for this test were fixed in a 10% formaldehyde solution and transported to the anatomo-pathology laboratory for analysis. The gastric biopsies were removed from the 10% solution and transferred into an accessioned cassette for dehydration, clearing, and impregnation with paraffin. The paraffin-embedded products were cut into tiny microscopic samples (4-μm thick) and stained with Giemsa for histological evaluation of *H. pylori* presence.

Study of oxidative stress. We evaluated oxidative stress by determining the content of malondialdehyde (MDA), a lipid peroxidation product; the content of reduced glutathione (GSH), a nonenzymatic antioxidant; the activity of SOD and catalase (CAT) activity in the serum of all the selected dyspeptic patients.

These parameters of oxidative stress were measured using the enzyme immunoassay method (ELISA) on spectrophotometer (URIT Medical Electronic Co. China).

Determination of serum catalase activity (CAT). Initially, the total protein content in the serum was determined according to the method described by Gornall using the bovine serum albumin as the standard.[21] The absorbance was read at 540 nm.

The enzymatic activity of catalase was assessed by the decrease of the hydrogen peroxide concentration in the spectrophotometer absorbance at 240 nm.[22] A hydrogen peroxide substrate solution of 50 mM was prepared with 0.1 mM phosphate buffer, pH 7.4 (750 μl). Then, 50 μl of serum was mixed with 200 μl of substrate solution and incubated at 25°C for 1 min. Then potassium dichromate in glacial acetic acid was added at the end and the absorbance was read at 240 nm in a spectrophotometer. The catalase activity was expressed in mM of hydrogen peroxide/min/mg of total protein.

Determination of serum glutathione (GSH). Glutathione (GSH) concentration in serum samples was determined using the Ellman's reagent.[23] Eighty microliters of distilled water and 20 μl of 50% trichloroacetic acid were added to 100 μl of serum for protein precipitation. After that, the sample was centrifuged at 3000 rpm for 15 min at 4°C. Then, 200 μl aliquots of the supernatant were mixed with 200 μl of 0.1 M phosphate buffer, pH 6.5, and 5 μl of DTNB (5,5-dithiobis-2-nitro-benzoic acid). The mixture was homogenized for 40 s and incubated for 60 min. The absorbance was read at 412 nm. The concentration of GSH was expressed in mol/g of protein.

Determination of serum level of malondialdehyde (MDA). Malondialdehyde (MDA) concentration was determined by means of lipid peroxidation.[24] A solution mixture was prepared with 250 μl of serum, 125 μl of 20% trichloroacetic acid, and 250 μl of 0.67% thiobarbituric acid in test tubes. The tubes were then covered using glass beads, heated to 90°C in a water bath for 10 min, and the content was centrifuged at 3000 rpm at room temperature for 15 min. The supernatant was collected and the absorbance read at 530 nm against the blank. Total serum concentration of MDA was expressed in mol/g of protein.

Determination of serum SOD activity. The activity of superoxide dismutase was determined based on its ability to inhibit the conversion of adrenaline to adrenochrome.[25] A volume of 1666 μl of carbonate buffer (0.05 M, pH 10.2) was added to 134 μl of serum sample in the test tube. The reaction was triggered by adding 200 μl of adrenaline (0.3 mM) in each test tube and the absorbance read at 480 nm. The results were expressed as number of units of SOD per milligram of protein (U/mg de protein).

Statistical data processing. Statistical data processing was performed using the SPSS software (version 22.0). The analysis of the conformity of the type of distribution to the law of normal distribution was done using Agostino and Pearson omnibus test. The range, mean ± standard deviation, median, and interquartile range (IQR) were calculated when describing the variable. Student's two-tailed t-test was used for pairwise comparison of groups. The level of significance was set at a P value ≤0.05.

Results

Sociodemographic and lifestyle characteristics of the study population. A total of 266 participants were enrolled in this study. Their mean age was 48.28 ± 17.29 years (range 15–88 years). Participants aged more than 60 years were the most represented (68 participants, 25.56%). The number of males and females was nearly similar (131 males and 135 females). More than the half of the participants had middle or high income level (53.76%), 58.27% of them were alcohol consumers, and 56.77% had family history of gastric cancer. Only 19 of the participants were smokers (7.14%) (Table 1).

Prevalence of* H. pylori *infection in the study population. One hundred and ninety-one participants were *H. pylori* infected, giving an infection rate of 71.80% in our sample population. Participants with low income level, those having history of gastric cancer, alcohol consumers, and smokers were more prone to *H. pylori* infection compared with nonexposed ones, but with a nonsignificant P value ($P > 0.05$) (Table 1).

