



بسم الله الرحمن الرحيم



Sudan University of Science and Technology  
College of Graduate studies

**Effect of *Helicobacter pylori* Infection on the Levels of Serum  
Zinc and Copper Among Sudanese Patients with Gastritis  
(In Khartoum state)**

تأثير الإصابة بالبكتريا الحلزونية علي مستويات الزنك و النحاس في مصل الدم  
لدي المرضى السودانيين الذين يعانون من إلتهاب المعدة  
(في ولاية الخرطوم)

A dissertation submitted in partial fulfillment for the requirement of  
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# الآية

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قال تعالى:

وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا

صدق الله العظيم

سورة الإسراء (الآية رقم ٨٥)

## **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. Ghada Abdelrahman Elfadil**

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 SaadRashwan Medical Center Staff, for their assistance in sample collection

To my friends, colleagues and relatives  
whomassisted me

## Abstract

**Background and Objectives:** *Helicobacter pylori* (*H.pylori*) colonize the human gastric and duodenal mucosa, and the infection may cause peptic ulcers and gastritis. Most studies indicated that *Helicobacter pylori* infection affects micronutrients zinc and copper. The objective of the study was to compare serum concentration of trace elements zinc (Zn) and copper (Cu) of gastritis *Helicobacter pylori*-infected patients with those of gastritis *H.pylori* negative.

**Materials and Methods:** This was cross sectional case control study. The study was conducted in 40 gastritis *H.pylori*-infected patients and 40 (age and gender) matched gastritis *H.pylori* negative control. Stool Antigen *H.pylori* test was used to determine *H.pylori*, and Flame Atomic Absorption Spectroscopy method was employed to analyze both serum zinc and copper concentrations.

**Results:** Compared to the control subjects, *H.pylori*-infected patients had significant decrease in both serum concentrations of Zn and Cu ( $p=0.000$ ), and BMI was insignificantly increased in patients compared to control ( $p=0.255$ ). According to recurrent occurrence (once or twice) there was insignificant difference between means of serum zinc and copper ( $p=0.824$ ,  $0.869$ ) respectively. Among patient group, Level of serum zinc was insignificant decreased in male compared with female ( $p=0.409$ ), while serum copper was significantly decrease in male compared with ( $p=0.013$ ). According to age groups both serum zinc and copper were insignificant difference between groups ( $p=0.221$ ,  $p=0.504$ ) respectively. And also there was significant weak positive correlation between zinc and age ( $p=0.016$ ,  $r=0.377$ ), and insignificant correlation between copper and age ( $p=0.148$ ,  $r=0.233$ ).

**Conclusion:** Serum concentrations of Zn and Cu were significantly decreased in patients with gastritis *Helicobacter pylori*.

## المستخلص

**الخلفية والاهداف:** البكتريا الحلزونية تستوطن في الغشاء المخاطي للمعدة و الإثني عشري الإنسان, هذه العدوي قد تسبب قرحة و إتهاب في المعدة, و تُظهر معظم الدراسات ان عدوي البكتريا الحلزونية تؤثر علي المغذيات الدقيقة مثل الزنك والنحاس. كان الغرض من الدراسة مقارنة مستويات العناصر الشحيحة (الزنك و النحاس) بين المرضى المصابين بالبكتريا الحلزونية و يعانون من إتهاب المعدة والأشخاص غير المصابين بالبكتريا الحلزونية ويعانون من إتهاب المعدة.

**المواد و الطرق:** هذه الدراسة المقطعية دراسة مقارنة. أجريت الدراسة علي 40 من المرضى المصابون بالبكتريا الحلزونية ويعانون من إتهاب المعدة, و 40 مطابقون لهم في ( العمر و النوع) غير مصابون بالبكتريا الحلزونية و يعانون من إتهاب المعدة. أُستخدم فحص البكتريا في البراز لإكتشاف البكتريا. واستخدم جهاز الامتصاص الذري لقياس مستويات الزنك و النحاس في مصل الدم.

**النتائج:** بالمقارنة مع مجموعة الضبط, وجد ان المرضى المصابون بالبكتريا الحلزونية و يعانون من إتهاب المعدة لديهم انخفاض ذو دلالة احصائية في معدلات الزنك والنحاس (القيمة المعنوية 0.000), و وُجد ان مؤشر كتلة الجسم زائد زيادة ذات دلالة غير احصائية في المرضى مقارنةً بمجموعة الضبط(القيمة المعنوية 0.255). فيما يتعلق بحدوث تكرار العدوي وجد ان المرضى الذين تكررت لديهم العدوي (مرة او مرتين) لديهم اختلاف ذو دلالة غير احصائية في مستويات الزنك والنحاس مقارنة (القيمة المعنوية 0.824 للزنك, 0.869 للنحاس). في مجموعة المرضى المصابون بالبكتريا الحلزونية و إتهاب المعدة وُجد ان؛ مستوي الزنك منخفض إنخفاض ذو دلالة غير احصائية لدي الرجال مقارنة بالنساء (القيمة المعنوية 0.409), بينما النحاس منخفض انخفاض ذو دلالة احصائية لدي الرجال مقارنة بالنساء (القيمة المعنوية 0.013). فيما يتعلق بالعمر ليس هنالك اختلاف احصائي في مستويات الزنك والنحاس (القيمة المعنوية 0.221 , 0.504) علي التوالي. و ايضا وجدت علاقة ارتباط ايجابي ضعيفة ذات دلالة احصائية بين الزنك والعمر (القيمة المعنوية 0.016, قوة الارتباط 0.377), وعلاقة ارتباط ايجابي ضعيفة ذات دلالة غير احصائية بين النحاس و العمر (القيمة المعنوية 0.148, قوة الارتباط 0.233) في مجموعة المرضى.

**الخلاصة:** مستويات الزنك والنحاس في مصل الدم تتخفض إنخفاض ذو دلالة احصائية في المرضى المصابين بالبكتريا الحلزونية و يعانون من إتهاب المعدة.

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## List of Abbreviation

Abbreviation	Full term
$^{13,14}\text{C}$	$^{13,14}\text{Carbon}$
Cu	Copper
<i>H.pylori</i>	<i>Helicobacter pylori</i>
Ig A	Immunoglobulin Alpha
Ig G	Immunoglobulin Gamma
LPS	Lipopolysaccharide
MALT	Mucosa Associated Lymphoid Tissue
MAO	Monoamine Oxidase
mg/L	Mil gram per liter
MTs	Metallothioneins
NK	Natural Killer
PPI	Protein Pump Inhibitor
<i>SLC39A4</i>	Solute Carrier Family 39 Member 4
Zn	Zinc

# **Chapter One**

# 1. Chapter One

## 1.1.Introduction:

*Helicobacter pylori* are a spiral-shaped, flagellated, microaerophilic Gram-negative bacillus which colonizes the gastric mucosa of more than 50% of human population. Chronic infection generates a state of inflammation which may evolve toward chronic gastritis, peptic ulcer, gastric mucosa-associated lymphoid tissue (GMALT) lymphoma, and gastric cancer (Ruggiero, 2010).

Trace elements are inorganic micronutrients present at very low concentrations in body fluids and tissue (Shenkin and Baines, 2008). Make up less than 0.01 per cent of the body's dry weight (Crook, 2012). The essential trace elements are usually associated with an enzyme (metalloenzyme) or another protein (metalloprotein) as an essential component or cofactor (Rockwood and Bakowska, 2010; Boosalis and Brickell, 2007).

An element is considered essential if a deficiency impairs a biochemical or functional process and replacement of the element corrects this impairment. Decreased intake, impaired absorption, increased excretion, and genetic abnormalities are examples of conditions that could result in deficiency of trace elements. Excess concentrations are associated with at least some degree of toxicity. Trace elements, such as iron, copper, and zinc, are found in mg/L concentrations (Rockwood and Bakowska, 2010).

Zinc (Zn) is an essential trace element in the body required for over 300 different cellular processes, including enzyme activity, DNA and protein synthesis and intracellular signaling (Kelleher *et al.*, 2011). Also involved in homeostasis, in immune response, in oxidative stress, in apoptosis and in aging (Stefanidou *et al.*, 2006)

Copper (Cu) is also an essential trace element, play an important role in redox (reduction and oxidation) reactions because of it is ability to convert easily between the oxidized ( $\text{Cu}^{2+}$ ) and the reduced ( $\text{Cu}^+$ ) state, Copper is transported mainly by ceruloplasmin; a copper-binding antioxidant protein that is synthesized in several tissues. Copper levels are low in malnutrition and malabsorption. High levels associated with infection, inflammation, Wilson's disease, excessive dietary intake(Russo, 2011).

