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Biochemical changes associated with *H. pylori* infection in humans

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Abstract

Since its first isolation, *H. pylori,* a Gram-negative flagellated bacterium, has been the subject of intensive research because of its widespread dissemination and associations with several disorders. The bacterium is proven to be a causative factor for a number of gastric diseases, such as gastritis, gastric adenocarcinoma, and MALT-lymphoma. While *H. pylori* infection has been reported to be linked to several extra-gastric diseases, this study investigated extra-gastric manifestation *of H. pylori* using specific biomarkers in humans. The GERDs questionnaire was used to randomly chose 150 (one hundred and fifty) participants, of which 75 (seventy five) were found to be *H. pylori* positive and another 75 (seventy five) were chosen as negative controls. Samples were gathered, and biochemical characteristics were examined. The study revealed a significant ($p<0.05$) reduction in the levels of oxidative stress markers, glutathione peroxidase (GPX), vitamin C (vit C) and no significant difference was observed in malondialdehyde (MDA) and Total iron concentrations, although with a mean increase and reduction respectively. For the lipid profile biomarker results were statistically ($p<0.05$) significant for triglyceride (TG) and low density lipoprotein(LDLc) while no significant difference was observed in total cholesterol (TC) and high density lipoprotein (HDLc). Kidney function assessment revealed a statistical ($p > 0.05$) no significant different with sodium (Na^+), potassium (K^+), chloride (Cl^-) and bicarbonate (HCO_2^-) concentrations while there was a significant difference in urea and creatinine concentrations when compared with the control subjects. In the liver function parameters, a statistical ($p<0.05$) significant difference was observed with the aminotransferases (ALT, AST, ALP) and conjugated bilirubin while total bilirubin had a no significant difference when compared with the control subjects. A correlation coefficient analysis of parameter in *H. pylori* positive subjects were significantly adjusted ($p<0.05$) for TG, total cholesterol, aminotransferases and bilirubin. The aim of the study was to investigate the biochemical changes of *H. pylori* infection in humans using specific biomarkers.

Keywords: *H. pylori;* Mucosa associated lymphoid tissue; Gastro-esophageal reflux disease.

1. Introduction

Ulcers are deep sores that penetrate both the muscularis mucosae and gastrointestinal tract (G.I.T) to their full thickness. Unquestionably, Peptic ulcer is undoubtedly a disease of the twentieth century. The incidence and frequency of this disease and its consequences have exhibited notable geographic variations according to epidemiological data. There are several different varieties of ulcers, but the two most prevalent types are peptic ulcers, also known as gastric ulcers, which appear to be caused by damage to the stomach's lining, and duodenal ulcers, which are linked to the stomach's excessive acid production. The cause of peptic ulcers was hotly contested. Mucin, bicarbonate, and prostaglandins are thought to operate as protective factors whereas *Helicobacter pylori (H. pylori),* non-steroidal anti-inflammatory medicines (NSAIDs), and stomach acid act as aggressive factors (24). Its proven that ulcer develop due to imbalance between the aggressive and protective factors. *Helicobacter pylori* are a helical shaped, gram negative, micro-aerophilic bacterium (2 – 4 µm long with diameter of 0.5 µm) that infects the stomach and duodenum of humans (38)

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and has been recognized as a class I carcinogen (38). They were initially noticed as clinically important by Robin Warren & Barry Marshall from the human gastric mucosa in 1982 and were both awarded a Nobel Prize for their immense contributions and the demonstration of its involvement in gastro duodenal pathologies which has radically changed people's perception of these diseases (29). Regional prevalence estimates suggest that approximately 4.4 billion people are infected with *H. pylori* worldwide (22). The countries with the highest incidence of *H. pylori* compared to the general population were Nigeria, Portugal, Estonia, Kazakhstan, India and Pakistan, while the lowest incidence was observed in Switzerland (20).

