Research Article

Effect of *Helicobacter pylori* Infection on Haematological Parameters in Kosti Teaching Hospital, Sudan

Elham Ali Ibrahim Elamin,[1] Mohammed Ahmed Suliman,[2] Mohammed Eltoum Azoz,[3] Elshazali Widaa Ali,[4] Livesey David Olerile,[5] Yulian Jiao,[6] and Yueran Zhao[6,*]

[1]Department of Clinical Laboratory Diagnostics, Shandong Provincial Hospital Affiliated to Shandong University, Shandong University, 44 Wenhua Xi Road, Jinan 250012, China
[2]Department of Parasitology, Faculty of Medical Laboratory Science, El Imam El Mahdi University, Kosti, Sudan
[3]Department of Surgery, Faculty of Medicine and Health Sciences, El Imam El Mahdi University, Kosti Teaching Hospital, Kosti, Sudan
[4]Department of Haematology, Faculty of Medical Laboratory Sciences, Al Neelain University, Khartoum, Sudan
[5]The School of Pharmaceutical Sciences, Shandong University, 44 Wenhua Xi Road, Jinan 250012, China
[6]Department of Central Laboratory, Shandong Provincial Hospital Affiliated to Shandong University, Shandong University, 324 Jingwu Road, Jinan 250021, China

[*] *Corresponding author*: Yueran Zhao, Department of Central Laboratory, Shandong Provincial Hospital Affiliated to Shandong University, Shandong University, 324 Jingwu Road, Jinan 250021, China. Tel: +86-15168867196, E-mail: yrzhao@sdu.edu.cn

Abstract

Background: *Helicobacter pylori* infection is a major global gastric infection and has been associated with changes in haematological parameters.

Objectives: The present study aimed at establishing the effect of *H. pylori* infection on haematological parameters.

Methods: We conducted a case-control study among 98 patients and 62 controls in Kosti teaching hospital in Sudan from July 2014 to July 2015. All patients and controls were investigated for complete blood count, peripheral blood picture, and *Helicobacter pylori* infection. Data were analysed using SPSS Version 23 at 95% confident interval.

Results: There was a significant effect of *H. Pylori* infection on all haematological parameters ($p \leq 0.001$), except for MCHC, WBCs, and total differential ($p \geq 0.001$), at the rate of 89.80%, infection was recorded in patients with gastritis. However, after one month follow-up, the Hb, PCV, MCV Retic, and platelet count for GIa were found to be not significant ($p \geq 0.001$) for all; 12.11 ± 1.11 g/dL, 40.33 ± 3.09 %, 81.36 ± 3.5 fL and 1.38 ± 0.84 %, $233700.0000 \pm 34947.7400$ cmm³, respectively. On the other hand, GIb, Hb, PCV, MCV, Retic and platelet counts were reported to be significant ($p \leq 0.001$), for all; 10.33 ± 1.82 g/dL, 33.99 ± 3.48%, 111.01 ± 7.89 fL, 3.20 ± 1.24 % and $112895.6000 \pm 41043.0500$ cmm³. In the GII, all were found to be significant ($p < 0.001$), 8.58 ± 1.93 g/dL, 30.86 ± 3.10 %, 60.13 ± 4.44 fL 4.53 ± 1.84%, $317818.2000 \pm 91442.7600$ cmm³, except for the retic and platelet count ($p \geq 0.001$).

Conclusions: There was a significant effect of *H. pylori* infection on haematological parameters. The complete blood count and peripheral blood picture revealed that group I_a, group I_b, and group II were normocytic anemia, macrocytic anemia, and microcytic anemia, respectively.

Keywords: Anemia, Endoscopy, *Helicobacter pylori*, Infection, Outpatients, Sudan

1. Background

According to world health organization (WHO), nearly 550,000 new cases of stomach cancer, attributed to Helicobacter pylori (*H. pylori*), are recorded annually. This represents about 55% of all cases of this cancer globally (1, 2). Nearly more than 50% of the adult population in developed countries and 90% of those in the developing countries are infected with this bacterium (3).

