بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Assessment of Serum High Sensitivity C-Reactive Protein, Lipid Profile and Magnesium among Sudanese Patients with H. Pylori Infection .

(In Khartoum State)

تقييم مستويات بروتين سي عالي الحساسية ومستوى الدهون والمغنسيوم في مصل الدم لدى المرضى السودانيين المصابين بالبكتريا الحلزونية (في ولاية الخرطوم)

A dissertation submitted in partial fulfillment for the Requirements of M.Sc. degree in Medical laboratory science -Clinical Chemistry

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بسم الله الرحمن الرحيم

الآيـــــة

قال تعالي :

﴿ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِينَ ﴾

صدق الله العظيم

سورة الفاتحة الآية (1)

Dedication

I would like to dedicate this firstly to my beloved Parents, brothers and sisters
Without your encouragement and heartfelt support, I would be lost.

To my Supervisor
Dr. GhadaAbdelrahmanElfadil

To My friends and colleagues
Your presence in my life itself is enough.

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Praise to Allah, the Almighty, who gave me the patience and power to finish this work

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For Saad Rashwan Medical Center Staff, and singa center health ,for their assistance in sample collection

To my friends, colleagues and relatives
whom assisted me

Abstract

Background and Aim : *Helicobacter pylori* (*H.pylori*) colonize the human gastric and duodenal mucosa, and the infection may cause peptic ulcers and gastritis. The aim of the study was to assess the level of serum concentration of HsCRP ,lipid profile and Magnesium of gastritis *Helicobacter pylori*-Infected patients with those of gastritis *H.pylori* negative.

Materials and Methods: This was cross sectional comparative study. The study was conducted in 40 gastritis *H.pylori*-infected patients and 40 matched gastritis *H.pylori* negative as control. Stool Antigen *H.pylori* test was used to determine *H.pylori*, and BS200 was employed to analyze total cholesterol ,LDL-C,HDL-C and ICHROMA 11 was employed to analyze Hs CRP and biosystem350 spectrophotometer to determinde Magnesium concentrations.

Results: Compared to the control subjects, there were significant increase in meams serum level of Hs.CRP, total cholesterol, and LDL-C, P value less than 0.05; for Hs.CRP mean patient vs control (8.5 ±2.1mg/l vs 4.60±2.20mg/l) (p=0.000), for total cholesterol mean patient vs control (150 ±38mg/dl vs 106±27.7mg/dL) (p=0.026), for LDL-C mean patient vs control (70.5 ± 20.1mg/dl vs 54.5±12.7mg/dL) (p=0.006) , there were insignificant decrease in mean of serum HDL-C and magnesium Level concentration, P. value more than 0.05, for HDL-C mean patient vs control (41.4 ±21.3 mg/dL vs 46.1±13.9 mg/dL) (p=0.990), for Magnesium mean patient vs control was (1.9 ± 0.2mg/dL vs 2.00±0.20mg/L) (p= 0.58).

Conclusion: Sudanese patient with H. pylori infection had increased level of serum Hs CRP, Total Cholesterol and LDL-C level, and had decreased level of serum HDL-C and magnesium.

المستخلص

الخلفية والأهداف: البكتريا الحلزونية تستوطن في الغشاء المخاطي للمعدة والإثني عشر في الإنسان، هذه ألعدوي قد تسبب قرحة والتهاب في المعدة، والهدف من الدراسة قياس بروتين سي عالي حساسية التفاعل, ومستوي الدهون والمغنسيوم بين المرضي المصابين بالبكتريا الحلزونية ويعانون من التهاب المعدة والأشخاص غير المصابين بالبكتريا الحلزونية ويعانون من التهاب المعدة.

المواد والطرق:هذه الدراسة المقطعية دراسة مقارنة. أجريت الدراسة علي 40 من المرضي المصابون بالبكتريا الحلزونية ويعانون من التهاب المعدة، و 40 مطابقون لهم في (العمر و النوع) غير مصابون بالبكتريا الحلزونية ويعانون من التهاب المعدة. أُستخدم فحص البكتريا في البراز لاكتشاف البكتريا. واستخدم جهاز(بي اس 200) المحلل الكيميائي الآلي بالكامل المنتج من قبل شركة ميندراي الألمانية لقياس الكلوسترول الكلي والبروتين ألدهني منخفض الكثافة البروتين ألدهني عالي الكثافة. وأيضا جهاز الاي كروما تو 11 لقياس بروتين سي عالي حساسية التفاعل و أيضا جهاز بايوسستم 350 لقياس الماغنسيوم . البيانات التي تم الحصول عليها عولجت وحللت باستخدام برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة 16.0 للحصول على النتائج

النتائج:بالمقارنة مع مجموعة الضبط أظهرت هذه الدراسة ان هناك زيادة ملحوظة في مستوى بروتين سي عالي حساسية التفاعل, والكلسترول الكلي والكلسترول الدهني منخفض الكثافه في المصابين بمرض بكتريا المعدة الحلزونية مقارنة بمستواه في الأشخاص غير المصابين بالبكتريا الحلزونية ويعانون من التهاب المعدة لبروتين سي عالي حساسية التفاعل احتمال إحصائي يساوي(000. 0) ووسط حسابي يساوي(2.1 ±8.5) مقارن بالكنترول (4.60±2.20mg/l)،وللكلسترول الكلي احتمال إحصائي يساوي(0.026) ووسط حسابي يساوي(±150 38.9)مقارنه بالكنترول (106±27.7mg/l) , وللبروتين ألدهني منخفض الكثافة احتمال إحصائي يساوي(0.006)ووسط حسابي يساوي (20.1 ±70.5)مقارنه بالكنترول (54,5±12.7mg/dL) .أيضا أظهرت هذه الدراسة ان هنالك نقصان في مستوي البروتين ألدهني عالي الكثافة والمغنيسيوم في المصابين بمرض بكتريا المعدة الحلزونية مقارنة بمستواه في الأشخاص غير المصابين بالبكتريا الحلزونية ويعانون من التهاب المعدة للبروتين الدهني عالي الكثافه احتمال إحصائي يساوي (0.99) ووسط حسابي يساوي (21.3 ±41.4)مقارنه بالكنترول, (46.1±13.9mg/dL)للمغنسيوم احتمال إحصائي يساوي(0.58) ووسط حسابي يساوي 0.2± 1.9) mg/l) مقارنه بالكنترول(2.00±0.20mg/L).

الخلاصة: مستويات بروتين سي عالي الحساسية والبروتين الدهني منخفض الكثافة والكولسترول الكلي في مصل الدم تزيد زيادة ذات دلالة إحصائية في المرضي المصابين بالبكتريا الحلزونية ويعانون من التهاب المعدة اما البروتين ألدهني عالي الكثافة والمغنسيوم فتنقص في ا لمرضي المصابين بالبكتريا الحلزونية ويعانون من التهاب المعدة.

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Chapter One

Introduction, Rationale and Objectives

1- Introduction, Rationale and Objectives

1.1. Introduction:

Helicobacter pylori (H. pylori) is a spiral-shaped, gram-negative bacterium that inhabits the stomach mucosa, and further more from child to elderly in developing countries This bacterium can elicit lifelong inflammatory and immune responses with release of various bacterial and host-dependent cytotoxic substances ,resulting in chronic gastritis, peptic ulcer, and gastric cancer (Ishida *etal*., 2008).

Isolated and cultured of H.pylori in early eighties by Warren and Marshall, and after few years they recognized its relationship to gastric and duodenal ulcers at all ages(salih *et al*., 2017).

Human C-reactive protein (CRP) is one of the so called acute phase proteins. Its concentration in blood increases rapidly as a response to inflammation. CRP is a 224 residue protein with a monomer molecular mass of approximately 25 kDa and,It be- longs to pentraxins, an evolutionally conserved family of proteins characterized by calcium dependent ligand binding and radial symmetry of five monomers forming a ring around central pore (Hirschfield *et al*., 2003) .

C-reactive protein is accepted in clinical use as a major, although rather non-specific, marker of inflammation. In generally healthy people, CRP levels are usually less than 5 mg/L. In pathology, CRP concentration has an enormous, 10,000-fold dynamic range (approximately 0.05–500 mg/L) (Lowe *et al*., 2006) .

