# Chapter One

# 1. Introduction

## 1.1 Background

*Helicobacter pylori* previously named *Campylobacter pylori*, is a Gram-negative spiral rod shaped, microaerophilic bacterium found in the stomach on the surface of epithelial cells. It was identified in 1982 by Australian scientists Barry Marshall and Robin Warren with further research led by British scientist Stewart Goodwin, who found that it was present in patients with chronic gastritis and gastric ulcers, conditions not previously believed to have a microbial cause (Giustic, 2004).

*Helicobacter pylori* is main causative agent of gastrointestinal disease including chronic gastritis, peptic ulcer, gastric and it is also linked to the development of duodenal ulcers lead to duodenal carcinoma and stomach cancer lead to morbidity and mortality in human (Black, 2004., Baik*et al.*, 2012).

In 1994 the national institute of health consensus development conference concluded that *H. pylori* infection is major cause of peptic ulcer, and patients with confirmed peptic ulcer associated with *H. pylori* should received antimicrobial agent (Yamada *et al.*, 2006).

However over 80% of individuals infected with the bacterium are asymptomatic and it may play an important role in the natural stomach ecology (Blaser, 2006).

More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is high prevalence was reported in developing countries (Baik*et al.*, 2012). The prevalence of Infection may be 80% in adult and more frequently in males than females (Yamoka, 2008). Person to person

transmission of *H. pylori* is likely because interfamilial clustering occurs (Carrol*et al*., 2010).

Infection of *H. pylori* was diagnosed by invasive technique used biopsy based test (urea's test, direct stain, culture) also western immune blotting, polymerase chain reaction and serological testes are non- invasive technique (Rocha *et al*., 2003).

Colonization of human gastric mucosa by *H. pylori* stimulates specific cellular and humeral immune responses to bacterium (Crabtree *et al*., 2003).

Specific immunoglobulin (IgM) antibody can be detected shortly after infection occurred, but IgA, IgG antibodies titer used to determine prevalence of acute and chronic infection (Crabtree *et al*., 2003).

Measurements of specific antibody in serum or antigen in stool by using immunochromatograophy have been used as non- invasive methods (Crabtree *et al*., 2003). The enzyme- linked immune assay (ELISA) test is rapid non expensive and high sensitive and specific and widely use in epidemiological studies (Rocha *et al*., 2003).

## 1.2 Rationale

*H. pylori* are human pathogen that causes several illnesses and it is commonest bacterial infection worldwide.

The annul incidence rate of it is 4-15% in developing countries (including Sudan) compared with 0.5% in industrial countries (according to Center for Disease Control and Prevention, 1998).

*H. pylori* occur in about 70% of patient with chronic active gastritis and are associated with ulcer condition in 60% - 90% of all cases.

*H. pylori* are one of important cause of gastric carcinoma. So proper and rapid laboratory assays are needed to diagnose infection in order to treatment and management (Rocha *et al*., 2003).

Several techniques including culturing and molecular method are present to identify the organism. The pathogen is difficult to grow and serological tests

are less expensive and less stressful for the patients especially for children. So serodiagnostic methods have been attempted to detect infection (Wood *et al.*, 2003).

## 1.3 Objective

### 1.3.1 General objective

To detect the *Helicobacter pylori*IgG and IgAantibodies in students with gastritis in different Universities in Khartoum State.

### 1.3.2 Specific objective

1. To detect bothIgG and IgA antibodies of *Helicobacter pylori* in students with gastritis in Khartoum State by using ELISA technique.
2. To determine the relationship between seropositive IgG, IgA of *Helicobacter pylori* and gender, close families, coffee, alcohol and smoking as risk factors.

# Chapter Two

# 2. Literature Review

## 2.1 Background and history

The presence of gastric spiral bacterium was first reported in1893 by Bizzozer and Italian pathologist who describe spiral bacteria in canine stomach (Marshall, 1989). Spiral bacteria were demonstrated for the first time in human in 1906(Warren and Marshall.,1983). Five year later spiral microorganisms in the stomach of patient with gastric and duodenal ulceration were reported (Rosenow and Sanford, 1915).

*H. pylori* previously named *Campylobacter pylori*, German scientist described bacteria that live in the luminal surface of the epithelial of all gastric ulcers. But they were not able to culture the organism. In 1982

*H. pylorus was* first successfully cultured from gastric biopsy tissue by Marshall and Warren (Black, 2004).