Distribution of oxidative stress biomarkers in study population. The distribution of oxidative stress indicators according to the characteristic of the population is summarized in Table 2. Our data showed a significant difference of GSH level related to income level ($t = 2.259$, $P = 0.0249$) and SOD activity related to family history of gastric cancer ($t = 2.437$, $P = 0.0156$). In fact, participants with low income level were with higher GSH level than those with high income, also higher SOD activity was recorded among participants with

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Table 1 Characteristics of the study population according to *Helicobacter pylori* infection

Variable	Number (%) $n = 266$	*H. pylori* (+) *n* (%)	*H. pylori* (−) *n* (%)	X^2 value; (*t*-value); *P* value
Age	48.28 ± 17.29	48.01 ± 1.537	48.75 ± 1.901	(0.2929); 0.7699
≤20	14 (5.26)	11 (78.57)	3 (21.43)	8.226; 0.1442
21–30	36 (13.53)	30 (83.33)	6 (16.67)	
31–40	46 (17.29)	28 (60.87)	18 (39.13)	
41–50	46 (17.29)	33 (71.74)	13 (28.26)	
51–60	56 (21.05)	36 (64.28)	20 (35.72)	
>60	68 (25.56)	53 (77.94)	15 (22.06)	
Gender				
Male	131 (49.25)	96 (73.28)	35 (26.72)	0.2785; 0.5977
Female	135 (50.75)	95 (70.37)	40 (29.63)	
Income level				
Low	123 (46.24)	91 (73.98)	32 (26.01)	0.5367; 0.4638
High	143 (53.76)	100 (69.93)	43 (30.07)	
Tobacco consumption				
Yes	19 (7.14)	14 (73.68)	5 (26.32)	0.03571; 0.8501
No	247 (92.86)	177 (71.66)	70 (28.34)	
Family history of gastric cancer				
Yes	151 (56.77)	115 (76.16)	36 (23.84)	3.271; 0.0705
No	115 (43.23)	76 (66.09)	39 (33.91)	
Alcohol consumption				
Yes	155 (58.27)	105 (67.74)	50 (32.26)	0.028; 0.0818
No	111 (41.73)	86 (77.47)	25 (22.53)	

X^2: chi-square, *n*: number, (+): positive, (−): negative.

family history of gastric cancer compared with nonexposed ones (Table 2).

As age of participant is concerned, the level of oxidative biomarkers increase with the age of participants, stating at 50 years old. So, participants older than 50 years old were chosen as reference group to assess the effect of age on the level of biomarkers. Higher levels or activity of the studied biomarkers of oxidative stress were recorded in elderly individuals (>50 years old) than in those aged less than 50 years old, even if the difference was nonsignificant ($P > 0.05$). Similarly, smokers were found with the higher level or activity of the studied biomarkers of oxidative stress compared with nonsmokers, but with nonsignificant difference ($P > 0.05$) (Table 2).

Helicobacter pylori *infection and oxidative stress.*

Table 3 shows the distribution of the different parameters used for the assessment of oxidative stress in relation to *H. pylori* status among the participants. The results obtained showed that all of the studied parameters were influenced by the presence of this bacterium, with the higher values recorded among infected individuals compared with noninfected ones. But the difference was significant only for MDA content ($t = 5.878$, $P < 0.0001$) and SOD activity ($t = 2.282$, $P = 0.0235$) (Table 3).

Impact of *Helicobacter pylori* positivity on oxidative stress distribution according to the characteristic of the study population: *H. pylori* infection has affected the distribution of oxidative stress indicators according to the characteristic of the population (Table 4). Infected smokers had higher MDA levels compared with nonsmokers ($P = 0.0461$), despite the fact that MDA content did not show a significant difference according to smoking status independently of infection status ($P = 0.1179$). Also, SOD activity was significantly higher among infected participants older than 50 years old than among their counterparts ($P = 0.0491$), despite the fact that the activity of this enzyme did not show a significant difference regarding age of participants independently of infection status ($P = 0.6122$). In addition, infected participants with family history of gastric cancer had higher activity of SOD, higher activity of CAT, and higher level of GSH compared with nonexposed ones but with marginal *P* value (0.0704, 0.0619, and 0.0957 respectively) (Table 4).