Although it is unknown how *H. pylori* first enters the stomach, it is thought to do so by being consumed with food and tainted water, which act as physical barriers to stomach acid. Geographic and socio-demographic distributions have been shown to affect the distribution of the organism's infection (30, 5) as proven with African mystery based on high prevalence and low incidence of gastric cancer within the black community. Their ability to adhere, invade, get past host defenses, and damage tissue is largely a result of the colonization and virulence factors they produce. According to some estimates, this pathogen may be responsible for up to 95% of duodenal and 70% of gastric ulcers, affecting subjects in their middle years (35). Although the relationship between *H. pylori* infection and pathologic conditions of the stomach is well established, the bacterium's effects on the entire host organism are still under debate because it is well-known that some pathogens can have systemic pathological effects as they remain locally within the body (27). *H. pylori* infection is also involved in the development and course of cardiovascular, respiratory, blood, metabolic, genitourinary, and skin diseases (14). However, the underlying mechanisms are poorly understood. It has been shown that specific *H. pylori* cytotoxins or inflammatory responses triggered by *H. pylori* may be the reason why *H. pylori* infection can cause these non-gastrointestinal diseases (33). Current research focuses on hematologic, cardiovascular, hepatic, renal and some selected antioxidants biomarkers which are counteractive against reactive oxygen species in the development of gastric and extra gastric *H. pylori* infection.

2. Materials and methods

2.1. Materials

2.1.1. Subjects

The study was carried out at Human Race Specialist, Medical Diagnostic, Cardiac and Neurosurgical Hospital Naze, Owerri, located at plot R111/112 Naze industrial cluster, Federal Polytechnic Nekede road, Owerri, Imo State, Nigeria, founded since 2014 by Dr Obioma Cosmas Iwuagwu, a United Kingdom practicing interventional radiologist.

Both written and verbal ethical considerations were sought from the respective groups in this study; consent was taken from Human Race Hospital management for approval for this study and the respondent's consent which mandated the confidentiality and privacy of the respondents.

The sample population comprises of one hundred fifty (150) adults within the ages of 25 to 65 years of which seventy five (75) were group as *H. pylori* positive and another seventy five (75) apparently healthy individuals who tested negative and confirmed with endoscopy were grouped as controls , subjects for the study were selected based on random sampling technique.

2.1.2. Recruitment of respondents

The recruitment of respondent was randomly selected based on the criteria standard of the Gastro-Esophageal Reflux disease questionnaire (GERDQ) in primary care centers (21). In this study *H. pylori* positive were defined as subjects who tested positive for serological antibodies for *H. pylori* and confirmed by a certified gastroenterologist through endoscopy.

The inclusive criteria for the selection of respondent for positive subjects were Hepatitis B, and Retroviral screening (RVS) negative, age brackets that have never had treatments or medication for ulcer or GERD with a persistent complain related to abdominal or stomach bite and a current symptom of gastroenteritis and heartburn while subjects controls are selected based on the facts that they tested serologically negative for *H. pylori* antibodies, Hepatitis B and RVS and are within age brackets without any sign and symptoms of GERD or gastroenteritis related to ulceration in the last 2 to 3 months.

The exclusion criteria for the selection of respondents for *H. pylori* positive subjects were hepatitis B and RVS positive and subjects with a recent history of GERD, gastroenteritis or heartburn and subjects who has been on *H. pylori*

treatment regimens currently or before and control subjects, apparently ill or tested positive for Hepatitis B and RVS or have been on *H. pylori* treatment regimen in the last 2 to 3months.

2.1.3. Ethical consideration

Both written and verbal ethical considerations were sought from the respective groups in this study, consent was taken from Human Race Hospital management for approval for this study and the respondent's consent mandated the confidentiality and privacy of the respondents.

2.1.4. Sample size/subjects

The sample population comprises one hundred and fifty (150) adults between the ages of 25 to 65 years of which seventy five (75) were grouped as *H.pylori* positive and another seventy five (75) healthy individuals who tested negative were grouped as controls, subjects for the study were selected based on random sampling technique using the GERD questionnaire .

2.2. Methods

Determination of serum total iron concentration (19), serum total iron level was determined colorimetrically using TECO diagnostic kits.

Total cholesterol concentration by (3). Total cholesterol was determined enzymatically using Biolabo France reagent kit. Values expressed in mg/dl. Triglycerides concentration was assayed using enzymatic hydrolysis (41) using randox assay kits (Randox laboratories Ltd) values expressed in mg/dl. High density lipoprotein cholesterol (HDL c) concentration. HDL –c was measured by the method described by (16), using Agappe kits (Agappe diagnostics Switzerland GmbH). Low density lipoprotein (LDL), the serum levels of (LDL-c) was calculated according to the protocol of (15).