The metallothioneins, is a metal binding proteinsdiscovered over 40 years ago, may have several diverse functions, including essential metal homeostasis and protection against metal toxicity. They have low molecular weights (about 6000 Da) and are rich in thiol ligands. These ligands provide the basis for high-affinity binding of several essential and nonessential but toxic metals such as Cd, Cu, Hg, Ag, and Zn. In most cases but not all, the metallothioneins are highly inducible by a number of metals and other stimulants. Metallothioneins can interact with metals in complex physiologic and biochemical pathways (Klaassen, 2001).



## **1.2. Rationale:**

The metabolism of trace elements is altered in infection or inflammation. *Helicobacter pylori* are causative in chronic gastritis, duodenal ulcer and gastric cancer. *H.pylori* can change the secretion and acidification functions of stomach, because it penetrates especially into the stomach. This situation can affect digestion and absorption of some components of the nutrients and micronutrients, Although nutrient absorption does not take place in the stomach, this organ contributes to the process by means of secretion of hydrochloric acid and several enzymes. These substances help not only release the micronutrients from the food matrix, but also in the case of the essential minerals, render them soluble during the digestive process. In the last few years, a number of studies have suggested that *H.pylori* infection may affect the homeostasis of different micronutrients.

### **1.3.Objectives:**

#### **1.3.1. General objective:**

To detect the effect of *Helicobacter Pylori* infection on the levels of serum Zinc and Copper among Sudanese patients with gastritis.

#### **1.3.2. Specific objectives:**

- 1- To measure serum levels of zinc and copper in *H.pylori*-infected patients and control.
- 2-To compare serum zinc and copper levels in *H.pylori*-infected patients with control.
- 3- To compare between mean of BMI among *H.pylori*-infected patients and control group.
- 4- To assess the levels of both serum zinc and copper with recurrent *H.pylori* infection.
- 5- To explore the effect of gender and age group in the levels of serum zinc and copper in *H.pylori*-infected patients.
- 6- To correlate between age and serum zinc and copper levels in *H.pylori*-infected patients.

## **Chapter Two**

## 2. Literature Review

### **2.1. *Helicobacter pylori*:**

#### **2.1.1. General Characteristics of *H. pylori*:**

*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, 3-5 $\mu$ m long and about 0.5 $\mu$ m 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 Maier, 2002).

#### **i) Acid Resistance in *H. pylori*:**

*H. pylori* neutralize the acid in its environment. It does this by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. The ammonia, which is basic, neutralizes stomach acid (Stinglet *et al.*, 2002). *H. Pylori* is also unique in having a large amount of urease in the cytoplasm at neutral pH (Hong *et al.*, 2003). Urease is synthesized constitutively by the microorganism accounting for about 10-15% of the total protein synthesized by the bacterium (Feyissa, 2015). The urease activity of intact cells increased nearly exponentially when the external pH decreases. With the activity of carbonic anhydrase also encoded in the genome of *H. pylori*, the CO<sub>2</sub> generated will be able to increase the buffering capacity of the cytoplasm to resist internal alkalization due to the generation of NH<sub>3</sub>. The NH<sub>3</sub> produced can act as a buffer leaving the bacterial cytoplasm and entering the bacterial periplasm by the formation of NH<sub>4</sub><sup>+</sup>. It was previously believed that urease located on the cell surface created a neutral microenvironment that was conducive to bacterial

survival; however, it has been shown that intracellular urease actually plays a key role in promoting acid resistance (Scott *et al.*, 2002).

## ii) Chemotaxis in *H. pylori*:

*H. pylori* use its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where there is a more neutral pH (Amieva and El-omer, 2008). *H. pylori* are able to sense the pH gradient in the mucus and move towards the less acidic region (a process called chemo taxis). *H. pylori* are found in the mucus, on the inner surface of the epithelium, and occasionally inside the epithelial cells themselves (Petersen and Krogfelt, 2003). It adheres to the epithelial cells by producing adhesions, which bind to lipids and carbohydrates in the epithelial cell membrane (Feyissa, 2015).

Concentration gradient of urea is formed in the gastric mucus layer, which should be sensed by *H. pylori*. The chemotactic response to urea could be crucial not only for acid resistance, but also for colonization in the hostile environment. *H. pylori* in the mucus layer may sense urea and move toward the epithelial cell surface, which must be important for persistent infection of this microorganism. Chemotaxis is significant for *H. pylori*, because amino acid metabolism is essential for *H. pylori* growth and this microorganism does not synthesize L-arginine (Gobert *et al.*, 2001), it must obtain that amino acid from extracellular sources. In this way chemotaxis to arginine could play a role allowing the bacteria to find the arginine source (Cerdeira *et al.*, 2003).

### **2.1.2. Classification 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).

### **2.1.3. Signs and Symptoms of *H.pylori*infection:**

Up to 85% of people infected with *H.pylori* never experience symptoms or complications (Bytzer *et al.*, 2011). Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea. It usually occurs two to three hours after a meal or in the middle of the night (when the stomach is empty) and is relieved by eating, drinking milk or taking antacid medications. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: stomach pains, nausea, and bloating, excessive burping (belching), sometimes vomiting or black stool (Ryan and Ray, 2010), flatulence (passing gas from the rectum), loss of appetite weight loss (Stanley and Swierzewski, 2008). Individuals infected with *H. pylori* have a 10 to 20% lifetime risk of developing peptic ulcer and a 1 to 2% risk of acquiring stomach cancer (Kusters *et al.*, 2006). Inflammation of the pyloric antrum is more likely to lead to duodenal ulcers, while inflammation of the corpus (body of the stomach) is more likely to lead to gastric ulcers (Suerbaum and Michetti, 2002).

### **2.1.4. Diagnosis of *H.pylori*infection:**

Testing for *H.Pylori*infection has become a very important part of the diagnostic process for gastric and duodenal inflammatory disease, since the presence or absence of infection determines the type of treatment to be applied. A number of different diagnostic test methods, both invasive and non-invasive are available.

i) Biopsy: The collection of biopsy specimens from inflamed or ulcerated regions of the stomach and duodenum during invasive endoscopy is considered to be the

reference method for diagnosing *H.pylori* infection. The biopsy material can be examined using different test methods. Such as histological examination: Staining and examination of the tissue samples allows both evaluation of cell damage and the detection of *H.pylori* cells. Its sensitivity is partly dependent on the accuracy of the biopsy procedure, since sampling tissue from areas where only low numbers of bacteria are present may give false-negative results (Logan and Waker, 2001). The biopsy is also placed in a urea solution or gel with a pH indicator; when *H.pylori* are present, the urea is hydrolyzed by its urease, resulting in a colour change and this is called Urease test. This method can be used to give a rapid indication of infection at the time of the biopsy. Urease testing is much more rapid and less costly, but still requires an invasive procedure to obtain the samples (Stenstrom *et al.*, 2008).

ii) Serological tests: non-invasive methods, People infected with *H.pylori* generally have specific IgG and IgA antibodies circulating in their blood and these can be detected by serological tests. Serological testing cannot be used to monitor the progress of antimicrobial therapy, since patients whose infection has been eradicated may continue to carry serum antibodies specific to *H.pylori* for several months after successful treatment (Kuster *et al.*, 2006).

iii) Urea breath test: non-invasive method, the breath test utilizes the ability of *H.pylori* to produce large quantities of urease as a diagnostic characteristic. The patient is required to drink a solution of urea labeled with  $C^{13}$ , or  $C^{14}$  isotopes. If *H.pylori* infection is present, the urea will be metabolized by the bacteria, producing ammonia and labeled carbon dioxide, which can be detected in the patient's exhaled breath by radioactive counting or mass spectrometry. Breath testing has been found to have a high sensitivity and specificity (94-98%) and can be applied at moderate cost. It is also suitable for monitoring the effectiveness of

treatment as it is specific to active infections and can be used to confirm eradication (Stenstrom *et al.*, 2008; and Kuster *et al.*, 2006).

iv) Stool antigen testing: new tests for *H.pylori* infection, rely on the detection of specific antigens in the stools of infected individuals, it is more reliable, sensitive and specific. It also has the advantage of indicating only active infection. This means that it can be used to monitor the eradication of infection by antimicrobial treatment and can also detect recurrent infections (Stenstrom *et al.*, 2008).

### **2.1.5. Mode of Transmission of *H.pylori* infection:**

*H.pylori* is contagious, person-to-person transmission by either the oral-oral or fecal-oral route is most likely. *H.pylori* may also be transmitted orally by means of fecal matter through the ingestion of waste-tainted water, so a hygienic environment could help decrease the risk of *H.pylori* infection (Brown, 2000).