Discussion

It is well known that *H. pylori* infection induces progressive inflammatory changes in the gastric mucosa and that this infection is implicated in the pathogenesis of gastritis and diverse gastrointestinal pathological conditions. However, the mechanisms leading from chronic active gastritis to other gastrointestinal manifestations remain unclear.[26] In this study, we hypothesize that the presence of *Helicobacter pylori* in the gastric mucosa leads to oxidative stress, which induce progressive changes in the histological aspect of the gastric lining, therefore causing diverse gastrointestinal pathological conditions. To verify this hypothesis, we assessed the amount of free radicals, the main cause of oxidative stress among *H. pylori*-infected individual in comparison with noninfected ones. The oxidation of fatty acids leads to the formation of aldehydes with malondialdehyde (MDA) being the most studied.[27] The host organism has a mechanism to protect itself from oxidative stress and lipid peroxidation products. One of the most important antioxidant enzymes for neutralization of exogenous ROS is SOD. SOD is responsible for catalyzing the dismutation of the superoxide radical into H_2O_2 and oxygen.[8] By strongly catalyzing this pathway, the

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Table 2 Mean values of oxidative stress markers according to the characteristic of the study population (sociodemographic and economic parameter, life style)

Variables	Oxidative stress markers Glutathione (mol/g of protein) Range	Mean	Median	IQ 25	IQ 75	*t*-value (*P* value)
Total value	0.001065–0.3609	0.09610 ± 0.04831	0.08180	0.06444	0.1162	
Age (years)						
≤20 *n* = 14	0.06240–0.1789	0.09095 ± 0.05389	0.07274	0.05335	0.1128	
21–30 *n* = 36	0.03275–0.2147	0.09007 ± 0.04334	0.08484	0.05695	0.1185	
31–40 *n* = 46	0.02664–0.2531	0.09018 ± 0.04521	0.07277	0.05275	0.1216	
41–50 *n* = 46	0.001065–0.2250	0.09193 ± 0.03605	0.09671	0.06585	0.1187	
51–60 *n* = 56	0.02377–0.2138	0.09950 ± 0.04264	0.08809	0.06258	0.1314	
>60 *n* = 68	0.006097–0.3209	0.09640 ± 0.06621	0.07895	0.05986	0.1092	
Age > 50 years						
Yes *n* = 124	0.001065–0.2531	0.09303 ± 0.04611	0.08342	0.06112	0.1204	0.06756 (0.9462)
No *n* = 142	0.006097–0.3209	0.09351 ± 0.05723	0.07658	0.05716	0.1146	
Gender						
Male *n* = 131	0.01355–0.3202	0.09449 ± 0.04947	0.08340	0.06197	0.1140	0.3401 (0.7341)
Female *n* = 135	0.001065–0.3209	0.09207 ± 0.05346	0.08131	0.05455	0.1218	
Income level						
Low *n* = 123	0.02377–0.3209	0.1004 ± 0.05691	0.08809	0.06355	0.1260	2.259 (0.0249*)
High *n* = 143	0.001065–0.2161	0.08445 ± 0.04249	0.07188	0.05362	0.1109	
Alcohol consumption						
Yes *n* = 155	0.001065–0.3209	0.09093 ± 0.04915	0.08018	0.05846	0.1140	0.7810 (0.4367)
No *n* = 111	0.006097–0.3202	0.09658 ± 0.05468	0.08484	0.06080	0.1229	
Smoking						
Yes *n* = 15	0.006097–0.2067	0.09405 ± 0.05158	0.08250	0.05938	0.1195	0.8019 (0.4236)
No *n* = 247	0.001065–0.3209	0.08298 ± 0.05012	0.07077	0.05575	0.1015	
Family history of gastric cancer						
Yes *n* = 151	0.006097–0.2856	0.09864 ± 0.05537	0.08962	0.06275	0.1184	1.346 (0.1799)
No *n* = 115	0.001065–0.3209	0.08901 ± 0.04795	0.07254	0.05416	0.1180	
Catalase (Mm of H_2O_2/min/mg of total protein**)**						
Total value	108.3–2063	1054 ± 298.4	1056	823.2	1239	
Age (years)						
≤20 *n* = 14	737.8–1692	1117 ± 269.5	1055	1009	1256	
21–30 *n* = 36	573.1–1704	1068 ± 268.4	1027	871.0	1272	
31–40 *n* = 46	203.6–1830	1037 ± 339.2	1052	801.5	1331	
41–50 *n* = 46	108.3–2056	966.5 ± 405.1	947.4	728.7	1170	
51–60 *n* = 56	538.9–1743	1092 ± 301.1	1123	839.9	1308	
>60 *n* = 68	338.1–2063	1018 ± 302.6	997.1	764.0	1188	
Age > 50 years						
Yes *n* = 124	338.1–2063	1057 ± 302.3	1044	836.7	1281	0.6771 (0.4991)
No *n* = 142	108.3–2056	1027 ± 338.6	1009	815.1	1235	
Gender						
Male *n* = 131	197.2–1830	1026 ± 303.0	984.4	827.5	1245	0.5683 (0.5704)
Female *n* = 135	108.3–2063	1052 ± 339.9	1055	806.7	1278	
Income level						
Low *n* = 123	108.3–2063	1043 ± 362.9	1037	808.2	1278	0.1471 (0.8832)
High *n* = 143	197.2–2056	1036 ± 285.6	1013	826.8	1219	
Alcohol consumption						
Yes *n* = 155	197.2–2063	1065 ± 339.9	1027	801.5	1308	1.371 (0.1720)
No *n* = 111	108.3–1741	1003 ± 292.2	1007	846.8	1181	
Smoking						
Yes *n* = 15	338.1–1830	1099 ± 360.0	1178	944.2	1351	0.7528 **(**0.4524)
No *n* = 247	108.3–2063	1034 ± 319.3	1018	812.7	1245	
Family history of gastric cancer						
Yes *n* = 151	203.6–2356	1045 ± 346.4	1052	794.7	1291	0.1499 (0.8810)
No *n* = 115	108.3–1863	1038 ± 285.1	1046	892.7	1172	