Serum electrolytes concentration (Na^+, K^+, CL^- and HCO_2^-), electrolytes were assayed using an automatic serum electrolyte analyzer precisely Audicom AC 9900 model as described by (39). Serum urea concentration was determined by a method described by (44) using Randox kit (Randox laboratories limited). Serum Creatinine concentration was determined as described by (4), using Agappe kits (Agappe diagnostics Switzerland GmbH).

Serum Glutamic Oxaloacetic Transaminase (SGOT) as described by (10), using Agappe kits (Agappe diagnostics Switzerland GmbH). Serum Glutamic Pyruvic Transaminase (SGPT) as described by (9), using Agappe kits (Agappe diagnostics Switzerland GmbH). Alkaline Phosphatase (ALP) colorimetrically as described by (34), using Randox kit (Randox laboratories limited). Conjugated and Total Bilirubin (B1 and B2) were determined as described by (28), using Biolabo SAS reagent kit France.

Glutathione Peroxidase (GPX) The activity of glutathione peroxidase was determined by the method of (36). Malondialdehyde Level (MDA). MDA level was determined by the colorimetric method of (17). Vitamin C was estimated by the method of (32). Statistical analysis: The data was analyzed by the Analysis of Variance (ANOVA) using SPSS program (version 16.0 SPSS Inc., Chicago, IL, USA). The results were expressed as mean ± standard deviation (SD). P values less than 0.05 was considered as significant ($P<0.05$). Events were considered related when the probability of absence of relationship was lower than 0.05 ($P< 0.05$)

3. Results

Table 1 Oxidative stress markers and serum total iron of *H. pylori* infected subjects

Parameter	Positive	Control	T-value	P-value	Comment
Malondialdehyde (nmol/mL)	3.13 ± 0.65	3.03 ± 0.62	0.557	0.5804	Not significant
Gluthathione peroxidase (U/mL)	1.05 ± 0.26	1.26 ± 0.33	2.499	0.0159	Significant
Vitamin. C (mg/dL)	1.31 ± 0.19	1.45 ± 0.25	2.229	0.0305	Significant
Total Iron (µg/dL)	58.82 ± 7.93	62.29 ± 7.63	1.577	0.1215	Not significant

Values are presented as mean ± standard deviation. Values are adjudged significant at $p \leq 0.05$. Sample size, n = 75

Table 2 Lipid profile of *H. pylori* infected subjects

Parameter	Positive	Control	T-value	P-value	Comment
Total cholesterol (mg/dl)	155.60 ±10.43	154.20 ± 10.14	0.4812	0.6326	Not significant
Triglyceride (mg/dl)	88.40 ± 7.92	94.12 ± 8.70	2.431	0.0188	Significant
HDL (mg/dl)	38.96 ± 5.55	39.16 ± 5.78	0.1248	0.9012	Not significant
LDL (mg/dl)	101.20 ± 6.48	96.36 ± 6.81	2.574	0.0132	Significant

Values are mean ± standard deviation. Values are adjudged significant at p ≤0.05. Sample size, n = 75

Table 3 Renal function profile of *H. pylori* infected subjects

Parameter	Positive	Control	T-value	P-value	Comment
Sodium (mmol/L)	141.50 ± 8.51	140.00 ± 5.34	0.7465	0.4590	Not significant
Potassium (mmol/L)	3.64 ± 0.37	3.72 ± 0.36	0.7748	0.4422	Not significant
Chloride (meq/L)	97.80 ± 6.53	100.60 ± 2.87	1.963	0.0555	Not significant
Bicarbonate (mmol/L)	26.36 ± 3.61	27.53 ± 3.50	1.163	0.2504	Not significant
Urea (mg/dl)	16.63 ± 2.33	18.84 ± 1.59	3.917	0.0003	Significant
Creatinine (mg/dl)	0.96 ± 0.10	0.81 ± 0.06	6.431	<0.0001	Significant

Values are mean ± standard deviation. Values are adjudged significant at p ≤0.05. Sample size, n = 75

