Association between *Helicobacter pylori* Infection and Iron Deficiency in Sudanese Population

العلاقة بين عدوى البكتيريا الملوية البوابية ونقص الحديد لدى السودانيين

A Dissertation Submitted in Partial Fulfillment for the Requirements of M. Sc. In Medical Laboratory Science (Hematology)

By

Amged Ameen Mohammed Abd Elhakeem

(B. Sc. Hematology- Sudan University of Science and Technology 2015)

Supervisor

Dr. Hisham Nour Aldayem Altayeb

(Assistant professor of Molecular Biology)

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Approval Page

(To be completed after the college council approval)

Name of Candidate: [Amad Ameen Mohammed Abd Elhakeem]

Thesis title: Association between Helicobacter pylori infection and Iron Deficiency in Sudanese Population

Degree Examined for: [M. Sc. Hematology]

Approved by:

1. External Examiner
   Name: Dr. Leena Babiker Nighani
   Signature: [Signature]
   Date: 29.6.2019

2. Internal Examiner
   Name: Dr. Maysar Mohammed Mansoor
   Signature: [Signature]
   Date: 29.6.2019

3. Supervisor
   Name: Hisham Nouradheem Almayouf
   Signature: [Signature]
   Date: 29.6.2019
قال تعالى:

(يرفع الله الذين آمنوا منكم والذين أوتوا العلم درجات واللهم بما تعملون
خبير)

صدق الله العظيم

سورة المجادلة: الآية (11)
Dedication

To our mothers
Source of love and Tenderness Sea

To our fathers
A source of struggle and tender

To our brothers, sisters, relatives and friends
Flowers of our lives

To My Lovely Wife

To all those wonderful persons

We are trying to say thank you
Acknowledgement

I thank Allah who gave me the health, strength and patience to accomplish this study

I would like to gratefully and sincerely thank My supervisor for his help and support Dr. Hisham Nour Aldayem Altayeb

And gratitude to all Students and Teachers in college of Medical Laboratory Science S.U.S.T and the Managers and colleagues in Saad Rashwan Medical Laboratory

Finally, I would like to appreciate and express my deepest feelings to all my colleagues, brothers, sisters, relatives and friends for encouragement And help.
Abstract

*Helicobacter pylori* infection is a major gastric infection worldwide and has been associated with many gastrointestinal and non-gastrointestinal diseases including hematological disorders.

This case control study was conducted in Sudan-Khartoum in the period from May 2018 to April 2019 and it was aimed to study the association between *H. pylori* infection and iron deficiency among Sudanese population.

For this study stool and blood samples were collected from 100 Sudanese subjects (50 were infected with *H. pylori* as cases and 50 were apparently healthy subjects as controls). Stool samples were tested for *H. pylori* Ag by commercially available kits (HanzouAllTest Biotech Co., Ltd, Germany), All blood samples were analyzed for complete blood count using (SYSMEX KX21N) automated analyzer and serum iron profile (iron, ferritin, and TIBC) using spectrophotometery and turbidimetry.

The results of *H. pylori* antigen were positive in all cases and negative in control samples. Serum iron level mean was significantly lower among *H. pylori* positive patient (62±18.1) than control group (91.3±16.7) (p-value 0.001), serum ferritin level mean was significantly lower in *H. pylori* infected patients (36.8±16.5) than control group (64±16.4) (p-value 0.003), hemoglobin level mean was significantly lower in *H. pylori* patients (12.5 ±1.1) than control group (13.8±1.0) (p-value 0.009), PCV level mean was lower in *H. pylori* patients (37.6±3.1) than control group (41±3.0) (p-value .036), TIBC mean was 313 in cases and 308 in control samples. All other parameters showed no significant difference between *H. pylori* positive patients and control subjects.
This study concluded that *H. pylori* infection is associated with iron deficiency in Sudanese patients.
ملخص الدراسة

عدوى البكتيريا الملولية البوابية هي العدوى المعدية الرئيسية في جميع أنحاء العالم وقد ارتبطت بالعديد من أمراض الجهاز الهضمي وغيرها بما في ذلك الإضطرابات الدموية.

تم إجراء هذه الدراسة في السودان - الخرطوم في الفترة من مايو إلى أكتوبر 2018 وكان الهدف منها دراسة العلاقة بين عدوى الملولية البوابية ونقص الحديد لدى السكان السودانيين.

تم جمع عينات البراز والدم من عدد 100 شخص سوداني (50 شخص مصاب بالملولة البوابية كحالات، و 50 منها عناصر بصحة جيدة كضوابط). تم اختبار عينات البراز للكشف عن أجسام بكتيريا الملولية البوابية بواسطة أشرطة الاستشراب المناعية المتوفرة تجاريا، تم تحليل جميع عينات الدم للإعداد الكامل للدم باستخدام محلل الدم الآلي (سمسكس)، وموجز الحديد في المصل (الحديد، الفيريتين، معدل الإرتباط الكلي لل الحديد) باستخدام الطيف الضوئي والتعكر.

كان متوسط مستوى الحديد في المصل أقل بشكل ملحوظ في المرضى المصابين بالملولية البوابية (18.1 ± 18.1) من مجموعة الأصحاء (16.7 ± 16.7)، كان متوسط مستوى الفيريتين في المصل أقل بشكل ملحوظ في المرضى المصابين بالملولية البوابية (16.5 ± 6.8) من مجموعة الأصحاء (16.4 ± 16.4)، كان متوسط مستوى الهيموغلوبين أقل بكثير في المرضى المصابين بالملولية البوابية (12.5 ± 1.1) من مجموعة الأصحاء (13.8 ± 1.0)، متوسط مستوى حجم الخلايا كان أقل في المرضى الملولية البوابية (37.6 ± 3.1) من مجموعة الأصحاء (41 ± 3.0). أظهرت جميع العوامل الأخرى عدم وجود فرق واضح بين المرضى المصابين بالملولية البوابية وغير المصابين.

