Effect of *H. pylori* Infection on Hematological Parameter among Patient with *H. pylori* Infection at Atbara City 2017

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Abstract

*H. pylori* is one of common infection of human. It is associated with chronic gastritis, peptic ulcer, mucosa related tissue lymphoma and gastric cancer. The platelets indices (MPV, PDW and PLCR) used as indicator of systemic inflammation. This is cross sectional study conducted in Atbara city from patient with *H. pylori* and compared with normal control groups according to selection Criteria. Venous blood (2.5ml) were collected in EDTA containers from (100) patient with *H. pylori* and (100) subject as normal control groups the platelets indices, neutrophils and lymphocytes were measured using Sysmex KX-21N analyzer. The data was analyzed by SPSS software using Independent test. The result of this study showed that mean SD of *H. pylori* patient neutrophils, lymphocytes, MPV, PDW and PLCR 55.9, 31.46, 8.9, 10.03 and 17.47 respectively. There was a significant mean difference between MPV, PDW and PLCR (P<0.05). However, there was insignificant MD between *H. pylori* patient and normal control groups for neutrophil and lymphocyte count (P>0.05). There was association between MPV, PDW and age among *H. pylori* patients r²=0.005 however there was negative association between PLCR and age among *H. pylori* patients r²=-0.008. The study concluded that platelets indices in Atbara city from patient with *H. pylori* and normal control groups There was insignificant neutrophil and lymphocyte count but there as correlation to age in MPV, PDW but not PLCR .

Keywords: *H. pylori* infection; Hematological parameters

Abbreviations: TNF: Tumor Necrosis Factor; MPV: Mean Platelet Volume; PDW: Platelet Distribution Width; P-LCR: Platelet Large Cell Ratio; PCT: Platelet-Crit

Introduction

Literature review

Helicobacter pylori infection is the most common bacterial infection Worldwide, with almost half of people in developed countries and three-quarters of people in developing countries infected. Although many infected individuals are asymptomatic, *H. pylori* is an important health problem [1]. Prevalence of *H. pylori* depends on geographical location and socioeconomical status but approximately 50% of the world adult population is infected by *H. pylori* [1].

Gastric mucosa it generally does not invade the gastroduodenal tissue but triggers a host immune response which leads to tissue injury. Although it is a noninvasive organism itself, the antigenic substances it produces such as heat shock protein, urosease and lipopolysaccharide activate T cells. With an enhanced antigen presentation, inflammatory cytokines such as IL-1, IL-6, IL-8, cytokines such as IL-1, IL-6, IL-8, tumor necrosis factor-alpha (TNF-alpha) are released. Besides, both local and systemic B cell response also occurs. Through these local and systemic immune responses, *H. pylori* is known as a causative agent for gastritis, peptic ulcers and probably by a chronic infection and immune stimulation, gastric cancer and mucosa-associated lymphoid tissue lymphoma. It has also been associated with diseases outside the gastrointestinal tract such as coronary heart disease, acne rosacea, chronic idiopathic urticaria, iron deficiency anemia and systemic autoimmune diseases [2]. Existence of *H. pylori* can be determined by noninvasive tests such as anti *H. pylori* antibodies in the blood or urea breath test in patients with symptoms of dyspepsia [3]. *H. pylori*is linked to ITP with an unclear mechanism. On eprobable explanation is molecular mimicry; an antibody induced by *H. pylori* may cross react with platelet glycoprotein antigens. Cag A positive strains are also suggested as pathogen- (i.e. causitives for ITP). One other probability is the enhancing effect of *H. pylori* on already present antiplatelet antibodies and platelet phagocytosis. Genetic factors of the host are also reported to provide a susceptibility for *H. pylori* related platelet destruction [4]. Eradication of *H. pylori* has
been associated with a platelet response, to a degree and in certain group of patients. Though still bearing controversy, this treatment is mentioned in ITP management guidelines [5].