H. pylori is a Gram-negative spiral-shaped bacterium that grows in the upper digestive tract (4). Previous studies have reported that *H. pylori* infection is a major cause of gastritis, gastric ulcer, duodenal ulcer, and increases the risk of gastric cancer (5-10).

In recent years, some researchers began to realize that *H. pylori* is also closely related to diseases such as ischemic cerebrovascular disease, primary immune thrombocytopenia (ITP), mucosa-associated lymphoid tissue (MALT) lymphoma, and iron deficiency anemia (IDA) (7, 11). Anemia is a condition characterized by a decrease in the number of red blood cells (RBCs) or low oxygen-carrying capacity, hemoglobin (Hb) < 13 g/d in males and Hb < 12 g/d in females. It is further associated with low serum iron or low serum vitamin B12 levels being defined as having iron or vitamin B12 deficiency (12-14). About 50% of all cases of ane-

mia are caused by iron deficiency, low iron intake, chronic blood loss, increased iron requirement, and poor absorption (15). *H. Pylori* infection is the most common cause of gastritis in Sudan (3).

We conducted this study to establish the effect of *H. pylori* infection on haematological parameters such as hemoglobin (Hb) concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), reticulocyte (retic) count platelet count, white blood cell (WBCs) count, and differential as well as peripheral blood picture (PBP). We hypothesized that there is a correlation between *H. pylori* and anemia based on the aforementioned haematological parameters.

2. Methods

2.1. Study Population and Design

We conducted a case-control study among 98 adult patients (27 males, 71 females) suffering from gastritis who underwent endoscopy for the first time. Also, 62 healthy control (HC) volunteers (19 males and 43 females) were enrolled in the study. This study was conducted in Kosti teaching hospital in city of Kosti, White Nile State, Sudan, from July 2014 to July 2015. The expected proportion in controls was 0.05, the assumed odd ratio 4, confidence level 0.95, power 0.8, and sample size per group was 98.

2.2. Inclusion and Exclusion Criteria

All patients having gastritis with or without *H. pylori* infection were included in the study. On the other hands, those who had other conditions, such as liver disease, renal disease, malaria parasitic infection, vitamin B_{12} deficiency, and pregnant women in all nine patients were excluded from the study.

2.3. Sampling Procedure

A total of 98 patients were screened for *H. pylori* infection as the first stage. Of them, 55 tested negative (Group I) and 43 tested positive (Group II). One month later, we conducted a follow-up screening, which revealed that out of 55 who tested negative during the first stage, 45 tested positive (group 1b) making 88 positives and leaving only 10 testing negative (group 1a). This result remained the same all through the end the study. Patients and HC were investigated for CBC, PBP, and *H. pylori*.

A 4 mL of venous blood was freshly collected from each participant at pre and post endoscopy. Of the 4 mL venous blood, 2 mL was added to a container with ethylene diamine tetra acetic acid (EDTA) and the other 2 mL was in a plain container for *H. pylori* test.

2.4. Estimation of Haematological Parameters

Automated hematology analyzer Sysmex KX-21 (Sysmex Corporation, Kobe, Japan) was used to analyse the blood samples after it has been calibrated to minimize instrumental errors. Immediately after this, we conducted estimation of haematological parameters, Hb, PCV, MCV, MCH, MCHC, retic count platelet count, WBCs count and differential as well as PBP. For retic count, equal volume of 50 μL of supra-vital stain, such as Azure B, was added to EDTA anticoagulated blood into 75 × 10 glass test tube and mixed gently. Then, the mixture was incubated at 37°C for 20 minutes, prepared proper smears, was air-dried and examined using Olympus microscope CH20 Japan by oil immersion lens and differential counter in 10 microscopic fields. The reticulocytes were calculated as the percentage of the total red cell count (16).

2.5. H. Pylori Screening

H. pylori was screened by rapid immune chromatography test. Serum was obtained by centrifugation of the blood for five minutes at 4000 rpm. Then, it was decanted into Eppendorf tubes and labeled with an identification number. All devices, serum, and controls were equilibrated to room temperature prior to testing. The devices were placed on the clean and level surface, the dropper was held vertically and 120 μL was added to the specimen (S) of the test device. The timer was started at 10 minutes. Two distinct red lines appeared for a positive test, and 1 red line for a negative test in the control region (C) as per manufacturer's instruction (17, 18).