### **2.1.6. Diseases caused by *H.pylori*:**

It is later confirmed that *H.pylori* infection is highly associated with gastritis and gastritis-associated diseases, peptic ulcer, gastric adenocarcinoma (Kuster *et al.*, 2006). *Helicobacter pylori* infection can cause severe inflammation of the stomach lining. Gastritis can damage glands that produce stomach acid and increase the risk for stomach cancer (gastric cancer). Gastritis caused by *H.pylori* infection must be monitored closely, because stomach cancer symptoms may not escalate until the disease is advanced. It is a common medical problem in many countries including the United States where the condition has been diagnosed in as much as 10% of patients seeking emergency medical care for abdominal pain. Such increased rate of prevalence of stomach cancer is believed to be due to *H.pylori* infection (Stanley and Swiezewski, 2008)

#### **2.1.6.1. 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:

i) 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.

ii) Establishment: after attachment they adhere themselves to lining of stomach using adhesion, then they neutralize their environments by secreting urease and start to grow.

iii) 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.

iv) 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.

v) 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).

#### **2.1.6.2. Factors involved in tissue damage:**

*H.pylori* produces and possesses phospholipase A enzymes; which can digest phospholipids of cell membranes, Urease; has a cytotoxic activity, Alcohol dehydrogenase; has been described to contribute to gastric mucosal injury. It also contains a toxin Vac A; which can produce vacuoles in gastric epithelial cells and has been related to peptic ulcer, severe gastritis and mucosal integrity. Lipopolysaccharide (LPS) in *H.pylori* has a low biological activity as compared to LPS from other gram-negative bacteria which may be explained by the unusual composition of lipid A. When the gastric epithelial barrier is weakened by disruption of the mucosal surface cells and the extracellular matrix, LPS is responsible for a marked increase in epithelial cell apoptosis (Feyissa, 2015).

### **2.1.7. Other factors that cause Gastritis:**

Gastritis are also caused by other risk factors such as stress ,spicy foods, pain killer, alcoholism, some foods, consumption of corrosive substances. These factors cause acute gastritis which may develop to chronic gastritis when untreated. According to (Ddineet al. (2012), in situations when there is high stress and anxiety, the secretion of HCl increases and causes erosion of the stomach. In the same way it is also explained that spicy foods like red pepper, black pepper, chilli pepper also increase the gastric acid secretion. Certain medications like aspirin and consumption of corrosive substance like poison also develop gastric ulcer. Some foods also increase gastric motility and due to these the secretions also increase. The risk factors of gastritis were alcohol, table salt, soy sauce and smoking (Rajashekhar,2000).

### **2.1.8. Survival Mechanism of *H.pylori*:**

*H.pylori* survives in the human body and other environments by different mechanism. Some factors thought to be involved in the colonization of *H.pylori* in the gastric mucosa, including urease activity and motility using flagella (Feyissa, 2015).

Motility using flagella; the curved morphology of *H.pylori* and the polar motility caused by flagella in one end cause screwlike movements, which may enable the organism to penetrate the mucin layer, but the motility is pH dependent and impaired at a pH below 4. Urease; is one of the key enzymes in *H.pylori* pathogenesis, Urease is necessary for *H.pylori* to maintain a pH neutral microenvironment around the bacteria, necessary for survival in the acidic stomach (Perez-Perez et al., 2004). Superoxide dismutase; has been isolated from *H.pylori*, which breaks down superoxide produced in polymorph nuclear leukocytes and macrophages and thereby prevents the killing of these organisms.

Catalase; protects *H. pylori* against the damaging effects of hydrogen peroxide released from phagocytes. Transforms into coccoid forms; *H. pylori* transforms into coccoid forms under certain conditions such as nutrient starvation and media containing growth inhibitors (bismuth, proton pump inhibitor, or certain antibiotics). These coccoid forms have been reported to survive for several years in river water and have been proposed by some to be an important factor for transmission by fecal excretion and for therapy failure. It has also been suggested that some cocci can revert to their original spiral shape (Feyissa, 2015).

### **2.1.9. Treatment of *H. pylori* infection:**

Treatment for *Helicobacter pylori* infection often involves medications and lifestyle changes.

i) 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)

ii) Quadruple therapy: An 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 (Pernaet *al.*,2007 and Hsu *et al.*, 2008)

#### **2.1.10. Prevention of *H.pylori*infection:**

*Helicobacter pylori* bacteria are present in contaminated food and water. Therefore, it is important to avoid these sources (e.g., floodwater, raw sewage). Washing the hands thoroughly with warm soapy water after using the restroom and before eating also may help prevent infection. Eating utensils and drinking glasses should never be shared, since the bacteria can be spread through saliva (Stanley and Swierzewski,2008).

#### **2.2.Trace Elements:**

Trace elements are important substances for biochemical reactions in the body; likewise, these take part in hormone synthesis, food digestion, cell reproduction and the immune system. Trace elements are inorganic compounds which are not synthesized in the body; these are contained in minimum quantity, and they are necessary in food consumption, are most important for human life (Calderon-Guzman,*et al.*, 2011).

Although the trace elements are the essential components of biological structures, they may show toxic effect when they are more concentration than the amount that are required for biological functions. In addition, the toxicity can be spread to other non essential elements of very similar atomic characteristics that can mimic the reactivity of trace elements. To cope with this essentiality/toxicity duality, biological system has developed the ability to recognize a metal, and bring it to the target without allowing the metal to participate in toxic reactions. Proteins are primarily account for such recognition and transport, and most of the associations

of trace element with other biomolecules lead to undesirable chemical modifications of these molecules (Pirincciet *al.*, 2013)

### **2.2.1. Zinc:**

Zinc is an essential mineral that is found in almost every cell. Deficiency results in severe health consequences. At the other extreme, excessive exposure to zinc is relatively uncommon and occurs only at very high levels (Klaassen, 2001). Total adult body content of zinc is about 2 to 2.5 g. About 55% of the total is found in muscle and approximately 30% in bone (Shenkin and Baines, 2008).

Zinc-binding proteins (metallothioneins, MTs), are protective in situations of stress and in situations of exposure to toxic metals, infections and low Zn nutrition. Metallothioneins play a key role in Zn-related cell homeostasis due to their high affinity for Zn, which is in turn relevant against oxidative stress and immune responses, including natural killer (NK) cell activity and ageing, since NK activity and Zn ion bioavailability decrease in ageing. Zinc is not stored in the body and excess intake result in reduced absorption and increased excretion (Stefanidou *et al.*, 2006).

#### **2.2.1.1. Health Effects of Zinc:**

Zinc is second only to iron in importance as an essential trace element. The main biochemical role of zinc is its influence on the activity of more than 300 enzymes (from the classes of Oxidoreductases, Transferases, Hydrolases, Lyases, Isomerases, and Ligases). Zinc can be essential for the structure, regulation, and catalytic action of an enzyme (Kelleher, 2011; Rockwood and Bakowska, 2010).

Zinc occurs in enzymes that realize the synthesis and metabolism of DNA and RNA. It influences the synthesis and metabolism of proteins, participates in glycolysis and cholesterol metabolism, maintains membrane structures, effects functions of insulin, and affects growth factor. Chronic oral zinc supplementation interferes with copper absorption and may cause copper deficiency. This ability to

interfere with copper absorption is also the basis for using zinc to treat Wilson's disease (Rockwood and Bakowska, 2010).

Zinc supports a healthy immune system; immune system is affected by even moderate degree of zinc deficiency, severe zinc deficiency depresses immune function. It is also required for the development and activation of T lymphocytes. Gastrointestinal infections are also strongly attenuated by ingestion of zinc, and this effect could be due to direct antimicrobial action of zinc ions in the gastrointestinal tract, or due to absorption of the zinc and re-release from immune cells or both. Zinc is also needed for wound healing, helps maintain the sense of taste and smell, and is needed for supports normal growth and development during pregnancy, childhood, and adolescence (Abdelsalam, 2010).

#### **2.2.1.2. Source of Zinc:**

Zinc is ubiquitous in the environment, it is present in: Seafood's, meats, whole grains, dairy products, nuts, and legumes which are high in zinc. In vegetables are lower, although zinc applied to soil is taken up by growing vegetables. Atmospheric zinc levels are higher in industrial areas (Klaassen, 2001).

#### **2.2.1.3. Absorption, Transportation and Excretion of Zinc:**

Gastrointestinal absorption of zinc is homeostatically controlled and is probably a carrier-mediated process. Within the mucosal cell, zinc binds to the metal-regulatory transcription factor 1 (MTF1) activates metallothionein (Mt) expression. This multifunctional, low molecular weight protein (9000 to 10,000 Da) has a high content of cysteine and reversibly binds zinc. Mt is important in intracellular zinc trafficking and helps to maintain intracellular zinc concentrations (Shenkin and Baines, 2008). When the cell is saturated; this may depress zinc absorption (Klaassen, 2001). Hepatic synthesis of Mt is induced by interleukin-1, interleukin-6, and glucocorticoids in response to infection, trauma, and other stressors (Shenkin and Baines, 2008).

In the blood, about two-thirds of the zinc is bound to albumin and most of the remainder is complexed with alpha<sub>2</sub>-macroglobulin. Zinc enters the gastrointestinal tract as a component of metallothionein secreted by the salivary glands, intestinal mucosa, pancreas, and liver. The normal basic physiologic requirement for absorbed zinc is 1.4 mg/day. Assuming 20 percent absorption, a daily diet of 7 mg will meet the basic requirement for males, but the requirement for females is greater during pregnancy and lactation (Klaassen, 2001).

