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Effect of *Helicobacter Pylori* on Some Haemostatic Parameters Among Students of a Tertiary Institution in Nigeria

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Abstract

The study evaluated the effects of *Helicobacter pylori* on haemostatic parameters of infected students of an educational tertiary institution in Nigeria. A total of 32 *Helicobater pylori* patients participated in this study comprising of 17 females and 15 males. 20 and 21 control subjects were established for females and males respectively. Blood was collected from the participants and analyzed for platelets (PLT), prothrombin time (PT) and activated partial prothrombin time (APPT) following standard protocol. Result showed that test and control subjects had mean values 186.24 x 10^9/L and 271.55 x 10^9/L respectively (PLT), 15.50 seconds and 11.75 seconds respectively (PT) and 34.53 seconds and 28.50 seconds respectively (APPT) for females, and 188.53 x 10^9/L and 274.81 x 10^9/L respectively (PLT), 13.86 seconds and 11.24 seconds respectively (PT) and 32.20 seconds and 29.05 seconds respectively (APPT) for males. There was significant difference ($P < 0.05$) among the test and control subjects in the three parameters of study. PT and APPT showed significant increase in the *H. pylori* students and PLT showed significant decline among test subjects when compared with the controls. Significant alteration in haemostatic parameters could predispose the *H. pylori* patients' to possible vascular complication.

Keywords: *Haematological Indices; Helicobater pylori; Human health; Bacteria infection*

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Introduction

Helicobacter pylori is a gram-negative, micro aerophilic spiral-shaped bacterium that grows in the mucus layer (Yahya., *et al*. 2017; NCI, 2013; Tamokou., *et al*. 2017). This bacterium could weaken the protective coat of the stomach, and allow the digestive juices to irritate the sensitive part of the stomach lining. During this process, the bacterium could cause ulcer and inflammation in the stomach (Yahya., *et al*. 2017).

H. pylori is one of the most common cause of gastric ulcers and gastritis, and 10% of those infected with *H. pylori* may develop an ulcer (WebMD, 2017). Furthermore, individuals infected by *H. pylori* are at increased risk of stomach cancer and lymphoma (WebMD, 2017). According to Yahya., *et al*. (2017), Hajimahmoodi *et al*. (2011), mucosa-associated-lymphoid-type lymphoma is one of other disease conditions that are associated with *H. pylori.*

Typically, the condition of the stomach is usually acidic *H. pylori* secrete urease enzymes that can convert the chemical urea to ammonia. The ammonia produced aid in neutralizing the acidic condition of the stomach thereby making it a habitable place for the bacterium (NCI, 2013). NCI (2013) also noted that helical shape of the bacterium aid the bacterium to burrow into the mucus layer of the stomach.

H. pylori infection occurs in over 50% of the world's population, though most of those infected have no symptoms (WebMD, 2017). A significant number of world populations that harbor the bacterium reside in the developing countries (Ananthakrishnan and Kate, 1998; Yahaya., *et al*. 2017; Amieva., *et al*. 2016). *H. pylori* infection can be transmitted through consumption of contaminated food and water and person-to-person (Karen., *et al*. 2007; WebMD, 2017; Yahya., *et al*. 2017). Since contamination of resources that are ingested are major route of transmission, hygiene level therefore play significant role in the prevalence of *H. pylori* infection.

The infection associated with *H. pylori* have varying symptoms such as upper abdomen pain, nausea, vomiting, weight loss, loss of appetite, heartburn, weakness and fatigue (Yahya., *et al*. 2017; MFMER, 2017; WebMD, 2017; Health line, 2017). *H. pylori* infections can lead to peptic ulcers which could lead to complications such as internal bleeding, obstruction, perforation and peritonitis (Health line, 2017).

Despite the deleterious effect of *H. pylori* in human, its presence in the stomach could decrease the prevalence of esophagitis due to reduction in stomach acid condition that refluxes back into the esophagus (WebMD, 2017). NCI (2013) also reported that epidemiologic evidence suggests that *H. pylori* infection could lead to reduction in the risk of esophageal adenocarcinoma. This could lead to reduction in the risk esophageal cancer among *H. pylori* infected patients.