In the original paper Marshall and Warren contented that most stomach ulcers and gastritis were caused by infection of bacteria not by stress or spice food as had assumed before (Goodwin *et al*., 1989).

In 1989 Goodwin was the first to propose the name *Helicobacter pylori* to this genus (Black, 2004).

The association of these bacteria with gastritis was first presented by Royal Australian College of physicians in 1983(Warren and Marshall., 1983).

## 2.2 Scientific Classification

Domain: Bacteria

Phylum: Proteobacteria

Class: Epsilonproteobacteria

Order: Campylobacterales

Family: Helicobacteraceae

Genus: *Helicobacter*

Species: *H. pylori*

(Goodwin*et al.*, 1989)

## 2.3 Bacteriology

*H. pylori* is a helix-shaped (classified as a curved rod), Gram-negative bacterium about 3 μm long with a diameter of about 0.5 μm. It is microaerophilic that is requires oxygen, but at lower concentration than is found in the atmosphere. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen ($H_2$) produced by intestinal bacteria it produces oxidase, catalase, and urease. It is capable of forming biofilms and can convert from spiral to a possibly viable but non culturablecoccoid form, both likely to favor its survival and be factors in the epidemiology of the bacterium (Stark *et al*., 1999).

*H. pylori* possess five major outer membrane protein families.The largest family includes known and putative adhesins. The other four families are porins, iron transporters, flagellum-associated proteins, and proteins of unknown function. Like other typical Gram-negative bacteria, the outer membrane of *H. pylori* consists of phospholipids and lipopolysaccharide (LPS). The O antigen of LPS may be fucosylated and mimic Lewis blood group antigens found on the gastric epithelium (Chang and Parsonnet, 2010).The outer membrane also contains cholesterol glucosides, which are found in few other bacteria (Chang and Parsonnet, 2010). *H. pylori* have four to six lophotrichous flagella all gastric and enterohepatic*Helicobacter* species are highly motile owing to flagella(Josenhans*et al*., 2000).

## 2.4 Microscopy

*H. pylori* can be demonstrated in tissue by Gram stain, Giemsa stain, haematoxylin-eosin stain, Warthin-Starry silver stain, acridine-orange stain, and phase-contrast microscopy (Brown, 2002).

## 2.5 *H. pylori* genome

*H. pylori* was originally classified in the genus *Campylobacter* but is now know to be different from *Campylobacter* in 16s RNA sequences.

The genetic diversity of *H. pylori* can be exploited by using the molecular typing based on DNA analysis. PCR technique which are able to isolate and replicate *Helicobacter* DNA in biological material and then detect the genetic variation between different strains of *Helicobacter* (Arora, 2006).

Study of *H. pylori* genome is contended on attempts to understand pathoginisity, ability of it to cause disease. Approximately 29% of the loci in the pathogenic category of the genome database. Both sequence strains have an approximately 40kb -10ng CagApathoginisity island (Medis and Marshall, 2008).

## 2.6 Host range

Man is principle host of *H. pylori* occasionally strain identified as *H. pylori* have been isolated from domenestic cats and other animals including pig and monkey (Owen *et al.*, 2001).

## 2.7 Signs and symptoms

Up to 85% of people infected with *H. pylori* never experience symptoms or complications (Brown, 2002). Acute infection may appear as an acute gastritis with abdominal pain or nausea (Bytzer*et al.*, 2001). Where this develops into chronic gastritis, the symptoms, if present those of non-ulcer dyspepsia: stomach pains, nausea, bloating, belching, and sometimes vomiting or black stool (Butcher and Graham, 2003).

Individuals infected with *H. pylori* have 10 to 20% lifetime risk of developing peptic ulcers and 1 to 2% risk of acquiring stomach cancer (Chang and Parsonnet, 2010). 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 and gastric carcinoma (Kusters*et al*., 2006). However, *H. pylori* possibly plays a role only in the first stage that leads to common chronic inflammation, but not in further stages leading to carcinogenesis (Yamoka, 2008).

*H. pylori* have also been associated with colorectal polyps and colorectal cancer (Suerbaum and Michetti, 2002).