Colonization with \textit{H. pylori} is not a disease in and of itself, but a condition associated with several disorders of the upper gastrointestinal tract [6]. Testing for \textit{H. pylori} is recommended if peptic ulcer disease or low-grade gastric MALT lymphoma is present, after endoscopic resection of early gastric cancer; first-degree relatives with gastric cancer; and in certain cases of dyspepsia [7], not routinely [8]. Several ways of testing exist. One can test noninvasively for \textit{H. pylori} infection with a blood antibody test, stool antigen test, or with the carbon urea breath test (in which the patient drinks 13C- or 14C-labelled urea, which the bacterium metabolizes, producing labeled carbon dioxide that can be detected in the breath) [9]. Also, a urine Elisa test with a 96% sensitivity and 79% specificity is available. None of the test methods is completely failsafe. Even biopsy is dependent on the location of the biopsy. Blood antibody tests, for example, range from 76% to 84% sensitivity. Some drugs can affect \textit{H. pylori} urease activity and give false negatives with the urea-based tests. The most accurate method for detecting \textit{H. pylori} infection is with a histological examination from two sites after endoscopic biopsy, combined with either a rapid urease test or microbrial culture [10].

Platelets are cytoplasmatic fragments of bone marrow megakaryocytes, with a diameter of 3-5μm and a volume of 4.5-11fL [11]. Single megakaryocytes release 1500-2000 of them to the blood stream, where they circulate for 7-10 days. Inactivated platelets in the blood are discoid shaped and do not contain a nucleus. Their cytoplasm contains three different types of granules (i.e. alpha granules, dense granules, and lysosomal granules), secretory vesicles that contain pre-formed molecules, and a complex membranous system.

Platelets have essential roles in thrombosis and hemostasis. The normal platelet count ranges from 100×10^9/L to 450×10^9/L. Circulating lifespan of platelets is 10 days and almost one third of platelets are sequestered in the spleen. Approximately 100×10^9 of platelets are released from megakaryocytes every day to maintain the adequate platelet count. Therefore, a regular and continuous balance between production and consumption is required. Generally defined as platelet count less than 150×10^9/L, thrombocytopenia is caused by increased destruction or consumption, splenomegaly, and decreased production due to bone marrow suppression or failure. Besides the contribution of genetic factors, age, gender and seasonal variations are known to affect the platelet count [12]. Existence of \textit{H. pylori} can be determined by noninvasive tests such as anti-\textit{H pylori} antibodies in the blood or urea breath test in patients with symptoms of dyspepsia. But these tests do not give us any information about intensity of \textit{H pylori} in gastric mucosa and severity of acute and chronic inflammation. We aimed specially to investigate the usability of MPV as a simple indicator that may reflect severity of inflammation in gastric mucosa [13].

Platelet size is a useful indicator to differentiate between hypo proliferative & hyper destructive thrombocytopenia. The combined interpretation of platelet (PLT) count, platelet-Crit (PCT), Mean Platelet Volume (MPV), Platelet Distribution Width (PDW) & Platelet Large Cell Ratio (P-LCR) by automated cell counters can be very useful parameters in differentiating thrombocytopenia due to various etiologies. Platelet count in the blood can be rapidly measured using an automated hematological analyzer. Platelet indices are biomarkers of platelet activation. They allow extensive clinical investigations focusing on the diagnostic and prognostic values in a variety of settings without bringing extra costs. Among these platelet indices, platelet-crit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) are a group of platelet parameters determined together in automatic CBC profiles; they are related to platelets morphology and proliferation kinetics.

An increase in both mean platelet volume (MPV) and platelet distribution width (PDW) due to platelet activation was suggested due to:

a. Platelet swelling.

b. Pseudopodia formation.

c. MPV and PDW are simple platelet indices, which increase during platelet activation

\textbf{Mean platelet volume (MPV)}

In MPV, the analyzer-calculated measure of thrombocyte volume is determined directly by analyzing the platelet distribution curve, which is calculated from a log transformation of the platelet volume distribution curve, to yield a geometric mean for this parameter in impedance technology systems. In some optical systems, MPV is the mode of the measured platelet volume [14]. The most commonly available derived parameter is the mean platelet volume (MPV), calculated by dividing the platelet-crit (PCT) (Platelet-crit is a measure of total platelet mass. Values vary depending on mean platelet volume resulting in overlap between normal platelets, thrombocytopenia and thrombocytosis). By the total number of platelets. This is analogous to the calculation for the mean red cell volume (MCV), in which the hematocrit is divided by the total red cell count.

