Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Serum Iron and Vitamin B12 Levels among *Helicobacter Pylori* Infected Patients in Kingdom of Saudi Arabia

تقييم مستويات الحديد وفيتامين ب12 لدي المرضى المصابين بالجرثومة الملوية البوابية في المملكة العربية السعودية

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

By

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الآية

قال تعالي :

{رَبِّ أَوْزِعْنِي أَنْ أَشْكُر نِعْمَتَكَ الَّتِي أَنْعَمْتَ عَلَيَّ وَعَلَى وَالِدَيَّ وَأَن أَعْمَل صَالِحًا تَرْضَاهُ وَأَدْخِلْنِي بِرَحْمَتِك فِي عِبَادِكَ الصَّالِحِينَ }

صدق الله العظيم

(سورة النمل : الآية 19)

DEDICATION

I dedicate my dissertation to:

My parents

My wife

Sisters & brothers

Lovely kids

&

Those who played a great role in my life

ACKNOWLEDGEMENT

Primary my prays and thanks granted to ALMIGHTY ALLAH, the most gracious and most merciful, who grated me the serenity, means of strength and patience to complete this work.

I deeply indebted to my supervisor Dr. Abu Elgasim Abass Awad Elkareem, for his help and supervision. I gratefully acknowledged him for keen supervision, support, patience and special insights.

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My heartful thanks to my loving and supportive wife for her help and support all the time.

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Abstract

This is a hospital-based analytical case-control study aimed to evaluate serum iron and vitamin B12 among *Helicobacter pylori* infected patients in Kingdom of Saudi Arabia, the study conducted during the period from January to July 2019.

Under aseptic conditions 5 mL of venous blood was collected from 100 participants who selected conviniously (50 were *H. pylori* infected patients as cases and 50 apparently healthy individuals as controls). *H. pylori* screening for antibodies was detected by strips, serum iron was measured using Humalyzer 3000 analyzer and vitamin B12 was measured by electrochemiluminescenc Cobas e411 immunoassay analyzers. The data obtained was analyzed by the SPSS program.

The age of *H. pylori* patients range for 11 to 75 years with mean age was (33.6) years and in controls range for 15 to 72 years with mean age was (38.0) years. The gender of *H. pylori* patients males were 14(28%), and females were 36(72%) and in healthy controls males were 15(30%), and females were 35(70%).

The level of serum iron among cases was (64.6 ± 25.4), and in controls was (95.0 ± 40.8), with statistically significant difference between cases and controls (*P. value* = 0.000). The level of vitamin B12 among cases was (532.1±233.0), and in controls was (657.96±351.9), with statistically significant difference between cases and controls (*P. value* = 0.015).

The relation between iron and vitamin B12 with treatment showed that the levels of iron and vitamin B12 were (73±34 and 595 ±273) in treated patients and (63±24 and 523 ±229) in non- treated, with statistically insignificant differences between treated and non-treated patients (*P. value* = 0.37 and 0.48) respectively.

The comparison of iron and vitamin B12 with sex among cases showed that the levels among males were (79.6±31.1 and 673.3 ±314) and females were (58.8.±20.5 ; 477.1 ±167.5), with statistically significant increases in males than females (P. value = 0.008 and 0.006) respectively.

The study found that there is no correlation between vitamin B12 and age (r= 0.09, sig= 0.519) and no correlation between iron and age (r = -0.027, sig= 0.85).

The study concluded that *H. pylori* causes decrease in iron and vitamin B12 with more decrease in females than males and with no effect with treatment and age.

المستخلص

هذه الدراسة عبارة عن دراسة حالة وحالة ضابطة تحليلية غير تداخليه مستشفوية هدفت إلى تقييم مستويات الحديد وفيتامين ب12 بين المرضى المصابين بعدوى الجرثومة الملوية البوابية في المملكة العربية السعودية خلال الفترة من يناير إلى يوليو 2019 م.

في ظل ظروف معقمة، تم جمع 5 مل من الدم الوريدي من 100 مشارك تم اختيارهم عشوائيا (50 منهم مصابين بمرض الجرثومة الملوية البوابية كعينة دراسية و 50 من المشاركين غير مصابين كعينة ضابطة).

تم الفحص عن الأجسام المضادة للجرثومة البوابية بواسطة شرائط، وتم قياس الحديد في الدم باستخدام جهاز Humalyzer 3000 وتم قياس فيتامين ب12 بواسطة جهاز المقايسة المناعية الكهربائية Cobas e411.

تم تحليل البيانات التي تم الحصول عليها بواسطة برنامج الحزمة الإحصائية للعلوم الاجتماعية(SPSS).

كان متوسط عمر مرضى الجرثومة البوابية 33.6 عامًا (11 - 75 عامًا) وكان عدد عناصر العينة الضابطة 38.0 عامًا (15 - 72 عامًا). كانت النسبة المئوية للجنس في ذكور المصابين بالجرثومة (28٪)، والإناث كانت (72٪) وفي العينة الضابطة كانت نسبة الذكور (30٪)، والإناث كانت (70٪).

كان مستوي الحديد في الحالات المصابة (25.4 ± 64.6)، وفي عناصر العينة الضابطة كان (40.8 ± 95.0)، مع وجود فروق ذات دلالة إحصائية بين الحالات الدراسية والحالات الضابطة (P.value = 0.000).

كان متوسط فيتامين B12 في مرضى الحالات المصابة (233.0 ± 532.1)، وفي عناصر العينة الضابطة كان (351.9 ± 657.9)، مع وجود فروق ذات دلالة إحصائية بين الحالات والضوابط (P.value = 0.015).

تبين العلاقة بين الحديد وفيتامين ب12 مع العلاج ان مستويات الحديد وفيتامين ب12 بين مرضى الجرثومة البوابية المعالجين كانت (34 ± 73) و(273±595) على التوالي، وعند مرضى الجرثومة البوابية لغير المعالجين كانت (229± 523 and 24±63)، مع عدم وجود فروق ذات دلالة إحصائية بين الحالات والعينات الضابطة (P.value = 0.37 and 0.48). تشير العلاقة بين الحديد وفيتامين B12 مع الجنس في الحالات المصابة إلي إن المستويات بين الذكور كانت (314± 673.3 and 31.1±79.6) والإناث كانت (20.5±58.8. 167.5± 477.1)، مع دلالة إحصائية زائدة عند الذكور، (p. value = 0.008 and 0.006) على التوالي .