The highest levels of CRP (above 30 mg/L) are observed in bacterial infection, such as septic arthritis, meningitis and pneumonia n 2003, the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) issued a statement that identified CRP as the inflammatory marker best suited for use in current clinical practice to assess cardiovascular risk (Ridke., 2003).

Many epidemiologic studies have indicated that CRP is a strong independent predictor of future cardiovascular events, including myocardial infarction, ischemic stroke, peripheral vascular disease, and sudden cardiac death without known cardiovascular disease (as reviewed by Clearfield (Clearfield., 2005).

CRP has been shown to participate in inflammatory as well as innate immunity processes. Important bioactivities of CRP are determined by its ability to bind to a variety of ligands, such as damaged cell membranes, apoptotic cells and fibronectin, with the highest affinity to phosphocholine residues. When CRP is ligand-bound, it can be recognized by the complement component C1q, which leads to activation of the classical complement pathway. On the other hand, via interaction with the complement factor H, CRP regulates the alternative complement pathway (Bıro *et al*., 2007) .

Lipids play a critical role in almost all aspects of biological life – they are structural components in cells and are involved in metabolic and hormonal pathways. The importance of having a knowledge of lipid disorders cannot be overstated, not least because they are common in clinical practice and, in some cases associated with atherosclerosis such as coronary heart disease, one of the biggest killers in urbanized societies(Raton *et al*., 2013).

Magnesium is the second most abundant intracellular cation and it plays a key role in a wide range of cellular functions(de Baaij *et al*., 2015).

Total body magnesium depends on dietary intake and recent studies showed that the vast majority of elderly do not consume the average dietary requirement for magnesium.2 In addition to a magnesium-insufficient diet, elderly are also at risk for hypomagnesaemia due to comorbidities and medication that increase urinary excretion of magnesium(de Baaij *et al*., 2015) .

The prevalence of hypomagnesaemia in the general population is estimated at 2%,3 but it may be as high as 53% in specific high-risk groups, such as patients

with chronic heart failure.4 Although hypomagnesaemia may have acute and chronic complications, serum magnesium is still measured relatively infrequently (de Baaij *et al*., 2015) .In recent studies, low serum magnesium has been associated with inflammation and disturbances in the regulation of vascular tone and endothelial function(Shechter et al., 2000). These mechanisms are thought to contribute to the development and progression of atherosclerosis, potentially worsening coronary heart disease (CHD) (Amighi *et al*.,200).

1.2 Rationale :

Helicobacter pylori (HP) is a gram-negative bacillus responsible for one of the most common infections found in humans worldwide**.**This gram negative bacillus is more prevalent in developing countries, more often among younger ages reaching up to 10% of the population in comparison to only 0.5% in more developed world. Helicobacter pylori (HP) usually acquired in children who develop peptic ulcer disease in adulthood but without proven evidence for causing gastrointestinal symptoms in children. H. pylori infection might be involved in the pathogenesis of coronary heart disease .The suspicion about H. pylori involvement in the pathological lesions is based on the following: local inflammation can have systemic effects, H. pylori gastric infection is a chronic process that lasts for decades; and persistent infection induces chronic inflammatory and immune responses that can induce lesions both local and remote sites from the primary infection. In the last few years, a number of studies have suggested that Helicobacter pylori (H. pylori) infection leads to changes in serum lipid profile and high sensitivity C-reactive protein (HsCRP) levels.

1.3Objective

1.3.1 General objective :

To assess the levels of serum Hs.CRP, lipid profile and serum Magnesium in study group.

1.3.2Specific objective :

1-to estimate and compare serum HsCRP , Total cholesterol ,LDL cholesterol ,HDL cholesterol and Magnesium levels in study group (patient and control).

2-corelate the parameters

Chapter Two

Literature review

2- Literature review

2.1 H .pylori

Helicobacter are non-spore-forming gram-negative bacteria. The cellular morphology may be curved, spiral, or species identity of the cells. In old cultures or those exposed to air, cells may become coccoid. Periplasmic fibers or an electron-dense glycocalyx or capsule-like layer has been observed on the cellular surface of several species ,Electron-dense granular bodies have been observed in H.pylori and H.rodentium,In H.pylori these bodies are known to be aggregates of polyphosphate and may serve as a reserve energy source. Helicobacter cells are motile, with a rapid cork-screw-like or slower wave-like motion due to flagellar activity. Strains of most species have bundles of multiple sheathed flagella with a polar or bipolar distribution. Other species have only a single polar or bipolar flagellum . However, flagellation can be peritrichous (H.mustelae) or nonsheathed (H.pullorum, H.rodentium, and "H.mesocricetorum") as well. commonly colonized in upper gastrointestinal (GI) tract, especially in the stomach.(Sulaiman *et al*., 2017)

2.1.2 General properties of H. pylori

Several unique properties contribute to *H. pylori* persistence. All *H. pylori* clinical isolates express urease . Urease converts urea to ammonia plus carbon dioxide, raising the pH of the surrounding area. This provides temporary protection against gastric acid, but *H. pylori* is not an acidophil. It requires the near-neutral pH found in the mucus layer directly adjacent to the gastric surface epithelium. The helical shape of *H. pylori* makes it easier for its polar flagella to propel *H. pylori* through viscous mucus. Chemotaxis systems direct *H. pylori* towards some amino acids, bicarbonate, and cholesterol, while acidic

pH serves as a repellent.This system keeps the organisms in the favorable milieu close to the surface epithelium (William *et al*., 2005).

Helicobacter pylori, formerly known as *Campylobacter pyloridis*, is gram negative, microphilic bacteria found in the stomach. It is a neutrophilic, motile bacterium which is unique in its ability to colonize the normal human stomach, spiral-shaped bacillus, 3-5µm long and about 0.5µm in diameter. It is microaerophilic; it requires oxygen, at lower concentration (5–15%) than is found in the atmosphere). *H. pylori* depends on the presence of various amino acids for growth, including arginine, histidine, isoleucine, leucine, methionine, phenylalanine, and valine (Olson and Maie., 2002).

2.1.3Classification of *H. pylori*

Domain – Bacteria, Phylum – Proteobacteria, Class – Epsilonproteobacteria, Order – Campylobacteriales, Family – Helicobacteraceae, Genus – *Helicobacter* (Pluym ., 2008).

Helicobacter species can be subdivided into two major lineages, the gastric *Helicobacter* species and the enterohepatic (nongastric) *Helicobacter* species. Both groups demonstrate a high level of organ specificity, such that gastric *Helicobacter* in general are unable to colonize the intestine or liver, and vice versa. The only known *Helicobacter* species that colonizes the gastric pouch is *H.pylori*(Solnick and Schaueri., 2001).

2.1.4Epidemiology of H. pylori:

Researchers had discovered that specific anti-H. pylori antibodies could be detected in the blood serum of individuals who were infected with the organism. That enabled the use of a simple blood test for the important epidemiological studies. Researchers were able to expedite the investigations because they did not have to collect new tissue samples from each person; instead,(Susser., 2009).

they used blood samples that had already been collected in large numbers at clinics and blood banks, often for other studies and tests. This early research on H. pylori characterized much of the work to come. The data that emerged from the study of all these samples were unexpected. It showed that H. pylori is a common bacterial agent and at least 30-50% of the world's population are colonized with it. Investigators discovered that the frequency of H. pylori presentation was highly variable from country to country and between socioeconomic and ethnic groups. Overall, they found a consistent pattern in most developing nations, where 70 to 90% of adults harbored the bacteria; most individuals acquired the infection as children, before age 10(Susser., 2009).

2.1.5 Pathogenicity of *H.pylori* infection:

Infection can cause chronic gastritis, both gastric and duodenal ulcers in adults and children, and also increases the risk of gastric cancer (Kusters *et al*., 2006). Infection can result in an immune reaction leading to a localized inflammation of the lining of the stomach and duodenum. When the bacterium is in the mucous lining of the stomach, the body's natural defenses cannot reach it. The immune system will respond to *H.pylori*infection but will not be able to kill the bacteria since they are hidden in the stomach lining. Ammonia and other toxic substances produced by the bacteria may also damage epithelial cells and contribute to the inflammation. Inflammation may also increase stomach acid production and damage the gastric mucus layer this increased exposure to stomach acids can cause ulceration and possibly stomach cancer in some cases (Feyissa., 2015).