Is average size of the platelets in Blood Normal range of MPV=(7.5-11.5fl). Usually MPV>13 occurs in hyper- destruction \\ & MPV<8 in hypo-production of platelet. When platelet production is decreased, young platelets become bigger and more active, and MPV levels increase. Increased MPV indicates increased platelet diameter, which can be used as a marker of production rate and platelet activation. During activation, platelets’ shapes change from biconcave discs to spherical, and a pronounced pseudopodia formation occurs that leads to MPV increase during platelet activation. Widely used marker of platelet function, mean platelet volume (MPV), is affected from the production step through activation and finally the production step through activation and finally sequestration. In patients with thrombocytopenia, platelet volume analysis [14].
May be practical in differential diagnosis. Platelet size does not depend on its age. It is rather determined as they are produced from the megakaryocytes as pre-platelets. Like their size and shape, their cytoplasmic characteristics are determined by the megakaryocytes from which they arise. Large and big platelets are more active and tend to aggregate. This type of platelets contains denser granules, secretes more serotonin and thromboglobulin and produces more thromboxane A2 compared to small platelets [15].

Recently, mean platelet volume (MPV) has been started to be used as a simple inflammatory indicator in some diseases. Normal MPV values are 7.2 to 10.1 fl. It has been observed in some studies that MPV level increased in myocardial infarction and cerebrovascular diseases [16]. Mean platelet volume (MPV) has been started to be used as a simple inflammatory indicator in some diseases. High MPV values indicate larger and more active platelets which contribute to the thrombotic events. Some studies yielded higher MPV values for diseases assumed to have low grade inflammation such as diabetes mellitus and coronary artery disease. It has been observed in some studies that MPV level increased in myocardial infarction and cerebrovascular diseases. However, it has been reported that MPV level has decreased in active rheumatic diseases such as rheumatoid arthritis and any losing spondylitis [17]. Similarly, in another study, reduced platelet volume in patients with ulcerative colitis was determined [18].

Platelet distribution width (PDW)

Is an indication of variation in platelet size which can be a sign of active platelet release the PDW median and was 13.3%, with a reference range of 10.0%-17.9% for the 5th-95th percentiles, with a confidence interval of 95. The PDW is a reasonably good tool to distinguish essential thrombocythemia (PDW increased) from reactive thrombocytosis (PDW normal) [19]. PDW is a distribution curve of platelets measured at the level of 20% relative height in a platelet-size distribution curve, with a total curve height of 100. PDW directly measures variability in platelet size, changes with platelet activation, and reflects the heterogeneity in platelet morphology indication of variation in platelet size which can be a sign of active platelet release the PDW median [20].

Platelet larger cell ratio (P-LCR)

Is an indicator of circulating larger platelets (>12 fl), which is presented as percentage? The normal percentage range is males 12.7-36.1%, females 10.9-38.8%. It has also been used to monitor platelet activity [21].

Complete blood count (CBC)

A complete blood count (CBC) is a blood test used to evaluate the overall health and diagnose a wide range of disorders, including anemia, infection, leukemia etc. Sysmex Automated Hematology Analyzer XT-2000i advanced instrument was used for CBC and platelet indices. The specific parameters included in the study were neutrophils, lymphocytes, MPV, PDW, P-LCR, and PLT-crit [22].

General studies has been done regarding the effect of H. pylori on hematological parameters among patient A study done by Lewis SM, -2006 Mean platelet volume: a useful marker of inflammatory bowel disease activity reported that no significant with agreement study [23]. Another study was associated with severity of HP infection with peripheral neutrophil/lymphocyte ratio and MPV concluded that moderate increase intensity of H.P does not lead to significant change in MPV [24].

A study in Turkey, concluded that H. pylori infection and normal platelet counts, it may be speculated that an ongoing and compensated platelet destruction-production may be responsible for the increase in MPV [25]. Other study [26] performed in MPV only, which reported there no relationship to the severity of the H.pylori according to the lymphocyte and neutrophil [26]. Other Turkey study [2010] concluded there were no correlation between MPV and HP intensity, acute and chronic inflammation [27].

