

# Assessment of Some Immune System Related Parameters on *Helicobacter pylori* Infected Students in a Nigerian Tertiary Educational Institution

Dennis Oju Allagoa<sup>1</sup>, Benjamin Onyema Elede<sup>2,3</sup>, Kingsley Excel Dunga<sup>2</sup>,  
Sylvester Chibueze Izah<sup>4,\*</sup>

<sup>1</sup>Department of Gynecology and Obstetrics, Federal Medical Centre Yenagoa, Bayelsa state, Nigeria

<sup>2</sup>Department of Medical Laboratory Science, Faculty of Health Sciences, Madonna University, Elele, Nigeria

<sup>3</sup>Haematology and Blood Transfusion Department Federal Medical Centre, Yenagoa, Nigeria

<sup>4</sup>Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Nigeria

## Email address:

chivestizah@gmail.com (S. C. Izah)

\*Corresponding author

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**Abstract:** This study evaluated the effect of *Helicobacter pylori* infection on total white blood cells and cluster of differentiation 4 (CD4) cells of students in a Nigerian tertiary educational institution. A total of 32 test subjects (comprising of 17 females and 15 males) and 30 control subjects (comprising of 16 females and 14 males) within the age of 18 – 32. The blood samples of the participants were analyzed for CD4 cells and total white blood cells counts using standard protocols. Results revealed that test and control subjects had mean values of  $8.89 \times 10^9/L$  and  $7.13 \times 10^9/L$  respectively (for females) and  $9.12 \times 10^9/L$  and  $7.25 \times 10^9/L$  respectively (for males) (total white blood cells), and 1369.76 cells/ $\mu l$  and 2169.55 cells/ $\mu l$  respectively for females and 1424.47 cells/ $\mu l$  and 2069.15 cells/ $\mu l$  respectively for males (CD4 counts). Significant variations ( $p < 0.001$ ) exist between test and control subjects for each of the immune system parameters under study. The significant increase in total white blood cells among the test subjects is a result of an immune response to an active infection and subsequent neutrophilia elicited by inflammation which is a major consequence of *Helicobacter pylori* infection. The decline in CD4 (though within normal range) may suggest that *Helicobacter pylori* infection may affect the immune system. However, caution should be exercised in the management of the infection especially among individual with other health challenges to avoid adverse health effects.

**Keywords:** Cluster of Differentiation 4, *Helicobacter Pylori*, Immune System, Infections, White Blood Cells

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## 1. Introduction

*Helicobacter pylori* is among the microorganism found in the interior part of the human system. Marshall and Warren [1], Xu *et al.* [2] reported that *Helicobacter pylori* is found on the gastric mucosa in the human body. Authors have variously reported that *Helicobacter pylori* is a pathogenic gram negative bacterium that is microaerophilic, and spiral and rod-like in shape [2 - 6].

Globally, the prevalence of *Helicobacter pylori* is affected

by geographic area, race, ethnicity, age, gender and socio-economic status [7] and it affects nearly 50% of global population [8]. The global prevalence is still high in many regions especially in developing nations. Campuzano-Maya [9] reported that *Helicobacter pylori* infection very common with wide range of disparity between developed and developing nations. According to Rahmani *et al.* [8], Campuzano-Maya [9], McMahon *et al.* [10] about 80% and

20 – 50% of *Helicobacter pylori* occurs in developing and developed regions of the world respectively. Carabotti *et al.* [11] reported prevalence *Helicobacter pylori* in the range of 8.7 – 85.5%. Rahmani *et al.* [8] reported the incidence of the infection to vary from 15 – 85%. Specifically, Şenkaynağı and Yıldız [6] reported *H. pylori* infection of about 43.34% in Isparta province.

*Helicobacter pylori* infection is among the cause of some upper digestive system infection since 1984 [8]. *Helicobacter pylori* infection plays an essential role among chronic gastritis [2, 9, 12], stomach cancer and etiology of stomach lymphoma “mucosa-associated lymphoid tissue” [6], duodenal and gastric peptic ulcers [9] and it has been reported to alter some haematological parameters [13, 14] though its involvement is not well understood [2]. According to Yahya *et al.* [4], Eledo *et al.* [13, 14], *Helicobacter pylori* has the capacity to cause ulcer and inflammation in the stomach. It also has the tendency to weaken the protective coat of the stomach, thereby enhancing the irritation of the sensitive part of the stomach lining [3, 13].