Table 4 Liver function markers of *H. pylori* infected subjects

Parameter	Positive	Control	T-value	P-value	Comment
AST (IU/L)	34.10 ± 4.26	25.91 ± 3.26	7.634	<0.0001	Significant
ALT (IU/L)	28.09 ± 4.45	33.70 ± 3.76	4.815	<0.0001	Significant
ALP (IU/L)	237.40 ± 11.78	202.78 ±7.45	12.42	<0.0001	Significant
Conjugated Bilirubin (mg/dl)	0.25 ± 0.07	0.34 ± 0.10	3.687	0.0006	Significant
Total Bilirubin (mg/dl)	0.82 ± 0.07	0.85 ± 0.06	1.627	0.1103	Not significant

Values are mean ± standard deviation. Values are adjudged significant at p ≤ 0.05. Sample size, n = 75

Table 5 Correlation Coefficient matrix (p-values) of total iron concentration and oxidative stress markers in H pylori infected subjects

Parameters	MDA	GPX	Vit. C	Total Iron
Malondialdehyde		-0.128 (0.543)	-0.304 (0.140)	-0.157 (0.455)
Gluthathion peroxidase	-0.128 (0.543)		-0.073 (0.728)	-0.096 (0.647)
Vitamin C	-0.304 (0.140)	-0.073 (0.728)		0.220 (0.290)
Total Iron	-0.157 (0.455)	-0.096 (0.647)	0.220 (0.290)	

Correlation coefficient (r^2) is adjudged significant at p ≤0.05.

The table above describes the relationship between biochemical biomarkers of oxidative stress and total iron concentration of *H. pylori* infected subjects, No significant correlation was seen established (p>0.05) between malondialdehyde , Gluthatione peroxidase, vitamin C and total iron concentration. Nonetheless gluthathione peroxidase and Ascorbic acid were significantly reduced in the experimental mean as levels of these useful oxidative stress markers was altered which might indicate depletion.

Table 6 Correlation Coefficient matrix (p-values) of lipid profile in *H. pylori* infected subjects

Parameters	T. Cholesterol	Triglyceride	HDL	LDL
T. Cholesterol		0.027 (0.898)	0.229 (0.272)	0.794 (0.000)*
Triglyceride	0.027 (0.898)		-0.298 (0.148)	-0.001 (0.997)
HDL	0.229 (0.272)	-0.298 (0.148)		-0.257 (0.215)
LDL	0.794 (0.000)*	-0.001 (0.997)	-0.257 (0.215)	

Correlation coefficient (r^2) is adjudged significant at $p \leq 0.05$.

The table above shows the correlation matrix of lipid profile biomarker of *H. pylori* infected subjects, there was a significant correlation established between Total cholesterol and Low density lipoprotein in the experiment $r^2 = 0.794$ (0.000), however statistical results obtained in the experiment proved not to be significant for Total Cholesterol and HDL, 155.60 ± 10.43 (mg/dl) compared to 154.20 ± 10.14 (mg/dl) and 38.96 ± 5.55 (mg/dl) compared to 39.16 ± 5.78 (mg/dl) respectively, in contrary to Triglyceride and LDL that were statistically significant 88.40 ± 7.92 (mg/dl) compared to 94.12 ± 8.70 (mg/dl) and 101.20 ± 6.48 (mg/dl) compared to 96.36 ± 6.81(mg/dl) but without significant relationship $r^2 = -0.001$ (0.997).

Table 7 Correlation coefficient matrix (P values) of renal function profile in *H. pylori* infected subjects.

Parameter	Sodium	Potasium	Chloride	Bicarbonate	Urea	Creatinine
Sodium		0.672 (0.000)*	0.671 (0.000)*	-0.204 (0.328)	0.333 (0.104)	-0.381 (0.060)
Potasium	0.672 (0.000)*		0.709 (0.000)*	-0.269 (0.194)	0.240 (0.248)	-0.474 (0.017)*
Chloride	0.671 (0.000)*	0.709 (0.000)*		-0.316 (0.123)	0.183 (0.382)	-0.470 (0.018)*
Bicarbonate	-0.204 (0.328)	-0.269 (0.194)	-0.316 (0.123)		-0.159 (0.448)	0.021 (0.920)
Urea	0.333 (0.104)	0.240 (0.248)	0.183 (0.382)	-0.159 (0.448)		0.288 (0.162)
Creatinine	-0.381 (0.060)	-0.474 (0.017)*	-0.470 (0.018)*	0.021 (0.920)	0.288 (0.162)	

Correlation coefficient (r^2) is adjudged significant at $p \leq 0.05$.