Iron at supplemental dosages (up to 65 mg/day) may decrease zinc absorption so that pregnant and lactating women taking iron may require zinc supplementation (Shenkin and Baines, 2008). Adaptation to low dietary zinc will increase gastrointestinal absorption to as much as 50 percent (Klaassen, 2001).

Bile is the major route of zinc excretion. Homeostatic control of zinc is maintained primarily by fecal excretion of endogenous zinc. Urinary excretion of zinc is low and not significantly influenced by dietary zinc. Liver concentration is influenced by humoral factors, including adrenocorticotropic hormone, parathyroid hormone and endotoxin (Klaassen, 2001).

#### **2.2.1.4. Deficiency of Zinc:**

The causes of zinc deficiency are:

Acrodermatitis enteropathica (a rare autosomal recessive condition associated with dermatitis, diarrhea and alopecia, defect of the *SLC39A4* gene, which encodes for a membrane zinc transporting protein), poor intake (e.g. parenteral nutrition or malabsorption), increased utilization, high levels of dietary phytates (Crook, 2012), digestive system diseases and poorly absorbed, also alcoholism because alcohol decreases the absorption of zinc and increases loss of zinc in urine. In addition many alcoholics do not eat an acceptable variety or amount of food, so their dietary intake of zinc may be inadequate. Also deficiency occurs when the body's requirement for zinc increases; human milk does not provide recommended



amount of zinc for older infants between the ages of 7-12 months, so breast-fed infants of this age should also consume age-appropriate foods containing zinc or be given formula containing zinc. Deficiency can also occur in vegetarians: vegetarians may need as much as 50% more zinc than non-vegetarian because of the lower absorption of zinc from plant foods (Abdelsalam, 2010).

Zinc deficiency may result in a number of clinical states, including:

Growth retardation, Alopecia, Dermatitis, Diarrhea, Poor wound healing, Infertility, increased risk of infections, Hair loss, Delay sexual maturation, Loss of appetite, Weight loss, Taste abnormalities, Mental lethargy and Death (Crook, 2012).

#### **2.2.1.5. Zinc Toxicity:**

Zinc toxicity has been seen in both acute and chronic forms. Even though zinc is an essential requirement for a healthy body, too much zinc can be harmful. Intake of 150 to 450 mg of zinc per day has been associated with:

Suppressed copper absorption, lead to hypocupraemia (low plasma copper) (Crook, 2012), Alter iron functions, Reduce immune functions, and reduce levels of high-density lipoprotein (Abdelsalam, 2010).

Also can result in; Pulmonaryoedema, Jaundice and Oliguria (Crook, 2012).

The free zinc ion in solution is highly toxic. Swelling zinc can cause damage to the stomach lining due to the high solubility of zinc ions in the acidic stomach. Zinc toxicity, mostly in the form of the ingestion causes hemolytic anemia (Abdelsalam, 2010).

#### **2.2.2. Copper:**

Copper is an essential nutrient for humans. It is a component of numerous enzymes that affect a wide variety of metabolic processes. Copper content in adult human body is estimated to range between 50 and 120 mg. copper is found in high

concentration in liver, brain, and to a lesser degree in kidney, heart, and pancreas (Shike, 2009).

In the body, though the majority of the body's copper is in the  $\text{Cu}^{2+}$  forms, copper shifts between the cuprous ( $\text{Cu}^+$ ) and the cupric ( $\text{Cu}^{2+}$ ) forms. The ability of copper to easily accept and donate electrons explains its important role in oxidation-reduction (redox) reactions and the scavenging of free radicals (Abdelsalam, 2010).

### **2.2.2.1. Health Effect of Copper:**

Copper is a component of all living cells and is associated with many oxidative processes. It is an essential component of several metalloenzymes, including type A oxidases and type B monoamine oxidases. Of the type B oxidases, cytochrome C oxidase is probably the most important because it catalyzes a key reaction in energy metabolism. Of the type A oxidases, lysyl oxidase plays a major role in elastin and collagen synthesis. There are two forms of superoxide dismutase, the copper/zinc superoxide dismutase is present in the cytosol of most cells, particularly brain, thyroid, liver, and erythrocytes. Both dismutases scavenge superoxide radical by reducing them to hydrogen peroxide. Impairment of the function of these enzymes is responsible for the various diseases associated with copper deficiency (Klaassen, 2001).

The following enzymes are including copper as an essential constituent:

Ferroxidases (including ceruloplasmin): which catalyze the oxidation of ferrous iron in the process of transferring iron from storage sites to bone marrow for hemoglobin synthesis, reduced activity results in anemia, which appears like iron deficiency. Lysyl oxidase: participates in cross-linking of collagen and elastin in connective tissues, deficiency results in bone formation abnormalities and defects in connective tissues and blood vessels. Dopamine-hydroxylase: catalyzes the conversion of dopamine to norepinephrine in the brain, deficiency causes neurologic abnormalities. Copper/zinc superoxide dismutase: functions as a free radical

scavenger; copper deficiency is associated with oxidative damage. Monoamine oxidase: is essential for serotonin synthesis. And Tyrosinase: participates in melanin synthesis, deficiency results in hypopigmentation (Shike, 2009).

#### **2.2.2.2. Functions of Copper:**

Copper is critical functional component of a number of essential enzymes, known as cuproenzymes. Some of the physiological functions known to be copper-dependant are:

**i) Energy production:** the copper-dependant enzyme plays a critical role in cellular energy production. By catalyzing the reduction of molecular oxygen ( $O_2$ ) to water ( $H_2O$ ), the enzymes generate an electrical gradient used by mitochondria to create vital energy-storing molecule, ATP (Abdelsalam, 2010).

**ii) Connective tissue formation:** Another cuproenzyme is required for the cross-linking of collagen elastin, which is essential for the formation of strong and flexible connective tissue. The action of this enzyme helps maintain the integrity of connective tissue in the heart and blood vessels and play a role in bone formation.

**iii) Iron metabolism:** two copper-containing enzymes (ferroxidase I and ferroxidase II) have the capacity to oxidize ferrous iron ( $Fe^{2+}$ ) to ferric iron ( $Fe^{3+}$ ), the form of iron that can be loaded onto the protein transferrin for transport to the site of red blood cell formation (Abdelsalam, 2010).

**iv) Central Nervous System:** A number of reactions essential to normal function of the brain and nervous system are catalyzed by cuproenzymes.

**v) Neurotransmitter synthesis:** dopamine- $\beta$ -monooxygenase catalyzes the conversion of dopamine to the neurotransmitter norepinephrine.

**vi) Metabolism of neurotransmitters:** monoamine oxidase (MAO) plays a role in the metabolism of the neurotransmitters norepinephrine, epinephrine and dopamine. MAO also functions in the degradation of the neurotransmitter

serotonin, which is the basis for the use of monoamine oxidase inhibitor as antidepressants (Abdelsalam, 2010).

**vii)** Formation and maintenance of myelin: the myelin sheath is made of phospholipids whose synthesis depends on copper-dependent enzymes activity.

**viii)** Melanin formation: the cuproenzyme, tyrosinase, is required for the formation of pigment melanin.

**ix)** Antioxidant functions.

**x)** Regulation of gene expression: copper-dependent transcription factors regulate transcription of specific genes. Thus cellular copper levels may affect the synthesis of proteins by enhancing or inhibiting the transcription of specific genes (Abdelsalam, 2010).

#### **2.2.2.3. Source of copper:**

Copper is found in high quantities in shellfish, nuts, liver, and legumes (Shike, 2009).

Also it is found in wheat bran cereals, whole grain, oysters, clams, crab meat, cashews, sunflower seeds, hazelnuts, almonds, peanut butter, mushrooms, shredded wheat cereal, chocolate and hot cocoa (Abdelsalam, 2010).

The fractional absorption from the gastrointestinal tract is regulated to maintain homeostasis (Shike, 2009).

#### **2.2.2.4. Absorption, Transportation and Excretion of Copper:**

Copper is absorbed mostly in the small intestine and to a lesser degree in the stomach (Shike, 2009). Absorption is reduced by other dietary components, such as zinc (via metallothionein), molybdenum and iron, and increased by amino acids (Shenkin and Baines, 2008).

Once absorbed through the enterocytes, copper is transported to the liver bound by albumin or transcuprein. In the liver, it is incorporated into ceruloplasmin then copper is released from the liver into the blood bound to ceruloplasmin and is

delivered in this form to the peripheral tissues (Shenkin and Baines, 2008 and Shike, 2009). Ceruloplasmin binds to its receptors on the cell surface then copper is released from its binding protein and enters the cell (Shike, 2009).