*Helicobacter pylori* infection has been implicated in the etiopathogenesis of certain non-gastrointestinal disease including atherosclerosis, diabetes mellitus, and insulin resistance [6, 14]. *Helicobacter pylori* also have the tendency to occur with other disease conditions. For instance, Rostami-Nejad *et al.* [15] reported that association of the bacterium with iron deficiency anaemia. Rahmani *et al.* [8] reported the occurrence of *Helicobacter pylori* with myocardial infarction in Iran. Evidence resulting from epidemiological and clinical studies have supported the association between some disease condition with *Helicobacter pylori* including anaemia [2], myocardial Infarction [8] though this is affected by areas and countries. Campuzano-Maya [9] further reported that since it was discovered that *Helicobacter pylori* could colonize the gastric mucosa it has been reported in medical literature of over 50 extragastric manifestations compromising of varieties of specialty including cardiology, dermatology, endocrinology, gynecology and obstetrics, haematology, pneumology, odontology, ophthalmology, otorhinolaryngology and pediatrics, and they involve conditions with clear indication of *Helicobacter pylori* infection and disease developments.

Basically, *Helicobacter pylori* test is carried out on individuals with stomach ache to confirm the presence of the infection and gastric ulcer. During the analysis, blood sample is usually required just like in many other haematological parameters investigation. According to Humeida and Abdalla [16], *Helicobacter pylori* infection has the tendency to alter some haematological parameters. Previous studies have reported the effects of *Helicobacter pylori* infection on some haemostatic parameters viz: platelets, prothrombin time and activated partial prothrombin time [3], haematological parameters viz: hemoglobin, packed cell volume and erythrocyte sedimentation rate [13]. Therefore, this present study focused on total white blood cells and cluster of differentiation 4 (CD4) cells among *Helicobacter pylori* infected patients in a tertiary educational institution in

Nigeria.

## 2. Materials and Methods

### 2.1. Study Area

This study was carried out among undergraduate students of an educational institution in Elele, Rivers state, Nigeria. The area lies in the sedimentary basin and farming is a major occupation of the indigenous people of the area. Other economic sources of livelihood include civil service jobs and petty trading. Like other Niger Delta region, there are two predominant seasons viz: wet (April to October) and dry (November to March of the following year).

### 2.2. Selection Criteria

Inclusion Criteria: Subjects for this research were students of Madonna University, Elele. *H. pylori* infection was determined using one step anti-HP rapid screen test kit (Lot. Number: 20161115) and positive patients including 17 female and 15 males within the age of 18 – 32 years participated in the study. Furthermore control (individual with no *Helicobacter pylori* infection) was also established using 14 males and 16 females within the same age grade.

Exclusion criteria: Pregnant women, lactating mothers, and individuals with known cases of HIV 1&2 hepatitis, tuberculosis, diabetics and cardiovascular diseases.

### 2.3. Blood Collection

The blood samples were collected through the antecubital or dorsal vein following venipuncture techniques from each of the subjects under study. The blood samples were dispensed into dipotassium EDTA tubes.

### 2.4. Laboratory Analysis

#### 2.4.1. Total White Blood Cell Estimation

The total white blood cells was carried out using Turk's solution and diluents as previously described by Eledo *et al.* [17]. The resultant leukocytes in the 1mm<sup>2</sup> areas of the top and lower extremes of the counting chamber as observed with the x10 objectives were calculated as:

$$TWBC = \frac{\text{No of cells counted}}{\text{Volume counted (ml)}} \times \text{Dilution} \times 10^6$$

#### 2.4.2. Enumeration of CD4 Count Using Partec Cyflow

The cluster of differentiation 4 was analyzed using PartecCyflow counter equipped with portable/mobile flow cytometry system for the identification and the enumeration of the CD4 helper/inducer T-lymphocyte subset. The methods have been described by Eledo *et al.* [17]. Approximately 20-µl of blood was added into a Partec tube and mixed with 20µl of CD4 monoclonal antibody, which is mixed and incubated for 15 minutes. Then 800µl of no lyse buffer was added to the Partec tube and it was mixed. The mixture was analyzed using Partec device. Sysmex CD4 easy count kit Lot no: 528576 antibody was used.

### 2.5. Statistical Analysis

Statistical Package for Social Sciences software version 20 was used for the statistical analysis viz: mean standard deviation and t-test. Significant difference between the subjects and control group was observed at  $\alpha = 0.05$ .