وجدت الدراسة إلى انه لا يوجد ارتباط بين فيتامين ب12 والعمر (r= 0.09, sig= 0.519) وبين الحديد والعمر(r = -0.027, sig= 0.85).

خلصت الدراسة إلى أن الجرثومة الملوية البوابية تسبب انخفاضا في الحديد وفيتامين ب12 مع انخفاض في الإناث أكثر من الذكور ومع عدم وجود تأثير مع العلاج والعمر.

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Abbreviations

Abbreviation	Meaning
CAB	Chromazurol B
COA	Co Enzyme A
CTMA	Cetyltrimethylammonium
FDA	Food and Drug Administration
H&E	Hematoxylin and Eosin
H. pylori	*Helicobacter pylori*
IARC	International Agency for Research on Cancer
ICT	Immune chromatographic Test
ID	Iron Deficiency
IDA	Iron Deficiency Anemia
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IF	Intrinsic Factor
LCF	Lipid Clearing Factor
MALT	Mucosa-Associated Lymphoid Tissue
MCV	Mean Cell Volume
PCR	Polymerase Chain Reaction
RBC	Red Blood Cells
RGT	Reagent
RUT	Rapid Urease Test
SD	Standard Deviation
SPSS	Statistical Package for Social Science
UBT	Urea Breath Test
WHO	World Health Organization

Chapter I

Introduction

CHAPTER I

INTRODUCTION

1.1 Introduction

Helicobacter pylori (*H. pylori*) is a spiral-shaped microaerophilic bacterium that colonizes the gastric mucosa and causes both acute (Morris and Nicholson, 1987), and chronic gastritis (Dixon *et al*., 1992). At least half of the world's populations are infected by *Helicobacter pylori* (Hocker and Hohenberger, 2003). However, most of the infected people (>70%) are asymptomatic, whereas only <30 are symptomatic, half of the symptomatic patients develop peptic ulcer diseases, lymphoproliferative disorders or gastric cancer. Some of the infected individuals develop duodenal ulcer whereas other develop gastric ulcer (Logan and Wolker, 2001). It is now established that *H. pylori* infection is a persistent condition that is probably related to the occurrence and relapse of peptic ulcer disease (Moss and Calm, 1992), and possibly to the risk of gastric cancer (Forman *et al*., 1991).

H. pylori, which was discovered in 1982, is the most frequently occurring chronic bacterial infection in developing countries (Ou *et al*., 2013). The prevalence of the disease is high >90% in developing countries, whereas the prevalence is less than 40% in developed countries excluding Japan (Garza-González, 2014).

Risk factors of *H. pylori* infection include socioeconomic status, the infection rates were high in a low socioeconomic group (Yucel *et al*., 2009). Other risk factors, such as water sources, peptic ulcer, lower socioeconomic status, consumption of restaurant food, meat, non-filtered water, smoking, typing of housing, presence/absence of sewage system and garbage collection (Parente *et al*., 2006).

H. pylori is contagious, although the exact route of transmission is not known, person-to-person transmission by either the oral-oral or fecal-oral route is most likely (Megraud, 1995).

H. pylori infection causes gastritis and it is associated with the development of peptic ulcer disease, gastric carcinoma and micronutrient deficiencies (Rothenbacher and Brenner, 2003). A recent review of a number of published studies on the influence of *H. pylori* on nutritional status revealed that the infection appeared to have a definite negative effect on iron, vitamin B12 and vitamin C metabolism (Akcam, 2010; Stabler, 2013).

Several theories about *H. pylori* infection can lead to IDA including: impairing iron absorption, competing with the host for uptake of iron, or elevating the pH and reducing vitamin C concentration (Daniel *et al*., 2019).

The mechanisms of vitamin B12 malabsorption caused by *H. pylori* infection are unclear but the followings are the possibilities; diminished acid secretion lead to a failure of vitaminB12splittingfrom food binders, a secretory dysfunction of the intrinsic factor (Liana, 2013), and decreased secretion of ascorbic acid from the gastric mucosa that lead to increased gastric pH (Del and Carmel, 1990).

H. pylori patients had significant decreases in vitamin B12 and serum iron. Eradication of *H. pylori* infection alone may correct vitamin B12 levels and serum iron (Kursad *et al*., 2000).

1.2 Rationale

H. pylori infection is one of the most common gastric infections worldwide. It is estimated that more than half of the adult population in developed countries and 90% of those in the developing countries is infected with this bacterium (Negrini *et al*., 1997). *H. pylori* is one of bacteria exists in Saudi Arabia and associated with gastric and peptic ulcer disease among patients. Furthermore, *H. pylori* seems to be an etiologic factor in iron and vitamin B12 deficiency (Malfertheiner *et al*., 2007 ; Egan *et al*., 2008). The primary goal of the study is to observe whether *H. pylori* infection in the gastric mucosa is responsible for the iron and vitamin B12 deficiency because the early detection and eradication of the H. pylori can prevent the development of complications as gastritis, gastric, duodenal ulcer, iron deficiency and megaloblastic anemia.

1.3 Objectives

1.3.1 General objective

To evaluate serum iron and vitamin B12 among *helicobacter pylori* infected patients in Saudi Arabia.

1.3.2 Specific objectives

1- To estimate serum iron and vitamin B12 in *H. pylori* infected patients and controls.

2- To compare mean of serum iron and vitamin B12 among patients and controls.

3- To correlate serum iron and vitamin B12 with patients variables (sex, age and treatment).