H.pylori weakens the protective mucous coating of the stomach and duodenum, allowing the stomach acid to get through to the sensitive lining beneath. Both the acid and the bacteria irritate the lining, causing gastritis

(stomach inflammation) and perhaps the formation of an ulcer. The ulcer is formed by the inflammatory response to the bacteria. The inflammatory response caused by bacteria colonizing near the pyloric antrum induces gastric cells in the antrum to secrete the hormone gastrin (Blaser and Atherton ., 2004). Gastrin stimulates the parietal cells to secrete more acid into the stomach lumen (Schubert and Peura ., 2008). The increased acid load damages the duodenum, which may eventually result in ulcers forming in the duodenum. This may also increase the risk of cancer development (Suerbaum and Michetti., 2002).

Mechanisms of pathogenesis which *H.pylori*may go through when establishing itself in the stomach include Attachment: The *H. pylori* bacteria must enter the stomach and attach themselves to the lining of the stomach to establish an environment in which to grow,Establishment: after attachment they adhere themselves to lining of stomach using adhesion, then they neutralize their environments by secreting urease and start to grow,Toxin production: *H.pylori*produce poisonous substances to increase the secretion of water and electrolytes in the stomach and cause cell death in the cells of the stomach lining. This will help the bacteria take over the stomach environment and will lessen the competition for required nutrients Cell invasion: The bacteria will enter the stomach lining cells for protection and will then kill the cells they are in (their host cells) so that they can move on to invade more stomach-lining cells. This process will continue, thus creating tissue damage. This tissue damage will become the ulcer formation in the stomach,Loss of microvilli/villi: The substances released into the host cell during the 'Cell Invasion' step cause a change in the stomach-lining cells. This change results in fewer calories getting absorbed by the stomach. The body will get fewer nutrients from the food eaten at every meal (Feyissa., 2015).

the immunogenic properties of Helicobacter pylori induce an inflammatory reaction with neutrophilic gastritis that ultimately results in the clinical manifestations of the infection. This process is mediated by host factors, including interleukins IL1, IL2, IL6, IL8, and IL12, interferon gamma, tumor necrosis factor, T and B lymphocytes and phagocytic cells. These factors mediate injury through release of reactive oxygen species and inflammatory cytokines. Helicobacter pylori additionally appear to increase the rate of mucosal-programmed cell death also known as apoptosis(Mehmood *et al.*, 2010).

2.1.6 Diagnosis of h. Pylori infection

2.1.6.1 Endoscopic Diagnostic Tests

In patients who have not been on a PPI within 1–2 wk or an antibiotic or bismuth within 4 wk of endoscopy, the rapid urease test (RUT) provides an accurate, inexpensive means of identifying H. pylori., For patients who have been taking a PPI, antibiotics, or bismuth, endoscopic testing for H. pylori should include biopsies from the gastric body and antrum for histology with or without rapid urease testing, Though culture or polymerase chain reaction (PCR) are the primary means by which antibiotic sensitivities can be determined, neither is widely available for clinical use in the United States and therefore, cannot be routinely recommended ,There are presently four biopsy-based diagnostic methods for H. pylori infection. These include the RUT, histology, culture, and PCR(van IJ *et al*., 2001).

2.1.6.2 Polymerase chain reaction(PCR):

With the advent of PCR, many exciting possibilities emerged for diagnosing and classifying Helicobacter pylori infection. PCR allows identification of theorganism in small samples with few bacteria present and entails no special requirements in processing and transport. Moreover, PCR can be performed

rapidly and cost- effectively, and it can be used to identify different strains of bacteria for pathogenic and epidemiologic studies. As suggested earlier, PCR also is being evaluated for its utility in identifying Helicobacter pylori in samples of dental plaque, saliva, and other easily sampled tissues. The major limitation of PCR is that relatively few laboratories currently have the capability to run the assay. In addition, because PCR can detect segments of Helicobacter pylori DNA in the gastric mucosa of previously treated patients, false-positive results can occur, and errors in human interpretation of bands on electrophoretic gels can likewise lead to false-negative results (Mehmood *et al*., 2010).

2.17 Rapid Urease Testing:

The method used for rapid urease test for the detection of pre-formed urease enzyme was as per. One biopsy sample was introduced in the rapid urease test solution in the endoscopy room. Development of a dark pink colour from the initial pale yellow was checked at 1 min, 5 min, ½ hour, 1 hour and 3 hours. If during these 3 hours the colour changed to pink, then rapid urease test was recorded as being positive for H. pylori infection **(**Dogra *et al*., 2014).

2.1.8 Histopathological examination

Histology has been considered by some to be the gold standard for detection of H.pylori ,All clinical specimens were processed for histopathological examinations using hematoxylin and eosin stain and Giemsa stain as described (van IJ *et al*., 2001) .

2.1.9 Stool Culture

is another highly specific method for identifying active H. pylori infection. Conceptually, culture is attractive because it not only provides a means by which to identify infection, but also allows characterization of antimicrobial sensitivities,Unfortunately, culture is not as sensitive as RUT or histology

.Furthermore, culturing techniques for H. pylori are demanding and costly and as a consequence, only available in a limited number of clinical laboratories. Nonculture-based means of determining antibiotic(William *et al., 2007).*

2.1.10Treatment of H.pylori

Treatment for *Helicobacter pylori* infection often involves medications and lifestyle changes. Triple therapy: used in most cases, this treatment consists of two antibiotics (Clarithromycin and Amoxicillin) to destroy the bacteria, and another type of medicine; proton pumps inhibitors such as Omeprazole to promote healing and reduce symptoms. This combination of medicines generally is taken for 10 to 14 days. Using a different proton pump inhibitor, as with Pantoprazole or Rabeprazole, or replacing Amoxicillin with Metronidazole for people who are allergic to penicillin (Malfertheiner *et al*.,2007) (Malfertheiner *et al*.,2012),Quadruple therapyAn increasing number of infected individuals are found to harbor antibiotic resistant bacteria. This results in initial treatment failure and requires additional rounds of antibiotic therapy or alternative strategies, such as a quadruple therapy, which adds a bismuth colloid, such as bismuth subsalicylate. Four medications are used; this treatment involves using triple therapy medications in combination with a proton pump inhibitor (PPI) to prevent the stomach from producing acid. Quadruple therapy usually is administered for 1 week. This treatment may decrease rates of treatment failure caused by resistance to antibiotics (Stanley and Swierzewski., 2008).

For the treatment of Clarithromycin-resistant strains of *H.pylori*, the use of Levofloxacin as part of the therapy has been suggested (Perna *et al*., 2007 and Hsu *et al*., 2008)

Third-line treatment： High-dose PPI, amoxicillin, tetracycline, levofloxacin, f urazolidone-containing regimen (65.5%) or rifabutin (Wannher., 2011).

2.2 C-reactive protein (CRP)

C-reactive protein (CRP) is a member of the pentraxin family of proteins. It is an acute phase reactant synthesized mainly by the liver. Serum CRP levels are elevated in response to acute infections, inflammatory conditions and trauma. In these clinical situations, the serum CRP levels rise rapidly generally beyond 10 mg/l with a concomitant elevation of erythrocyte sedimentation rates (ESR)(Kushnerand Samols.,2004) .

2.2.1 The Structure of Human CRP

CRP circulates in the human serum as a noncovalently bound disc-shaped pentamer consisting of five identical subunits .It presents two faces: a binding side where it binds calcium-dependent to its widely recognized specific ligands and an effector side. Each subunit consists of 206 amino acids with a molecular weight of 23 kDa and carries 2 calcium ions essential for the pentameric isoform Under physiological circumstances, that is, calcium present in the extracellular environment and a physiological pH, it remains quite stable unless it binds to one of its specific ligands .It has a particularly high affinity for phospholipids, especially lysophospholipids found on the surface of damaged or apoptotic cells .Upon binding to one of its ligands it dissociates into monomers (Deepak *et al*., 2015) .

