

A correlative study of peptic ulcer and thrombocytopenia in Remo land of Ogun State, Nigeria

John Cletus Ihongbe^{*1}, Ayodele Ilesanmi², Isaac Oluwole Adediji¹,
Ufuoma Laju Otomewo¹ and Adewumi Olasebikan¹

¹Babcock University, Department of Medical Laboratory Science, Ilisan Remo, Ogun State, Nigeria

²Kwara State University, Department of Medical Laboratory Science, Malete, Kwara State, Nigeria.

*Correspondence Info:

Prof. John Cletus Ihongbe
Professor of Medical Microbiology,
Babcock University,
Department of Medical Laboratory Science,
Ilisan Remo, Ogun State, Nigeria
E-mail: ihongbej@yahoo.com

Abstract

Introduction: *Helicobacter pylori* infection is one of the commonest bacterial infections in human world-wide. *H.pylori* is a significant cause of a number of intra-digestive and extra-digestive disorders with increased prevalence in the developing countries.

Objectives: This study investigated the relationship between platelet count and peptic ulcer and also compared the results of the test subjects with the controls.

Methodology: Sixty subjects (age: 27.5±3.2) were enrolled for the study which included forty newly diagnosed peptic ulcer patients from the Medical Outpatient Clinic of a tertiary health institution in Ogun State, Nigeria and twenty age-matched, apparently healthy individuals from the same geographical location were selected as controls. Platelet count analysis was done with BC-5300 Auto Haematology analyzer and the detection of serum *H.pylori* antibodies was done using diaspot rapid chromatographic immunoassay method. Data obtained were statistically analysed using ANOVA, Post-Hoc and Pearson's correlation. P<0.05 was considered significant.

Results: The platelet count was significantly low in *H.pylori* infected peptic ulcer subjects when compared with both *H.pylori* negative peptic ulcer subjects and controls (p<0.05). There was no significant difference in the platelet count of *H.pylori* negative peptic ulcer subjects when compared with controls. There was a significant negative correlation between platelet count and *H.pylori* seropositivity (p<0.05) and also there was a significant positive correlation between platelet count and *H.pylori* seronegativity (p<0.05).

Conclusion: The study concluded that, not all cases of peptic ulcer are associated with thrombocytopenia however, thrombocytopenia in peptic ulcer subjects, is solely associated with *H.pylori* infection.

Keywords: *Helicobacter pylori*, Peptic ulcer, Thrombocytopenia, Immunoassay

1. Introduction

Helicobacter pylori previously called *Campylobacter pylori*, is a Gram negative spiral microaerophilic bacterium that colonizes the mucous layer of the stomach [1].

H.pylori is the causative agent of gastritis, gastric ulcer and duodenal ulcer. It has also been implicated in the pathogenesis of gastric cancer, mucosa-associated lymphoid tissue lymphoma and also a number of extra-digestive disorders including cardiovascular, haematologic and autoimmune diseases. [2,3]

H.pylori was first discovered in 1982 by Australian scientists Barry Marshall and Robin Warren, who detected this organism in patients with

chronic gastritis and gastric ulcer, as these are conditions, which were previously believed to have a microbial cause [3]. The discovery of *H.pylori* is one of the greatest achievements in the modern history of gastroenterology and this has led to fundamental changes in the approach to pathogenesis and treatment of peptic ulcer disease.

H.pylori infection is one of the most common bacterial infections in human world-wide[4,5]. More than 50% of the world's population harbour *H.pylori* in their upper GIT. The prevalence of *H.pylori* infection varies from country to country and it is related to demographic and socio-economic factors [6].

The incidence of *H.pylori* infection is estimated to be approximately 0.5% per year in adults of developed countries. However, the incidence of *H.pylori* infection in developing countries is estimated to be approximately 10-25% per year [7, 8].

The increased prevalence of *H.pylori* infection in the developing countries has been attributed to increased consumption of food from street vendors, lack of proper sanitation, lack of basic hygiene, inadequate diet and overcrowding [9]. Studies conducted in various parts of South Western region of Nigeria reported *H.pylori* infection prevalence rates of 60.5% to 73% [10, 11]. Seroprevalence studies conducted in the same region revealed prevalence rate as high as 88% to 94.5% [12,13].