CHAPTER II
LITERATURE REVIEW

CHAPTER II

LITERATURE REVIEW

2.1 *Helicobacter pylori*

2.1.1 General characteristic

H. pylori is a microaerophilic bacterium which means that it requires oxygen to function. However, *H. pylori* require much lower concentrations of oxygen than those found in our atmosphere. This bacterium contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (in the form of H2) produced by intestinal bacteria. *H. pylori* also produces oxidase, catalase, and urease. It has an outer-membrane consisting of phospholipids and lipopolysaccharide which are characteristic of typical Gram-negative bacteria (Baldwin *et al*., 2007). Under certain circumstances, *H. pylori* can be U-shaped or coccoid (Enroth and Wreiber,1999).

2.1.2 Classification of *H. pylori*

The genus *H. pylori* belongs to the subdivision of the *proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella, Flexispira*, *Sulfurimonas, Thiomicrospira*, and *Thiovulum*. To date, the genus *Helicobacter* consists of over 20 recognized species, with many species awaiting formal recognition, It resides naturally in the gastrointestinal tract of humans and animals (Fox, 2002).

2.1.3 Epidemiology of *H. pylori*

Prevalence rates of *H. pylori* infection varies by age, country of origin, and socioeconomic status. Prevalence of infection affects 50% of the population (Correa and Piazuolo, 2008). And is higher in developing countries than that of developed nations (Logan and Wolker, 2001).

The prevalence of *H. pylori* infection is stated to be as high as 80% in the developing countries. The infection penetrates especially during childhood and continues lifelong. The prevalence of *H. pylori* infection in Saudi Arabia was 46.5% reported by (Akeel *et al*.,2018) and 54.6% by (Ayoola *et al*., 2004).

2.1.4 Mechanisms of infection with *H. pylori*

In the stomach, the majority of *H. pylori* can be found in the gastric mucosa; however a few are found adhered to the gastric mucosal epithelium. The bacterium is highly adapted to survive in the hostile environment of the stomach where few other organisms can survive. Although, *H. pylori* is considered to be an extracellular bacteria, there is evidence suggesting that the bacteria has a mechanism for intracellular invasion (Kusters *et al*., 2006).

H. pylori colonizes the human stomach. It colonizes approximately half of the world's population and its infection of the gastric mucosa has been associated with various diseases of the upper gastrointestinal tract, such as chronic gastritis, peptic ulcer, mucosa-associated lymphoid tissue (MALT) lymphoma and gastric adenocarcinoma (Roesler *et al.*, 2014).

H. pylori usually causes asymptomatic gastric infection. Chronic gastritis, peptic ulcer disease, and atrophic gastritis are recognized consequences of this infection. Although *H. pylori* infection causes gastric inflammation virtually in all infected subjects, the majority of infected subjects remain asymptomatic, while certain subset of patients develops atrophic gastritis (Suerbaum and Michetti, 2002)**.**

During its course, the disease can have several manifestations including acute gastritis, chronic atrophic gastritis, intestinal metaplasia, dysplasia, growth failure, malnutrition and finally cancer (Akcam*et al*., 2007;Windle*et al*., 2007*). H pylori* is the major cause of histologic gastritis and also plays an important role in the development of peptic

ulcers, gastric ulcers, gastric carcinoma, and primary gastric B-cell lymphoma (Dixon, 1991;Tytgat*et al*., 1993). *H. pylori* colonizes the entire gastric epithelium, and has an important urease activity, that leads to the ammonia production in order to protect itself from gastric acidity. It produces also other enzymes, such as phospholipase A2 and C, and glycosulfatase, which play a role in the development of the gastric mucosal damage (Dzierzanowska-Fangrat and Dzierzanowska, 2006*). H. pylori*induces an inflammatory response through the gastric epithelium, with production of pro-inflammatory cytokines, such as interleukin 1β and interleukin 8.*H. pylori* is etiologically associated with non-atrophic and atrophic gastritis and peptic ulcer (especially duodenal ulcer). Moreover, there is a deep association between *H. pylori* and primary gastric B-cell lymphoma (mucosa-associated-lymphatic-tissue or MALT-lymphoma) and gastric adenocarcinoma.

H. pylori has been therefore classified by IARC/WHO as ―group 1 carcinogen‖ (Pandey *et al*., 2010).

2.1.5 Diagnostic technique of *H. pylori* infection

2.1.5.1 Invasive tests

2.1.5.1.1 Histology

A routine hematoxylin and eosin (H&E) stain detects *H. pylori* and inflammation (gastritis type) (Rajindrajith *et al*., 2009), when this stain has produced inconclusive results, special stains, such as Giemsa, Warthin-Starry, acridine orange, Toluidine blue, Dieterle, Genta, Romanowski and McMullen stains, or immunochemical methods can be utilized (Garza-González *et al*., 2014).

2.1.5.1.2 Rapid urease test

The test uses the ability of the organism to produce large quantities of urease enzyme for diagnosis of the infection in tissue biopsy specimens. Being rapid, inexpensive, commonly available, and highly

specific are the advantages of RUT as a preferred diagnostic test to be used for detecting *H. pylori* infection (Garza-González *et al.*, 2014).

2.1.5.1.3 Culture

The culture that requires an endoscopy is the gold standard and the most specific method for diagnosing *H. pylori.* It is used for determining antibiotic susceptibility of *H. pylori* in clinical practice (Garza-González *et al*., 2014).

2.1.5.1.4 Molecular methods

The PCR test has been reported to be the most reliable and accurate methods to detect *H. pylori* (Belda *et al*., 2012).It can be performed rapidly and cost-effectively to detect different bacterial genotypes. The PCR can be carried out on tissue and stool specimens and helps identify genes related to antibiotic resistance and virulence (Guarner *et al.,* 2010).

2.1.5.2 Noninvasive tests

2.1.5.2.1 Serology

Serological tests are commonly used to detect immunoglobulin IgG, IgA, or IgM antibodies to *H. pylori* infection, and are accepted as first-line noninvasive diagnostic methods. However, serology does not indicate whether or not the infection is active or past (Czinn, 2005). They cannot be used to observe the success of eradication therapy. A positive IgG test can result in several months or even years after the infection and is not reliable for diagnosis or treatment outcomes (Guarner *et al*., 2010).