According to Humeida and Abdalla (2017), Helicobacter pylori infection is associated with several hematological disorders. Therefore, this study aimed at evaluating the effect of haemostatic parameters among *H. pylori* infected students in a tertiary educational institution in Nigeria.

Materials and Methods

Study Area

Subjects of this study were students of Madonna University, Elele, Nigeria. Typically, Rivers state is one of the states in the Niger Delta region of Nigeria. The Rivers state shares boundary with neighboring Niger Delta states including Bayelsa in the Western and southern region, Abia in the East. The climatic condition of the area is similar to other Niger Delta region previously described by Ben-Eledo., *et al*. (2017a,b); Seiyaboh., *et al*. (2017a-c); Seiyaboh and Izah (2017a,b); Ogamba., *et al*. (2015a,b,c; 2017a,b). Farming of food crops such oil palm and cassava is a major economic activity of the resident of the area.

Selection Criteria for Subjects

Inclusion Criteria: Subjects for this research were students of Madonna University, Elele. Patients that tested positive for *H. pylori* using one step anti-HP rapid screen test kit (Lot. Number: 20161115) were selected for the study. A total of 17 female Helicobacter pylori infected patients and 15 male *Helicobacter pylori infected* patients within the age of 18-32 years participated in this study. Another 21 and 20 age-matched control subjects for males and females respectively who were negative for *H. pylori* were selected.

Exclusion criteria: Pregnant women, lactating mothers, and individuals with known cases of HIV/AIDs, hepatitis, tuberculosis, diabetics and cardiovascular diseases.

Blood Collection

Blood samples was collected following standard venipuncture technique previously described by Eledo., *et al.* (2017a, b). Approximately 5 ml of blood was collected from each subject from the anticubital or dorsal vein. About 2.25 ml of blood meant for prothrombin time and activated partial thromboplastin time was dispensed into plastic tube containing 0.25 ml of trisodium citrate, while the remaining 2.75 mls of blood mean for platelets analysis was dispensed into dipotassium EDTA bottles.

Laboratory Analysis

Prothrombin time

The methodology previously applied by Eledo., *et al*. (2017a,b) was adopted for the determination of prothrombin time. The kit used was from Agappe Diagnostics Switzerland. The PT REAGENT was pre-warmed to 37ºC for 10 minutes. Then 0.1 ml of plasma was dispensed into test cuvette at 37ºC and incubated for 3 minutes. About 0.2 ml of pre-warmed PT REAGENT was forcibly added into the test cuvette. The timer was started simultaneously and the time for the first clot to appear was recorded in seconds.

Activated Partial Thromboplastin time

The methodology previously applied by Eledo*., et al*. (2017a,b) was adopted for the determination of activated partial prothrombin time. Reagent 1 ($Cacl_2$) and reagent 2 (APTT REAGENT) were pre-warmed at 37ºC. Then 0.1 ml of the plasma was dispensed into test cuvettes at 37ºC. Then after, 0.1 ml of the pre-warmed reagent was added into the test cuvettes, which was well mixed and incubated at 37ºC for approximately 3 minutes. In addition, 0.1 ml of pre-warmed reagent 1 was added into the test cuvettes. The timer was started simultaneously and the time for the first clot to appear was taken.

Platelets count

Platelets count was determined based on the Cronkite's ammonium oxalate method previously applied by Eledo., *et al*. (2017a,b). Ammonium oxalate was used as the diluent. A 1:20 dilution of blood was made by adding 0.1 ml of blood to 1.9 ml of diluents. The suspension was thoroughly mixed in an improved Neubauer counting chamber. The mixed settled suspension was viewed under x40 objective and the platelets counted. The number of platelets per liter was calculated from the formula:

$$\text{Platelets} = \frac{(\text{Number of cells counted} \times \text{dilution} \times 10^6)}{(\text{volume } (\mu l))}$$

Statistical analysis

SPSS software version 20 was used to carry out statistical analysis. Data were presented and mean ± standard error. The haemostatic values obtained among *H. pylori* patients were compared with the control subjects using "t" test at $P < 0.05$.