Most copper excretion and loss is through the bile but small amounts are excreted through urine, sweat, and menstrual blood (Shike, 2009). Patients with cholestatic jaundice or other forms of liver dysfunction are therefore at risk of copper accumulation caused by failure of excretion (Shenkin and Baines, 2008).

#### **2.2.2.5. Manifestations of Copper Deficiency:**

Copper deficiency is rare in humans. It has been reported in patients with prolonged severe malabsorption, premature infants, children recovering from severe malnutrition, and patients receiving parenteral nutrition (PN) without copper supplementation (Shike, 2009). In some cases, excessive intake of oral zinc supplements has caused copper deficiency by zinc induction of metallothionein in the intestinal mucosa, which then sequesters dietary copper, blocking its absorption (Rockwood and Bakowska, 2010).

Manifestations of copper deficiency include:

Anemia (often hypochromic, microcytic), Leucopenia, variety of bone abnormalities, including osteoporosis, new subperiosteal bone formation, and fibrosis of epiphysis also. It has been suggested, but not well proven, that prolonged marginal copper deficiency can result in cardiac diseases, arthritis, loss of hair pigmentation, and neurologic abnormalities, mimicking vitamin B12 deficiency (Crook, 2012).

**Menkes disease:** is a rare fatal X-linked disorder, an inborn error of copper transport resulting in low copper concentrations and abnormal hair that has a characteristic 'kink' appearance (Crook, 2012). In which copper accumulates in intestinal mucosa and is not transported to other sites. This leads to copper deficiency in the liver and other tissues. The serum copper and ceruloplasmin levels

are low. The disease is manifested as defective arteries, degeneration of brain cells, severe mental retardation, and death. The anemia and neutropenia is seen in nutritional copper deficiency do not occur in Menkes disease (Shike, 2009).

Copper deficiency can be treated with certain copper salts, but care is needed in case there is an effect on zinc and iron absorption(Crook, 2012).

#### **2.2.2.6. Manifestations of Copper Toxicity:**

Acute copper toxicity is rare in humans and occurs mostly from consumption of beverages stored in copper vessels or from consumption of contaminated water. Acute copper toxicity results in abdominal pain, liver and kidney failure, and death(Shike, 2009).

**Wilson's disease:** Chronic copper toxicity, an inborn error of copper metabolism characterized by high levels of copper in liver, brain, kidney, and other organs (Shike, 2009).

There are two defects of copper metabolism in Wilson's disease: Impaired biliary excretion leads to deposition in the liver and deficiency of ceruloplasmin results in low plasma copper concentrations. Most is in a loosely bound form and is therefore deposited in tissues; more than normal is filtered at the glomeruli and urinary copper excretion is increased (Crook, 2012).

Excessive deposition of copper in the eyes, basal ganglia of the brain, liver and renal tubules produces: Kayser–Fleischer rings at the edges of the cornea due to deposition of copper in Descemet's membranes, Neurological symptoms due to degeneration of the basal ganglia, Liver damage leading to cirrhosis and Renal tubular damage with any or all of the associated biochemical features, including amino aciduria (Fanconi's syndrome) (Crook, 2012;Shike, 2009).

Idiopathic copper toxicosis, which mostly manifests as cirrhosis of the liver, a high dietary copper level appears to play a role in addition to an inherited disposition (Shike, 2009).

The penicillamine test is sometimes used in the diagnosis of Wilson's disease and is based on the principle that penicillamine solubilizes copper and enhances copper urinary excretion. Following a dose of oral penicillamine, urinary copper excretion is more than 25  $\mu\text{mol/day}$ , indicative of Wilson's disease (Crook, 2012).

### **2.2.3. Association between Zinc and Copper with *H.pylori*:**

Trace elements are essential for life. They act as essential cofactors for enzymes and as organizers of the molecular structures of the cell. Deficiencies of micronutrients influence immune homeostasis and thus affect infection-related morbidity and mortality. Micronutrient as copper is powerful antioxidants and has significant impact on infection related morbidity in humans (Akcem, 2010). Zinc contribute to host defense mechanism by maintaining the structure and function of the membrane barrier, that is especially important in the gastro-intestinal tract, which is continuously exposed to plenty of pathogens and noxious agents (Finamore *et al*, 2008).

Subclinical deficiencies are known to impair biological and immune functions in the host. *H.pylori* can change the secretion and acidification functions of stomach. This situation can affect digestion and absorption of some components of the nutrients and micronutrients. Although nutrients absorption does not occur in the stomach, secretion of hydrochloric acid and several enzymes secreted by this organ contribute in absorption by releasing micronutrients from food matrix and render them soluble during digestive process (Akcem, 2010).

### **2.2.4. Estimation of Zinc and Copper:**

The most commonly used analytical methods are:

#### **2.2.4.1. Spectrophotometry:**

When applied to the analysis of trace elements, spectrophotometric methods are based on the use of a color-forming reagent; however, they lack specificity. Interferences also occur in hemolyzed, lipemic, and icteric samples. In practice, the technique is only sensitive for the more abundant trace elements, such as iron, zinc, and copper (Shenkin and Baines, 2008).

#### **2.2.4.2. Atomic Absorption Spectrophotometry (AAS):**

Atomic Absorption is an absorption spectrophotometric technique in which a metallic atom in the sample absorbs light of specific wavelength. However, the element is not appreciably excited in the flame, but is merely dissociated from its chemical bonds (atomized) and placed in an unexcited or ground state (neutral atom). Thus, the ground state atoms absorb radiation at a very narrow band width corresponding to its own line spectrum. A hollow cathode lamp with the cathode made of the material to be analyzed is used to produce a wavelength of light specific for the atom. When the light from the hollow cathode lamp enters the flame, some of it is absorbed by the ground-state atoms in the flame, resulting in a net decrease in the intensity of the beam from the lamp (Shenkin and Baines, 2008).

#### **2.2.4.3. Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES):**

ICP-OES is replacing AAS in some laboratories. Major changes to ICP-OES instrumentation have led to lower limits of detection. It also offers a wide dynamic range (e.g., three orders of magnitude for most elements) that allows simultaneous analyses to be obtained on a single diluted aliquot of sample. The high temperature of the plasma (7500°C) renders the technique largely free of chemical interferences, but matrix effects, background, and spectral interferences are greater than those in AAS (Shenkin and Baines, 2008).

#### **2.2.4.4. Inductively Coupled Plasma-Mass Spectrometry (ICP-MS):**



This technique is more sensitive than either AAS or ICP-OES and is now the method of choice for ultratrace elements. Polyatomic interferences are more likely at masses less than 80. ICP-MS also has been used to measure stable isotopes and for conducting stable isotope tracer experiments and isotope dilution analysis (Shenkin and Baines, 2008).

# **Chapter Three**

## Materials and Methods

## Chapter Three

### 3. Materials and Methods

#### **3.1. Study approach:**

Quantitative and Qualitative methods were used to estimate serum Zinc and serum Copper levels in adult Sudanese patients with *Helicobacter pylori* infection in Khartoum state during the period from January to September 2017.

#### **3.2. Study design:**

This was Cross sectional case control study. A total of 80 samples were included in this study, 40 *H.pylori*-positive Adult with Gastritis as cases and 40 *H.pylori*-negative Adult with Gastritis as control.

#### **3.3. Study area:**

This study was conducted in Al-egairbab Health Center in Khartoum State.

#### **3.4. Target population:**

The study was included *H.pylori*-Infected adult Patients (males and females).

#### **3.5. 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.

#### **3.6. Exclusion criteria:**

*H.pylori* positive or negative adult under 18 year or more than 60 year and Patients with diabetes mellitus, Hypertension, liver disease, heart disease, kidney disease, anemia, Malignancy, and pregnancy were excluded.

#### **3.7. Ethical consideration:**

This study was approved by department of clinical chemistry. Verbal informed Consent was taken from participant to participate in the study and reassurance of confidentiality. Before the sample was collected, the donors knew

that this specimen for research and the objectives of the research was explained to each patient.

### **3.8. 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.

### **3.9. Sample collection and processing:**

i) For serum: about 5 ml of venous blood were collected from each participant (both cases and control). The samples collected under aseptic conditions and placed in sterile plain containers, stand in room temperature for 30 minutes and then centrifuged for 10 minutes at 3500 rpm to obtain serum, then the serum were kept at -80°C till used.

ii) For feces: the test specimens and buffer were allowed to reach room temperature (27 °C) prior to testing, (2 ml or 2 g) of feces was collected in clean, dry specimen collection container to obtain maximum antigens (if present), fecal specimens were processed as for solid specimens: the cap of specimen collection tube was unscrew, then randomly the specimen collection applicator was stabbed into the fecal specimen in at least 3 different sites to collect approximately 50 mg of feces (equivalent to ¼ of a pea). For liquid specimens: the dropper was Hold vertically, fecal specimens were aspirated, and then 3 drops were transferred (approximately 80 µL) into the specimen collection tube containing the extraction buffer, the cap was Tightened onto the specimen collection tube, then the specimen collection tube was shaken vigorously to mix the specimen and the extraction buffer. The tube was left alone for 2 minutes, the pouch was brought to room temperature before opening it.