(*Continues*)

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Table 2 (Continued)

Variables	Oxidative stress markers Glutathione (mol/g of protein) Range	Mean	Median	IQ 25	IQ 75	*t*-value (*P* value)
	Malondialdehyde (mol/g of protein)					
Total value	0.0775–0.3465	0.1725 ± 0.04885	0.1688	0.1387	0.1970	
Age (years)						
≤20 *n* = 14	0.1088–0.2675	0.2064 ± 0.05535	0.2249	0.1517	0.2610	
21–30 *n* = 36	0.07967–0.2786	0.1775 ± 0.05043	0.1745	0.1469	0.2082	
31–40 *n* = 46	0.08482–0.3031	0.1799 ± 0.04859	0.1906	0.1563	0.2044	
41–50 *n* = 46	0.09929–0.2629	0.1830 ± 0.04326	0.1833	0.1490	0.2205	
51–60 *n* = 56	0.1003–0.3465	0.1946 ± 0.05253	0.1937	0.1541	0.2192	
>60 *n* = 68	0.07755–0.2942	0.1892 ± 0.05525	0.1790	0.1557	0.2336	
Age > 50 years						
Yes *n* = 124	0.07755–0.3465	0.1925 ± 0.05418	0.1898	0.1551	0.2247	1.370 (0.1720)
No *n* = 142	0.07967–0.3031	0.1829 ± 0.04735	0.1836	0.1513	0.2136	
Gender						
Male *n* = 131	0.07755–0.3031	0.1900 ± 0.05049	0.1910	0.1541	0.2258	0.7415 (0.4592)
Female *n* = 135	0.07967–0.3465	0.1848 ± 0.05109	0.1799	0.1536	0.2161	
Income level						
Low *n* = 123	0.07755–0.3465	0.1807 ± 0.05564	0.1801	0.1457	0.2166	1.731 (0.0850)
High *n* = 143	0.08482–0.3031	0.1928 ± 0.04588	0.1920	0.1606	0.2205	
Alcohol consumption						
Yes *n* = 155	0.08592–0.3465	0.1893 ± 0.05158	0.1904	0.1536	0.2205	0.6420 (0.5216)
No *n* = 111	0.07755–0.3187	0.1847 ± 0.05027	0.1794	0.1531	0.2193	
Smoking						
Yes *n* = 15	0.08592–0.3465	0.1889 ± 0.05040	0.1889	0.1538	0.2205	1.570 (0.1179)
No *n* = 247	0.07755–0.2927	0.1676 ± 0.05273	0.1532	0.1434	0.1874	
Family History of gastric cancer						
Yes *n* = 151	0.07755–0.3031	0.1863 ± 0.04696	0.1858	0.1537	0.2154	0.3566 (0.7218)
No *n* = 115	0.08482–0.3465	0.1888 ± 0.05541	0.1834	0.1522	0.2267	
	Superoxide dismutase (U/mg of protein)					
Total value	3.324–76.05	28.43 ± 14.19	25.97	17.22	76.05	
Age (years)						
≤20 *n* = 14	3.306–51.37	30.12 ± 15.19	28.39	25.30	41.25	
21–30 *n* = 36	0.8869–52.34	28.22 ± 14.65	25.73	15.20	42.63	
31–40 *n* = 46	1.532–76.05	28.38 ± 16.44	26.21	16.29	37.58	
41–50 *n* = 46	1.049–76.05	28.18 ± 20.35	25.85	8.065	48.04	
51–60 *n* = 56	1.129–76.05	30.92 ± 19.15	25.73	15.92	47.86	
>60 *n* = 68	1.774–74.11	25.23 ± 16.29	22.00	12.36	37.06	
Age > 50 years						
Yes *n* = 124	1.129–75.57	27.69 ± 16.83	25.85	14.30	40.00	0.5078 (0.6122)
No *n* = 142	0.8869–76.05	26.50 ± 17.23	23.31	13.15	37.07	
Gender						
Male *n* = 131	1.049–76.05	27.25 ± 17.70	24.27	15.03	37.22	0.08873 (0.9294)
Female *n* = 135	0.8869–61.34	27.04 ± 16.36	25.48	13.39	40.63	
Income level						
Low *n* = 123	0.8869–74.11	25.34 ± 15.56	24.88	13.75	34.98	1.384 (0.1680)
High *n* = 143	1.049–76.05	28.60 ± 18.00	24.76	14.86	44.60	
Alcohol consumption						
Yes *n* = 155	1.129–76.05	27.01 ± 16.35	24.52	15.32	37.10	0.1283 (0.8980)
No *n* = 111	0.8869–76.05	27.32 ± 17.95	25.61	13.27	42.17	
Smoking						
Yes *n* = 15	1.952–76.05	27.30 ± 16.99	25.00	14.05	40.48	0.4920 (0.6233)
No *n* = 247	0.8869–75.57	25.06 ± 17.39	21.20	14.86	35.78	
Family history of gastric cancer						
Yes *n* = 151	1.049–76.05	29.65 ± 17.80	25.73	15.32	44.72	2.437 (0.0156*)
No *n* = 115	0.8869–74.11	23.95 ± 15.39	23.91	9.819	34.38	