## 2.8 Route of transmission

*H. pylori* is contagious, although the exact route of transmission is not known (Smoot, 1997). Person-to-person transmission by either the oral-oral or fecal-oral route is most likely. Consistent with these transmission routes, the bacteria have been isolated from feces, saliva and dental plaque of some infected people. Findings suggest *H. pylori* is more easily transmitted by gastric mucus than saliva (Yamoka, 2008).Transmission occurs mainly within families in developed nations, yet can also be acquired from the community in developing countries.

*H. pylori* may also be transmitted orally by means of fecal matter through the ingestion of waste-tainted water, so a hygienic environment could increase the risk of *H. pylori* infection (Yamoka, 2008).

Iatrogenic spread through contaminated endoscopes has been documented as rout of transmission (Dunn *et al*., 2001).

## 2.9 Pathophysiology

To avoid the acidic environment of the interior of the stomach (lumen),

*H. pylori*uses its flagella to burrow into the mucus lining of the stomach to reach the epithelial cells underneath, where the pH is more neutral (Amieva and EL-omer, 2008). *H. pylori* are able to sense the pH gradient in the mucus

and move towards the less acidic region (chemotaxis). This also keeps the bacteria from being swept away into the lumen with the bacteria's mucus environment, which is constantly moving from its site of creation at the epithelium to its dissolution at the lumen interface (Schreiber *et al*., 2004).

*H. pylori*are found in the mucus, on the inner surface of the epithelium, and occasionally inside the epithelial cells themselves. It adheres to the epithelial cells by producing adhesins, which bind to lipids and carbohydrates in the epithelial cell membrane(Mahdavi*et al*., 2002).

In addition to using chemotaxis to avoid areas of low pH, *H. pylori* also neutralizes the acid in its environment 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 then neutralizes stomach acid (Ilver*et al*., 1998).

## 2.10 Pathogenicity

### 2.10.1 Peptic ulcer

At present it well accepted that *H. pylori* are an important factor in development of peptic ulcer. Although, antral gastritis develops in virtually all patients with infection, the relationship between *H. pylori* and peptic ulcer disease has been more difficult to establish.

Ulceration develops in only few people harboring the organism. The reason of these is unknown, but possibilities include difference in individual host defenses, in strain of *H. pylori* and environmental factors (Torres *et al*., 2002).

The pathologic mechanism of *H. pylori* to cause peptic ulceration is unknown but several factors may operate such as production of ammonia by ureas enzyme which cause ionic change in mucus layer with consequent back diffusion of hydrogen ions in the mucosa, production of toxin or other substance such as lipopolysaccarides that activate inflammatory cells, stimulation of autoimmune response by the production of antigen that cross

react with internal gastric antigens and degeneration mucous by protease (Arora, 2006; Wood *et al*; 2003).

In review articles that in two cohort studies, one with 10 years and the other with 18 years follow up period duodenal ulcer develop far more frequently in persons infected with *H. pylori* than non infected persons (Torres *et al*., 2002).Recurrent rat of duodenal ulcer decrease from 70% to 5% after adequate treatment of infection (Torres *et al*., 2002).

## 2.10.2 Gastric cancer

Epidemiologic studies link *H. pylori* infection with both adeno carcinoma and lymphomas of stomach.Data from both American and Europeanstudies indicate that the presence of the organism confers about six fold increased risk ofgastric cancer and infection may be implicated in half of causes of adenocarcinoma of the stomach (Malfartheiver*et al*., 2007).

Suggested mechanism isthat chronic inflammation related to *H. pylori* infection leads to atrophic gastritis with decreased in gastric acid secondary in the number of bacteria which carcinogenic nitrosamine from dietary nitrates (Webb *et al*., 2004).

Recent study demonstrated that patients with *H. pylori* infection and esophagitis, treated with proton pump inhibitors had siginfically increased risk of atrophic gastritis compared with these undergoing anti reflex surgery. Thus, the risk of gastric carcinoma may increased with wide spread use these agents (Webb *et al.*, 2004).

Mucosa associated lymphoid tissue lymphoma is also associated with *H. pylor*i infection. This disease may induce by going *H. pylori* antigen stimuli and thus in theory should regress when the organism is eradicated (Chey and Wong, 2007).

### 2.10.3 Acute gastritis

The acute phase of colonization with *H. pylor*i may be associated with transient non specific dyspeptic symptoms, such as fullness, nausea and

vomiting and with considerable inflammation of both the proximal and distal stomach mucosa and gastritis. This phase is often associated with hypochlohydria witch can last for months (Prez- Prez*et al*., 2003).