## 3. Results and Discussion

The total white blood cell and CD4 count of males and females in a Nigerian tertiary educational institution with *Helicobacter pylori* infection is presented Table 1 and 2 respectively. In the *Helicobacter pylori* infection and control subjects, the total white blood cell were  $8.89 \times 10^9/L$  and  $7.13 \times 10^9/L$  respectively (females) (Table 1), and  $9.12 \times 10^9/L$  and  $7.25 \times 10^9/L$  respectively (males) respectively (Table 2) (Figure 2). Significant variation ( $P < 0.001$ ) between test and control subjects exists. This trend of white blood cells being higher in patients with some health conditions compared to the control have been reported in wide range of health conditions including post-menopausal women [18]. However, the significant higher white blood cell counts suggest the ability of the body system to fight infections. According to Eledo et al. [17], white blood cells is produced in the stem of the bone marrow and it consist of granulocytes (neutrophils, eosinophils, and basophils) and non-granulocytes (lymphocytes and monocytes) help the body against infections (viz pathogenic microbes and other infections).

The CD4 cells counts for the test and control subjects were 1369.76 cells/ $\mu$ l and 2169.55 cells / $\mu$ l respectively for females (Table 1) and 1424.47 cells/ $\mu$ l and 2069.15 cells / $\mu$ l respectively for males (Table 2) (Figure 2). Significant variation ( $P < 0.001$ ) exist between the test and control subjects. The CD4 play essential role in the body's immune system and are found in certain immune cells including T-cells, macrophages, and monocytes [17]. The CD4 T-cells

have been considered as "helper" cells probably due to its ability to trigger body's response against infections [17]. Hence, the lower CD4 counts among the *Helicobacter pylori* infected patients suggests lowering of the T-cell mediated immune system. Though the values of CD4 *Helicobacter pylori* infected patients are within normal limits just as the control.

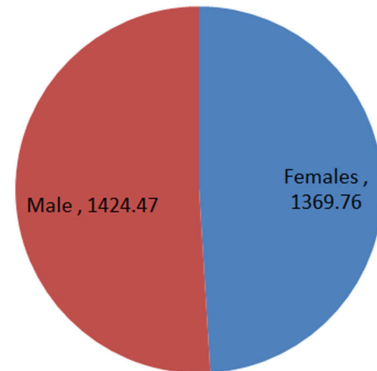


Figure 1. Sex based distribution of CD4 on *Helicobacter pylori* patients.

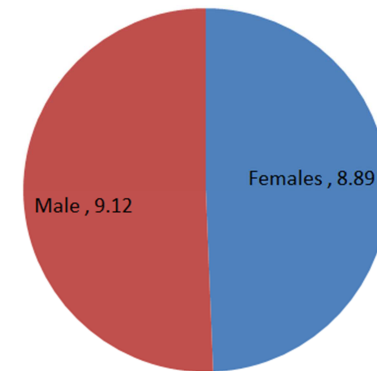


Figure 2. Sex based distribution of total white blood cell on *Helicobacter pylori* patients.

Table 1. Effect of *Helicobacter pylori* on total white blood cells and cluster of differentiation 4 in females of a tertiary educational institution in Nigeria.

Parameters	Mean $\pm$ standard error		t-value	P-value
	Subjects (n=17)	Control (n=16)		
Cluster of differentiation 4, cells/ $\mu$ l	1369.76 $\pm$ 102.99	2169.55 $\pm$ 93.77	-5.748	0.000
Total white blood cells, $\times 10^9/L$	8.89 $\pm$ 0.20	7.13 $\pm$ 0.23	5.678	0.000

Table 2. Effect of *Helicobacter pylori* on total white blood cells and cluster of differentiation 4 in males of a tertiary educational institution in Nigeria.

Parameters	Mean $\pm$ standard error		t-value	P-value
	Subjects (n=15)	Control (n=14)		
Cluster of differentiation 4, cells/ $\mu$ l	1424.47 $\pm$ 102.53	2069.15 $\pm$ 88.03	-4.777	0.000
Total white blood cells, $\times 10^9/L$	9.12 $\pm$ 0.22	7.25 $\pm$ 0.25	5.356	0.000

## 4. Conclusions

This study investigated the effect of *Helicobacter pylori* infection on total white blood cells and CD4 cell counts amount students of a Nigerian tertiary institution. The results of the study showed that *Helicobacter pylori* infection causes an increase in total white blood cells while the CD4 cell

counts showed a slight decrease in both males and females. Despite the significant decline the values are still within normal limits. Hence caution should be exercised in the management of *Helicobacter pylori* infection especially when associated with other health conditions that could also lower the immune system.

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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