*Significant: *t*-value and *P* value comparing the mean values of oxidative stress markers among groups of characteristic parameter tested in the sample population.
n: number, IQ 25: 25th percentile, IQ 75: 75th percentile.

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Table 3 Oxidative stress according to *Helicobacter pylori* status

H. pylori status	Oxidative stress markers					*t*-value (*P* value)
	Range	Mean	Median	IQ 25	IQ 75	
	Glutathione (mol/g of protein)					
Positive $n = 191$	0.06032–0.3609	0.09887 ± 0.05170	0.08955	0.06533	0.1187	1.111 (0.2679)
Negative $n = 75$	0.001065–0.1886	0.09114 ± 0.04143	0.07752	0.06090	0.1149	
	Catalase (Mm of H_2O_2/min/mg of total protein)					
Positive $n = 191$	603.6–2063	1075 ± 318.2	1066	799.2	1245	1.364 (0.1741)
Negative $n = 75$	108.3–1470	1017 ± 257.2	1023	831.2	1237	
	Malondialdehyde (mol/g of protein)					
Positive $n = 191$	0.09205–0.3465	0.1863 ± 0.04966	0.1798	0.1516	0.2209	5.878 (< 0.0001*)
Negative $n = 75$	0.07755–0.2031	0.1479 ± 0.03628	0.1505	0.1175	0.1820	
	Superoxide dismutase (U/mg of protein)					
Positive $n = 191$	10.00–76.05	30.09 ± 13.43	27.14	21.25	37.58	2.282 (0.0235*)
Negative $n = 75$	3.324–61.69	25.47 ± 15.08	21.20	13.87	40.63	

*Significant.
n: number, IQ 25: 25th percentile, IQ 75: 75th percentile.

enzyme plays a key role in preventing the development of more toxic molecules, such as peroxynitrite.[28] Working in close concert with SOD is the antioxidant enzyme catalase. Catalase promotes the decomposition of hydrogen peroxide, the product of SOD activity into water and oxygen.[7] Thus, catalase provides a final step in neutralizing ROS before they can damage cellular components.[29] In addition to enzymatic antioxidants that protects the organism against free radicals, there are also nonenzymatic antioxidants such as glutathione that plays the same role.[8] So, in the present study, the activity of antioxidant enzymes catalase and SOD, the content of nonenzymatic antioxidant glutathione, and that of malondialdehyde, product of lipid peroxidation, was determined in relation to *H. pylori* status in order to assess the free radical formation and oxidative stress.