Results and Discussion

The effect of on some *H.pylori* on haemostatic parameters in females and males students from a tertiary educational institution in Nigeria is presented in Table 1 and 2 respectively. In female, *H.pylori* infected patients and control subjects had platelets mean platelets

counts of 186.24 x 10^9/L and 271.55 x 10^9/L respectively, being significantly different (P < 0.001) among the test subjects (Table 1). While in males, *H.pylori* infected patients and control subjects had platelets mean platelets counts of 188.53 x 10^9/L and 274.81 x 10^9/L respectively, being significantly different (P < 0.001) among the test subjects (Table 2). The platelet count increased among the *H. pylori* infected patients.

The trend reported in this study, in which the test subjects had higher platelets counts were contrary to the trend previously reported among patients of health status including diabetes (Eledo., *et al*. 2017a), after vigorous exercise (Eledo., *et al*. 2017b), marijuana smoking (Eledo., *et al*. 2015a), but comparable to the trend reported during pregnancy (Eledo., *et al*. 2015b). The significant decline of platelet among *H.pylori* patients suggests possible platelets dysfunction which could predispose the patient to risk of vascular complications.

In female, H.pylori infected patients and control subjects had mean Prothrombin time counts of 15.50 seconds and 11.75 seconds respectively, being significantly different (P < 0.001) among the test subjects (Table 1). While in males, *H.pylori* infected patients and control subjects had mean prothrombin time counts of 13.86 seconds and 11.24 seconds respectively, being significantly different (P < 0.001) among the test subjects (Table 2). The trend in prothrombin time reported in this study is comparable to the trend reported among marijuana smokers as reported by Eledo., *et al*. (2015a), but contrary to the trend reported in different health issues such as diabetes (Eledo., *et al*. 2017a), and after vigorous exercise (Eledo., *et al*. 2017b).

Furthermore, in female, *H.pylori* infected patients and control subjects had mean activated partial prothrombin time counts of 34.53 seconds and 28.50 seconds respectively, being significantly different (P < 0.001) among the test subjects (Table 1). While in males, *H.pylori* infected patients and control subjects had mean activated partial prothrombin time of 32.20 seconds and 29.05 seconds respectively, being significantly different (P < 0.004) among the test subjects (Table 2).

Both activated partial prothrombin time and prothrombin time showed significant increase among the *H. pylori* patients. Typically, prothrombin time, activated partial thromboplastin time play essential role in blood coagulation. Therefore, significant variation among the *H.pylori* patient and control suggest possible vascular complications risk by patients over a prolong period of time.

Parameters	Mean ± standard error		t-value	P-value
	Subjects (n = 17)	Control (n = 20)		
Prothrombin time (PT), secs	15.50 ± 0.18	11.75 ± 0.19	14.232	0.000
Activated partial thromboplastin time (APTT), sec	34.53 ± 0.42	28.50 ± 0.65	7.440	0.000
Platelets counts (PLT)(x10^9/L)	186.24 ± 7.14	271.55 ± 11.71	-5.958	0.000

Table 1: *Effect of H.pylori on some haemostatic parameters in females of a tertiary educational institution in Nigeria.*

Parameters	Mean ± standard error		t-value	P-value
	Subjects (n = 15)	Control (n = 21)		
Prothrombin time (PT), secs	13.86 ± 0.27	11.24 ± 0.54	3.861	0.000
Activated partial thromboplastin time (APTT), sec	32.20 ± 0.84	29.05 ± 0.62	3.091	0.004
Platelets counts (PLT)(x10^9/L)	188.53 ± 10.72	274.81 ± 11.15	-5.385	0.000

Table 2: *Effect of H.pylori on some haemostatic parameters in males of a tertiary educational institution in Nigeria.*

Conclusion

This study evaluated the effect of *H. pylori* on some haemostatic parameters among patients from an educational tertiary institution. The findings of this study showed that *H. pylori* infection leads to significant increase in activated partial prothrombin time and prothrombin time and decline in platelets counts. The variations suggest possible vascular complication. As such caution should be exercise toward effective management of the infection so as to reduce possible risk of other disease condition associated with *H. pylori* infection.

Ethical Consideration

Permission was obtained from the ethics committees of the Medical Laboratory Science Department of Madonna University, Elele, Nigeria. Informed consent was obtained from the patients prior to sample collections.

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