Table above shows a matrix of correlation between electrolytes, urea and creatinine as a function of renals in *H.pylori* positive subjects, there was a positive correlation between the electrolytes ($p<0.05$) with an exception of biocarbonate ($p> 0.05$), though there was no statistical significant differences observed in the experiment with the electrolytes. There was a significant negative correlations observed between creatinine, potassium and chloride. There was no correlation observed between urea and creatinine as a measure of GFR $r^2 = 0.288(0.162)$ but experimental results presented with a mild lower mean value of urea 16.63 ± 2.33 (mg/dl) compared to 18.84 ± 1.59 (mg/dl) and significant increase in Creatinine 0.96 ± 0.10 (mg/dl) compared to 0.81 ± 0.06 (mg/dl).

Table Correlation coefficient matrix (P values) of Liver function profile in *H. pylori* infected subjects

parameter	AST	ALT	ALP	Conj. Br	Total Br
AST		0.823 (0.000)*	0.727 (0.000)*	-0.212 (0.308)	0.015 (0.942)
ALT	0.823 (0.000)*		0.803 (0.000)*	0.084 (0.689)	0.181 (0.385)
ALP	0.727 (0.000)*	0.803 (0.000)*		-0.074 (0.724)	-0.061 (0.771)
Conj. Br	-0.212 (0.308)	0.084 (0.689)	-0.074 (0.724)		0.598 (0.002)*
Total Br	0.015 (0.942)	0.181 (0.385)	-0.061 (0.771)	0.598 (0.002)*	

Correlation coefficient (r^2) is adjudged significant at $p \leq 0.05$.

The Table above describes the correlation matrix of liver function parameters of *H. pylori* infected subjects, there was a positive significant correlation observed within the Transaminases, between the transaminases and alkaline phosphatase and between conjugated and total bilirubin $p< 0.05$, while no significant correlation was observed between bilirubin and transaminases $p>0.05$.

4. Discussion

The results of biochemical studies related to hematological abnormality reveals that total serum iron for positive group was slightly lower than the control group 58.82 ± 7.93 ug/dl when compared to 62.29 ± 7.63 ug/dl) respectively ($p >0.05$) . This observation was in agreement with results obtained by (45). (13) reported no significant difference when compared between positive and control subjects, but the slight variation in the mean values of both groups suggested that *H. pylori* infection impaired iron update by direct competition with the host for availability or impairing iron absorption. How *H. pylori* can cause ID/IDA has not yet been determined. However, stomach acid is required for iron absorption by reducing ferric iron to the more soluble and absorbable form of ferrous iron (46). While some studies in developing countries have found no such link. A contemporary report from Bangladesh concluded that *H. pylori* are not the cause of IDA, iron deficiency, or iron deficiency in Bangladeshi children (37). This study did not find a statistically significant association between *H. pylori* infection and anemia or low iron levels. This study suggested that the presence of anemia and low iron status in developing countries might be contributed by many factors put together. Quite a Population in less developed countries is frequently susceptible to deficiency of various micronutrients needed for haemoglobin synthesis other than iron. These may include folic acid, vitamin B_{12}, vitamin A and low protein intake from poor quality diet might have contributed to the development of anemia or low iron status among these populations

Malondialdehyde was seen to be statistically $p > 0.05$ not significant, though there was a remarkable increase in the mean value of the positive when compared to the control subjects (3.13 ± 0.65 nmol/ml when compared to 3.03 ± 0.62 nmol/ml) respectively, these mean value increase indicates increase lipid peroxidation and cellular membrane turnover in positive subject as described by (43), who reported that severe chronic inflammation in *H. pylori* infections correlate with high serum malondialdehyde. Contrary result was still described by (25) on the "Comparison of Serum Malondialdehyde Concentration between *H. pylori* Positive and *H. pylori* negative Gastritis Patients", a cross sectional study of 40 dyspepsia discovered that serum malondialdehyde level was significantly higher in patients with positive *H. pylori* gastritis when compared to *H. pylori* negative gastritis. Up until now, MDA concentration still do not have a specific value which can be used as standard during measurement, because MDA concentration can be affected by age, enzyme activities (superoxide dismutase, catalase, and glutathione perioxidase), antioxidant supplement (vitamin C, E, βeta-karoten, etc), diseases and environment (pollution and radiation) (25).