2.1.5.2.2 Urea breath test

The UBT is a reliable and noninvasive technique and widely used for determining of *H. pylori* infection in adults and to confirm or monitor the eradication therapy. However, it has less accuracy for the detection of *H. pylori* infection in infants and young children (Ou *et al.,* 2013).

2.1.5.2.3 Stool antigen test

Although *H. pylori* stool antigen test is an excellent technique compared with other techniques, its sensitivity and specificity depend on types of commercial test used, treatment status, cut-off value, and the interpretation of weakly positive results (Koletzko *et al.*, 2011).

2.1.6 Treatment of *Helicobacter pylori* infections

Therapy for *H. pylori* infection consists of 10 days to 2 weeks of one or two effective antibiotics, such as amoxicillin, tetracycline,. metronidazole, or clarithromycin, plus either ranitidine bismuth citrate or bismuth subsalicylate. Currently, eight *H. pylori* treatment regimens are approved by the Food and Drug Administration (FDA); antibiotic resistance and patient noncompliance are the two major reasons for treatment failure. Overall, triple therapy regimens have shown better eradication rates than dual therapy. A longer length of treatment (14 days versus 10 days) results in better eradication rates (Soll, 1996). *H. pylori* eradication has also been shown to improve the absorption of other nutrients, including iron, and produce more rapid and complete clinical responses in patients with iron deficiency anemia (Choe *et al*., 1999; Annibale *et al*., 2002).

2.2 Iron deficiency anemia

2.2.1 Definition

Iron deficiency anemia is a decrease in the total hemoglobin levels caused by insufficient iron to maintain normal physiologic functions. Iron deficiency anemia results from inadequate iron absorption to a accommodate an increase in requirements attributable to growth or resulting from a long-term negative iron balance either of these situations leads to a decrease in iron stores as measured by serum ferritin concentrations or bone marrow iron content (Baker and Greer, 2010).

2.2.2 Causes of iron deficiency anemia

Iron deficiency anemia can be the consequence of several factors, stomach ulcer, piles, ulcerative colitis, and bowel cancer may cause bleeding in the gut and result in anemia. Kidney or bladder disease can cause bleeding that can result in anemia. Certain medical conditions, such as rheumatoid arthritis or cancer, can lead to iron deficiency anemia. Long-term aspirin taking is associated with iron deficiency anemia (Bermejo and Garcia-Lopez, 2009 ; Goldberg, 2013).

2.2.3 Symptoms of iron deficiency anemia

Common symptoms of IDA include breathlessness, tiredness, dizziness, tachycardia, headache, and paleness (Zhu *et al*., 2010 ; Goldberg, 2013).

2.2.4 Diagnosis of iron deficiency anemia

Iron deficiency is usually diagnosed with a laboratory. Low hemoglobin in the setting of a reduced MCV is usually the initial finding on a routine complete blood count. Then, ferritin level <10 ng/dl diagnosed as IDA tests (Bermejo and Garcia-Lopez, 2009 ; Short and Domagalski, 2013).

2.2.5 Association between iron deficiency and *H. pylori*

Several epidemiological studies have revealed an association between *H. pylori* and iron deficiency anemia (Akcam, 2007). *H. pylori* related gastritis and its effects on gastric physiology, affecting the normal process of iron absorption, may possibly explain this phenomenon (Annibale *et al*.,2000); however, *H. pylori* is probably responsible of iron deficiency anemia through several mechanisms. Hypochlorhydria might induce the conversion of ascorbic acid to dehydroascorbic acid – a less active form –hampering the promotion of iron absorption; moreover, the reduction of the ferric to ferrous form, which is fundamental for the absorption of non-heme iron, might be impaired by *H. pylori* infection, that cause an increase of gastric pH and consequent decreases in gastric

acid secretion. Since iron is an essential growth factor for *H. pylori*, it also competes with the host for iron absorption (Capurso *et al*., 2001).

H. pylori possesses some proteins of the outer membrane that play a role in bacterial iron absorption as well as intracellular storage proteins with similar characteristics as ferritin (Perez and Israel, 2000). The association between iron deficiency anemia and *H. pylori* infection is strongly recommended by Maastricht III European guidelines in patients with unexplained sideropenic anemia (Malfertheiner *et al.*, 2007)

2.3 Vitamin B12

2.3.1 Definition and structure

Vitamin B12 or cyanocobalamin is a relatively large and complex water-soluble vitamin. The molecular weight of vitamin B12 is equal to 1355.4 (Watanabe, 2007). Vitamin B12 represents all potentially biologically active cobalamins in this review. Cobalamin is the term used to refer to a group of cobalt-containing compounds (corrinoids) that have a lower axial ligand that contains the cobalt-coordinated nucleotide (5,6-dimethylbenzimidazole as a base); cyanocobalamin, which is used in most supplements, is readily converted to the coenzyme forms of cobalamin (methylcobalamin and 5- deoxyadenosylcobalamin) in the human body (Ball, 1998).

2.3.2 Sources of vitamin B12

Vitamin B12 is synthesized only in certain bacteria (Scheider and Stroin˜ski, 1987). The vitamin B12 synthesized by bacteria is concentrated mainly in the bodies of higher predatory organisms in the natural food chain system. Animal foods (i.e., meat, milk, egg, fish, and shellfish) but not plant foods are considered to be the major dietary sources of vitamin B12 (Ball,1998). Some plant foods, such as edible algae or blue-green algae (cyanobacteria), however, contain large

amounts of vitamin B12.Vitamin B12 compounds in algae appear to be inactive in mammals (Watanabe *et al*., 2002).

2.3.3 Body requirements of vitamin B12

The recommended dietary allowance of vitamin B12 for adults is set at 2.4 μg/day in the United States and Japan; however, daily body loss of the vitamin is estimated to be between 2 and 5 μg/day (Institute of Medicine, 1998). (Bor *et al*., 2006) reported that a daily vitamin B12 intake of 6 μg appears to be sufficient to maintain a steady-state concentration of plasma vitamin B12 and vitamin B12-related metabolic markers.