Rationale

H. pylori is an important health problem. The prevalence of H. pylori depends on geographical location and socioeconomic status but approximately 50% of the world adult population is infected by H. pylori. Platelet indices as inflammatory indicator which help for diagnosis of many disease such as rheumatoid arthritis, myocardial infarction and bowel disease. There is limited, or none publish data regarding the Platelet indices and it is relation with H. pylori. Therefore, this study was conducted to determine the relationship between Platelet indices and H. pylori infection.

Objectives

General objective
To determine the Effect of H. pylori infections on platelet indices among patients with H. pylori infections in Atbara city 2017.

Specific objectives
A. To estimate the platelet indices (MPV, PRW and P-LCR) among the patient with H. pylori infection and normal control in Atbara city using SYMEX KX21N.
B. To compare the result of platelet indices between the H. pylori patients and normal control group.
C. To know the effect of age and gender on and platelets indices H. pylori infected people.
D. To count lymphocytes and neutrophils in H. pylori patients and normal control.
E. To compare of lymphocytes and neutrophils of H. pylori patients to the normal control.
F. To know the effect of H. pylori infection on lymphocytes and neutrophils.

Materials and Methods

Study design
Cross sectional study.
Study area
Atbara city.

Study duration
From August-October 2017.

Study population
H. pylori positive patient and healthy control group.

Ethical consideration
The individuals included in the study were notified well about the objective and the need of this study and they accept to give blood samples before the start of the collection process.

Sample size formula
Sample size was 100 sample H.P patient and 100 sample healthy control group

Selection criteria
Inclusion criteria:
1. Live in Atbara locality.
2. Positive H. pylori for case and negative H. pylori for control group.
3. From both gender and any age.

Exclusion criteria: For control group diabetes mellitus, coronary artery disease, renal failure, liver failure, cardiac failure, inflammatory disease such as rheumatoid arthritis, systemic lupus erythematosus and malignancies.

Data collection: The primary data was collected by a standard questionnaire and the secondary data was analyzed.

Blood sample collection
First, the patient identity has been investigated for ICT for H. pylori and found that it corresponds to the details on request form. Recommended that skin must be cleaned by 70% alcohol or by 0.5% chlorhexidine and allowed to dry before being punctured. Blood best withdrawn from antecubital vein or other visible vein in the forearm by either an evacuated tube or a syringe. The tourniquet has been applied just above the venipuncture site and released as soon as the blood has been flown into syringe or evacuated tube. If a syringe was used for blood collection the piston of syringe should be withdrawn slowly. Anticoagulated specimen was mixed by harvesting the containers several times. After the blood was obtained and released the tourniquet, the needle was removed and then pressed applying pressure on the swab, Then the punctured site was covered with a small adhesive dressing.

Test procedure
The MPV is calculated by the following formula: MPV (fl)= ((platelet (%) / Platelet count (x109/l)) [22]. PCT is the ratio of the platelet volume in relation to blood volume. PDW is the width of the size distribution curve in femtoliter (fl). An increase in both mean platelet volume (MPV) and platelet distribution width (PDW) due to platelet activation, and platelets destruction. The P-LCR indicator of circulating larger platelets (>12 fl), which is presented as percentage.

6.10. Data analysis: Data was analyzed by SPSS variation 22 using an Independent t test and simple linear regression.

Results
A total of (200) subject was enrolled in this study (100) subjects positive H. pylori and (100) normal control groups, the patients and normal control groups were selected according to inclusion criteria in Atbara city. The mean age was 38 years old. Table (3.1) shows the comparison of mean (SD), mean difference, lower and upper 95% confidence interval of neutrophils, lymphocytes count, platelets indices (MPV, PDW and P-LCR) between H. pylori patient and normal control groups. There was a significant mean difference between MPV, PDW and P-LCR (P<0.05). However, there was insignificant MD between H pylori patient and normal control groups for neutrophils and lymphocytes count (p<0.05). These results showed reflect the effect noise on the above hematological parameters among subjects exposed to noise Figure 1-3 and Table 1.