Our results showed that infected individuals had significantly elevated MDA level than uninfected ones ($t = 5.878$, $P < 0.0001$) (Table 3). Our findings are in agreement with previous studies revealing a significant higher tissue level of malondialdehyde in *H. pylori* positive patients compared with negative ones.[30,31] Such an observation indicate that *H. pylori* infection is a risk factor for elevated lipid peroxidation, since malondialdehyde (MDA) is one of the final products of lipid peroxidation. MDA reacts with proteins and amino acids causing their carbonylation.[32,33] Carbonylation of proteins lead to the dysfunction of proteins, which in turn lead to oxidative damage in cells, tissues, and organs.[34]

Although reactive substances are essential for a variety of cellular defense mechanisms and metabolic pathways,[35,36] they may cause oxidative damage in biomolecules[37] when present in numbers above their neutralization via antioxidant defense system.[38,39] This defense system is constituted of enzymatic components,[40] among which SOD partitions the highly reactive superoxide radicals into H_2O_2 and O_2.[20] Our data showed a significantly higher SOD activity among infected individuals compared with noninfected ones ($t = 2.282$, $P = 0.0235$) (Table 3). As elevated activity of SOD represents a compensatory mechanism against ROS attack, the current higher activity of this antioxidant enzyme is suggestive of an excessive free radical species formation related to *H. pylori* infection. Taking into account our result, *H. pylori* infection can be considered as a risk factor for overproduction of ROS and oxidative stress. Elevated levels of ROS have been reported in the gastric mucosae of *H. pylori*-infected patients.[41–43] Some authors suggest that oxidative stress can be modulated by *H. pylori* eradication.[44] Several mechanisms by which *H. pylori* induces oxidative stress have been reported including the overproduction of ROS. Sources of ROS in case of this infection include those generated by the pathogen itself,[45] those originating from activated phagocytic leukocytes in the gastric mucosa,[41] and those originating from various cell types under the induction of pro-inflammatory cytokines.[46] The human immune system usually fails to eradicate *H. pylori* completely, thereby leading to a long-term infection.[47] In this prolonged infectious condition, *H. pylori* exhibits chemotactic activity for neutrophils continuously.[48] This situation triggers the activated neutrophils to release excessive ROS, reactive nitrogen species (RNS),[49] and monochloramine (NH_2Cl), a potent oxidant[50] in the gastrointestinal tract. These oxidizing agents contribute to the immediate development of the inflammatory process and oxidative stress. Other mechanisms that could contribute to a pro-oxidative environment in *H. pylori* infection could be the decrease in ascorbic acid levels associated with this infection,[51] as well as the polyamines abundance observed within epithelial cells colonized by this pathogen. Indeed, ascorbic acid is a known antioxidant in that it scavenges free radical. In situations of polyamines abundance, the expression and activity of spermine oxidase, the enzyme that catalyzes the oxidation of polyamines to hydrogen peroxide, are multiplied.[52] This largely contributes to the overproduction of hydrogen peroxide and thus to oxidative stress.

The presence of *H. pylori* virulence factors may also contribute to the overproduction of hydrogen peroxide. In fact, *H. pylori* strains contain multiple virulence factors such as CagA, VacA, BabA, sialic acid binding adhesion, and g-glutamyl transferase that may contribute to the host's production of ROS and oxidative stress. Increased hydrogen peroxide levels and oxidative DNA damage have been reported in infected patients with

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Table 4 Impact of *Helicobacter pylori* positivity on oxidative stress markers distribution according to the characteristic of the study population (sociodemographic and economic parameter, life style)