Gluthathione peroxidase in this study was seen to be statistically ($p<0.05$) significant, when compared between mean value of the infected subjects with control subjects (1.05 ± 0.26 U/ml) compared to (1.26 ± 0.33 U/ml), there was a significant reduction in the mean value of positive subjects when compare to the control, T-value was 2.499. (40) in his study termed Glutathione peroxidase level in patients with *H. pylori* associated gastritis, had a contrary result that projected a no significant difference in positive and negative subjects but with a reduction in the mean (115 ± SD U/g HGB) of the positive group when compared to the control subject (125.5 ± SD U/g HGB), which is in line with this study. A mean reduction of GPX in positive subject indicate a reduced functionality or activity of this useful peroxidase enzyme that help neutralize and mob up free radicals in *H. pylori* infection, therefore its empirical to say, from the findings that *H. pylori* infection causes a decrease detoxification capacity not only due to less detoxification enzymes but because of high production of reactive oxygen species that are in massive turnover with these enzymes, which indirectly affects their concentration and activity.

Vitamin C was also seen to be statistically ($p<0.05$) significant when compared between subjects, there was a mean value reduction in the positive subjects as compared to the control, T value 2.229, a greater evidence against the Null hypothesis, it is therefore clear that the bioavailability of vitamin C can be significantly reduced by *H. pylori* infection, this agrees with the reports of (31) which revealed that *H. pylori* can oxidize and neutralize ascorbic acid in the stomach, in his study vitamin C levels was significantly reduced with decreased vitamin C intake, due to increase oxidation and impaired or absent of ascorbate secretion in the gastric mucosa of positive subjects. Apart from its antioxidant, biosynthetic functions and more, , it is believed that in the presence of Fe, high ascorbic acid concentrations in gastric juice will inactivate and vitiate urease secreted by *H. pylori* under H_2O_2 – mediated low pH conditions, thus preventing *H.pylori* from surviving and colonizing acidic surfaces (23). Finally our result shows that there is an association between *H. pylori* infection and oxidative stress status as reflected by the mean increase in MDA but didn't differ significantly and a total significant decrease in total antioxidant capacity as seen with GPX and vitamin C. We therefore suggest that *H. pylori* causes a considerable levels of oxidative stress in the body and recommend antioxidant treatment should be considered as supplementary therapy in the prevention and treatment of *H. pylori* dependent oxidative damage.

The lipid profile parameters of positive subjects were compared to that of negative control subjects and it was discovered that the total cholesterol and high density lipoprotein was statistically ($p>0.05$) not significant while the Triglyceride and Low density lipoprotein was statistically ($p<0.05$) significant. It is important to equally remember that LDL-c and Total cholesterol in the study shows a very positive correlation in the experiment $r^2=0.794$ as described in the correlation analysis. (6), provided similar results that showed evidence that *H. pylori* infection was associated with increased LDLc and Triglyceride levels but no effect on levels of both total cholesterol and HDL. (1), was able to observe remarkable changes in lipid profile in *H. pylori* seropositive subjects, he observed high triglyceride, total cholesterol and LDL-c when compared to healthy subjects , his findings supports the hypothesis that chronic infection may modify lipid profile metabolism in a way that could increase the risk of atherosclerosis hence CVD. Generally any chronic infection causes atherosclerosis via distributed lipid and lipid protein metabolism (2). (18), also discovered a lower HDL-c, higher Triglycerides and Total cholesterol in *H. pylori* seropositive, he went further to say that the reason for the changes in lipid profile metabolism is inflammation caused by *H. pylori* infection. It is of no doubt that chronic infections causes inflammation by increasing expression of inflammatory cytokines such as TNF-α, TNF-α has been shown to inhibit lipoprotein lipase which provides fatty acid the passage from blood to the tissues, this result in mobilization of TGs from tissue to blood circulation and thus elevated triglyceride in circulation is observed. After *H. pylori* treatment with antibiotics decreasing LDLc, TG, TC levels and increasing HDL-c level shows that *H. pylori* eradication is important for prevention of CVD (18).