2.2.2 The Function of Human CRP

Since neither a deficiency of CRP is known nor a therapeutical inhibitor has yet been tested *in vivo*, the role of CRP in physiological or disease settings remains elusive. As it binds to phospholipids, especially lysophospholipids, and recognizes bacterial lipids, it has been suggested that it functions as part of the innate immune system. Once CRP has bound to one of its ligands and dissociated into its monomers, it presents properties not shared with the circulating pentameric CRP. The pentameric CRP appears to have no

interaction with complement or the regulatory complement factor H .whereas the monomeric CRP can directly activate the complement cascade through C1q fixation and induce platelet and monocyte activation(Deepak *et al*., 2015)

2.2.3 Role of hs-CRP in Atherogenesis

C-reactive protein binds the phosphocholine of oxidized low-density lipoprotein (LDL), C-reactive protein increases LDL uptake into macrophages and enhances the ability of macrophages to form foam cells, C-reactive protein inhibits endothelial nitric oxide synthase expression in endothelial cells. Nitric oxide has important antiatherogenic effects, including decreased platelet aggregration, vasoconstriction, and smooth muscle cell proliferation (Weliam *et al*., 2010).C-reactive protein increases plasminogen activator inhibitor-1 expression and activity. C-reactive protein activates macrophages to secrete tissue factor,a powerful procoagulant. C-reactive protein upregulates the expression of adhesion molecules in endothelial cells that will attract monocytes to the site of injury. High concentrations of CRP mRNA have been demonstrated to be present in atherosclerotic plaques.28 Two research groups revealed independently that CRP is produced by human artery smooth muscle cells of atherosclerotic lesions in response to inflammatory cytokines.29,30 Taking all this evidence into account, locally produced CRP may actually participate directly in aspects of atherogenesis, promoting the development of cardiovascular complications(Weliam *et al*., 2010).

2.2.4 Role of hs-CRP in Cardiovascular Risk Prediction

A number of large, prospective epidemiologic studies have examined inflammatory markers as predictors of CVD events in different clinical settings. At present, the best characterized biomarker is hs-CRP. Low-level increases in C-reactive protein appear to be a strong independent predictor of future cardiovascular events, including myocardial infarction, ischemic stroke,

peripheral vascular disease, and sudden cardiac death among individuals with and without prior evidence of cardiovascular disease(Weliam *et al*., 2010). High-sensitivity assay techniques such as immunonephelometry, immunoturbidimetry, high-sensitivity enzyme-linked immunosorbent assay (ELISA) and resonant acoustic profiling (RAP) can detect CRP with a sensitivity range of 0.01 to 10 mg/ l (Roberts.,2004) .

2.2.5 The Synthesis of Human CRP

CRP is mostly synthesised in the liver—although extrahepatic transcription of CRP has been described upon inflammatory stimuli as interleukin-1, interleukin-6, and tumor necrosis factor α,It can rise from baseline to its 10,000-fold upon bacterial inflammation. Following myocardial infarction, an increase in CRP levels is also observed .This response is very quick and happens within 12 hours reaching its peak at about 50 hours after stimuli .Actually, it seems that most forms of adverse stress are associated with an increase in CRP levels (Deepak et al., 2015)

2.3 Lipid

Lipids are often broadly ,andpoorly, defined as bio molecules that are insoluble in water but soluble in organic solvents. They are structurally quite diverse ,covering pigments ,vitamins, fatty acids, cholesterol ,phospholipids, sphingolipids, and many others**.(**Kresge *et al*., 2010).

2.3.1 Type of lipid :

2.3.1.1 fatty acids

These are straight-chain carbon compounds of varying lengths. They may be saturated, containing no double bonds, monounsaturated, with one double bond, or polyunsaturated, with more than one double bond .Fatty acids can esterify with glycerol to form triglycerides or be non-esterified (NEFAs) or free. Plasma NEFAs liberated from adipose tissue by lipase activity are

transported to the liver and muscle mainly bound to albumin. The NEFAs provide a significant proportion of the energy requirements of the body. Summary diagrams of fatty acid synthesis and oxidation. Triglycerides are transported from the intestine to various tissues, including the liver and adipose tissue, as lipoproteins. Following hydrolysis, fatty acids are taken up, re-esterified and stored as triglycerides. Plasma triglyceride concentrations rise after a meal, unlike that of plasma cholesterol. Phospholipids are complex lipids, similar in structure to triglycerides but containing phosphate and a nitrogenous base in place of one of the fatty acids. They fulfi l an important structural role in cell membranes, and the phosphate group confers solubility on nonpolar lipids and cholesterol in lipoproteins. A family of nuclear receptors that are activated by fatty acids – called peroxisome proliferator-activated receptors (PPARs) – has been described and implicated in insulin resistance and dyslipidaemia. The PPARs can be subdivided into a-PPARs, which are activated by fi brate drugs, and g-PPARs, which are activated by thiazolidinedione drugs, for example pioglitazone or rosiglitazone(Crook., 2013).

2.3.1.2 cholesterol

Cholesterol is a steroid alcohol found exclusively in animals and present in virtually all cells and body fluids. It is a precursor of numerous physiologically important steroids, including bile acids and steroid hormones. A summary of the cholesterol synthetic pathways is shown in Figure 13.4. The rate-limiting enzyme is 3-hydroxy3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), which is controlled by negative feedback by the intracellular concentration. About two-thirds of the plasma cholesterol is esterified with fatty acids to form cholesterol esters(Crook.,2013).

2.3.1.3 lipoproteins

Because lipids are relatively insoluble in aqueous media, they are transported in body fluids as, often spherical soluble protein complexes called lipoproteins. Lipids can be derived from food (exogenous) or synthesized in the body (endogenous). The water-soluble (polar) groups of proteins, phospholipids and free cholesterol face outwards and surround an inner insoluble (nonpolar) core of triglyceride and cholesterol esters. Lipoproteins are classified by their buoyant density, which inversely reflects their size. The greater the lipid to protein ratio, the larger their size and the lower the density. Lipoproteins can be classified into five main groups .The fi rst three are triglyceride rich and, because of their large size, they scatter light, which can give plasma a turbid appearance (lipaemic) if present in high concentrations ,Chylomicrons are the largest and least dense lipoproteins and transport exogenous lipid from the intestine to all cells, Very low-density lipoproteins (VLDLs) transport endogenous lipid from the liver to cells. Intermediate-density lipoproteins (IDLs), which are transient and formed during the conversion of VLDL to low-density lipoprotein (LDL), are not normally present in plasma. The other two lipoprotein classes contain mainly cholesterol and are smaller in size:Low-density lipoproteins are formed from VLDLs and carry cholesterol to cells.High-density lipoproteins (HDLs) are the most dense lipoproteins and are involved in the transport of cholesterol from cells back to the liver (reverse cholesterol transport)(Crook., 2013).

These lipoproteins can be further divided by density into HDL2 and HDL3. If a lipaemic plasma sample, for example after a meal, is left overnight at 4°C, the larger and less dense chylomicrons form a creamy layer on the surface. The smaller and denser VLDL and IDL particles do not rise, and the sample may appear diffusely turbid. The LDL and HDL particles do not contribute to

this turbidity because they are small and do not scatter light. Fasting plasma from normal individuals contains only VLDL, LDL and HDL particles. In some cases of hyperlipidaemia, the lipoprotein patterns have been classified (Fredrickson's classification) according to their electrophoretic mobility. Four principal bands are formed, based on their relative positions, by protein electrophoresis, namely a (HDL), pre-b (VLDL), b (LDL) and chylomicrons(Crook., 2013).

2.3.2 biochemical importance of lipid

Lipids are served as efficient source of energy when stored in adipose tissue and of great importance in biochemical processes. They are components of cell membrane and thus concerned with phenomenon of cell permeability and cell organization. Fat gives 9 cal of energy per gram on oxidation of CO2 and water. In addition to providing flavors and satiety to diet, fat serve as vehicle for fat soluble vitamins A, D, E and K. Fats provide building blocks for different high molecular weight substances. Fatty acids form substances, which are essential for maintaining cellular integrity such as lipoproteins and glycolipids. Fats provide essential fatty acids, which are not synthesized by human body(Maheshwari *et al*., 2008).