استنتجت هذه الدراسة أن هناك علاقة بين عدوى بكتيريا الملولية البوابية ونقص الحديد في المرضى السودانيين.
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List of abbreviations

CBC: Complete Blood Count
CFU: Colony Forming Unit
DNA: Deoxyribo Nucleic Acid
EDTA: Ethylene Diamine Tetra Acetic acid
ELISA: Enzyme Linked Immuno-Sorbent Assay
GIT: Gastro-Intestinal Tract
GI: Gastro-Intestinal
Hb: Hemoglobin
IDA: Iron Deficiency Anemia
LPS: Lipo Poly Saccharide
MALT: Mucosal Associated Lymphoid Tissue
MCH: Mean Cell Hemoglobin
MCHC: Mean Cell Hemoglobin Concentration
MCV: Mean Cell Volume
PCR: Polymerase Chain Reaction
PCV: Packed Cell Volume
Plts: Platelets
QC: Quality Control
RDW: Red cell Distribution Width
ROS: Reactive Oxygen Species
TBE: Tris Buffered EDTA
TIBC: Total Iron Binding Capacity
UBT: Urea Breath Test
WBC: White Blood Cell
CHAPTER I

1. Introduction

1.1 Introduction

Iron is one of the most common elements in the Earth’s crust, yet iron deficiency is the most common cause of anemia, affecting about 500 million people worldwide. Organic dietary iron is partly absorbed as heme and partly broken down in the gut to inorganic iron. Absorption occurs through the duodenum. In developed countries, chronic blood loss, especially uterine or from the gastrointestinal tract, is the dominant cause of iron deficiency and dietary deficiency is rarely a cause on its own (Hoffbrand et al., 2006).

Gluten induced enteropathy, partial or total gastrectomy and atrophic gastritis (often autoimmune and with Helicobacter pylori infection) may predispose to iron deficiency. The cause of iron deficiency is done according to the patient case. In premenopausal women the most cause is menorrhagia or pregnancy, while in postmenopausal women and men the most cause of iron deficiency is gastrointestinal loss in these cases the deficiency may be investigated either by: occult blood test, endoscopy for GIT, tests for parietal cell antibodies and detection of H. pylori infection (Hoffbrand et al., 2006).

When gastroenterological evaluation fails to disclose a likely cause of IDA, or in patients refractory to oral iron treatment, screening for celiac disease, autoimmune gastritis, and H. pylori is recommended (Turgeon, 2012).

Epidemiological studies of H. pylori show acquisition in early childhood. However, infection often remains asymptomatic in children and, except for peptic ulcer disease (which is rare in childhood), a relationship between abdominal pain and H. pylori infection is not demonstrated. At the same
time several gastrointestinal and non-gastrointestinal diseases has been associated with this infection (Malaty and Nyren, 2003). There is a strong association between the presence of *H. pylori* infection and duodenal ulceration (Jawetz *et al.*, 2016).

*H. pylori* infection is a major gastric infection in the world. Approximately more than 50% of the adult population in the developed countries and 90% of those in the developing countries are infected with this bacterium.

*H. pylori* associated gastritis can result in many extra gastric complications like vitamin B12 and iron deficiency, megaloblastic anemia, and iron deficiency anemia respectively and other hematological changes (Abbass *et al.*, 2016).

*H. pylori* associated chronic gastritis has emerged as a potential cause of iron deficiency anemia that is unresponsive to iron therapy. Knowledge into the pathogenesis of the anemia is still lacking. The refractoriness to iron treatment and the finding that the eradication of the bacterium may reverse anemia and normalize the iron profile, have been demonstrated in a few studies (Kurekci *et al.*, 2005).

There are several literature studies found that *H. pylori* infection can lead to diminished iron in the body which ends by IDA. Gastric *H. pylori* infection cause of IDA of previously unknown origin in adult patients (Monzón, 2013).

Any previous *H. pylori* infection can be associated with higher prevalence of anemia and reduction of hemoglobin level and red cell indices in school-age children independent of socioeconomic variables (Taye *et al.*, 2015). Infection with *H. pylori* has a role in iron deficiency and the subsequent IDA in infected patient also in puberty and childhood (Caseem, 2011 ; Choe, 2003).
1.2 Rationale

*Helicobacter pylori* infect greater than 50% of the world population’s stomachs, therefore constituting one of the most common infection of the human population. *Helicobacter pylori* infection has been worldwide distributed specially in the developing countries (Campuzano, 2014) and Sudan is one of them. *H. pylori* infection is the most common cause of gastritis in the Sudan (Abass *et al.*, 2016).

*H. pylori* infection related gastritis and ulcers can lead to many pathological complications if not detected and treated, at the same time most infections start asymptptomatically (Malaty and Nyren, 2003 ; Jawetz *et al.*, 2016 ; Tamokou *et al.*, 2017).

There is an international literatures suggested that there is an association between the infection with *H. pylori* and iron deficiency (Kurekci *et al.*, 2005 ; Monzón, 2013).

The mechanism by which infection with *H. pylori* causes iron metabolism disturbance is of considerable interest. However, despite the importance of this, relatively little research has been done yet. In Sudan, a few studies conducted to study the relationship between the infection with *H. pylori* and hematological disorders. A study by Abbass *et al.* in 2016 found that infection with *H. pylori* can lower vitamin B12 level and serum ferritin.

Both, *H. pylori* and iron deficiency are common in the Sudan, and according to our knowledge there is few studies done to detect the association between them. The studies done also did not estimate all parameters of iron profile. So, we conducted this case control study to detect if there is an association between the infections with *H. pylori* and iron deficiency in Sudanese population.
1.3 Objectives

1.3.1 General Objective

To study the association between *Helicobacter pylori* infection and iron deficiency.

1.3.2 Specific Objectives

1- To detect *H. pylori* antigen in stool samples by the commercially available kits from (Hanzou AllTest Biotech Co., Ltd, Germany).

2- To estimate complete blood count (Hemoglobin, red cells and their indices, hematocrit, white blood cells and Platelets) in cases and controls by the full automated hematology analyzer (sysmex KX21N).