The renal function parameters were compared between positive and negative control subjects and there were no statistical ($p>0.05$) difference seen between the electrolytes. Sodium, Potassium, Chloride and bicarbonate the P values are 0.4590, 0.4422, 0.0555 and 0.2504 respectively results were statistically not significant, this signifies that *H. pylori* infection has little or no relationship in the alteration of electrolytes concentration, it is equally important to note that there exist a positive correlation in the study between the electrolytes except for bicarbonate .The mean ± SD of Urea between groups are 16.63 ± 2.33 compared to 18.84 ± 1.59, P = 0.0003 showed a statistical significant reduction in their mean values. *H. pylori* are known to be urease positive, this enzyme help colonization by making its environment less acidic through generation of ammonia and CO_2. Is it possible that the high level of urease production might be crucial in the reduction of serum urea concentration during infection? In a study conducted by (42), with 117 patients on dyspeptic complain of which 53(45%) of the 117 patients were *H. pylori* positive, discovered a high serum urea nitrogen level to correlate with a low prevalence of *H. pylori* infection, in his study gastric urease production might reflect serum urea blood nitrogen concentration in positive subjects this is in agreement with this study as the reduction in the mean value of blood urea concentration in infected subjects reflects increase urease production. Serum creatinine level was statistically ($p>0.05$) significant when compared between the two groups, a significantly increase in the mean value of the groups 0.96 ± 0.10 (mg/dl) compared to 0.81 ± 0.06 (mg/dl). Serum creatinine (SC) is widely used as a measure of kidney function and so the serum level reflects renal excretion, as well as the generation, intake, and metabolism of creatinine (26). Serum creatinine is used to calculate glomerular filtration rate (GFR), which is the most accurate way to measure renal function, the association between *H. pylori* infection and estimated GFR is unclear as Age, sex and body mass index are the main confounders between estimated (e) GFR and *H. pylori* and so the importance of *H. pylori* infection and renal function remains unclear. *H. pylori* are an etiological factor in endothelial dysfunction and may be involved in causing proteinuria by this mechanism but little is known about this mechanism. In fact there is a paucity of information regarding *H. pylori* and Nephritis, besides large sample size is required and more accurate indicators or biomarkers should be evaluated to elucidate a proper relationship between *H. pylori* and kidney complication in patients with gastrointestinal disease.

Changes within the liver enzyme and biomarkers was determined and compared between the two study groups, there was a statistical $p<0.05$ significant difference seen with the transaminases, Alkaline phosphatase and conjugated bilirubin. While no significant difference was noticed in the Total bilirubin. In ALT and Direct bilirubin there were significant reductions in the mean value while a significant increase in mean value was observed in AST and Alkaline phosphatase. Previous studies on the relationship between *H. pylori* infection and liver disease have been inconsistent. Various systematic reviews and randomized controlled trials have revealed a positive association between *H. pylori* infection and nonalcoholic fatty liver disease (NAFLD) (7, 12), but a negative association has also been reported in large and small cross-sectional studies, retrospective studies and randomized. A study by (18), on *H.pylori* infection may increase the severity of nonalcoholic fatty liver disease via promoting liver function damage, had a somewhat contradictory result for liver function measurements, 26.37% of NAFLD patients had abnormal ALT elevations ranging from 41 to 79 U/L, which was higher than the normal reference range of 5 to 40 U/L. There was an abnormal rise of total bilirubin (TBIL) in 10.99% of NAFLD patients, and abnormal increase in direct bilirubin (DBIL) in 7.69% becoming one among the early few to associate *H. pylori* and NAFLD. There appear to be a temporal association between *H. pylori* and liver enzyme abnormality in the development of NAFLD, but a direct link is yet to be established and so the underlying mechanism of liver injury is unclear and so further studies are required to investigate such association and its clinical significant. Furthermore, the evaluation of individuals with liver and biliary tract disease includes serum