2.3.3 lipid metabolism

Cholesterol, in both free and esterified forms, and triglycerides are the two main lipids in plasma. They are transported in lipoproteins, pseudomicellar lipid–protein complexes, in which the main apolipoproteins, apo B-100/48, apo A-I, apo A-II, apo E, and the apo Cs, are integral components. Apo B is a component of all atherogenic lipoproteins (chylomicron remnants, VLDL and their remnants, IDL, lipoprotein(a) [Lp(a)] and LDL), whereas apo A-I and apo A-II are components of HDL. The apo B-containing lipoproteins and the apo A-I/A-II lipoprotein classes are closely interrelated via several metabolic

pathways . In dyslipidaemic patients with cardiometabolic risk, increased free fatty acid flux may represent a significant abnormality driving increased hepatic assembly and secretion of VLDL, IDL, and/or LDL particles, although other mechanisms are also implicated.26,27 Low plasma levels of HDL-apo A-I are associated with its increased fractional removal.26 This is driven by both cholesteryl ester transfer protein (CETP)-mediated heteroexchange of triglycerides from apo B lipoproteins with cholesteryl ester from apo A-I lipoproteins, and dissociation of apo A-I from triglyceride-enriched HDL with clearanceviathekidney. Such metabolic perturbations are frequently associated with insulin resistance, which may in turn influence the activities of lipoprotein lipase (LPL), CETP and potentially, hepatic lipase (HL), phospholipids transfer protein, and endothelial lipase. Within this large dyslipidaemic group, however, many patients do not exhibit insulin resistance but nonetheless display a mixed dyslipidaemia characterized by elevated levels of TRL and LDL. This lipid phenotype typically involves subnormal concentrations of HDL-C and increased cardiovascular risk. Finally, although there are close links, both path physiologic and genetic, between the dyslipidaemia of insulin resistance and the phenotype of familial combined hyperlipidaemia (FCHL), some individuals with elevated levels of TRL remnants do not have insulin resistance or T2DM. Such individuals are at increased risk for premature CVD, although it is not clear if this risk is higher or lower than in those with the dyslipidaemia of insulin resistance and/or T2DM. Pharmacological correction of hypertriglyceridaemia in T2DM does not usually normalize low apo A-I levels, which probably reflects the complex mechanisms involved. The complexity of HDL metabolism is clearly relevant when strategies to raise HDL-C are reviewed; indeed, HDL-C concentration is

at most an indirect marker of the anti-atherogenic activities that are associated with this lipoprotein.(Chapman1*et al*., 2011)

2.3.4 Disorders of Lipid Metabolism:

2.3.4.1 Defects In Fatty Acid Oxidation Metabolism

Mitochondrial oxidation of fatty acids provides the chief source of energy during prolonged fasting as well as for skeletal muscle during exercise and for cardiac muscle. Ten genetic defects in this pathway have been recognized in infants and children, including: a. Long Chain Acyl CoA Dehydrogenase (LCAD) deficiency, b. Medium Chain Acyl CoA Dehydrogenase (MCAD) deficiency, c. Short Chain Acyl CoA Dehydrogenase (SCAD) deficiency, d. Deficiency of the plasma-membrane carnitine transporter, e. Carnitiepalmitoyltransferase I (CPT I) deficiency, f. Carnitin epalmitoyltransferase II (CPT II) deficiency. Patients with these defects present with coma after a period of starvation and have hypoketosis, i.e., their serum ketone concentrations are low. They may also have cardiomyopathy and muscle weakness. Urinary excretion of products of fatty acid oxidation through alternate pathway (e.g. omega-oxidation) is specific for each kind of disorder and urinary analysis looking for these acids makes a major tool in the diagnosis. Myoglobinuria may also be accompanied with the muscular features particularly in the acute phase of the disease(Chatterjea et al., 2010).

2.3.4.2 acyl-coa dehydrogenase deficiency

β-oxidation of fatty acids takes place inside the mitochondria for which the activated fatty acids (acyl CoAs) are transported across the mitochondrial membranes with the help of carnitine. Inborn errors of mitochondrial fatty acid βoxidation include the deficiency of β-oxidationenzymes, particularly the first enzyme of the pathway i.e. Acyl CoA dehydrogenase (ACD). Different chain length fatty acyl CoAs are acted upon by different enzymes because the

fatty acyl CoA can only accept a limited range of carbon atoms in the fatty acids. Therefore, the deficiency of different enzymes leads to slightly varying signs and symptoms; the enzymes with known inherited deficiency are short-chain acyl-CoA dehydrogenase deficiency,medium-chain acyl-CoA dehydrogenase deficiency, longchain acyl-CoA dehydrogenase deficiency andvery long-chain acyl-CoA dehydrogenase deficiency, In a prospective tandem mass spectrometry screening of 9,30,078 blood spots from neonates in the US population, a frequency of MCAD deficiency of 1 in 15,001 was documented(Chatterjea et al., 2010).

2.3.4.3Very long chain acd deficiency (VLCAD)

VLCAD deficiency can be classified clinically into 3 forms: a. Severe early-onset form: Presents within 4 months of birth with high incidence of cardiomyopathy and high mortality. All patients would have liver dysfunction. b. Intermediate childhood onset form: Usually presents with hypoketotic hypoglycemia. This is the form with more favorable outcome. c. The adult-onset myopathic form: Presents with isolated skeletal muscle involvement, rhabdomyolysis, and myoglobinuria after exercise or fasting (Andresen et al., 1999).

2.3.4.4 Long chain acd deficiency (LCAD)

Nonketotic hypoglycemia and episodes of cardiorespiratory arrest associated with fasting are characteristic. Other features included hepatomegaly, cardiomegaly, and hypotonia. Total plasma carnitine concentration is low,Specific assay show that the activity of longchain acyl-CoA dehydrogenase is very low (<20%) compared to control values in fibroblasts, leukocytes and liver. Treatment with frequent low-fat highcarbohydrate feedings, riboflavin and carnitine reduced the frequency and intensity of crises (Chatterjea et al., 2010).

2.3.4.5 (B) medium chain acd deficiency (MCAD)

Reported mostly from children and young adolescents with unexplained episodes of lethargy and unconsciousness and C6-C10 dicarboxylicaciduria. Inherited deficiency of medium-chain acyl-CoA dehydrogenase is characterized by intolerance to prolonged fasting, recurrent episodes of hypoglycemic coma with medium-chain dicarboxylicaciduria, impaired ketogenesis, and low plasma and tissue carnitine levels. The disorder may be severe, and even fatal, in young patients. As in long chain ACD deficiency, dicarboxylic acids and 3-hydroxydicarboxylic (3OHDC) acids can be demonstrated in the urine arising from the alternate (omega) oxidation of fatty acids and their intermediates. Adipic and monounsaturated sebacic, seburic and ozeleic acids are among those elevated in urine and serum(Chatterjea et al., 2010).

2.3.4.6(C) short chain acd deficiency (SCAD)

Two distinct clinical phenotypes of hereditary short-chain acyl-CoA dehydrogenase deficiency have been identifiedOne type has been observed in infants with acute acidosis and muscle weakness,The other has been observed in middle-aged patients with chronic myopathy, SCAD deficiency is generalized in the former typeand localized to skeletal muscles in the latter. Cases with neonatal onset have a variable phenotype that includes metabolic acidosis, failure to thrive, developmental delay, and seizures, as well as myopathy. There are no episodes of nonketotic hypoglycemia, which are characteristic of mediumchain and long chain acyl dehydrogenase deficiencies. All patients with SCAD have neurologic deficits: hypotonia/hypertonia, hyperactivity, and/ or developmental delay. Ethylmalonicaciduria, is commonly found in short chain ACD deficiency, but the disorder cannot be taken as confirmatory to short chain ACD deficiency. It

appears to be a complex multifactorial/polygenic condition where a number of other genetic and environmental factors are involved.(Chatterjea et al., 2010).