3- To estimate serum iron, serum ferritin and total iron binding capacity in cases and controls by the commercially prepared reagents of (biosystems), using turbidimetry and spectrophotometry.

4- To compare mean results of CBC and iron profile between cases and controls.
CHAPTER II

2. Literature review

2.1 Iron

2.1.1 Physiology of iron

Humans have 35 to 50 mg of iron per kilogram of body weight and the average adult has 3.5 to 5.0 g of total iron. Normal iron loss is very small, amounting to less than 1 mg/day and it lost from the body through exfoliation of intestinal epithelial and skin cells, the bile, and urinary excretion. To compensate for this loss, the adult male has a replacement iron need of 1 mg/day. However, additional iron is needed during the growth years, pregnancy, and lactation. Some women require supplementary iron because of heavy menstrual blood loss (Turgeon, 2012).

2.1.2 Dietary Iron

There are two broad types of dietary iron. Approximately 90% of iron is referred to as non-heme iron. The extent to which this type of iron is absorbed is highly variable and depends both on the person’s iron status and on the other components of the diet. The other 10% of dietary iron is heme iron, which is derived primarily from the hemoglobin and myoglobin of meat. Heme iron is well absorbed (Turgeon, 2012).

2.1.3 Iron absorption

Following oral intake of iron in the ferric (Fe3+) state, stomach secretions (reducing agents, which include glutathione, ascorbic acid, and sulfhydryl groups of proteins and digestion products) reduce the iron to the ferrous (Fe2+) state. Acidic pH of gastric juice makes iron available from
hemoglobin-containing meat in the diet and other sources. However, very little iron is absorbed by the stomach. Most of the iron passes from the stomach to the duodenum and upper jejunum, where it can be absorbed readily (Turgeon, 2012).

Ferrireductase present at the apical surface converts iron from the Fe3+ to Fe2+ state and another enzyme, hephaestin (ferrioxidase), converts Fe2+ to Fe3+ at the basal surface prior to binding to transferrin (Hoffbrand et al., 2006).

Most absorbed iron becomes attached to the plasma protein transferrin, which is formed in the liver. Transferrin chelates iron within the intestinal lumen and shuttles it into the mucosal cells of the small intestine. In iron deficiency, any increase in iron supply must come from the GI tract, because body tissues are already depleted of storage iron. At the time that iron has been depleted from these iron stores, an iron deficiency will manifest itself (Turgeon, 2012).

2.1.4 Iron Deficiency Anemia

2.1.4.1 Etiological factors for IDA

2.1.4.1.1 Inadequate Intake

IDA can develop when the erythron is slowly starved for iron. Each day, approximately 1 mg of iron is lost from the body, mainly in the mitochondria of desquamated skin and sloughed intestinal epithelium. Daily replacement of 1 mg of iron from the diet maintains iron balance and supplies the body’s need for RBC production as long as there is no other source of loss. When the iron in the diet is consistently inadequate, over time the body’s stores of iron become depleted. Ultimately, RBC production
slows as a result of the inability to produce hemoglobin. With approximately 1% of cells dying naturally each day, the anemia becomes apparent when the production rate is insufficient for replacement of lost cells (Keohane et al., 2016).

2.1.4.1.2 Increased iron utilization:

An increased demand for iron that is not met, such as during pregnancy, the growth years, or periods of increased blood regeneration (Turgeon, 2012).

2.1.4.1.3 Excessive loss of iron:

(Physiological or pathological) an excessive loss of iron can result from acute or chronic hemorrhage or heavy menstruation.

2.1.4.1.4 Faulty or incomplete iron absorption:

Conditions of faulty or incomplete iron absorption can be caused by achlorhydria in certain disorders or following gastric resection or chronic diarrhea. If a gastroenterological evaluation fails to disclose a likely cause of IDA, or in patients refractory to oral iron treatment, screening for celiac disease, autoimmune gastritis, and *Helicobacter pylori* is recommended. Twenty seven percent of patients with unexplained IDA have autoimmune gastritis, 50% have evidence of active *H. pylori* infection, and 4% to 6% have celiac disease (Turgeon, 2012).

2.1.4.1.5 Pathological iron loss

In adult males and postmenopausal females with iron deficiency. An evaluation of abnormal occult bleeding, especially gastrointestinal (GI) bleeding, is needed (Turgeon, 2012).
2.1.4.2 Pathogenesis of IDA

Iron deficiency anemia develops slowly, progressing through stages that physiologically blend into one another but are useful delineations for understanding disease progression. For a period of time as an increase in demand or increased loss of iron exceeds iron intake, essentially normal iron status continues. The body strives to maintain iron balance by accelerating absorption of iron from the intestine through a decrease in the production of hepcidin in the liver. This state of declining body iron with increased absorption is not apparent in routine laboratory test results or patient symptoms. The individual appears healthy. As the negative iron balance continues, however, a stage of iron depletion develops (Keohane et al., 2016).

Stage (1):
This stage is characterized by a progressive loss of storage iron. RBC development is normal; however, because the body’s reserve of iron is sufficient to maintain the transport and functional compartments through this phase (Keohane et al., 2016).

Stage (2):
This stage is defined by the exhaustion of the storage pool of iron. For a time, RBC production continues as normal, relying on the iron available in the transport compartment. Quickly the hemoglobin content of reticulocytes begin to decrease, which reflects the onset of iron deficient erythropoiesis, but because the bulk of the circulating RBCs were produced during the period of adequate iron availability, the overall hemoglobin measurement is still normal. Thus, anemia is still not evident, although an individual’s hemoglobin may begin dropping, and the RBC distribution width (RDW)
may begin increasing as some smaller RBCs are released from the bone marrow (Keohane *et al*., 2016).