assays for biochemical markers. Traditional markers associated with hepatocellular injury and biliary tract disorders include aminotransferases, ALP (Alkaline phosphatase), γ-GGT (Gamma glutamyltransferase), prothrombine time and bilirubin. In general, the size of the serum aminotransferase increase reflects the relative extent of active hepatocellular damage, but not necessarily its aggregate severity. However, even when combined with markers of hepatic synthetic function, such as serum albumin and prothrombine time. ALT and AST are relatively poor indicators of centrilobular hepatocellular injury because of their uneven distribution (11). In common with ALP and γ -GGT, ALT and AST are distributed mainly within the periportal area and substantial centrilobular necrosis can occur without a concomitant increase in serum aminotransferases. An additional limitation of using aminotransferases as markers for hepatocellular injury is their comparatively long plasma half-lives (17 hrs for AST; 47hrs for ALT). Thus, during acute liver damage, abnormalities in serum aminotransferase activities often lag behind changes in hepatocellular integrity, making them un-useful as early hepatic biomarkers. The ALT, AST and ALP show a very positive relationship in the progression of *H.pylori-related* NAFLD $P<0.05$, conjugated and total bilirubin equally shows a positive relationship $r^2 = 0.598$.

5. Conclusion

The result of the study revealed that *H. pylori* infection is not associated with the development of iron deficiency anemia (IDA), though there was a mean reduction in the value of positive subjects when compared to control subjects, it effects in the total body iron concentration cannot be overemphasized because it reflects total body iron pool, but the low iron concentration in infected subjects is purported not enough to initial IDA. The reduction might have been mitigated by mal-absorption associated with the activities of *H. pylori* in the mucosa.

The study also showed that *H. pylori* has a negative effect on the level of antioxidants as seen with vitamin C and gluthathione peroxidase as concentration was significantly reduced in infected subjects when compare to the controls, this reduction explains depletion which might have been propagated by increase production of ROS during infection, free radicals effects on cellular membrane peroxidation was established by the increased mean value of Malondialdehyde in *H. pylori* infected subjects when compared to the control.

The result of the study also showed an abnormal distribution of lipid profile parameters in seropositive subjects which might further results to cardiovascular related diseases as the infection progresses, there was a significant increase in the concentration of LDLc and a mean reduction for HDLc, LDL and HDL, which are important markers for atherogenic disease, the ratio of LDL/HDL in infected subjects is higher when compared to the controls. LDL and HDL ratios are important determinant in abnormal lipid distribution and in cases of hyperlipidemia, heart diseases and stroke complicated by *H. pylori* infection.

The study further revealed that *H. pylori* has a positive effects on GFR as seen with the significant ($P> 0.05$) increase in serum creatinine and mean value decrease in urea concentrations in infected subjects, creatinine is regarded basically as the standard for kidney filtrate capabilities, changes related to GFR might not be prompt but will complicate as the infection progress. The high urease production reflects reduced serum urea concentration in infected subject as *H. pylori* reduces urea to ammonia and CO_2 in the plasma. No statistical difference was observed with the electrolyte concentration in positive subjects, as *H. pylori* has little or no effects on the electrolyte concentration but this can only be said in an intact renal functionality presumably in an early stage of this infection because it is not possible to rule out the overwhelming contributions of the kidney in maintaining and stabilizing the electrolytes concentration.

There is a statistical ($p<0.05$) significant increase and reduction in liver biomarkers which elucidates *H. pylori* effects on the activities of these enzymes and biomarkers, (AST, ALT, ALP and conjugated bilirubin), the study further revealed a positive correlation established between these biomarker. The alterations and changes of these enzyme activities during *H. pylori* infection clearly implicate it in the etiogenesis of Non-alcoholic fatty liver disease. (NAFLD)

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

Approval was granted by federal university of technology project research committee prior to study commencement.

Statement of informed consent

Both written and verbal consent was sorted from the respondent's and ethical approval from Human Race Specialist, Medical Diagnostic, Cardiac and Neurosurgical Hospital, the confidentiality of the respondents was strictly mandated.

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