2.3.4 Functions of vitamin B12

Vitamin B12 comprises a number of forms including cyano-, methyl-, deoxyadenosyl- and hydroxy-cobalamin. The cyano form, which is used in supplements, is found in trace amounts in food (Scott, 1997). The other forms of cobalamin can be converted to the methyl- or 5-deoxyadenosyl forms that are required as cofactors for methionine synthase and L-methyl-malonyl-CoAmutase. Methionine synthase is essential for the synthesis of purines and pyrimidines. Vitamin B12 is responsible for RBCs maturation, a deficiency of vitamin B12 and the interruption of this reaction leads to the development of megaloblastic anemia. Folate deficiency independent of vitamin B12 also causes megaloblastic anemia (Gibson, 2005).

2.3.5 Deficiency of vitamin B12

Vitamin B12 deficiency is usually caused by the malabsorption of vitamin B12 although dietary inadequacy is common in the elderly or vegans. Causes can also relate to inadequate intrinsic factor production, atrophic gastritis, drug-nutrient interactions as well as some less common genetic defects (Food and Nutrition Board Institute of Medicine, 1998 ; Park and Johson, 2006). Pernicious anemia is the end stage of

autoimmune gastritis and results in the loss of synthesis of IF. It is this loss of IF that causes vitamin B12 deficiency and if untreated, megaloblastic anemia and neurological complications develop (O'Leary and Samman, 2010; Stabler, 2013).

2.3.6 Association between vitamin B12 and *H. pylori*

Helicobacter pylori have been suggested as an etiologic factor in vitamin B12 deficiency, in people with a high prevalence of vitamin B12, the frequency of vitamin B12 deficiency and its clinical consequences can be estimated to be high (Carmel *et al*., 2001*)*.

A mechanism that has been proposed to explain this association is that the action of *H. pylori* decreases gastric acid secretions which leads to hypochlorhydria (Annibale *et al*., 2002). The action of gastric acid in the stomach is required to release protein bounded vitamin B12 on the one hand while hypochlorhydria itself leads to an increase in the bacterial population of the stomach and intestines. These bacteria consume the vitamin B12 themselves (Baik and Russell, 1999). Hypochlorhydria also reduces the release of vitamin B12 bounded to proteins thereby preventing binding to intrinsic factor and absorption. Intrinsic factor decreases due to atrophy of the gastric mucosa which decreases the ability to absorb vitamin B12 in the intestines. In addition, it has been proposed that vitamin B12 deficiency is secondary to decreased production of an intrinsic factor due to atrophic gastritis (pernicious anemia) which results from chronic *H. pylori* infections (Carmel, 1995; Stopeck, 2000; Lahner, 2012).

Chapter III

Materials and Methods

CHAPTER III

MATERIALS AND METHODS

3.1 Study design

This is a hospital-based analytical case-control study.

3.2 Study area and duration

This study was conducted in Al Rafig National Hospital and Al Mikhwah National Hospital (Saudi Arabia) during the period from January to July 2019.

3.3 Study population:

Patients attended to the two hospitals, both females and males suffering from *H. pylori* infection were enrolled in this study.

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria

Patients of both sexes known to have *H. pylori* infection were enrolled to participate in this study.

3.4.2 Exclusion criteria

- Patients with history of inflammatory bowel disease, resection of stomach or small bowl surgery, strict vegetarian, pregnancy, and patients under *H. pylori* eradication therapy.
- Patients with malabsorption syndrome and folic acid deficiency.
- Patients who are receiving immunosuppressive or chemotherapeutic drugs.

3.5 Ethical consideration

All participants were informed verbally and satisfied with the study objectives recruited in this study. Privacy and confidentiality for each participant were guaranteed.

3.6 Sample Size

The sample of the study consists of 100 participants divided equally into two groups: cases group consist of 50 patients with positive *H. pylori*infection, and controls group consist of 50 healthy individuals.

3.7 Data collection

Questionnaires were filled for personal information and history of treatment that collected from medical file with the help of physician.

3.8 Specimen collection and processing

3.8.1 Specimen collection

Under aseptic conditions 5 mL of venous blood was collected from each subject (cases and controls). The blood was drawn in a plain container; serum was separated then *H. pylori* detection was performed. The rest of the serum was stored at -20˚C for later iron and vitamin B12 levels estimation.

3.8.2 Methods

3.8.2.1 Serological test for *Helicobacter pylori*

H. pylori investigation was determined by colored chromatographic immunoassay using ICT rapid test. A rapid one step test for the qualitative detection of antibodies to *H. pylori* in human serum or plasma.

3.8.2.1.1 Principle

The one step *H. pylori* test device (serum/plasma) is a qualitative membrane based immunoassay for the detection of *H. pylori* antibodies in serum or plasma. In this test procedure, anti-human IgG was immobilized in the test line region of the test. After specimen was added to the specimen well of the device, it reacted with *H. pylori* antigen coated particles in the test. This mixture migrated chromatographically along the length of the test and interacted with the immobilized anti-human IgG. If the specimen contained *H. pylori* antibodies, a colored line would appear

in the test line region indicating a positive result. If the specimen did not contain *H. pylori* antibodies, a colored line would not appear in this region indicating a negative result. To serve as a procedural control , a colored line would always appear in the control line region. If the control line did not appear, the test result was not valid.

3.8.2.1.2 Procedure

The test, serum specimen, controls and device were allowed to pouch to reach room temperature prior to testing. The test device was removed from the sealed pouch and was used as soon as possible. The test device was placed on a clean and level surface, the dropper vertically and 3 drops of serum or plasma were transferred to the specimen well(S) of the test device, and the timer was started. Trapped air bubbles were avoided in the specimen well. Results were read at 10 minutes.

3.8.2.1.3 Interpretation of results

The test was considered positive when two distinct colored lines appeared. One line in the control line region (C) and the other line in the test region (T). The intensity of the color in the test line region (T) varied depending on the concentration of *H. pylori* antibodies in the specimen. Therefore, any shade of color in the test line region (T) also was considered positive. Negative was considered when one colored line appeared in the control line region (C) without apparent red or pink line in the test line region (T).