Stage 3:
Stage 3 of iron deficiency is frank anemia. The hemoglobin concentration and hematocrit are low relative to the reference intervals. Depletion of storage iron and diminished levels of transport iron prevent normal development of RBC precursors. The RBCs become microcytic and hypochromic as their ability to produce hemoglobin is restricted. As expected, serum ferritin levels are exceedingly low. Results of other iron studies are also abnormal. In this phase, the patient experiences the non-specific symptoms of anemia, typically fatigue, weakness, and shortness of breath, especially with exertion. Pallor is evident in light skinned individuals but also can be noted in the conjunctivae, mucous membranes, or palmar creases of dark-skinned individuals. More severe signs include a sore tongue (glossitis) due to iron deficiency in the rapidly proliferating epithelial cells of the alimentary tract and inflamed cracks at the corners of the mouth (angular cheilosis). Koilonychia (spooning of the fingernails) may be seen if the deficiency is long-standing. Patients also may experience cravings for non food items, called pica. The cravings may be for things such as dirt, clay, laundry starch, or, most commonly, ice (craving for the latter is called pagophagia) (Keohane *et al*., 2016).

2.1.4.3 Diagnosis of IDA

Hematological laboratory investigations are firstly important in diagnosis of IDA which include complete blood count (CBC) all parameters (RBCs, Hb, PCV, MCV, MCH, MCHC, and RDW) and also white blood cells and
platelets are important. All red cell parameters will drop in IDA (Turgeon, 2012).

Iron studies (biochemical investigations) remain the backbone for diagnosis of iron deficiency. They include assays of serum iron, total iron-binding capacity (TIBC), transferrin saturation, and serum ferritin. Serum iron is a measure of the amount of iron bound to transferrin (transport protein) in the serum. TIBC is an indirect measure of transferrin and the available binding sites for iron in the plasma. Ferritin is not truly an extracellular protein because it provides an intracellular storage repository for metabolically active iron. However, ferritin is present in serum, and serum levels reflect the levels of iron stored within cells. Serum ferritin is an easily accessible surrogate for stainable bone marrow iron (Keohane et al., 2016).

2.1.4.4 Treatment

The first therapy for iron deficiency is to treat any underlying contributing cause, such as hookworms, tumors, or ulcers. As in the treatment of simple nutritional deficiencies or increased need, dietary supplementation is necessary to replenish the body’s iron stores (Turgeon, 2012).

2.2 Helicobacter pylori

*Helicobacter pylori* are non-spore forming, curved, spiral or fusiform Gram negative bacteria, typically 0.2 to 1.2 um in diameter, and 1.5 to 10.0 um in length. In old cultures it appears coccoid. It commonly colonized in upper gastrointestinal (GI) tract, especially in the stomach. It has been estimated that 50 % of global population are infected with *H. pylori* due to its highly contagious nature. In 1982 it was classified as *Campylobacter pyloridis*, it resemble *Campylobacter* in many aspects but it differ in important feature
such as flagellum morphology, fatty acid contents and 16s rRNA sequence. It named *Helicobacter pylori* in 1989 (Ibrahim, 2017).

*H. pylori* are ubiquitous, found worldwide. *H. pylori* infection occurs more frequently in developing countries than in the developed countries (Parija, 2012). The association of *H. pylori* with gastritis, peptic ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid type (MALT), and B-cell lymphoma is recognized worldwide (Parija, 2012).

### 2.2.1 Morphology of *H. pylori*

*Helicobacter pylori* in vivo and under optimum in vitro conditions is an S-shaped bacterium with 1 to 3 turns, 0.5x5 um in length, with a tuft of 5 to 7 polar sheathed flagella (Ibrahim, 2017).

### 2.2.2 Coccoid Forms

In common with many other spiral-shaped bacteria, members of the *Helicobacter* genus "ball up" and form coccoid cells as they age. After 3 to 4 days of bacterial culture coccoid cells dominate, and this is associated with a dramatic decrease in culturability as determined by CFU counts (Ibrahim, 2017).

### 2.2.3 *H. pylori* and the gastric mucosal barrier

In the upper gastrointestinal tract, the gastric mucosa considered as the first line defense mechanism, normally bacteria been trapped in mucus and excreted with feces. Urease expression and motility permit *H. pylori* to survive and colonize the mucus layer (Ibrahim, 2017).
2.2.4 Virulence factors

There are several factors that affect the gastric colonization, tissue damage and survival.

2.2.4.1 Flagella

The curved morphology and the polar motility caused by flagella in one end cause screw-like movements, which may enable *H. pylori* to penetrate the mucin layer (Ibrahim, 2017).

2.2.4.2 Urease

Urease is one of the key enzymes in *H. pylori* pathogenesis. It has a molecular weight of 550 kDa and consists of three subunits (Ure A), (Ure B), and (Ure C). Urease is necessary for *H. pylori* to maintain a pH-neutral micro environment around the bacteria, necessary for survival in the acidic stomach (Ibrahim, 2017).

2.2.4.3 Outer Membrane Proteins

*H. pylori* adhere to mucin and binds specifically to gastric mucosa epithelial cells both in vivo and in vitro. This adhesion pattern is different in children and in adults. Several gastric tissue receptors have been described for *H. pylori* such as: sialoglycoconjugates in gastric mucins and on epithelial cells, phagocytes, and extra-cellular matrix, sulfated glycoconjugates such as heparan sulfate and other glycosaminoglycans, and sulfatides (Ibrahim, 2017).
2.2.4.4 Factors involved in tissue damage and survival factors

2.2.4.4.1 Enzymes

It seems probable that *H. pylori* glycosulfatase degrades gastric mucin. *H. pylori* possess phospholipase A, which can digest phospholipids of cell membranes. Urease has a cytotoxic activity. Recently, alcohol dehydrogenase has been described to contribute to gastric mucosal injury (Ibrahim, 2017).

2.2.4.4.2 Toxins

Vacuolating cytotoxin A, lipopolysaccharide been related to peptic ulcer, severe gastritis, and mucosal integrity. Lipopolysaccharide (LPS) in *H. pylori* has a low biological activity as compared to LPS from other gram negative bacteria, which may be explained by the unusual composition of lipid A (Ibrahim, 2017).