There are plethoras of evidences which have implicated *H.pylori* in the development of peptic ulcers, in the absence of the use of non-steroidal anti-inflammatory drugs (NSAIDs). About 80% of duodenal ulcers and 70% of gastric ulcers are associated with *H.pylori* infection [14]. Hopkins *et al* (1996) reported that ulcer recurrence was significantly less common among *H.pylori* cured versus non-cured patients (60% versus 67% for patients with duodenal ulcers; 4% versus 59% for patients with gastric ulcers). It was however revealed by the study of Hopkins *et al*, (1996), that there is a relationship between *H.pylori* eradication and reduced recurrence of duodenal and gastric ulcers. [15]

H.pylori employs molecular mimicry to evade recognition by the host immune system, thereby establishing itself within the gastric mucosa. For instance, *H.pylori* flagellar proteins avoid being recognised by toll-like receptors [16]. Also, *H.pylori* lipopolysaccharides mimic host molecules such as Lewis antigens [17].

During infection, when the bacterium invades the gastric lumen, *H.pylori*-urease functions mainly as a protective buffering enzyme which allows the survival of the organism within the gastric acid environment. *H.pylori* injects CagA (Cytotoxin associated gene A) into host cells and releases other toxic factors such as VacA and HP-NAP (HP-NAP). Injected CagA triggers the release of proinflammatory cytokines which elicit recruitment of lymphocytes and consequent release of reactive oxygen intermediates (ROIs) which amplify inflammation. HP-NAP elicits the recruitment of neutrophils and monocytes, thus bringing about tissue damage by releasing ROIs. The combined toxic activity of CagA and ROIs leads to toxic tissue damage which is enhanced by loosening of protective mucus layer and acid permeation [18].

Stasi *et al* (2009) reported that *H.pylori* detection and eradication have been associated with unexplained iron deficiency anaemia and thrombocytopenia. Thrombocytopenia can however occur due to either decreased production or increased destruction of platelets [19].

Due to the mimicry of the host immune system mechanism exhibited by *H.pylori* virulence factors, the organism has been found to be closely associated with immune thrombocytopenia purpura (ITP). ITP is characterised by opsonisation of platelets by autoreactive antibodies and these opsonised platelets, are destroyed eventually by the reticuloendothelial system [20].

Elizade *et al* (1997) reported that *H.pylori* platelet activating factor promotes thrombotic occlusion of surface capillaries with consequent aggregation of circulating platelets and as a result of this, thrombocytopenia ensues [21].

Due to the variability of *H.pylori* strain and its infection which is strain-dependent, this study therefore aimed to determine if there would be any relationship between platelet counts and *H.pylori* infection in peptic ulcer patients in Remo- land, Ogun State, and to also compare these patients' results with those from apparently healthy subjects without peptic ulcer. This study was the first to investigate if there would be any relationship between platelet counts and *H.pylori* peptic ulcer in the South West region of Nigeria.

2. Materials and Methods

2.1 Subjects

A total of sixty subjects were randomly recruited which included, 40 newly diagnosed peptic ulcer patients at the Medical Out Patients' Clinic of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State (6°50'North, 3°39'East). These participants were divided into 20 *H.pylori* positive peptic ulcer patients (Group A) and 20 *H.pylori* negative peptic ulcer patients (Group B). 20 apparently healthy subjects were selected as controls and they were all seronegative for *H.pylori* (Group C). Individuals who had previously been diagnosed of peptic ulcer were excluded from the control group. All the subjects belonged to the same socio-economic class of the society. Informed, written consent was obtained from the participants. The study was approved by Babcock University ethical committee.

2.2 Sample collection and analysis

Blood sample (5ml) was collected by venepuncture from each of the subjects using sterile disposable needles and syringes.

Three millilitres (3ml) of the blood was dispensed into properly labelled ethylene diamine

tetra acetic (EDTA) container and two millilitres (2ml) was dispensed into a plain container. The samples in EDTA were analyzed for platelet count within 3 hours of collection with BC-5300 Auto Haematology analyzer. The samples in the plain containers were allowed to retract, to obtain sera. *H.pylori* antibodies were detected in the sera using diaspot rapid chromatographic immunoassay method.