### **3.10. Estimation of serum Zinc and serum Copper levels:**

#### **3.10.1. Principle of Atomic Absorption Spectrophotometer:**

Atomic Absorption is an absorption spectrophotometric technique in which a metallic atom in the sample absorbs light of specific wavelength. However, the element is not appreciably excited in the flame, but is merely dissociated from its chemical bonds (atomized) and placed in an unexcited or ground state (neutral atom). Thus, the ground state atoms absorb radiation at a very narrow band width corresponding to its own line spectrum. A hollow cathode lamp with the cathode made of the material to be analyzed is used to produce a wavelength of light specific for the atom. When the light from the hollow cathode lamp enters the flame, some of it is absorbed by the ground-state atoms in the flame, resulting in a net decrease in the intensity of the beam from the lamp (Shenkin and Baines, 2008).

#### **3.10.2. Procedure of serum Zinc and Copper measurement:**

Sample preparation: For the determination of serum copper, the sample was diluted with an equal volume of deionized water (100  $\mu$ l serum with 100  $\mu$ l dH<sub>2</sub>O). For the determination of serum zinc, the sample was diluted 1:5 with deionized water (100  $\mu$ l serum with 500  $\mu$ l dH<sub>2</sub>O).

The concentration of copper and zinc determined using the conditions listed in the table (3-1) (Appendix II). Copper standards are prepared by diluting the copper stock standard solution with 10% (v/v) glycerol. A 10% (v/v) glycerol solution also used as a blank solution when copper determined. Zinc standards are prepared by diluting the stock standard solution with 5% (v/v) glycerol. A 5% (v/v) glycerol solution used as a blank solution when zinc determined.

#### **3.10.3. Calculation of Analyze Concentration:**

The analyzer automatically calculated the analyze concentration, after the monochromator selects the wavelength to be used for the analysis, then signal is amplified and sent to the readout. After that concentrations of copper and zinc was

multiplied by dilution factor (2) for copper and (6) for zinc to obtain final results in mg/L for both.

### **3.11. Detection of *H.pylori* Stool Antigen:**

#### **3.11.1. 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).

#### **3.11.2. Procedure of *H.pylori* Stool Antigen:**

The test cassette was removed from the foil pouch and used within one hour, and then the specimen collection tube was held upright and the cap open onto the specimen collection tube. The specimen collection tube was inverted and 2 full drops of the extracted specimen were transferred (approximately 80 $\mu$ L) to the specimen well (s) of the test cassette, the timer was started then results were read at 10 minutes after dispensing the specimen (leaflet of *H.pylori*Ag test strip).

#### **3.11.3. 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.

### **3.12. Quality Control:**

For serum zinc and copper: The standard with different concentrations was run and repeated after running every 10 samples reading to check the stability of instrument, beside the reading of the reference sample to check the accuracy of readings.

For stool antigen *H.pylori*: a colored line was appearing in the control line region indicating a proper volume of specimen has been added and membrane wicking has occurred.

### **3.13. Data analysis:**

Data was analyzed to obtain means standard deviations and correlation of the sampling using statistical package for social science (SPSS) computer Programmed version 16. Student t test, person correlation and one-way ANOVA test were used for comparison and correlation.

## **Chapter Four**



## Chapter Four

### 4. Results

#### Characteristics of study participants:

**(Figure 4-1):** Frequency of sex in *H.pylori*-infected patients was 60% female and 40% male.

**(Figure 4-2):** Age distribution in *H.pylori*-infected patients was divided into three groups, (18-31) years, (32-45) years and (46-60) years, each group contain 47.5% (19), 32.5% (13), and 20% (8) of patients, respectively.

And frequency of recurrent *H.pylori* was 71.4% (10) once recurrent and 28.6% (4) twice recurrent.

**Table (4-1):** Shows mean concentration of serum zinc and serum copper in patients and control groups. The levels of zinc and copper were significantly decreased in *H.pylori*-infected patients compared to control group, p.value (0.000) for zinc and copper.

**Table (4-2):** shows mean of BMI in *H.pylori*-infected patients compared to control group. BMI was insignificant increased in *H.pylori*-infected patients compared to control group, p.value (0.255).

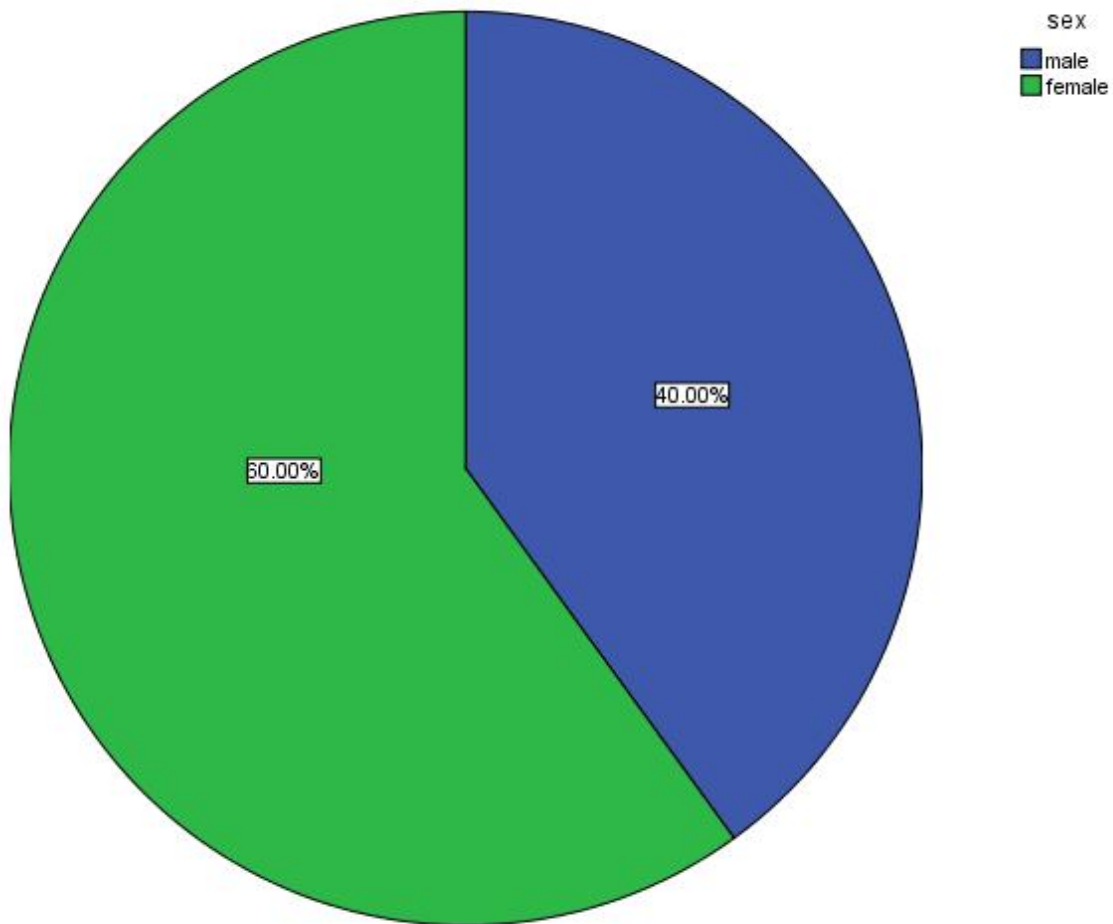
**Table (4-3):** shows mean of serum zinc and serum copper in patients with recurrent *H.pylori* infection, the levels of zinc and copper were insignificant decreased in twice recurrent infection compared with once recurrent infection, p.value (0.824) , (0.869) respectively.

**Table (4-4):** shows mean of serum zinc and serum copper in male and female in *H.pylori*-infected patients: the levels of zinc was insignificant decreased in male compared with female p.value (0.409), and the level of copper is significantly decreased in male compared with female, p.value (0.013).