Table 2. Virulence factors of *H. pylori*

<table>
<thead>
<tr>
<th>Virulence factor</th>
<th>Biological function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urease</td>
<td>Helps in colonization of the organism in gastric mucosa; the enzyme also stimulates monocytes and neutrophil chemotaxis and stimulates production of cytokines</td>
</tr>
<tr>
<td>Flagella</td>
<td>It helps the organism to penetrate into gastric mucous layer, hence protects the bacteria from acid environment of the stomach</td>
</tr>
<tr>
<td>Adhesins</td>
<td>Facilitate binding of <em>H. pylori</em> to gastric mucosa</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Both mucinase and phospholipase break down gastric mucus, while superoxide dismutase and catalase</td>
</tr>
<tr>
<td><strong>Pathogenesis of H. pylori</strong></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>The pathogenesis of <em>H. pylori</em> infection is extremely complex because of multi virulence factors possessed by the bacterium like Cytotoxin-associated gene A (CagA), Cag pathogenicity island (Cag PAI), lipopolysaccharide (LPS), urease, and Vacuolating cytotoxin A (VacA). Nevertheless, sustained inflammation, immunomodulation and oxidative stress (in the host) are the major reason for <em>H. pylori</em>-related diseases like peptic ulceration, gastritis, gastric mucosa-associated lymphoid tissue lymphoma and gastric cancer. Attachment of <em>H. pylori</em> with the host cell (epithelial cells) is the first step in the initiation of the infection. Once it has been attached to cell, it releases various virulence factors especially CagA, LPS and Vag A, which trigger the inflammatory process by elevating infiltration of sub epithelial lamina by macrophages and neutrophils, and generation of reactive oxygen species (ROS). Although the human immune system is capable of creating a robust innate and adaptive immune response to the infection, it usually fails to clear <em>H. pylori</em> completely, thereby resulting in a persistent infection. This prolonged infection results in chronic inflammation, oxidative stress, and DNA damage. There are several <em>H. pylori</em> virulence factors that contribute to</td>
<td></td>
</tr>
</tbody>
</table>
its ability to evade the immune system one of the most studied factors is cytotoxin associated gene A which is injected into the host cell where it can affect the cell’s shape, motility and proliferation. Vacuolating cytotoxin A (VacA) is a toxin secreted by *H. pylori* and able to induce inflammatory cytokines after entering the host cell. These are a few of the virulence factors that *H. pylori* uses to maintain a prolonged pro-inflammatory response while evading self-destruction (Parija, 2012).

**2.2.6 Host Damage and Gastric Cancer**

*H. pylori* was the first bacterial pathogen to be recognized as a carcinogen. The long lag time between the initial infection and carcinogenesis combined with the late-stage diagnosis results in a low survival rate (Parija, 2012).

**2.2.7 Host immunity**

*H. pylori* infection induces the production of IgM, IgG, and IgA antibodies and also cellular immunity, but they do not appear to confer any protection against the disease (Parija, 2012).

**2.2.8 Diagnosis of *H. pylori***

Many methods are developed to detect *H. pylori* infection which they are both high sensitivity and specificity, exceeding 90%, are necessary for accurate diagnosis of *H. pylori* infection in clinical practice. Each method has its own advantages, disadvantages, and limitations. The choice of one method or another could be depended on availability and accessibility of diagnostic tests, level of laboratories, clinical conditions of patients, and likelihood ratio of positive and negative tests on different clinical circumstances. Diagnostic tests are usually divided into invasive
(endoscopic-based) and noninvasive methods. Invasive diagnostic tests include endoscopic image, histology, rapid urease test, culture, and molecular methods. Non-invasive diagnostic tests included urea breath test, stool antigen test, serological, and molecular examinations (Wang, 2015).

2.2.8.1 Microscopy

*H. pylori* are demonstrated by microscopic examination of gastric biopsy specimen stained with Gram, Giemsa, Warthin Starrysilver, and hematoxylin & eosin stains. All these stains show organisms of *H. pylori* adhered to gastric mucosa. These methods are highly specific (Parija, 2012).

2.2.8.2 Culture

Diagnosis of *H. pylori* infection is made by culture of clinical specimens on freshly prepared chocolate agar and Skirrow’s campylobacter selective media followed by incubation at 35–37°C in a microaerophilic environment (5% O2, 10% CO2, and 85% N2) for 3–5 days. *H. pylori* produces convex, circular, and large colonies on these selective media (Parija, 2012).

2.2.8.3 Identification of bacteria

Identification of bacterial isolates is made on the basis of typical growth characteristics of *H. pylori* on selective medium, morphology, and oxidase, catalase, and urease test (Parija, 2012).

2.2.8.4 Biochemical reactions

*H. pylori* produce the enzyme urease, which is almost 100 times more active than that produced by *Proteus vulgaris*. Urease production is the most
important feature of *H. pylori*. And it is catalase and oxidase positive *H. pylori* is biochemically inactive and does not ferment or oxidize sugars, although they can metabolize amino acids by fermentative pathways (Parija, 2012).

### 2.2.8.5 Enzyme Linked Immuno-Sorbant Assay

ELISA is used for demonstration of serum antibodies to *H. pylori* in the patient’s serum. But since the antibody titers continue to remain elevated a long time after *H. pylori* eradication, antibody-based test cannot distinguish between recent and old infection. ELISA shows a high sensitivity and specificity (Parija, 2012).

### 2.2.8.6 Fecal antigen test

This is an immune-chromatographic test, which uses monoclonal antibody for direct detection of *H. pylori* antigen in stool samples. This test is very sensitive (94%) and specific (98%) (Parija, 2012).