2.3.5 diseases related to lipid metabolism

Steatorrhoea: Maldigestion of fats due to inadequate secretion of pancreatic lipase or bile salts or even may be defective absorption due to intestinal diseases like coeliac disease results in excessive excretion of fat in feaces. This is called as Steatorrhoea,Obesity is another disorder due to accumulation of excess of body fat. ,Lipidosis: This denotes the abnormal lipoproteins in blood or specific lipids in tissues , Hyperlipidemia: This is a condition in which plasma cholesterol or plasma triglyceride level is increased. This condition occurs due to inherent genetic defects (Maheshwari *et al.*, 2008).

2.4 Magnesium :

Magnesium (Mg) is the fourth most abundant cation in human body. Mg is predominantly intracellular and about 1% of body Mg2+ presents in the extracellular fluid. The normal serum level of Mg is usually in the range of 1.7- 2.2 mg/dL. Mg metabolism and excretion (which are predominantly renal) are impaired in kidney failure and dialysis patients. When glomerular filtration rate falls below 30 mL/min, Mg excretion decreases and serum Mg level increases subsequently (Navarro-Gonzalez JF,et al,2008), Parathyroid hormone (PTH) and vitamin D affect intestinal Mg absorption, and also its bone and renal re-absorption. Mg is an essential cofactor for several enzymes in human body and also an essential element for pathogens such as Helicobacter. Some recent studies have shown an association between H. pylori infection and serum Mg level in ESRD patients (NasriH., 2007).

Magnesium is predominately an intracellular divalent cation and is important for optimal cell function. It is an essential cofactor to many enzymes, as well as being important for membrane function. Furthermore, it can act as an

antagonist to calcium in cellular responses and has a structural role within the cell. The body contains about 1 mol (approximately 25 g) of magnesium, mostly in the bone and muscle. The recommended daily allowance of magnesium for adults is about 4.5 mg/kg; rich dietary sources include cereal, nuts and vegetables. Magnesium is largely absorbed in the upper small intestine but the large intestine may also be important; unlike calcium, its absorption is not vitamin D dependent. As much as 70 per cent of magnesium from dietary intake is not absorbed but eliminated in the faeces. The major excretory route is via the kidneys, and about 65 per cent of glomerular-fi ltered magnesium is reabsorbed in the loop of Henle. The exact mechanisms of magnesium homeostatic control are unclear, although PTH, insulin and calcitonin are important. Parathyroid hormone can increase magnesium reabsorption, although hypercalcaemia can increase the renal excretion of magnesium. About 35 per cent of plasma magnesium is protein bound, and the plasma concentration is normally 0.7–1.2 mmol/L(Martin., 2012) .

2.4.1 Hypermagnesaemia:

Hypermagnesaemia can result in cardiac arrhythmias, such as heart block and inhibition of atrioventricular conduction leading to cardiac arrest, seizures, altered nerve conduction, reduced tendon reflexes, paralytic ileus, nausea, respiratory depression and hypotension. Clinical features do not usually manifest until the plasma magnesium concentration exceeds 2 mmol/L(Martin., 2012).

2.4.2 Hypomagnesaemia:

Some causes of hypomagnesaemia are shown in Box 6.6. The symptoms of hypomagnesaemia are very similar to those of hypocalcaemia. If the plasma calcium concentrations (allowing for that of albumin) and blood pH are normal in a patient with tetany, the plasma magnesium concentration should

be assayed. Hypomagnesaemia can result in cardiac arrhythmias, including torsade de pointes, and digoxin sensitivity.(Martin., 2012).

2.4.3 Treatment of hypomagnesaemia :

Patients with mild to moderate deficiency (1.2 mg/dL to 1.7 mg/dL) should be treated with diet or oral magnesium supplements.(Augus 1999). Symptomatic patients should receive 3 g to 4 g (24 mEq to 32 mEq) of intravenous magnesium sulfate slowly over 12 to 24 hours. This dose can be repeated as necessary to maintain serum magnesium level above 1.2 mg/dL. Rapid intravenous push administration raises the serum magnesium concentration above physiologic levels, causing a large percent of magnesium to be excreted in the urine. Establishment of adequate kidney function is required before administering any magnesium supplementation. Patients with renal insufficiency should receive 25% to 50% of the initial dose recommended for patients with normal kidney function(Augus., 1999).

2.5Association between h.pylori infection and lipid :

H. pylori infection leads to chronic inflammation of the gastric mucosa. This leads to systemic release of inflammatory cytokines, one of the contributory factors of atherosclerosis. Alteration in lipid profile is another consequence of this systemic inflammatory state(Dogra *et al*., 2014).

H .pylori infection has been suggested to influence the development of atherosclerotic changes in coronary arteries, indicating a damaging effect of this bacterium or its products (e.g., cytokines, endotoxins, cytotoxins, and other virulence factors) on the coronary endothelium.On the other side, chronic HP infection is known to increase the pH level of the gastric juice and to decrease ascorbic acid levels, both of which will cause folate absorption reduction. Low folate hampers the methionine synthase reaction. This will

increase blood hemocysteine concentration which results in damage of endothelial cells (Corrado and Novo., 2005).

2.6 Association between h.pylori infection and hs CRP :

Hence, H. pylori infection may lead to increase in risk of coronary artery disease. It is well documented that a rise in inflammatory cytokine-interleukin 6 is primarily responsible for a rise in hsCRP production by the liver (Dogra *et al*., 2014).

Previous study found that the mean serum level of hs-CRP was significantly higher in H.pylori infected subjects in H.pylori infected subjects were significantly higher than that in healthy controls, supporting that infection with H.pylori may increase serum hs-CRP levels(Jafaezadeh *et al*., 2009)

Chapter Three

Materials and methods

3- Materials and methods

3.1 Study design:

This was across sectional comparative study.

3.2 Study area:

This study was conducted in Khartoum North Teaching Hospital- Khartoum State during the period February 2017 to April 2017.

3.3 Study population:

Forty Sudanese H .pylori patients and Forty Sudanese individuals with gastritis and h. pylori negative were enrolled in the study as cases and control respectively.

3.4 Inclusion criteria:

H. pylori positive adult aged from 18-60 year old with Gastritis and *H.pylori* negative adult with Gastritis as control were included in this study.

3.5 Exclusion criteria:

Any patients with H .pylori infection with any factors which may affect the results such as cardiac disease or hypertensive or mixed infection.

3.6 Participants' personal data collection:

An interview to obtain the clinical data was done to each participant in this study and questionnaire (Appendix I) was specifically designed to obtain data which help in either including or excluding certain individuals in or from the study, respectively. Clinical history and examination of test and control groups were done by physician to help exclusion or inclusion of the study subject.

3.7 Ethical approval:

Ethical approval for conducting the research was obtained from the college of Medical Laboratory Sciences-SUST also a permission was obtained from the

administration of Khartoum North Teaching Hospital for the same purpose. A verbal consent was obtained from all the participants after they had been informed about the aim of the study, expected outcome, confidentiality of the results and the procedure of blood collection.

3.8 Sample collection:

Venous blood was collected using sterile disposable plastic syringe after cleaning the vein puncture area with 70% ethanol.venous blood samples were obtained and stored at 4°C. plasma was acquired by centrifugation of blood samples at 2000 r/min for 15 minutes, immediately after sampling

3.9.1 Principle of Hs CRP estimation:

The i-CHROMA hs CRP Test is based on fluorescence immunoassay technology. The i-CHROMA hs CRP Test uses a sandwich immunodetection method, such that the fluorescence-labeled detector antibody or antigen binds to the target protein in blood specimen. Signal intensity of fluorescence reflects amount of the CRP captured and is micro processed from i-CHROMA Reader to show the CRP concentration in blood specimen.(pepys and hirschfied., 2003).

3.9.2 HS CRP procedure :(appendix 11)

3.10.1 Principle of LDL estimation

The auto LDLTM Cholesterol Reagent is a two-part, liquid stable method for directly measuring LDL-C levels in serum or plasma. The method depends on the properties of a unique detergent which eliminates the need for any off-line pretreatment or centrifugation steps. This detergent (Reagent 1) solubilizes only the non-LDL lipoprotein particles. The cholesterol released is consumed by cholesterol esterase and cholesterol oxidase in a non-color forming reaction. A second detergent (Reagent 2) solubilizes the remaining LDL

particles and a chromogenic coupler allows for color formation. The enzyme reaction with LDL-C in the presence of the coupler produces color that is proportional to the amount of LDL cholesterol present in the sample (Badimon, J.J*et al*., 1990).