2.6. Statistical Analysis

Data from the study were exported into the statistical package for social Sciences software (SPSS Version 23, Chicago, IL, USA) from Microsoft Excel 7. All data were expressed as the mean ± standard deviation (SD). Statistical differences between groups were evaluated using two-tailed independent student's t test; $P < 0.05$ was considered statistically significant.

2.7. Ethical Statement

The study protocol was reviewed and approved by Kosti teaching hospital ethics committee (No.K.T.H.1.1) (White Nile State, Sudan) in accordance with the declaration of Helsinki. An ethical clearance letter was also obtained from the ethics committee of the ministry of health in Sudan. Eligible participants were asked to provide written consent and they were ensured of the confidentiality of their information. Also, they were assured that their participation would not affect their relationships with health institutions at the time of the study or in the future and that refusal to participation would not have any penalty.

3. Results

3.1. Comparison of Demographic and Haematological Parameters Between Patients and Control Groups

This study included a total of 98 patients, 27 (27.55%) were males and 71 (72.45%) females. As many as 88 (89.80%) of those with gastritis were tested positive for *H. pylori* infection. Of the 62 matched healthy volunteers, 19 (30.64%) were male and 43 (69.35%) were female. The mean age for patients and control groups was 47.21 ± 14.97 and 43.11 ± 20.92, respectively, $P = 0.15$.

The results revealed that red blood cell parameters (Hb, PCV, MCV, and MCH) were low, while the platelet and retic count were significantly increased in patients with gastritis compared to those without gastritis. In contrast, no statistically significant difference was found in MCHC, total differential, and leukocytes count (Table 1).

Table 1. Comparison of Demographic and Haematological Parameters Between Patients and Control Groups[a]

Parameter	Patients (N = 98)	Control (N = 62)	P Value[b,c]
Age, y	47.21 ± 14.88	45.63 ± 10.27	0.15
Gender, M/F	27/71	27/46	-
***H. pylori*, with/without**	88/10	0/98	-
Gastritis, yes/no	yes	no	-
Hb, g/dL	12.05 ± 1.45	14.98 ± 1.11	0.001
PCV, %	38.15 ± 4.36	45.88 ± 3.09	0.001
MCV, fL	83.69 ± 4.73	91.05 ± 1.57	0.001
MCH, pg	26.16 ± 1.82	28.05 ± 1.50	0.001
MCHC, g/dL	33.85 ± 1.70	34.20 ± 1.22	0.16
Retic count, %	2.58 ± 0.88	1.69 ± 0.53	0.001
Platelet count, c/mm³	274320 ± 160672.9	202250 ± 37170.296	0.001
WBCs, c/mm³	5900 ± 1850	5830 ± 1900	0.82
Neutrophil, %	59.69 ± 9.59	58.93 ± 7.57	0.60
Eosinophil, %	4.75 ± 2.93	4.33 ± 1.72	0.31
Monocyte, %	5.67 ± 1.92	5.67 ± 1.35	1.00
Lymphocyte, %	29.75 ± 5.81	28.53 ± 6.80	0.23

Abbreviations: Hb, Hemoglobin; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; PCV, Packed Cell Volume; Retic, Reticulocyte; WBCs, White Blood Cells.
[a]Value are expressed as Mean $\pm$ SD
[b]$P < 0.05$ was considered statistically significant.
[c]Student's t-test.

3.2. Evaluations of Haematological Parameters Between Patients, Control Groups, and Their Correlation with H. Pylori

Table 2 displays patient groups, controls (62 participants), GI$_a$ (10 patients), GI$_b$ (45 patients) and GII (43 patients). GI$_b$ and GII tested positive for *H. pylori* infection.

However, previously GI$_b$ was tested negative. As presented in Table 2, Hb and PCV levels were significantly lower after 1 month. MCV was significantly increasing for group I$_b$ and decreasing but it remained within normal range for group I$_a$. However, GII increased.