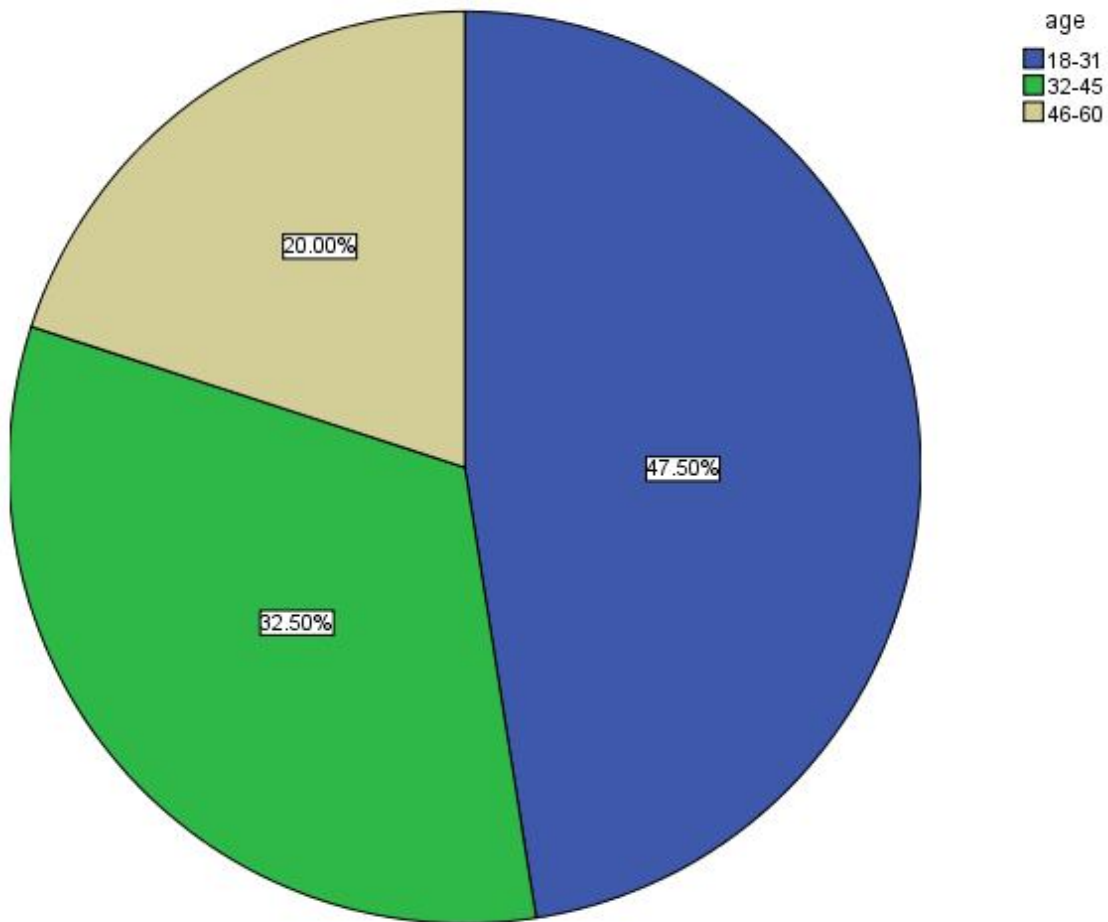
**Table (4-5):** shows mean of zinc and copper in age group in *H.pylori*-infected patients: the level of both serum zinc and copper show insignificant difference between age group. P.value (0.221), (0.504) respectively.

**Figure (4-3):** Show correlation between the levels of serum zinc and age in *H.pylori*-infected patients, there was significant weak positive correlation (p.value=0.016, r=0.377)

**Figure (4-4):** show correlation between the levels of serum copper and age in *H.pylori*-infected patients, there was insignificant correlation (p.value=0.148, r=0.233)



**Figure (4-1):** Percentage of Gender among Sudanese patients with *H.pylori*.



**Figure (4-2):** Percentage of Age distribution among Sudanese patients with *H.pylori*.

**Table (4-1): Comparison between the meanlevels of serum Zinc and Copper in *H.pylori*-infected patients and control group:**

Variables	Patients No=40 Mean $\pm$ SD	Control No=40 Mean $\pm$ SD	P.value
Zinc mg/L	0.206 $\pm$ 0.110	0.621 $\pm$ 0.266	0.000*
Copper mg/L	0.305 $\pm$ 0.144	0.709 $\pm$ 0.176	0.000*

\*Significant level at ( $P \leq 0.05$ ).

\*Result express as (mean $\pm$ SD), Student t test was used to comparison.

**Table (4-2): Comparison between means of Body mass index (BMI) in *H.pylori*-infected patients andcontrol group:**

Variables	Patients No=40 Mean $\pm$ SD	Control No=40 Mean $\pm$ SD	P.value
BMI Kg/m <sup>2</sup>	23.59 $\pm$ 3.56	22.79 $\pm$ 2.56	0.255

\*Significant level at ( $P \leq 0.05$ ).

\*Results express as (mean $\pm$ SD), Student t test was used to comparison.

**Table: (4-3):Comparison betweenmeans of serum Zinc and serum Copper in recurrent *H.pylori*-infected patients:**

Variables	Recurrent once No=10 mean $\pm$ SD	Recurrent twice No=4 mean $\pm$ SD	P.value
Zinc mg/L	0.205 $\pm$ 0.137	0.189 $\pm$ 0.054	0.824
Copper mg/L	0.254 $\pm$ 0.109	0.245 $\pm$ 0.053	0.869

\*Significant level at ( $P \leq 0.05$ ).

\*\*Results express as (mean $\pm$ SD), Student t test was used to comparison.

**Table (4-4): Comparison between means of serum Zinc and serum Copper in male and female patients with *H.pylori*-infection:**

Variables	Male No=16 mean±SD	Female No=24 mean±SD	P.value
Zinc mg/L	0.188±0.098	0.118±0.117	0.409
Copper mg/L	0.244±0.071	0.345±0.166	0.013*

\*Significant level at ( $P \leq 0.05$ ).

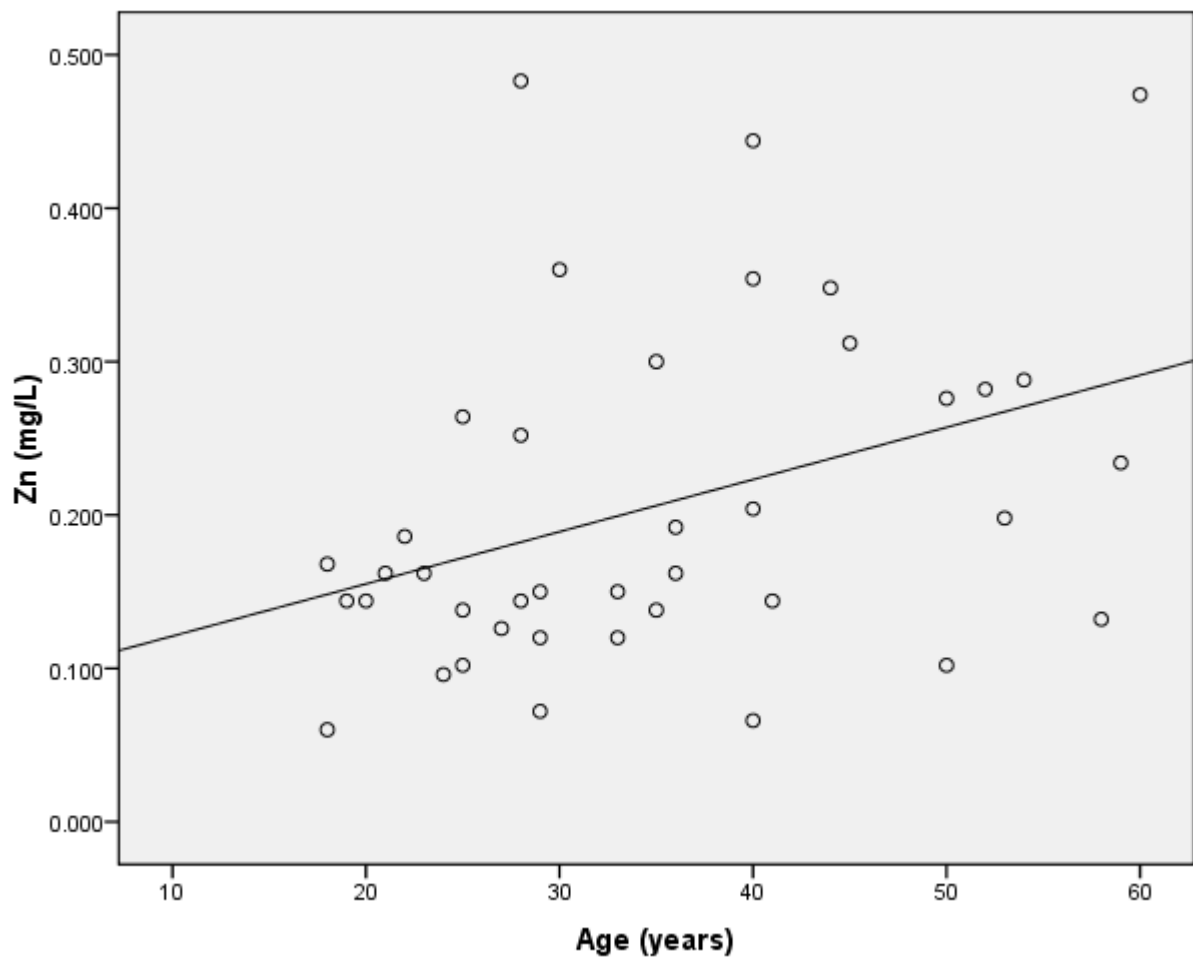
\*\*Results express as (mean±SD), Student t test was used to comparison.