3.10.2 Procedure of LDL estimation :(appendix 111)

3.11.1 Principle of HDL estimation :

The Liquid auto HDLTM Cholesterol assay is a homogeneous method for directly measuring serum HDL-C levels without the need for any off-line pretreatment or centrifugation steps. The method is in a two-reagent format. The first reagent contains α-cyclodextrin and dextran sulfate to stabilize LDL, VLDL, and chylomicrons. The second reagent contains PEG modified enzymes that selectively react with the cholesterol present in the HDL particles. Consequently, only the HDL cholesterol is subject to cholesterol measurement(Warnick Get al., 1995).

3.11.2 Procedure of HDL estimation : (appendix 1v)

3.12.1 Principle of magnesium estimation :

Magnesium ion forms a colored complex with xylidyl blue under alkaline conditions. The intensity of the developed color is proportional to the magnesium ion concentration of the sample(Rice EW et al., 2013).

3.12.2 Procedure of magnesium estimation: (appendix v)

3.13.1 Principle of *H.pylori* Stool Antigen:

The *H.pylori* Antigen Rapid Test Cassette (Feces) is a qualitative, lateral flow immunoassay for the detection of *H.pylori* antigens in human feces specimens. Membrane is pre-coated with anti-*H.pylori* antibodies on the test line region of the test. During testing, the specimen reacts with the particle coated with anti-*H.pylori* antibodies. The mixture migrates upward on the membrane by the

capillary action to react with anti-*H.pylori* antibodies on the membrane and generate a colored line (leaflet of *H.pylori*Ag test strip).

3.13.2 Procedure of *H.pyori* Stool Antigen:(appendix v1)

3.13.3 Interpretation of *H .pylori* stool antigen results:

Positive: two lines appear in control line region(C) and another appears in test line region (T).

Negative: one colored line appears in the control line region (C). No line appears in test line region (T).

Invalid: control line fails to appear.

3.14 Quality control:

Using manufacturing control bring to room temperature before analysis then run the control if all parameters fall within manufactures recommended range, then proceed with patient samples. During each run one control is run every 20 sample using sampler mode. If the machine does not produce WBCs differential or when in doubt of some parameters, make a peripheral blood smear, stain and perform WBC differential count.

3.15 Data analysis:

The participants' characteristics were analyzed qualitatively. Values were given as mean ± SD. Student T-test was used to test the effect of H.pylori onlevel of Hs CRP,total cholesterol ,LDL-C and HDL-C and the variation in these responses with gender of the patients. The significance level was set at $P < 0.05$. All statistical analyses were performed using SPSS version 16.

Chapter Four

Results

4. Results

(Table 4.1): Comparison between the mean concentration of Hs CRP, total cholesterol ,HDL cholesterol ,LDL cholesterol ,Magnesium and BMI in patient and control group.

(Table 4.2): Comparison between the mean concentration of hs CRP, total cholesterol ,HDL cholesterol , LDL cholesterol and magnesium in study group according to gender.

Table (4-3): Comparison between the mean concentration of Hs CRP, total cholesterol ,HDL cholesterol ,LDL cholesterol and Magnesium according to age groups among patients with H. pylori infection.

Table (4-4) The correlation (P-value, r) of total cholesterol , HDL cholesterol , LDL cholesterol , Magnesium ,and Hs CRP in H. pylori infected group.

Table (4-5): Comparison between serum Hs CRP and H .pylori and H .pylori with HS CRP Cross tabulation.

Figure (4.1) : Distribution of age among Sudanese patients with H.pylor,. was divided into three groups, (18-31) years, (32-45) years and (46-60) years, each group contain 45% (19), 35% (13), and 20% (8) of patients, respectively.

Figure (4-2):Percentage of gender among Sudanese patients with H.pylori, was 58.75% female and 41.25% male.

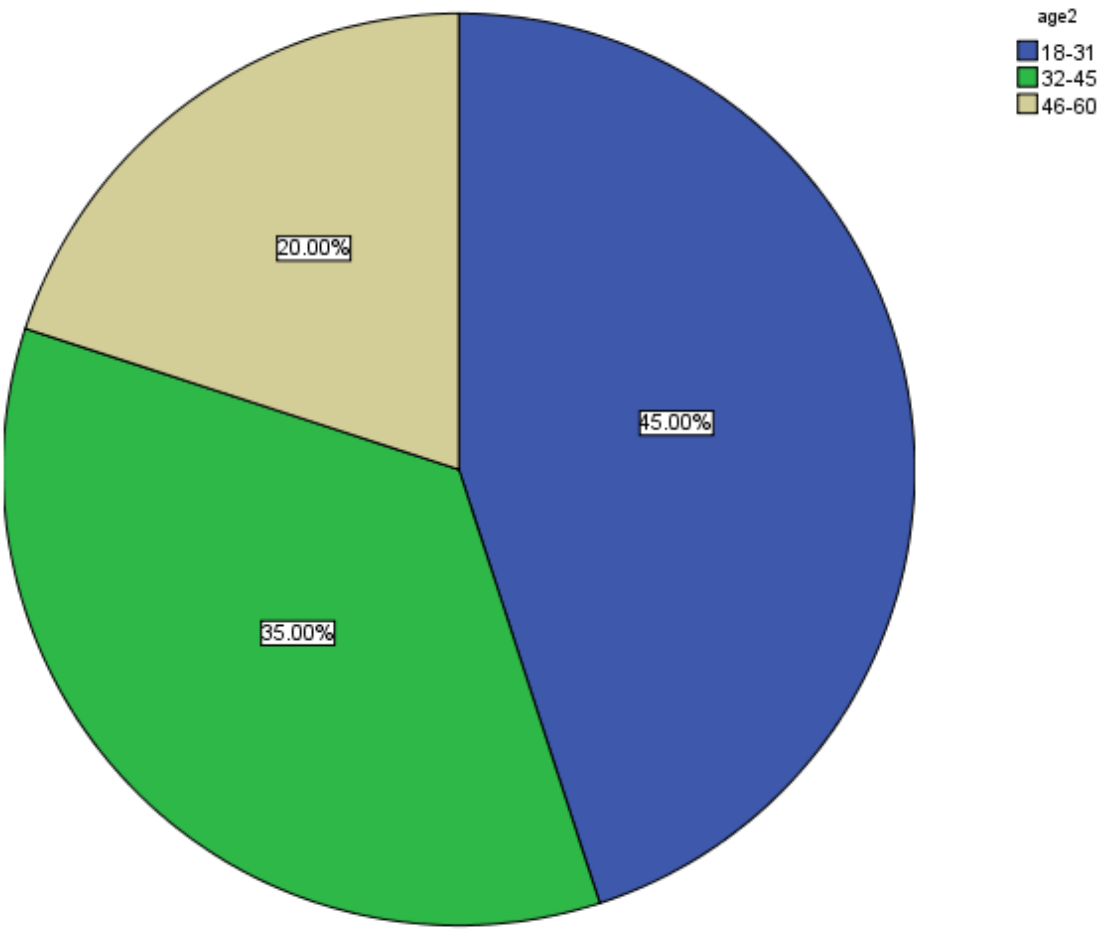
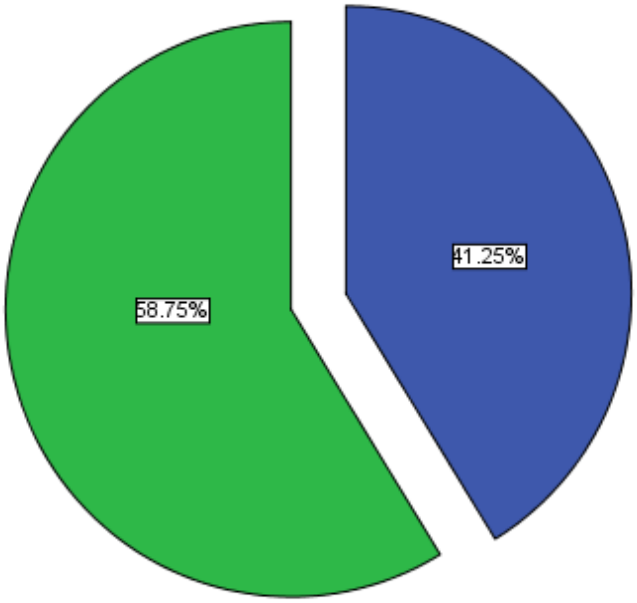


Figure (4.1) Distribution of age among Sudanese patients with *H.pylori.*



Male =41.25%

Female =58.75%

Figure (4.2)Percentage of gender among Sudanese patients with *H.pylori*.