	Age > 50 years		Gender		Smoking		Alcohol		Low Income		History of GC	
Oxidative markers	Yes	No	M	F	Yes	No	Yes	No	Yes	No	Yes	No
Glutathione												
Range	0.06032–0.3609	0.06098–0.2531	0.06098–0.3202	0.06032–0.3609	0.06097–0.2067	0.06032–0.3609	0.06032–0.3202	0.06097–0.3609	0.06032–0.3609	0.06098–0.3202	0.06047–0.3609	0.06032–0.2856
Mean	0.09811 ± 0.05917	0.09954 ± 0.04471	0.1006 ± 0.04763	0.09715 ± 0.05579	0.09873 ± 0.04940	0.09889 ± 0.05208	0.09630 ± 0.04852	0.1020 ± 0.05563	0.1041 ± 0.05454	0.09302 ± 0.04807	0.1081 ± 0.06204	0.09285 ± 0.04301
Median	0.07138	0.08152	0.08499	0.07001	0.07709	0.07752	0.07688	0.07833	0.08746	0.06983	0.1004	0.07026
IQ 25	0.06515	0.06529	0.06707	0.06418	0.06410	0.06558	0.06446	0.06609	0.06571	0.06449	0.06564	0.06517
IQ 75	0.1093	0.1223	0.1140	0.1205	0.1223	0.1152	0.1134	0.1223	0.1205	0.1099	0.1204	0.1118
t-value; *P* value	0.1591; 0.8738		0.3839; 0.7017		0.009285; 0.9926		0.6374; 0.5249		1.238; 0.2180		1.678; 0.0957	
Catalase												
Range	617.7–2063	603.6–2056	619.2–1943	603.6–2063	628.4–1355	603.6–2063	603.6–2063	620.2–1828	617.7–2056	603.6–2063	654.7–2063	603.6–2056
Mean	1072 ± 323.4	1082 ± 315.1	1050 ± 282.7	1101 ± 350.4	1050 ± 267.3	1078 ± 322.8	1108 ± 354.8	1036 ± 263.7	1079 ± 317.0	1071 ± 322.0	1139 ± 318.7	1034 ± 312.8
Median	1065	1067	1065	1068	1094	1066	1074	1058	1068	1055	1083	1013
IQ 25	779.5	826.9	826.5	788.4	861.0	797.9	780.9	834.1	799.8	785.6	956.6	780.4
IQ 75	1219	1268	1191	1280	1296	1236	1351	1177	1219	1272	1266	1231
t-value; *P* value	0.1934; 0.8470		0.9124; 0.3632		0.2624; 0.7934		0.6374; 0.1935		0.1518; 0.8796		1.883; 0.0619	
Malondialdehyde												
Range	0.09205–0.3465	0.09374–0.2835	0.09205–0.2786	0.09374–0.3465	0.1062–0.2430	0.09205–0.3465	0.09205–0.3465	0.09374–0.3187	0.1086–0.2835	0.09205–0.3465	0.09374–0.2942	0.09205–0.3465
Mean	0.1892 ± 0.05418	0.1832 ± 0.04543	0.1880 ± 0.04729	0.1846 ± 0.05222	0.1887 ± 0.04980	0.1570 ± 0.03890	0.1821 ± 0.04895	0.1915 ± 0.05045	0.1917 ± 0.04245	0.1803 ± 0.05644	0.1815 ± 0.04344	0.1938 ± 0.05754
Median	0.1786	0.1819	0.1856	0.1720	0.1833	0.1532	0.1810	0.1798	0.1856	0.1692	0.1786	0.1833
IQ 25	0.1536	0.1513	0.1513	0.1517	0.1536	0.1347	0.1488	0.1541	0.1564	0.1472	0.1504	0.1543
IQ 75	0.2287	0.2102	0.2287	0.2126	0.2221	0.1732	0.2109	0.2301	0.2221	0.2098	0.2084	0.2433
t-value; *P* value	0.6979; 0.4865		0.9124; 0.6940		1.964; 0.0461*		1.092; 0.277		1.326; 0.1870		1.409; 0.1611	
Superoxide dismutase												
Range	10.00–61.34	10.00–76.05	11.05–76.05	10.00–61.34	12.66–37.58	10.00–76.05	10.00–76.05	10.00–76.05	10.00–76.05	10.00–61.34	10.00–76.05	10.00–52.10
Mean	27.76 ± 11.89	32.23 ± 14.35	29.95 ± 13.72	30.23 ± 13.24	26.39 ± 8.966	30.39 ± 13.71	30.35 ± 12.80	29.76 ± 14.27	31.65 ± 13.86	28.33 ± 12.82	31.78 ± 14.65	27.49 ± 10.95
Median	25.97	28.59	26.69	28.55	26.94	27.14	27.03	27.50	28.06	25.73	27.82	25.48
IQ 25	20.35	22.94	20.79	21.41	19.30	21.68	21.80	18.04	23.31	19.76	22.22	19.09
IQ 75	34.19	41.30	37.10	38.31	36.09	38.63	37.22	38.13	41.45	35.78	41.15	34.31
t-value; *P* value	1.954; 0.0491*		0.1192; 0.9053		0.9040; 0.3676		0.2536;0.8002		1.431; 0.1549		1.824; 0.0704	

*Significant, *t* value and *P* value comparing the mean values of oxidative stress markers for each tested characteristic parameter among *H. pylori* positive individuals.

n: number, M: male, F: female, GC: gastric cancer, IQ 25: 25th percentile, IQ 75: 75th percentile.