Table 1: Comparison of mean difference of hematological parameters between H. pylori patients and normal control n=50.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (SD)</th>
<th>MD (95% CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutrophils in H. pylori patient</td>
<td>59.99 (11.01)</td>
<td>1.50 (-1.79, 4.79)</td>
<td>0.37</td>
</tr>
<tr>
<td>Neutrophils in control</td>
<td>58.49 (12.53)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LYM in H. pylori patient</td>
<td>31.46 (10.24)</td>
<td>-1.00 (-4.02, 2.02)</td>
<td>0.515</td>
</tr>
<tr>
<td>LYM control</td>
<td>32.46 (11.42)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPV in H. pylori patient</td>
<td>8.90 (1.06)</td>
<td>-0.40 (-0.68, -0.12)</td>
<td>0.006</td>
</tr>
<tr>
<td>MPV control</td>
<td>9.30 (0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDW in H. pylori patient</td>
<td>10.03 (1.89)</td>
<td>-1.35 (-1.90, -0.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDW control</td>
<td>11.38 (2.04)</td>
<td></td>
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</tbody>
</table>
Table 1: Comparison of PLCR in H. pylori patients and control group.

<table>
<thead>
<tr>
<th></th>
<th>PLCR H. pylori patients</th>
<th>PLCR Control</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLCR</td>
<td>17.47 (7.00)</td>
<td>20.14 (6.77)</td>
<td>0.007</td>
</tr>
<tr>
<td>Mean (95% CI)</td>
<td>-2.67 (-4.59, -0.75)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Independent t-test was applied. P-value as 0.05 is significant.

Figure 1: shows positive association between MPV and age among H. pylori patients $r^2=0.005$.
It shows the relationship between MPV level and age among H. pylori patients in Atbara city (n=40)

Figure 2: shows positive association between PDW and age among H. pylori patients $r^2=0.001$.
It shows the relationship between PDW level and age among H. pylori patients in Atbara city (n=40)

Figure 3: The relationship between PLCR level and age among H. pylori patients.
It shows negative association between PLCR and age among H. pylori patients $r^2=0.008$
Discussion

Helicobacter pylori infection is the most common bacterial infection Worldwide, with almost half of people in developed countries and three-quarters of people in developing countries infected. Although many infected individuals are asymptomatic, although the relation of H. pylori and ITP is thoroughly investigated, MPV has been the subject of only 5 small studies. These studies focused on the practicability of MPV in the prediction of H. pylori infection intensity and observed no correlation. However, they have not referred to the effect of H. pylori infection on platelet counts or MPV.

A study done by Lewis SM, -2006 Mean platelet volume: a useful marker of inflammatory bowel disease activity reported that no significant between MPV and inflammatory bowel disease which agree with our study [24]. Another study was associated of severity of H.P infection with peripheral neutrophil/lymphocyte ratio and MPV concluded that moderate increase intensity of H.P does not lead to significant change in MPV which agreement with our study [25].

A study in Turkey, concluded that H. pylori infection and normal platelets count, it may be speculated that an ongoing and compensated platelet destruction-production may be responsible for the increase in MPV [26]. Other study in Turkey 1996 performed in MPV only, which reported there no relationship to the severity of the H. pylori according to the lymphocyte and neutrophil and this result was agreed to this study. [27]. Other Turkey study (2010) concluded there was no correlation between MPV and HP intensity, acute and chronic inflammation which agreement to this study.

The report of this study showed that mean (SD) of Neutrophils 59.99 lymphocyte 31.46, MPV 8.9, PRW 10.0, P-LCR17.47, respectively. There is insignificant variant for Neutrophil and lymphocyte and H. pylori infection in this study. The platelets indices which reflect the effect on H. pylori. There are no significant differences among the genders with the platelet indices.

Conclusion

This study concluded that H. pylori had little effect on platelets indices and there was no effect to of H. pylori on lymphocytes and neutrophils.

Recommendation

We recommend that other studies with large sample size should be done to exclude or confirm the effect of H. pylori on platelets indices and lymphocytes and neutrophils.

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References