Invalid result was considered when no line appears in the control line region (C). If this occurred, the direction was read again and the test was repeated with a new test. If the result was still invalid, the test kit was stopped using immediately.

3.8.2.2 Determination of serum iron

Serum iron was measured using the iron liquicolor on photometric colorimetric test for iron with Lipid Clearing Factor (LCF) CAB method by using Humalyzer 3000 analyzer.

3.8.2.2.1 Principle

Iron (III) reacted with chromazurol B (CAB) and cetyltrimethylammonium-bromide (CTMA) to formed a colored complex with an absorbance maximum at 623 nm. The intensity of the color produced is directly proportional to the concentration of iron in the sample.

3.8.2.2.2 Contents

RGT :

CAB	0.18 mmol/l
CTMA	2.2 mmol/l
Guanidinium chloride	2.6 mol/l
Sodium acetate buffer (pH 4.7)	45 mmol/l

STD : 5 ml standard reagent 100mg/dl

3.8.2.2.3 Procedure

Pipette into cuvettes	Rb	Sample / STD
Sample / STD	---	50 µL
Distilled Water	50 µL	---
RGT	1000 µL	1000 Ml

mixed well, incubated for 15 minutes at R.T. The absorbance of the sample and the standard against the reagent blank were measured after 15 minutes.

3.8.2.2.4 Calculation

$$\text{Iron [µg/dl]} = \frac{\Delta A \text{ Sample}}{\Delta A \text{ Std/Cal}} \times \text{Conc. of Std [µg/dl]}$$

3.8.2.3 Determination of serum vitamin B12

Serum vitamin B12 was determined quantitative in human serum using the electrochemiluminescence immunoassay is intended for use on Cobas e411 immunoassay analyzers.

3.8.2.3.1 Principle

Competition chemiluminescent enzyme immunoassay.1st incubation by incubated the sample with the vitamin B12 pretreatment1 and 2. 2nd incubation by incubated the pretreated sample with the ruthenium labeled intrinsic factor.3rdincubation, after added of streptavidin-coated microparticles and vitamin B12 labeled with biotin, with formation of a ruthenium labeled intrinsic factor-vitamin B12 biotin complex. The entire complex became bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Reagents: working solution

- Pretreatment reagent 1, 4 ml: Dithiothreitol; stabilizer.
- Pretreatment reagent 2, 4 ml: Sodium hydroxide; sodium cyanide.
- Streptavidin-coated microparticles, 6.5 ml; preservative.
- Intrinsic factor, 10 ml: Ruthenium labeled porcine intrinsic factor; cobinamidedicyanide; stabilizer; human serum albumin; phosphate buffer, pH 5.5; preservative.

- Vitamin B_{12}-biotin, 8.5 ml: Biotinylated vitamin B_{12} ; biotin; phosphate buffer; pH 7.0; preservative.

3.8.2.3.2 Assay

For the optimum performance of the assay the direction given was followed in document for the analyzer concerned. Resuspention of the microparticles takes place prior was used. The test-specific parameters were read via barcode. The 15-digit sequence of numbers were entered. The cooled reagents were brought to 20˚C and placed on the reagent disk of the analyzer. Foam formation was avoided. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

3.9 Statistical analysis

Data collected was analyzed by Statistical Package for Social Science for a computer program (IBM- SPSS). Mean, frequency, independent t-test and Pearson's correlation were applied. *P-value* less than (0.05) was considered significant.

CHAPTER IV

RESULTS

CHAPTER IV

RESULTS

4. Results

The study sample consist of 100 participants divided equally into two groups: 50 cases with positive *H. pylori* infection diagnosed clinically and confirmed by laboratory diagnosis and 50 healthy controls.

The study showed that 36 (72%) of cases were females and 14 (28%) were males, 35(70%) of controls were females and 15(30%) were males (table 4.1). The age ranged between 11 – 75 years (mean ages was 33.6 years) in cases and 15 – 72 years (mean ages was 38.0 years) in controls (table 4.2).

There were statistically significant decreases in the mean serum iron level between cases (64.6 ±25.4) and controls (95.0 ± 40.8), (P. value= 0.000) (table 4.3).

There were significant decreases in the mean serum vitamin B12 level between cases (532.12 ±233.02) and controls (657.96 ±351.9), (P. value= 0.015) (table 4.3).

There were statistically insignificant difference in the mean levels of iron and vitamin B12 between treated (73±34 and 595 ±273)and non- treated (63±24 and 523 ±229) patients, P. value (0.48 and 0.37) respectively (table 4.4).

There were statistically significant increases in the mean levels of iron and vitamin B12 between males (79.6±31.1 and 673.3 ±314) and females (58.8.±20.5 and 477.1 ±167.5) in patients, P. value (0.008 and 0.006) respectively (table 4.5).

We found no correlation between iron and age among patients (r = -0.027, sig= 0.85) as indicated in (fig 4.1), and between vitamin B12 and age among patients (r= 0.09, sig= 0.519) as indicated in (fig4.2).

Table 4.1: Distribution of sex among study population:

Sex	Cases N = 50	Controls N = 50
Males	14(28%)	15 (30%)
Females	36(72%)	35 (70%)
Total	50 (100%)	50 (100%)

Table 4.2: Age mean and range among study population:

Age (mean and group)	Cases N = 50	Controls N = 50
Ages mean/year	33.6	38.0
Ages range/year	11 – 75	15 – 72

Table 4.3: The relation between serum iron, vitamin B12 in *H. pylori* patients and controls:

Parameter	Cases N = 50	Control N = 50	P. value
	Mean ± SD	Mean ± SD	
Iron (µg/dl)	64.6 ±25.4	95.02 ± 40.8	0.000
B12 (pg/ml)	532.12 ±233.02	657.96 ±351.9	0.015

Table 4.4: Comparison of iron and vitamin B12 mean level among patients with previous treatment history and those with no-treatment:

Parameter	Treatment N = 6	Non-treatment N = 44	P. value
	Mean ± SD	Mean ± SD	
Iron (µg/dl)	73 ±34	63 ±24	0.37
B12 (pg/ml)	595 ±273	523 ±229	0.48

Table 4.5: The relation between iron, vitamin B12 and sex among cases:

Parameter	Males N = 14	Females N = 36	P. value
	Mean ± SD	Mean ± SD	
Iron (µg/dl)	79.6 ±31.1	58.8 ±20.5	0.008
B12 (pg/ml)	673.3 ±314	477.1 ±167.5	0.006

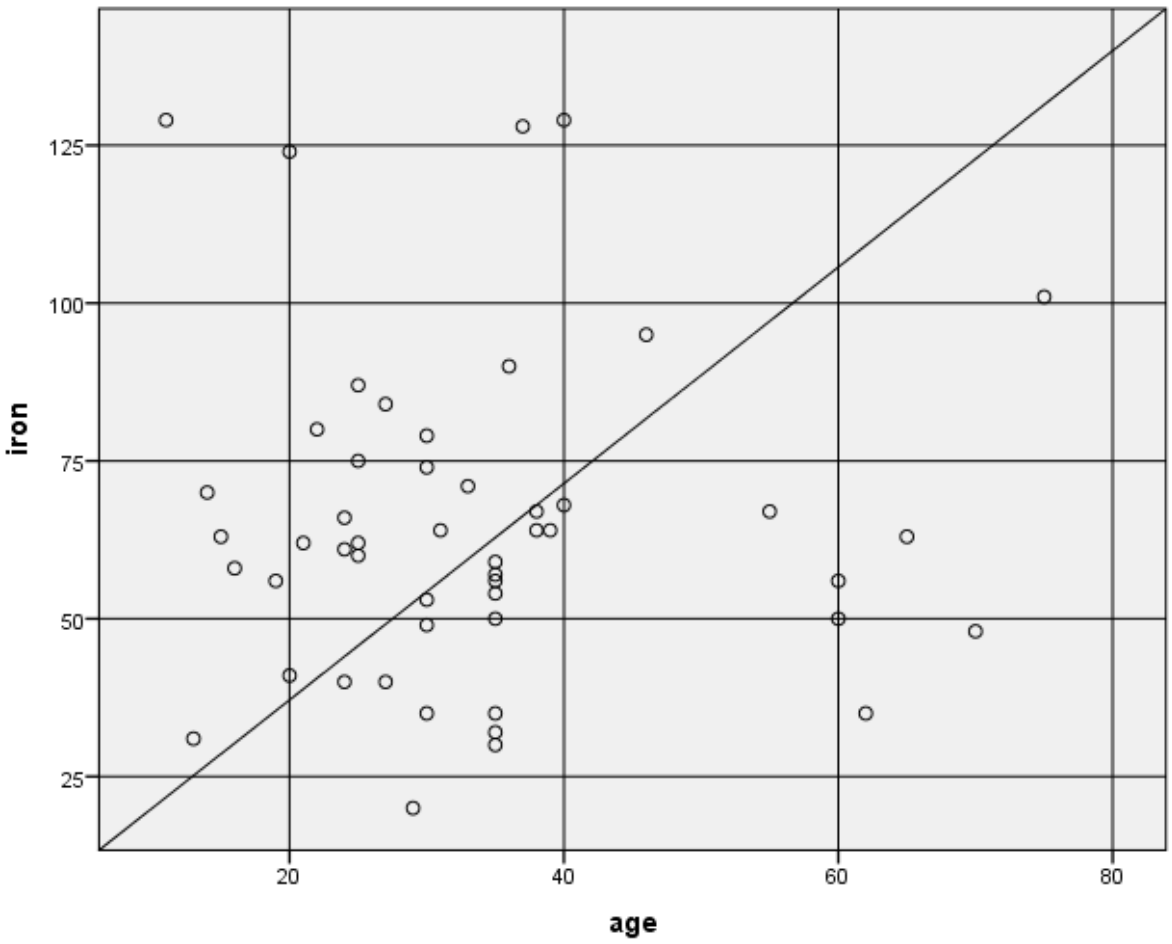

Fig 4.1: Correlation between iron and age among *H. pylori* patients (sig=0.85, r= -0.027).