### 2.2.8.7 Rapid Urea Breath Test

UBT is based on detection of the products of urea degraded by *H. pylori*. In this method, patients drink a beverage that contains urea labeled with a carbon isotope, such as carbon 13 or carbon 14. After a short period of time, the concentration of labeled carbon is measured in the breath. The concentration is high only when urease present in *H. pylori* found in the stomach breaks down the urea. In normal human host, the concentration of the labeled carbon in breath would not be high because the human stomach does not contain any urease (Parija, 2012).
2.2.8.8 Polymerase Chain Reaction

Since the application of polymerase chain reaction (PCR) to detect *H. pylori* infection, PCR has been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens, saliva, stool, gastric juice and variable specimens. PCR provides excellent sensitivity and specificity, greater than 95%, as compared with other conventional tests and has more accurate results of detecting *H. pylori* in patients with bleeding (Wang, 2015).

2.2.9 Treatment

Triple therapy with metronidazole and either bismuth subsalicylate or bismuth subcitrate plus either amoxicillin or tetracycline for 14 days eradicates *H. pylori* infection in 70–95% of patients. An acid-suppressing agent given for 4–6 weeks enhances ulcer healing. Proton pump inhibitors (PPIs) directly inhibit *H. pylori* and appear to be potent urease inhibitors. The preferred initial therapy is 7–10 days of a PPI plus amoxicillin and clarithromycin or a quadruple regimen of a PPI metronidazole, tetracycline, and bismuth for 10 days (Jawetz et al., 2016).

2.2.10 Prevention and Control

Improved personal hygiene is important in prevention of the disease (Jawetz et al., 2016).
2.3 Previous studies

There are many previous studies done to detect the association between *H. pylori* and iron deficiency, with various objectives and findings. Most of these studies agree with the theory that there is association between *H. pylori* infection and iron deficiency.

Baysoy et al. has investigated *H. pylori* related-changes in gastric physiology and histology in children. They have reported that *H. pylori* infection is associated with low serum iron levels and with a decrease in gastric juice ascorbic acid concentration. (Baysoy *et al*, 2004). Also, a study carried out in Iraq by Jasem *et al* (2011) found that *H. pylori* infection might have a role in causing iron deficiency anemia. Hence, *H. pylori* infection has to be looked for, in cases of recurrent iron deficiency anemia, as this condition is very common in our country (Jasem *et al*, 2011).

Emin *et al* found that *H. pylori* infection is associated with ID and IDA in children, and that complete recovery of iron deficiency and iron deficiency anemia can be achieved with the treatment of *H. pylori* infection (Emin *et al*, 2005).

A similar study to ours conducted in Iran by Qujeq *et al* and found that serum iron and total iron-binding capacity in *H. pylori* positive group were lower than in the control group. The results suggested that *H. pylori* infection impaired iron uptake. This role is may be related to directly competing with the host for available iron or by impairing iron uptake (Qujeq *et al*, 2011).

Monzón study showed that *H. pylori* infection is a frequent cause of IDA in men and postmenopausal women with either iron refractoriness or iron dependency. *H. pylori* eradication therapy produces long-term resolution of IDA in such patients.
At the other hand, Kermati et al found an opposite results despite the high prevalence of *H. pylori* and IDA in Iran. They reported that there is no association between *H. pylori* infection and iron deficiency (Keramati *et al*, 2007).
CHAPTER III

3. Materials and methods

3.1 Study design

Analytical case control study.

3.2 Study area

This study was conducted in Sudan, Khartoum state Omdurman locality, this area characterized with high prevalence of H. pylori infection and IDA. Laboratory analysis done in Saad Rashwan Medical Center.

3.3 Study period

The study was conducted during the period between 1st of May to 30th of October, 2018.

3.4 Samples

A total of 100 samples from Sudanese population with different gender and age were included in this study; 50 of them were positive for H. pylori antigen as cases; the remaining 50 were negative for H. pylori antigen used as controls. From each we requested an stool sample for detection of H. pylori Ag by commercially available kits (HanzouAllTest Biotech Co., Ltd, Germany), and 5 ml of blood collected (2.5 ml in EDTA ,and 2.5ml in plain tube) for analysis of CBC parameters and iron profile.
3.5 Selection criteria

3.5.1 Inclusion criteria

The criteria to be included in this study as cases is that the person should be positive for *H. pylori* by detection of its antigen in the stool sample. Controls criteria should be negative for *H. pylori* antigen.

3.5.2 Exclusion criteria

The exclusion criteria were patient under treatment within 10 days prior to the study with drugs have known effect on iron metabolism, iron drugs, or blood transfused patients. Subjects with any history of GI surgery, peptic ulcer, systemic disease, hematological disease, diabetes mellitus and smokers were eliminated from the study.

3.6 Ethical consideration

Ethical approval to carry out this study was obtained from college of graduate studies – (SUST) and from Saad Rashwan laboratory management. Verbal consent was obtained from all subjects of the study.

3.7 Samples collection

3.7.1 Stool samples collection

Stool samples were collected in clean dry and sterile container with wide mouth and screw cap.
3.7.2 Blood samples collection

The requirements: Syringe, tourniquet, cotton, disinfectant, EDTA and Plan blood containers. After applying tourniquet and by sterile plastic syringe 5 ml of blood was drained. 2.5 ml placed immediately in the EDTA container and mixed gently with the anticoagulant. The other remaining blood placed in the plan container and after it has been clotted the serum was separated in other container and preserved in the refrigerator for less than 24 hours till it has been analyzed for iron profile parameters.

3.8 Tests performed

3.8.1 Detection of *H. pylori* Ag in stool sample

This was done by the commercially available kits from (HanzouAllTest Biotech Co., Ltd, Germany) in which we followed the manufacturer instructions to emulsify amount of stool sample in buffer which prepared for *H. pylori* antigen extraction and incubated for minutes for satisfactory extraction and then three drops from this extract added to the cassette provided with the kit and then the result readed within 15 minutes.

3.8.2 Complete Blood Count

CBC parameters were analyzed using the automated hematology analyzer Sysmex KX21N. After the analyzer turned on, whole blood mode is selected then the sample ID No. is inputted, then sample was sufficiently mixed and the plug removed and the sample setted to the probe and start switch of the analyzer pressed. The result displayed in LCD screen and then printed out by the internal printer of the analyzer  (Sysmex KX-21., 2000).
3.8.3 Iron profile

To analyze the serum iron, serum ferritin and TIBC we used Biosystems reagents (Spain).