(Table 4.1): Comparison between the means concentration of measured parameters in gastritis patient with H .pylori and gastritis patient without H .pylori control group:

Variables	Mean H.pylori positive± SD	Mean H.pylori negative± SD	P value
HsCRP mg/L	8.50 ±2.10	4.60 ±2.00	0.000
Total cholesterol mg/dL	150 ±38.9	106 ±27.7	0.026
LDL cholesterol mg/dl	70.5 ±20.1	54.5 ±12.7	0.006
HDL cholesterol mg/dl	41.4±21.3	46.1 ±13.9	0.990
Mg mg/dL	1.90 ±0.20	2.00 ±0.20	0.580

* independent T test was used for comparison.

*Result given in mean ± SD, *P-Value* ≤ 0.05 considered significant.

(Table 4.2): Comparison between the mean concentration of measured parameters in study group according to gender:

Variables	Male No=16 mean±SD	Female No=24 mean±SD	P.value
Total cholesterol mg/dl	141.1±33.59	155.9±41.8	0.225
HDL cholesterol mg/dl	43.7±29.03	39.88±14.7	0.627
LDL cholesterol mg/dl	71.81+22.85	69.58+18.65	0.748
HsCRP mg/l	8.59+2.09	8.09+2.25	0.746
Magnesium mg/dl	1.96+0.17	1.90+0.15	0.269

* independent T test was used for comparison.

*Result given in mean ± SD, *P-Value* ≤ 0.05 considered significant.

Table (4-4):): Comparison between the mean concentration of measured parameters according to age groups among patients with *H.pylori*infection:

Age groups / Parameters	(18-31) years No=18Mean±SD	(32-45) years No=14 Mean±SD	(46-60) years No=8 Mean±SD	P.value
Total cholesterol mg/dl	144.39±37.69	157.86±40.83	148.88±41.34	0.634
HDL cholesterol mg/dl	28.3±0.100	30.5±0.170	35.5±0.189	0.904
LDL cholesterol mg/dl	68.5±23.64	73.79±17.90	69.12±16.81	0.765
Hs CRP mg/l	9.01±1.77	7.61±2.72	8.66±1.60	0.187
Magnesium mg/dl	2.01±0.14	1.84±0.12	1.92±0.16	0.004

*Results express as (mean±SD), One-way ANOVA test was used to comparison.
*Significant level at ($P \leq 0.05$).

Table (4-3): The correlation (P-value, r) of total cholesterol , HDL cholesterol , LDL cholesterol , Magnesium ,and Hs CRP in H. pylori infected group :

Variable	Person correlation(r)	p.value
Correlation B/w Hs CRP ,LDL	0.06	0.712
Correlation B/w total cholesterol ,Hs CRP	0.18	0.340
Correlation b/w total cholesterol and LDL cholesterol	0.64	0.000
Correlation b/w Mg and LDL cholesterol	0.020	0.906
Correlation b/w Mg and HDL cholesterol	0.149	0.359
Correlation b/w Mg and total cholesterol	0.069	0.671
Correlation b/w Mg and Hs CRP level	0.197	0.223

*Result given as p-value and r=person correlation.

**P-Value* ≤ 0.05 considered significant.

Table (4-5): Comparison between serum Hs CRP and H .pylori and H .pylori with HS CRP Cross tabulation:

			HS CRP			P value
			positive	negative	Total	
H.pylori	positive	Count	35	5	40	0.000
		% of Total	43.8%	6.2%	50.0%	
	negative	Count	16	24	40	
		% of Total	20.0%	30.0%	50.0%	
Total		Count	51	29	80	
		% of Total	63.8%	36.2%	100.0 %	

* Chi-Square Test was used to comparison.
*Significant level at ($P \leq 0.05$).
*Hs.CRP positive more than 5.0 mg/l
*Hs.CRP negative less than 5.0 mg/l

Chapter Five

Discussion, Conclusion, and Recommendations

5- Discussion, Conclusion, and Recommendations

5.1 Discussion

H .pylori infection may result in stomach inflammation that alter gastric secretion and cause tissue injury leading to peptic ulcer, gastritis, and other consequences.

In the present study mean serum level of hs-CRP was significantly higher in H.pylori infected subjects in comparison with the mean serum level of hs-CRP in gastritis subjects with H.pylori negative ($p<0.000$). These results are similar to that recently reported by Saad Al-Fawaeir *et al*.The production of proinflammatory cytokines such as tumor necrosis factor (TNF), interleukin (IL)1-6 and IL-8 stimulates by H.pylori infection. Elevated serum levels of TNF, IL-1, IL-6 and IL-8 was found in H.pylori-infected individuals. Production of CRP is regulated by cytokines, principally interleukins, as well as hormones such as cortisol and insulin. Therefore the association between CRP concentration and H.pylori infection could be explained by the action of cytokines, hormones or both of them.

In the present study we found that the mean serum level of LDL cholesterol was significantly higher in H.pylori infected subjects ($p =0.006$ t-test) in comparison with the mean serum level of LDL cholesterol in healthy subjects (pv 0.01by t-test). These results are similar to that recently reported by (Corrado and Novo., 2005).the role of these risk factors and cytokines were adjusted, therefore, the remained higher chance may be due this adjusting and reveal the independent role of H.pylori infection in atherosclerosis process. (Corrado and Novo., 2005)

HDL level there was no change (p-value=0.99). So there is no different between its level in study and control group . This agree with the result of (Dogra AC et al 2014) in which there was a significant decrease in its level (P = 0.17) .

-This study showed that total cholesterol level was significantly increased in H.pylori infected group (p-value= .026) .this is totally agree with the result of (Corrado and Novo., 2005). which there was a significant increase in its level but disagree with (Fairouz., 2010) which there was no significant difference.

Magnesium level there was no change (p-value=0.580). So there is no different between its level in study and control group . This disagree with the result of (Öztürk N et al., 2015) in which there was a significant decrease in its level (P = 0.000) due to life style.

-The study showed a strong positive significant correlation between total cholesterol and LDL-C in H. pylori infected patient (P-value= .000) (r= .658).

The study showed insignificant weak positive correlation between the level of serum HS CRP and serum Magnesium in *H.pylori*-infected patients

5.2Conclusion

Sudanese patient with H. pylori infection had increased serum level of Hs CRP, Total Cholesterol and LDL-C level, and had decreased in serum level of HDL-C and magnesium.

5.3 Recommendation

Further studies with a larger sample size are recommended to :

1.elucidate the utility and clinical importance of these parameters in H .pylori infection .

2. ensure that H. pylori infection was the most likely cause of the observed alterations by post- treatment evaluation.

3-educate age level (18-31) locking for healthy food

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Appendix

Sudan University of Science and Technology

College of Medical Laboratory Science

Questionnaire form

Personal Data:

Name:…… .....Serial No:……..

Age:…….. Gender: Male ( ) Female ( )

Weight:……. Kg Height:……..m BMI:…….. kg/m^2

Medical History of:

*****Diabetes Mellitus: yes ( ) No ( )

*Hypertension: yes ( ) No ( )

*Heart disease: yes ( ) No ( )

*Kidney disease: yes ( ) No ( )

*Liver disease: yes ( ) No ( )

*Anemia: yes ( ) No ( )

*Malignancy: yes ( ) No ( )

*Gastritis: yes ( ) No ( )

Pregnancy: yes ( ) No ( )

Laboratory Investigation:

Stool Antigen *H.pylori*: positive ( ) Negative ( )
Total Cholesterol :…… mg/dl
HDL Cholesterol:……mg/dl
LDL Cholesterol:……mg/dl
HS CRP……mg/l
Magnesium ……mg/dl