2.3 Statistical analysis

Statistical analysis was done using statistical package for social sciences (SPSS) version 13.0 Results were expressed as Mean±S.D. Post-hoc was used for comparison and Pearson's correlation was

used for correlation analysis. P of less than 0.05 was considered significant.

3. Results

Table 1 shows the comparison of platelet counts in *H.pylori* positive peptic ulcer subjects, *H.pylori* negative peptic ulcer subjects and the control subjects. The platelet counts of *H.pylori* positive peptic ulcer subjects were significantly lower than the platelet counts of *H.pylori* negative peptic ulcer subjects and the control subjects. However, no significant difference was observed in the platelet counts of both *H.pylori* negative peptic ulcer subjects and control subjects.

Table 1: Comparison of platelet counts for all groups using ANOVA and Post-Hoc

	Group A (n=20)	Group B (n=20)	Group C (n=20)	F-value	p-value
Platelet count (mean±s.d)	91.2±65.6 ^{x,y}	216.0±36.7 ^z	218.2±49.8	38.4	0.000*

Group A= *Helicobacter Pylori* positive peptic ulcer subjects.

Group B= *Helicobacter Pylori* negative peptic ulcer subjects.

Group C= Control subjects (non-peptic ulcer subjects and seronegative for *H.pylori*)

x= significantly different from control subjects (Post-Hoc)

y= significantly different from *H.pylori* negative subjects (Post-Hoc)

z= not significantly different from control subjects (Post-Hoc)

* Significant at P< 0.05

Table 2 shows that there was a significant negative correlation between platelet count and *H.pylori* seropositivity. Also, there was a significant positive correlation between platelet count and *H.pylori* seronegativity.

Table 2: Relationship between platelet counts, *H.pylori* seropositivity and *H.pylori* seronegativity using Pearson's correlation

Platelet count	r	p
<i>H.pylori</i> seropositive peptic ulcer subjects	-0.865	0.000*
<i>H.pylori</i> seronegative peptic ulcer subjects	0.826	0.000*
Controls (<i>H.pylori</i> seronegative)	0.880	0.000*

* Significant at P< 0.05

4. Discussion

The results of the present study showed that there was no significant difference in the platelet counts of *H.pylori* negative peptic ulcer subjects and control subjects. Meanwhile, there was a significant decrease in the platelet counts of *H.pylori* infected peptic ulcer subjects when compared with the platelet counts of *H.pylori* negative peptic ulcer subjects and control subjects. The observed decrease could be attributed to destruction of platelets and suppression of platelet production by autoreactive antibodies. The presence of cross-reaction between antibodies against *H.pylori* cytotoxin-associated gene (Cag-A) and

platelet surface antigens, leading to accelerated platelet clearance, is the major mechanism underlying *H.pylori* associated thrombocytopenia [22, 23].

Furthermore, there was a significant negative correlation between platelet counts and *H.pylori* seropositivity, which indicated an inverse relationship between platelet counts and *H.pylori* seropositivity. There was a significant positive correlation between platelet counts and *H.pylori* seronegativity which indicated that *H.pylori* seronegativity was found to be associated with normal platelet counts. This is however, in agreement with the report of Ando *et al* (2013) who demonstrated a significant association between the disappearance of anti-CagA antibodies in serum and improvement of *H.pylori* associated thrombocytopenia. [14]

Zahran *et al* (2013) also reported that pre-treatment platelet counts of *H.pylori* infected peptic ulcer patients was lower than the post-treatment platelet counts obtained, after the eradication of *H.pylori* in test subjects.[25]

5. Conclusion

The findings in this study showed that not all cases of peptic ulcer are associated with thrombocytopenia, it is only *H.pylori* related peptic ulcer that is associated with thrombocytopenia. Though the pathogenic mechanism of *H.pylori* induced thrombocytopenia still remains controversial,

prospective studies on a large number of subjects need to be done to explain this association especially among individuals in the developing countries.

Clinical laboratory investigation of *H.pylori* should be considered in every suspected case of thrombocytopenia in order to facilitate treatment and eradication of *H.pylori* and consequently ameliorate the health status of the patient.

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