3.8.3.1 Serum Iron estimation

Serum iron analyzed by iron ferrozine method. In which, the blood sample collected in plain tube used, serum is separated and the following procedure is followed:

In labeled test tubes pipette

<table>
<thead>
<tr>
<th></th>
<th>Reag. Blank</th>
<th>Sample blank</th>
<th>sample</th>
<th>standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. W</td>
<td>200 ul</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sample</td>
<td>-</td>
<td>200 ul</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Iron standard (200ug/dl)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>200 ul</td>
</tr>
<tr>
<td>Reagent A</td>
<td>-</td>
<td>1 ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Working reagent</td>
<td>1 ml</td>
<td>-</td>
<td>1 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

All tubes mixed thoroughly, and incubated at room temperature for 5 minutes. The absorbance of sample blank, sample and standard measured at 560 nm using spectrophotometer (biosystem 350) the concentration of iron calculated using the general formula:

\[
\frac{A_{\text{sample}} - A_{\text{sample blank}}}{A_{\text{standard}}} \times C_{\text{standard}} = C_{\text{sample}}
\]
3.8.3.2 Total Iron Binding Capacity

TIBC measured by reagents of biosystems, in which 0.5 ml of serum is added to 1 ml of reagent A in a labeled tube, mixed thoroughly and incubated at room temperature for 30 minutes, one spoonful of reagent B is added and mixed and incubated at room temperature for 60 minutes, the samples then were centrifuged at 3000 rpm for 10 minutes and the supernatant is used to measure iron concentration as in iron ferrozine method. TIBC calculated as follow:

TIBC = iron conc. In the supernatant x 3 (dilution)

3.8.3.3 Serum ferritin estimation

Latex turbidemetric method used in the estimation of ferritin, in which the full automated chemistry analyzer biosystems A5 used. After the analyzer became ready, sample identification is interred and ferritin test is selected and the analyzer started. At the end we gained results of ferritin from result review in the analyzer software.

3.8.4 Quality control

Standards and quality control materials provided with kits were used to verify the precision and accuracy of the reagent and to assure the reliability of all results. For CBC, a control sample from the laboratory administration of Khartoum state was used for quality control purposes.
3.9 Statistical Analysis

All data was analyzed using Statistical Package for Social Science (SPSS version 16.0) computer software. Significant level was set at p-value equal or less than 0.05 and the results were presented in form of tables and figures.
CHAPTER IV

4. Results

4.1 Demographic data

A total of 100 Sudanese individuals with different ages (between 10-75 years) and sex were included in this study. Of them, 50 were infected with *H. pylori* as cases (50%), and 50 were healthy individuals negative for *H. pylori* antigen as controls (50%). 58% of the cases were females (29 subjects) and 42% of them were males (21 subjects). 58% of the controls were females (29 subjects) and 42% of them were males (21 subjects). The age of cases was between 10-75 (mean 31.8 years), while controls between 10-65 years with (mean 32.3 years).

We used the following sample size equation to calculate the sample size and we selected 100 samples due to the high cost of reagents and devices.

\[ n_0 = \frac{z^2 \cdot p \cdot q}{d^2} \]
Figure 4.1 Distribution of the study population according to gender
Figure 4.2 Frequency of age (years) among cases and controls
### 4.2 Complete Blood Count

The mean hemoglobin concentration and PCV of cases was lower than that of controls which was (12.5 ±1.1 , 13.8±1.0 ) for hemoglobin and (37.6±3.1 , 41±3.0) for PCV. No difference detected on the other CBC parameters between cases and controls.

**Table 4.1 Means and standard deviations of CBC among cases and controls**

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean ± Std.D</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb (g/dl)</td>
<td>PCV (%)</td>
</tr>
<tr>
<td>Cases</td>
<td>12.5 ±1.1</td>
<td>37.6±3.1</td>
</tr>
<tr>
<td>Controls</td>
<td>13.8±1.0</td>
<td>41±3.0</td>
</tr>
</tbody>
</table>
4.3 Iron study

The concentration of serum iron and serum ferritin was lower in cases than controls (62±18.1, 91.3±16.7) for serum iron and (36.8±16.5, 64±16.4) for ferritin. Total iron binding capacity was not affected in cases and controls.

Table 4.2 Means and standard deviations of serum iron profile among cases and controls

<table>
<thead>
<tr>
<th>Population</th>
<th>Mean ± Std.D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S.Iron</td>
</tr>
<tr>
<td>Cases</td>
<td>62±18.1</td>
</tr>
<tr>
<td>Controls</td>
<td>91.3±16.7</td>
</tr>
</tbody>
</table>
4.4 Comparison of CBC between infected and non infected persons

There was significant difference in hemoglobin and PCV in the infected persons with *H. pylori* and non infected persons (p-value less than 0.05) while all other parameters not affected by the infection with *H. pylori* (p-value more than 0.05).

**Table 4.3 CBC parameter in relation to *H. pylori* infection**

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>H. pylori</em></th>
<th>No.</th>
<th>Mean ± SD</th>
<th>P. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>Positive</td>
<td>50</td>
<td>12.5 ±1.1</td>
<td>.009</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>13.8±1.0</td>
<td></td>
</tr>
<tr>
<td>RBCs</td>
<td>Positive</td>
<td>50</td>
<td>4.6±0.013</td>
<td>.22</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>4.8±0.01</td>
<td></td>
</tr>
<tr>
<td>PCV</td>
<td>Positive</td>
<td>50</td>
<td>37.6±3.1</td>
<td>.036</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>41±3.0</td>
<td></td>
</tr>
<tr>
<td>MCV</td>
<td>Positive</td>
<td>50</td>
<td>81.6±6.3</td>
<td>.4</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>82±2.7</td>
<td></td>
</tr>
<tr>
<td>MCH</td>
<td>Positive</td>
<td>50</td>
<td>27±2.4</td>
<td>.6</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>29±1.9</td>
<td></td>
</tr>
<tr>
<td>MCHC</td>
<td>Positive</td>
<td>50</td>
<td>32±2.1</td>
<td>.07</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>34±1.5</td>
<td></td>
</tr>
<tr>
<td>WBCs</td>
<td>Positive</td>
<td>50</td>
<td>6.8±2.8</td>
<td>.5</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>6.4±1.8</td>
<td></td>
</tr>
<tr>
<td>Plts</td>
<td>Positive</td>
<td>50</td>
<td>298±76</td>
<td>.054</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>318±73</td>
<td></td>
</tr>
</tbody>
</table>

Independent sample T test, P. value ≤ 0.05 is significant
4.5 The association between *H. pylori* and iron profile

There was a significant difference in serum iron and serum ferritin between infected people with *H. pylori* and healthy people (p-value less than 0.05). While there was no difference in TIBC between them.