### 2.10.4 Chronic gastritis

When colonization dose become persistent close correlation exists between the level of acid secretion and the distribution of gastritis, this correlation result from the contraction affects of acid on bacterial growth versus those of bacterial growth and association mucosal inflammation on acid secretion and regulation. This interaction is curricle in the determination of outcomes of *H. pylori* infection. In subject with intact acid secretion *H. pylori* in particularly colonize the gastric atrium, where few acid secretary partial cells are present. Histopathology evaluation in these cause reveal limited chronic inactivate inflammation and low number of superficially colonization *H. pylori* bacteria (Kuipers*et al*., 1995).

Subject in when acid secretion is impaired dueto whatever mechanism have more even distribution of bacteria in antrum and corpus, bacteria in corpus are in closer contact with mucous lead to corpus predominant gastritis disease(Kuipers *et al*., 1995).

## 2.11 Diagnosis

Multiple invasive and non invasive methods are available for detection of *H. pylori*(Dohil*et al*., 1999).

### 2.11.1 Invasive tests through endoscopy

#### 2.11.1.1 Biopsy and histopathology

The definitive diagnosis of *H. pylori* and the evidence of the consequence of infection can be made reliably only is endoscopy with multiple biopsy specimens obtained from one or more regions of the stomach (Dohil*et al*., 1999).

Histopathology evaluation is currently the “gold stander” for diagnosing of *H.pylori* infection. The organism can defined on routine haematoxilin- eosin stain also especial stains e.g. Warthin- Starry silver stain.

At least two samples should be evaluated, regardless of the stain used. Because *H. pylori* causeantral inflammation, some investigators suggested that the absence of inflammation on biopsy is the most dependable finding to use for exclusion of infection (Elomar*et al*., 2001).

### 2.11.1.2 Culture

Culturing of *H. pylori* from biopsy specimen is possible, but the organism is fastidious and difficult to grow (Kusters*et al.*, 2006).

The Current recommendations are to culture specimen only when the procedure is necessary to determine antibiotic sensitivity (Perez- Prez*et al*., 2003).

Biopsy specimens may be cultured on Colombia blood agar plate contain 7%defibrinated hours blood in atmosphere of 5% $O_2$ and 10% $Co_2$ Organism can be identified as *H. pylori* on the basis of colonial morphology, Gram stain and production of urease, oxidase and catalase.

Culture of organism is the least sensitive diagnostic test (approximate 70-80% positive) though been 100% specific (Perez- Prez*et al.*, 2003).

### 2.11.1.3 Rapid urease test

Measuring urease production can be accomplished with several expensive kits that use a pH- sensitive colorimetric indicator (e.g. phenolphthalein) mixed in to agar gel containing urea (Elomar*et al*., 2001).

When a biopsy specimen from patient with of *H. pylori* infection is tested bacterial urease hydrolyze the urea, resulting in production of bicarbonate and increasing the pH. Result indicates by the colure of medium are often available within 20 minutes and always within 24 minutes (Elomar*et al*., 2001).

### 2.11.2 Non invasive tests

Both European consensus and American Gastroenterological Association Position Statement recommended the use of non invasive testes for diagnosis of *H. pylori* (Walsh and Peterson, 2005).

### 2.11.2.1 Immunochromatography test (ICT)

The ICT for anti- *H. pylori* antibody detection is based on the principle of reverse – flow immunochromatography. The kits included high molecular mass- cell associated protein (HM-CAP) on antigen highly specific to *H. pylori*, as the target antigen to detect *H. pylori*- specific IgG antibodies in the serum (Manfred *et al.*, 2001).

### 2.11.2.2 Immune blotting technique

Immune blotting is sensitive serological test that enables the detection of antibodies against specific *H. pylori* antigen (Abusiyanik*et al*., 2004).

In these method electrophoreticaly separated components are transferred from gel to solid support and probed with reagent that is specific for particular sequences of amino acid. Western Immune blotting is there for extremely use full for identification and quantification of specific protein in complex mixtures of protein that not radio labeled. Because electrophoretic separation of proteins is almost carried under denaturizing condition, any problem in solublization, aggregation and co-precipitation of the target protein are eliminated (Abusiyanik*et al.*, 2004).