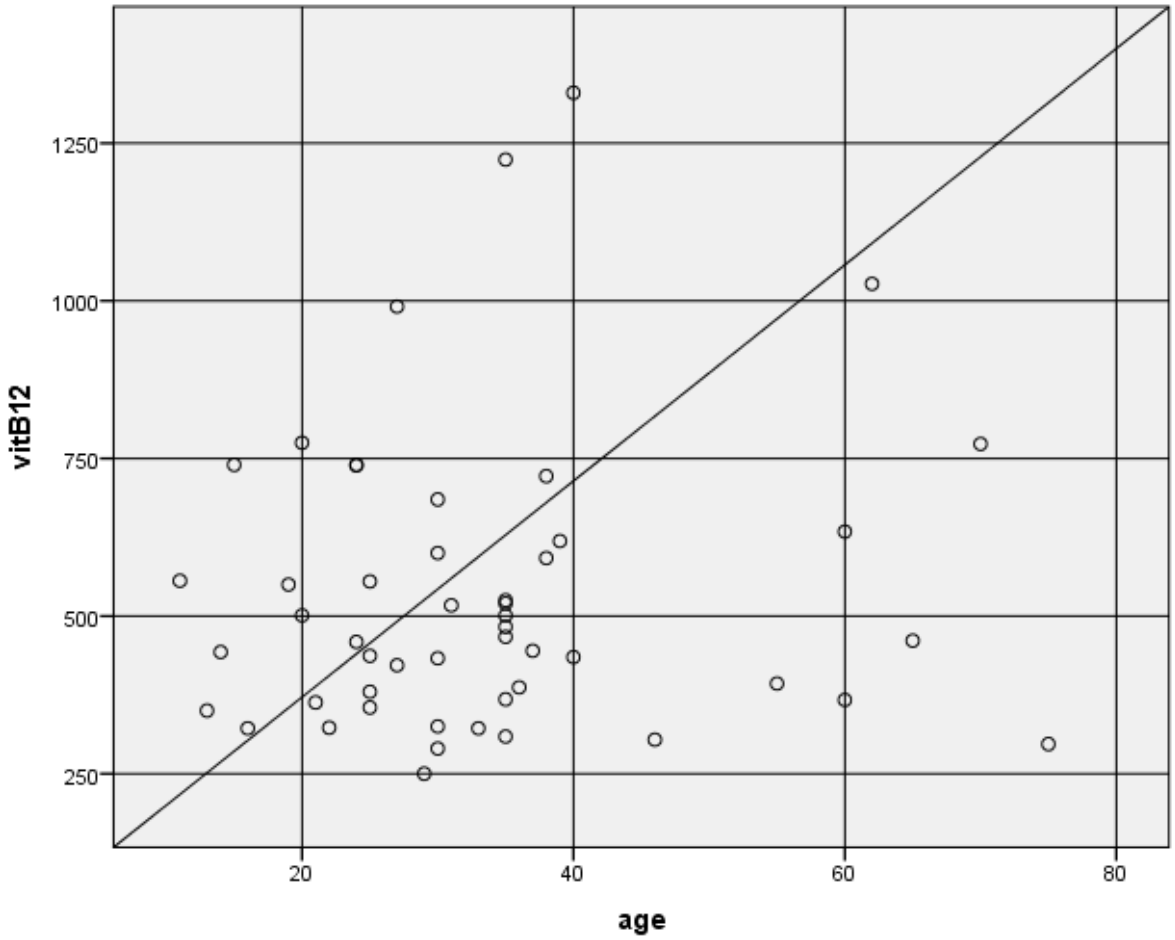

Fig 4.2: Correlation between vitamin B12 and age among *H. pylori* patients (sig=0.519, r=0.09).

Chapter V

Discussion, Conclusion and Recommendations

CHAPTER V
DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

H. pylori is a common gastrointestinal tract infection that affects a majority of the population. The malabsorption of vitamin B12, which is observed in gastritis due to overgrowth of *H. pylori* in the stomach, could result in hypochlorhydria. Thus, lack of an intrinsic factor may play a role in causing malabsorption of vitamin B12 in most patients with atrophic gastritis (Kaptan *et al*., 2000). Previous study suggested that *H. pylori* was an important factor in the development of atrophic gastritis, and the eradication of *H. pylori* infection may be beneficial in treatment of cobalamin deficiency (Carmel *et al*., 1994). Iron deficiency anemia has confirmed the etiological role of *H. pylori*, but the relationship remains controversial. Some previous studies have reported that *H. pylori* associated with iron deficiency anemia; since *H. pylori* colonization in the gastric mucosa may disturb some functions of the mucosa, it leads to a decrease in iron absorption and increases iron loss (Muhsen and Cohen, 2008 ; Monzón *et al*., 2013).

Regarding the age of study subjects we found that the *H. pylori* infects people at any age group. This result agrees with Khan,(1998) who concluded that *H. pylori* infection is acquired early in life, and there is no rise in the incidence with advancing age.

The study showed that serum iron was lower among *H. pylori* infected person, this result agrees with Bohr *et al*, (2007) and Hershko and Ronson, (2009) they reported that ferritin levels were lower in patients with *H. pylori*-positive gastritis than in those with *H. pylori* negative gastritis or healthy individuals. And also agrees with Valiyaveettil *et al*,

(2005) and Gessner *et al*, (2006) they described a direct relation between *H. pylori* and iron deficiency (ID) .

The study showed that vitamin B12 level was significantly lower among *H. pylori* infected person. This result is in agreement with Gümürdülü *et al*, (2003), Devrajani *et al*, (2011) and Ayesh *et al*, (2013) they reported that there was a statistically significant relation between *H. pylori* infection and serum vitamin B12 deficiency.

Our study showed that no differences in levels of serum iron and vitamin B12 among patients with previous treatment history & those with non-treatment. This study disagrees with Serin *et al*, (2002) and Saleh and Wesam, (2018), they reported that the serum iron and vitamin B12 were restore of the patients following eradication of *H. pylori* infection.

Regarding to the gender, our study showed that, there was significant low levels of serum iron among females compared to males in cases group. This study agrees with Choe *et al*, (2001), who reported that the association between iron status and *H. pylori* were largely restricted to girls rather than boys, and contradicted with Meroj *et al*, (2011) who reported that no significant differences of iron and ferritin between women and men.

We found that vitamin B12 levels was low in females than males in patients. This result is agrees with Gümürdülü et al, (2003)**,** who reported that vitamin B12 levels was low in females than males in *H. pylori* patients.

The study showed no correlation between serum iron and age in patients. This result agrees with Frank and Thomas, (2013), who reported that serum iron was not affected by age class. Also, there was no correlation between vitamin B12 and age. This result agrees with Shuval-Sudai and Granot, (2003), and Serin *et al*, (2002), whose concluded that vitamin B12 level did not correlate with age.

5.2 Conclusion

From these results, we concluded that:

- The iron and vitamin B12 levels were low among *H. pylori* patients.
- The iron and vitamin B12 levels were significantly low in females among *H. pylori* patients.
- No significant differences in levels of iron and vitamin B12 between treated and non-treated patients.
- No correlation between iron and age and between vitamin B12 and age.

5.3 Recommendations

On the basis, we recommend the following:

- Further studies about large sample size.
- Iron and vitamin B12 testing and are recommended in *H. pylori* patients.

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Appendix

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Evaluation of Serum Iron and Vitamin B12 among *Helicobacter Pylori* Infected Patients in Saudi Arabia

1. Personal information's:

1. Name:…….....2. Age:……

3. Gender Male ☐ Female ☐

4. Address:……5. Telephone…….

5. Treatment Yes ☐ No ☐

2. Laboratory Tests:

Serum vitamin B12 : ……pg / ml

Serum iron: ……mg / dl