**Table 4.4 Iron profile in relation to *H. pylori* infection**

<table>
<thead>
<tr>
<th>Parameter</th>
<th><em>H. pylori</em></th>
<th>No.</th>
<th>Mean ± SD</th>
<th>p. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron</td>
<td>Positive</td>
<td>50</td>
<td>62±18.1</td>
<td>.001</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>91.3±16.7</td>
<td></td>
</tr>
<tr>
<td>Serum ferritin</td>
<td>Positive</td>
<td>50</td>
<td>36.8±16.5</td>
<td>.003</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>64±16.4</td>
<td></td>
</tr>
<tr>
<td>TIBC</td>
<td>Positive</td>
<td>50</td>
<td>313±67</td>
<td>.094</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>50</td>
<td>308±20.7</td>
<td></td>
</tr>
</tbody>
</table>

Independent sample T test, P. value ≤ 0.05 is significant
CHAPTER V

5. Discussion, conclusion and recommendations

5.1 Discussion

*Helicobacter pylori* infection is considered a worldwide problem and it is the most common cause of chronic gastritis, and has been strongly linked to peptic ulcer disease and gastric cancer. Several gastro-intestinal and non-gastrointestinal diseases have been reported to have a significant association with *H. pylori* infection. *H. pylori* associated gastritis has emerged as a potential cause of iron deficiency anemia that is unresponsive to iron therapy (Malaty and Nyren, 2003).

In Baghdad a study conducted by Jasem *et al.* (2011) found that *H. pylori* infection has a role in iron deficiency and subsequently IDA. Another study in India done by Umakiran *et al.* (2011) found that there is an association between *H. pylori* infection and IDA, and they also suggested that even asymptomatic infection can impair iron absorption, and treatment of the infection along with iron supplements can improve IDA. In Iran, Qujeq *et al.* (2011) concluded that *H. pylori* may lower iron profile and it may impair iron metabolism, it competes the host in iron uptake. On the other hand, there are some studies did not support that, for example in Iran, a study done by Keramati *et al.* (2007) found that there is no correlation between *H. pylori* infection and iron deficiency.

We enrolled this research to study this hypothesis in Sudan, since *H. pylori* and iron deficiency both were commonly distributed. Our results showed that hemoglobin, PCV, serum iron and serum ferritin were significantly lower in patients infected with *H. pylori* than control
group whom were negative for *H. pylori* (p-value less than 0.05) which confirm that theory there is an association between *H. pylori* infection and iron deficiency and subsequently iron deficiency anemia. All other parameters showed no difference between *H. pylori* infected patients and healthy subjects. This may be due to some factors, it may be due to that most patient were newly diagnosed cases and the change in these parameters is time dependent according to the phases of IDA.

In this study most parameters values were near or in the permissible limits, this also may be due to the time of diagnosis or also may be due to the type of *H. pylori* strain, some strains have a virulence factor cause iron deficiency and other strains lack that factor.

Several possible mechanisms for the association between *H. pylori* infection and iron deficiency must be considered. Chronic bleeding that may result from the peptic ulcer can be a cause of deficiency. *H. pylori* may act as an iron-acquisition mechanism in vivo; it competes with the host for iron. *H. pylori* infection may progress into diffuse corpus gastritis. These conditions may play an important role in gastric hypoacidity. On the other hand, as high gastric acidity facilitates the solubilization of non-heme iron, iron uptake may be impaired in subjects with *H. pylori* infection due to loss of iron. Despite all of that, the mechanisms by which *H. pylori* infection may lead to iron deficiency and anemia remains unclear and need more work.
5.2 conclusion

This study found that hemoglobin, PCV, serum iron and ferritin were lower in *H. pylori* patients than healthy subjects (p-value < 0.05). The study concluded that there is an association between *H. pylori* infection and iron deficiency and subsequent iron deficiency anemia (Odds ratio 4.4).
5.3 Recommendations

As we found that *H. pylori* infection is associated with iron deficiency, we recommend that:

1. There may be a clinical value to regularly check any patient with this bacterium if he complained any sign of anemia by measuring complete blood count and iron profile.
2. A further studies with a large sample size to study; with more details the etiological mechanism of association.
3. Also more studies should be done in Sudan to detect *H. pylori* strains that associated with iron deficiency and study if *H. pylori* eradication with or without iron supplementation reveals iron deficiency or not.
References:


APPENDICES
Appendix (1)
Questionnaire
Sudan University of Science and Technology
College of Post Graduate Studies

Association between *Helicobacter pylori* Infection and Iron Deficiency in Sudanese Population

Date: / /2018
Name: .................................................................
Age: .............years

Sex: Male: [ ] Female: [ ]

Smoking: Yes: [ ] No: [ ]

Chronic disease: Yes [ ] No: [ ]

Result :
H. pylori antigen:
Hb: .................g/dl.
PCV: .................% 
MCV: ................. fl
MCH: ................. pg
MCHC: .................
Serum iron: ..............
Serum ferritin: ...........
TIBC: ...................

Date: …/…/……. Signature: …
Appendix (2)

Manufacturer procedures
Appendix (3)

Equipments and devices