### 2.11.2.3 Saliva and urine test for *H. pylori* antibodies

Similar to serological tests saliva based test also detected the presence of *H. pylori* specific IgG antibodies. The test is easy to perform, painless, but expensive.

Saliva test are less sensitive there assay of serum or whole blood (Fallone*et al.*, 1996). The protein concentration of saliva appears to affect the accuracy of the test results. Urine- based assay are easy to performed require minimal

labor for collection and painless (Almohammed*et al.*, 1993). However, these assays are highly variable and are not yet commercially available.

Therefor Saliva and urine assay for the detection of *H. pylori* antibodies cannot be recommended (Almohammed*et al.*, 1993).

### 2.11.2.4 Stool test for *H. pylori* antigen

Testing of *H. pylori* antigens in stool has show promising result in adults for the non invasive diagnosis of gastric infection using commercial available kit (Vaira*et al*., 1999). Testing of *H. pylori* antigens in feces also appears too accurate for using in monitoring the success of eradication therapy. However patients may be re lucentant to collect stool samples and refrigerated stool more difficult to test (Oderda*et al*., 2000).

Additional pediatric studies evaluator the accuracy of stool antigen testing of both initial diagnosis and post treatment follow- up are required before specific recommendation can be considerable (Oderda*et al*., 2000).

### 2.11.2 .5 Polymerase Chain Reaction (PCR)

The polymerase chain reaction (PCR) is able to isolate and replicate *H. pylori* DNA in biological material. PCR technique has been used to detect genetic variation between different strains of *H. pylori* (Wood *et al*., 2003).

### 2.11.2.6 Enzyme Linked Immune Assay (ELISA).

It is most extensively used in serological detection of *H. pylori* and had been show to produce constant and reliable results (Abusiyanik*et al*., 2004).

It is based on purified *H. pylori* antigen coated on the service of micro wells. Diluted patients serum is added to the well and *H. pylori* immunoglobulin specific antibody, if present, bind to the antibody-antigen complex. Excess enzyme conjugate is washed off and tetra methyl benzaldehyde (TMB) substrate is added. The enzyme conjugate cataletic reaction is stopped at specific time. The intensity of the color generating is proportional to the amount of antigen specific antibody in the sample.

The result is read by micro well reader compared in parallel manner with calibrator and control (Graham, 2004).

## 2.11.2 .7 Breath test

The Breath test utilizes the ability of *H. pylori* to produce large quantities of urease as diagnostic characteristic the patient is required to drink solution of urea lablled with $C^{13}$ or $C^{14}$ isotopes (Tsuji *et al.*, 2003).

If an *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 patients' exhaled breath by radioactive counting or mass spectrometry.

Breath test based on $C^{13}$ labeled urea and using infrared spectrophotometer to measure the ratio of $C^{13}$ or $C^{12}$ in breath sample.

Breath test has been found to have high sensitivety and specifity 94-98% and can be applied at moderate cost. It is suitable for monitoring the effectiveness of treatment. As it is specific to active infections and can be used to confirm eradication (Tsuji *et al.*, 2003).

## 2.12 Immunity

Patient infected with *H.pylori* develops IgM antibody response to the infection. Subsequently IgG, IgA are produced and persist. Both systemically and at the mucosa in high titer infected persons (Wood *et al.*, 2003).

The most common result from the immunity is chronic superfacial gastritis also the host could be harmed by the immune response due to direct damage of epithelial cells, which affect their function (Wood *et al.*, 2003).

## 2.13 Other body site *H. pylori* mayfound

Stomach was supposed to be the only reservoir of infection in human. Nevertheless*H. pylori* infection was detected in other site recently it was found in dental plaque and oropharyngeal lymphatic tissue. The question of direct contribution of *H. pylori* to oral and oropharyngeal disease was not resolve (Fallone*et al.*, 1996).

## 2.14 Epidemiology

At least half the world's population is infected by the bacterium, making it the most widespread infection in the world. Actual infection rates vary from nation to nation; the developing world has much higher infection rates than the West (Western Europe, North America, Australasia), where rates are estimated to be around 25%.The age at which this bacterium is acquired seems to influence the possible pathologic outcome of the infection: people infected with it at an early age are likely to develop more intense inflammation that may be followed by atrophic gastritis with a higher subsequent risk of gastric ulcer, gastric cancer, or both. Acquisition at an older age brings different gastric changes more likely to lead to duodenal ulcer (Yamoka, 2008). Infections are usually acquired in early childhood in all countries (Chang and Parsonnet, 2010).However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions, perhaps combined with lower antibiotics usage for unrelated pathologies. In developed nations, it is currently uncommon to find infected children, but the percentage of infected people increases with age, with about 50% infected for those over the age of 60 compared with around 10% between 18 and 30 years (Smoot, 1997). The higher prevalence among the elderly reflects higher infection rates in the past when the individuals were children rather than more recent infection at a later age of the individual (Chang and Parsonnet, 2010).