3.3. Platelet Count Between Control and Patients' Groups

Table 3 shows platelet count for patients' groups and controls. Statistically, no significant difference was found pre and post study for group I$_a$, group II, and the control group. On the other hand, group I$_b$ was significantly decreased. When comparing patients with controls, it was found that platelets were significantly increased ($P < 0.0001$), except for group I$_b$, which was significantly decreased at the end ($P < 0.0001$).

3.4. Morphological Examination of Reticulocyte Count

The reticulocytes are juvenile red blood cells that have a remnant of ribonucleic acid (RNA). These remnants were stained with a supra-vital stain, such as Azure B, to give them dark blue color, which appears as granules (Figure 1).

Figure 1. A Morphological Examination of Reticulocyte Count
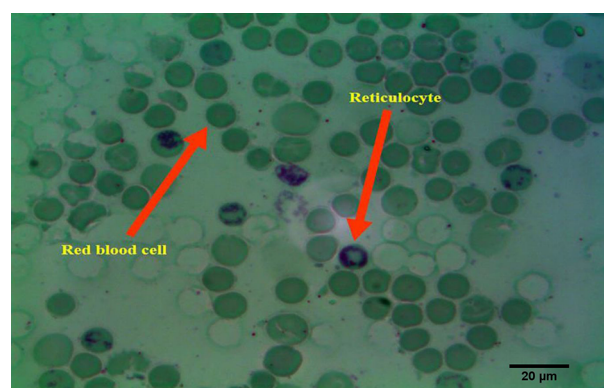
Reticulocyte and red blood cells are indicated by red arrow.

3.5. Morphological Blood Picture

Peripheral blood picture in the diagnosis of anemia (Figure 2) indicated that group I$_a$ showed normocytic normochromic red blood cells, the normal morphology of white blood cells, and adequate platelets. For group I$_b$, macro-ovalocytes, anisocytosis, poikilocytosis with teardrop, and target red cells were observed. In addition, group II showed dimorphic hypochromia with pencil shape and few target cells. The white cells were normal with an increased number of platelets.

Table 2. Evaluations of Haematological Parameters Between Control, Patients' Groups and its Correlation with *H. Pylori* (N = 3)[a]

Parameter	Hb, g/dL			PCV, %			MCV, fL			Retic, %		
	p[b]	E[c]	P Value	p[b]	E[c]	P Value	p[b]	E[c]	P value	p[b]	E[c]	P Value[d]
Control (n = 62)	14.70 ± 1.4	15.20 ± 2.2	0.13	44.02 ± 3.36	45.10 ± 3.18	0.07	89.02 ± 3.04	90.00 ± 4.50	0.16	1.39 ± 0.50	1.40 ± 0.73	0.93
GI$_a$ (n = 10)	12.96 ± 1.02	12.11 ± 1.11	0.09	39.97 ± 2.78	40.33 ± 3.09	0.79	81.36 ± 3.5	83.36 ± 3.44	0.56	1.33 ± 0.91	1.38 ± 0.84	0.99
GI$_b$ (n = 45)	12.98 ± 1.22	10.33 ± 1.82	0.001	40.09 ± 3.32	33.99 ± 3.48	0.001	78.27 ± 1.75	111.01 ± 7.89	0.001	1.76 ± 0.49	3.20 ± 1.24	0.001
GII (n = 43)	10.51 ± 1.11	8.58 ± 1.93	0.001	33.06 ± 2.30	30.86 ± 3.10	0.001	64.13 ± 5.88	60.13 ± 4.44	0.001	4.13 ± 0.68	4.53 ± 1.84	0.18

Abbreviations: GI$_a$, group I$_a$; GI$_b$, group I$_b$; GII, group II; Hb, hemoglobin; MCV, mean corpuscular volume; PCV, packed cell volume; Retic, reticulocyte.

[a] Control, pre and at the end of the study with no *H. pylori* and haematological abnormalities, No statistical significance (P > 0.05) in all parameters. Group I$_a$, patients having gastritis and negative for *H. pylori*. No statistical significance (P > 0.05) was noted in all parameters. Group I$_b$, patients having gastritis, negative for *H. pylori* pre -endoscopy. At the end of the study, *H. pylori* were positive with high MCV and reticulocyte count; Statistical significance (P < 0.05) was recorded in all haematological parameters. Group II, patients having gastritis and *H. pylori* pre-endoscopy. At the end of the study, Hb, MCH, and MCV were lower except reticulocytes.