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CagA positive strains.[53] In addition, there is an increase in tumor necrosis factor-α and IL8, which are inflammatory and oxidative stress markers in patients infected with CagA positive strains.[54] VacA-positive strains induce the generation of ROS that results in the activation of nuclear factor-kB, thereby increasing pro-inflammatory immune response.[54] BabA-positive strains induce a strong IL8 and weak IL33 cytokine response.[55,56] In granulocytes, sialic acid binding adhesin induces oxidative bursts.[57] G-glutamyl transferase contributes to IL8 production and nuclear factor-kB activation.[58] It also stimulates the production of H_2O_2 from the gastric epithelium.[59]

In the current study, we also found that being both *H. pylori* infected and smokers was significantly associated with higher level of MDA ($P = 0.0461$) (Table 4). Our data also showed significant higher SOD activity among infected participants older than 50 years ($P = 0.0491$) (Table 4). Such observations indicate that the presence of this pathogen in elderly individuals or in smokers increases risk for oxidative stress due to combined and cumulative exposure to both risk factors. Aging is a dynamic, progressive, and irreversible process, characterized by the occurrence of morphological, biochemical, functional, and psychological changes in the organism.[60,61] Evidence suggests that cellular oxidation may occur in the aging process, participating in the genesis of many non-transmittable chronic diseases that affect elderly individuals.[38] The accumulation of oxidative damage with age may occur via an increase in the generation of oxidized substances, reduction in antioxidant capacity, reduction in repairs to oxidative damage, or a combination of these mechanisms.[62,63]

Our finding concerning tobacco smoking and oxidative stress agrees with reports revealing that ROS can accumulate in the organism when exposed to tobacco smoking.[64,65] Cigarette smoke contains 10^{17} oxidant molecules per puff.[66] The oxidants in cigarette smoke cause lung injury by a number of mechanisms including the depletion of antioxidants among which is glutathione.[67] Donohue found that older smokers with long-term smoking histories had excessive protein carbonyls, a biomarker of protein oxidation and accumulated glutathione disulfide in sputum and broncho-alveolar lavage fluid.[68]

Our data also showed a significantly higher GSH level among participants with low income level ($t = 2.259$, $P = 0.0249$) and a significantly higher activity of SOD among those with family history of gastric cancer ($t = 2.437$, $P = 0.0156$) compared with nonexposed ones (Table 3). These results suggest that low income level as well as having history of gastric cancer alone can be considered as potential risk factors for oxidative stress independently of *H. pylori* status. When examining having history of gastric cancer or low income level among *H. pylori* participants, we noticed that exposed infected patients had higher GSH content, SOD and CAT activity compared with nonexposed ones, but with marginal ($P = 0.0704$, 0.0619 and 0.0957) and stronger P-values ($P > 0.1$) for having history of gastric cancer and income level, respectively. Such an observation highlights that these individual factors alone is sufficient to produce oxidative environment and oxidative stress.

Conclusion

In this study, we found significantly higher lipid peroxidation product and SOD activity among *H. pylori*-infected individuals. Our data also showed a significantly higher activity of SOD in participants older than 50 years old, a higher lipid peroxidation among smokers only when they were *H. pylori* infected. This finding suggests that *H. pylori* infection is associated with overproduction of ROS and oxidative stress and that the presence of this pathogen in elderly individuals or in smokers increased their risk for oxidative stress.

We hypothesize that this finding may be perhaps explained by presumably virulence factors in *H. pylori* strains circulating in our milieu that may contribute to the host's production of oxidative stress. Ongoing work in our laboratory has implicated the genotyping of virulence factors in *H. pylori* strains.

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Ethics approval statement

The study has been performed in accordance with the Declaration of Helsinki of 1975 and its later amendments or comparable ethical standards. The protocol was approved by the local Ethical Committee of Medical Sciences from the Bafoussam Regional Hospital (Approval 2330/L/MINSANTE/SG/DRSPO/ HRB/D), the Yaoundé General Hospital (Approval 07-19/HGY/DG/DPM/ NC-TR), and the Centre Médicale la Cathédrale (Approval 01-19CMC/TSM/LMS/ AutoRech /2019/10/03) and from the National Ethical Committee on Human Health Research in Cameroon (Approval 1476/ CE/CNERSH/SP).

Patient consent statement

Each potential participant received an information notice, oral explanation of the study, and was clearly informed on the potential risks and benefits of the study and measures taken for confidentiality. Only potential participants who accepted to participate and provided a written informed consent were enrolled. So, participation was voluntary and a written informed consent was obtained from all subjects and their legal guardian(s) before including him or her into the study. The written informed consent were enrolled of the current study are available from the corresponding author on reasonable request.

Data availability statement. The datasets used and/or analyzed during the current study are not publicly available because they are confidential but are available from the corresponding author on reasonable request.

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