**Table (4-5): Comparison between means of serum Zinc and serum Copper according to age groups among patients with *H.pylori* infection:**

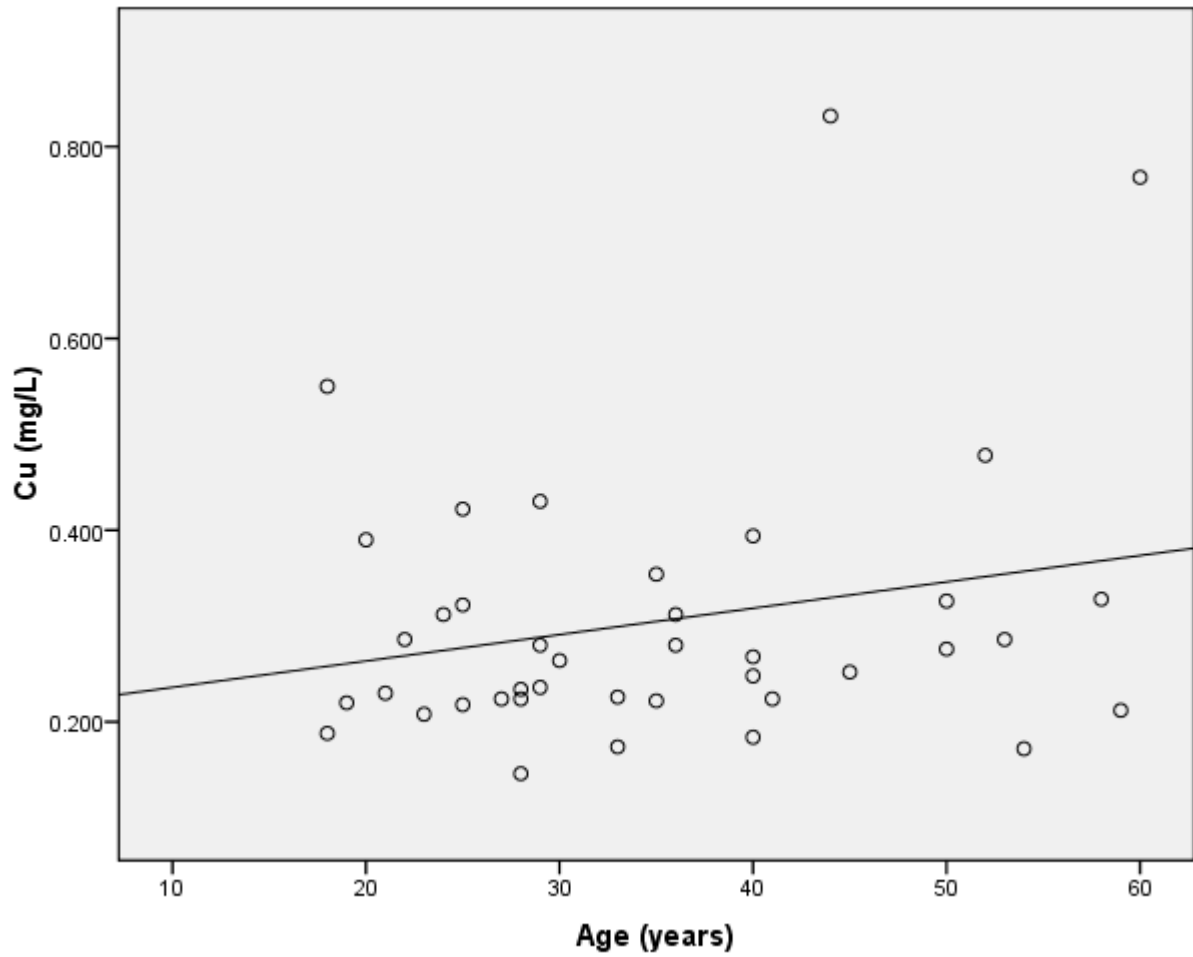
Age groups Parameters	(18-31) years No=19 Mean±SD	(32-45) years No=13 Mean±SD	(46-60) years No=8 Mean±SD	P.value
Zinc	0.175±0.102	0.225±0.113	0.248±0.114	0.221
Copper	0.283±0.100	0.305±0.170	0.355±0.189	0.504

\*Significant level at ( $P \leq 0.05$ ).

\*\*Results express as (mean±SD), One-way ANOVA test was used to comparison.



**Figure (4-3):** Correlation between the levels of serum Zinc and age of *H.pylori*-infected patients. Significant weak positive correlation ( $p.v=0.016$ ,  $r=0.377$ ).



**Figure (4-4):** Correlation between the levels of serum Copper and Age of *H.pylori*-infected patients. Insignificant weak positive correlation (P.v=0.148, r=0.233).



## **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. *H.pylori* also leads to hypochlorhydria in *H.pylori*-related gastritis. The change of gastric environment may affect the absorption of trace elements (Akcem, 2010).

This study was conducted to investigate the effect of *H.Pylori* infection in the level of serum Zinc and Copper.

Present study show that the mean of serum Zinc concentrations was significantly decreased: This agree with the finding of Almamory (2014), the former researcher suggest that the decrease in zinc concentration in *H.pylori*-infected patients is due to a protein that strongly binds to zinc, that protein has been identified on the membrane and in the cytosol of *H.pylori*. Because zinc is absorbed mainly in the small intestine, by binding dietary zinc in the stomach, *H.pylori* may possibly contribute to serum zinc deficiency. Also *H.pylori* infection had a significant decrease on the level of total protein and albumin according to Furuta *et al* (2002), and because most of zinc in the serum is bound to albumin, when there is a decrease in serum albumin, the level of zinc was also decrease.

This study showed that the mean of serum copper concentration was significantly decreased in *H.pylori*-infected patients: suggest that decrease in copper concentration in *H.pylori*-infected patients is due to finding that, a gene, *copA*, associated with copper transport, has been isolated from *H.pylori*. The adenosine triphosphate-derived copper-transporting mechanism is employed by various *H.pylori* strains. As cofactor in various reduction-oxidation enzymes and an essential trace metal required for the synthesis of metalloproteins, copper plays a role in the pathogenesis of *H.pylori* (Yakoob *et al*; 2003).

This study shows there was insignificant increase in the mean of BMI of *H.pylori*-infected patients compared to control. This disagrees with Zhang, (2015) that found significant increase in the mean of BMI in *H.pylori*-infected patients compared to control. This may be due to socioeconomic status, hygiene and life style of the population.

The mean of both serum zinc and copper levels were insignificant decreased in twice recurrent infection compared with once recurrent infection in *H.pylori*-infected group.

According to the gender there was insignificant decrease in the mean of serum zinc in male compared with female, and the mean of serum copper was significantly decreased in male compared with female in *H.pylori*-infected group this could be due to the difference in the lifestyles and habits.

Age group less than 31years had high prevalence of *H.pylori* infection; this may be due to their life style in consuming fast food and contaminated water from forwarders. The mean of both serum zinc and copper were insignificant difference between age groups of *H.pylori*-infected patients.

In comparing the Age with serum zinc levels there was a significant weak positive correlation, and insignificant weak positive correlation between the level of serum copper and Age in *H.pylori*-infected patients.

## **5.2. Conclusion:**

Serum Zinc and serum copper were significantly decreased in *H.pylori*-infected patients. The level of serum zinc and serum copper were insignificantly decreased in twice recurrent infection compared with once recurrent infection in *H.pylori*-infected group. Age exhibited in significant weak positive correlation with serum zinc level, and insignificant correlation with serum copper level.

### **5.3. Recommendations:**

From the findings of this study it is recommended that:

1. In *H.pylori* infections serum zinc and copper should be monitored regularly to avoid hypozincaemia and hypocupremia.
2. *H.pylori* Patients should uptake adequate zinc and copper rich nutrition, or receive zinc and copper supplements.
3. More studies should be carried out on the effect of *H.pylori* infection on serum zinc and copper levels with concerning duration of infection.

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## **Appendices**

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<sup>2</sup>

**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 ( )

Recurrent *H.pylori*: once ( ) twice ( ) more ( )

**Laboratory Investigation:**

Stool Antigen *H.pylori*: positive ( ) Negative ( )

Serum Zinc:..... mg/L

Serum Copper:.....mg/L

**Table (3-1)**

Table (3-1): Analytical Conditions for Atomic Absorption Spectrometry  
(Flame Atomic Absorption Ranges)

Metal	Wavelength (nm)	Slit (nm)	Detection limit (mg l <sup>-1</sup> )	Sense Check (mg l <sup>-1</sup> )	Linear Range (mg l <sup>-1</sup> )	Flame Type Color
Cu	324.8	0.7	0.005	2.00	5.0	A-A, lean/blue
Zn	213.9	0.7	0.005	0.50	2.50	A-A, lean/blue