[b] p, pre endoscopy.

[c] E, at the end of the study.

[d] Student's t-test.
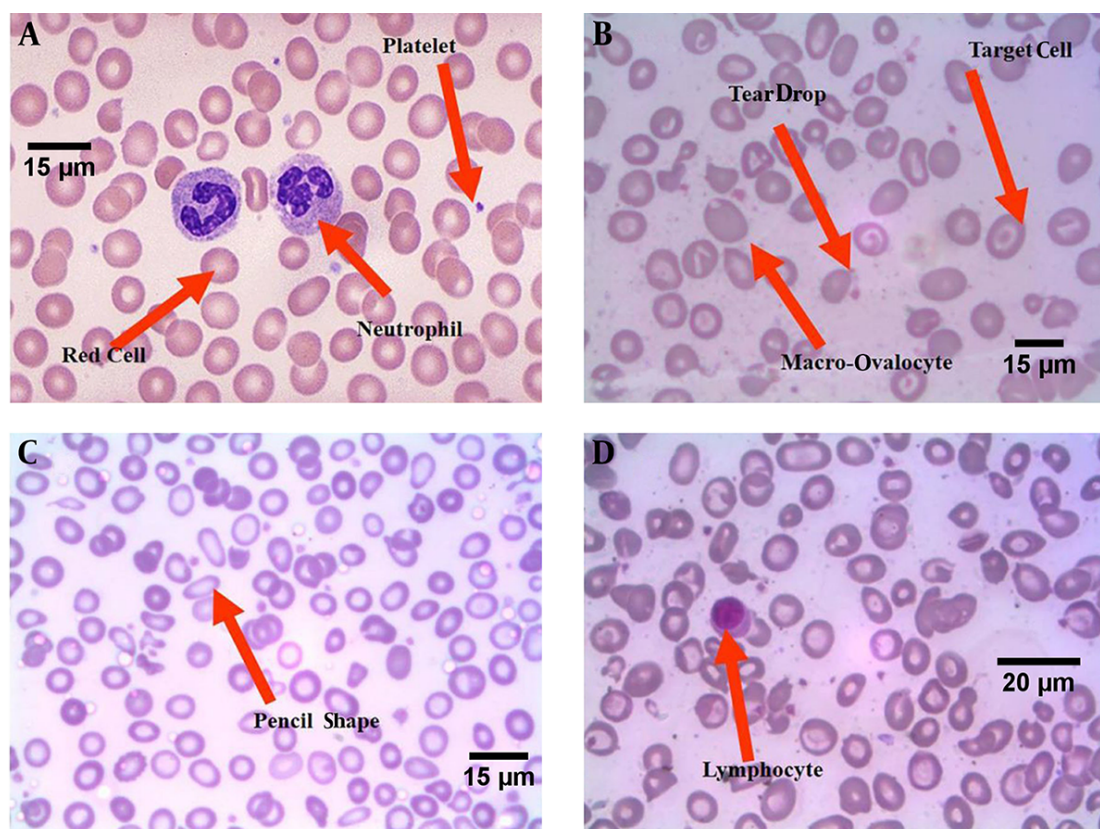
Figure 2. Morphological Blood Picture for A. Normal Morphology; B, Macro- ovalocyte; C, Microcytic Hypochromic with Pencil Shape and D, Lymphocyte

4. Discussion

Gastric mucosal biopsy by upper gastrointestinal endoscopy and histological examination are the actual gold standard for diagnosis of *H. pylori* infection, with the sensitivity and specificity of 95% and 98%, respectively (19). In this study, the rate of *H. pylori* infection in patients with gastritis was as high as 89.80%. A part of *H. pylori*-negative gastritis patients was converted to be *H. pylori*-positive after 1 month. This could be due to the following reasons:

a, *H. pylori*-negative patients were infected with *H. pylori* during this period; b, at the beginning of infection, *H. pylori* antibodies were below the detection limit and with the progress of infection, the *H. pylori* antibodies were also increased accordingly. Overall, the percentage of *H. pylori* infection in patients with gastritis was about 90%, which was much far higher than that in the normal population just as reported in a study (3). This implies that when gastric acid production is impaired, iron absorption is greatly re-

Table 3. Comparison of Platelet Count Between Control and Patients' Groups (N = 3)

Variables	Platelet Count, cmm³		
	Pre Endoscopy	At the End of the Study	P Value[a]
Control (n = 62)	203322.6000 ± 35039.4500	205000.0000 ± 27070.2200	0.77
GI$_a$ (n = 10)	236666.8000 ± 35118.9000	233700.0000 ± 34947.7400	0.85
GI$_b$ (n = 45)	260000.0000 ± 46806.6000	112895.6000 ± 41043.0500	0.001
GII (n = 43)	344740.0000 ± 70761.8800	317818.2000 ± 91442.7600	0.131

Abbreviations: GI$_a$, group I$_a$; GI$_b$, group I$_b$; GII, group II.
[a]Student's t-test.

duced. In addition, gastritis condition triggers immune system response, which produces neutrophils. Presence of neutrophils activates lactoferrin. However, *H. pylori* has lactoferrin-binding protein receptor and thus it binds to lactoferrin, and this gives rise to increase in *H. pylori* turnover as reported in earlier studies (20, 21).

The complete blood count is highly important in the diagnosis of diseases (22). We found abnormalities within the cases, as a haematological parameter in both G1b and GII were below that of the control group. This could be explained by significant low Hb and PCV levels after 1 month. This was probably due to the immune-mediated response, which works for an increased *H. pylori* turnover. Also, this is in line with a study conducted in the United States in which they assessed whether Helicobacter pylori infection is associated with iron deficiency and iron-deficiency anemia (20). MCV was significantly increasing for group Ib, which suggests that megaloblastic and hyperchromasia that are associated with thrombocytopenia on blood smear (macrocytic) anemia was a causative as indicated in a case report by Mishra Vikas et al. (23). This fact is further confirmed by other studies (13, 24-26). MCV was significantly increasing for group I$_b$ (Table 2), an indicative of megaloblastic (macrocytic) anemia (27, 28). Meanwhile, for GII, MCV was significantly decreased, suggesting microcytic anemia (29).

With respect to reticulocyte count results, group I$_b$ showed a significant increase in reticulocyte count. This may be due to the followings: a, hemolysis; b, hemorrhage; and c, after haematinics therapy due to erythropoietic activity in the bone marrow in response to the treatment. It is worth noting that both hemolysis and hemorrhage conditions stimulate mechanisms that produce RBCs. Both GIb and GII results are in agreement with red cell indices (MCV, MCH, and MCHC) results, as reported in the literature (30).

Haematological parameters of RBCs, WBCs, and platelets were normal for group I$_a$ (Figure 2A). According

to morphological examination, group I$_b$ showed oval shaped macrocytes and tear drops (Figure 2B), a characteristic feature of megaloblastic anemia Hypochromia with pencil-shaped RBCs, which was present in group II (Figure 2C). This is an indicative of iron deficiency anemia (microcytic anemia), which is consistent with an earlier study (31).

The strength of the study was that it was the first of its kind at the hospital. Also, it used original primary data with robust statistical analysis method. Moreover, the teaching hospital receives referrals from its catchment regions. This makes the data more representative and generalizable. However, the following limitations were encountered. The rapid immune chromatography test used in this study could not have been the best test to be used alone. Perhaps, there could not have been positive *H. pylori* results after 1 month of negative results, if a more sensitive test like ELISA had been used.

4.1. Conclusion

We can conclude that there is the effect of *H. pylori* infection on haematological parameters. This is because lower Hb and PCV indicates the presence of anemia. Our red-cell indices findings indicated that most of gastritis patients in the catchment area of Kosti hospital have megaloblastic anemia and microcytic anemia. The peripheral blood picture confirmed the types of anemia. However, there is the need to further investigate those who tested negative to find other causes of gastritis.

Acknowledgments

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