In the United States, prevalence appears to be higher in African-American and Hispanic populations, most likely due to socioeconomic factors .The lower rate of infection in the West is largely attributed to higher hygiene standards and widespread use of antibiotics. Despite high rates of infection in certain areas of the world, the overall frequency of *H. pylori* infection is declining. However, antibiotic resistance is appearing in *H. pylori*; many

metronidazole- and clarithromycin-resistant strains are found in most parts of the world (Smoot, 1997).

## 2.15 Previous studies of *H. pylori* in Sudan

The studycompared*H. pylori positive* Sudanese patients with dyspepsia comparable group matched of age, sex and endoscopy finding from United Kingdom. It was found that 17% of British patients have intestinal metaplasia compared to only 2.4% of Sudanese patients (Nuha and Fedail, 2000).

Cluter and Havestad in their study done in 192 patients evaluate the accuracy of ICT kit compared with ELISA. They found that sensitivity of ICT, specifityand accuracy was 99%, 86.6%, and 93.3% respectively (Culter and Havestad, 2000).

Another study conduct by study on the officy of triple therapy in eradication of *H. pylori* in Sudanese patients with peptic ulcer. In these study 33 patients with endoscopically confirmed peptic ulcer and how had positive rapid urease test were include, amoxicillin, metronidazole and ranitidine were used in treatment . 75% of patients had complete healing ulcer, 15% patients had reported healing ulcer and 5% had no healing ulcer (Ahlam and Fedail, 2001).

Mirghani*et al* studied the prevalence of *H. pylori* in 100 Sudanese subjects with gastroduodinal inflammation. In this study two methods were used to detect*H.pylori* (rapid urease test and culture).

*H. pylori* were found in 80% of patients with gastritis and 60% of duodenitis, 56% with duodenal ulcer and 16% in normal individual (Mirghani*et al*., 2002).

Francies and his worker in their study culture and susceptibility testing of *H. pylori* strain was performed in multicenter randomized clinical trial.

Culture was carried out in gastric biopsy samples obtained from 516 patients. When urea breath test was used as reference (Mergrand*et al*., 2003).

## 2.16 Treatment

Once *H. pylori*were detected in a person with a peptic ulcer, the normal procedure is to eradicate it and allow the ulcer to heal. The standard first-line therapy is a one-week "triple therapy" consisting of proton pump inhibitors such as Omeprazole and the antibiotics Clarithromycin and amoxicillin. Variations of the triple therapy have been developed over the years, such as using a different proton pump inhibitor, as with Pantoprazole or Rabeprazole, or replacing amoxicillin with Metronidazole for people who are allergic to penicillin. Such a therapy has revolutionized the treatment of peptic ulcers and has made a cure to the disease possible. Previously, the only option was symptom control using antacids, $H_2$-antagonists or proton pump inhibitors alone (Malfertheiver*et al*., 2007).

An increasing number of 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 (Tsuji *et al.,* 2003). For the treatment of Clarithromycin-resistant strains of *H. pylori,* the use of Levofloxacin as part of the therapy has been suggested (Malferteiver*et al*., 2007).

Ingesting lactic acid bacteria exerts a suppressive effect on *H. pylori* infection in both animals and humans, and supplementing with *Lactobacillus*- and *Bifidobacterium*-containing yogurt improved the rates of eradication of *H. pylori* in humans. Symbiotic butyrate-producing bacteria which are normally present in the intestine are sometimes used as probiotics to help suppress *H. pylori* infections as an adjunct to antibiotic therapy. Butyrate itself is an antimicrobial which destroys the cell envelope of *H. pylori* by inducing regulatory T cell expression and synthesis of an antimicrobial peptide,against the *H. pylori*(Tsuji *et al*., 2003).

## 2.17 Prevention

*H. pylori* are a major cause of certain diseases of the upper gastrointestinal tract. Rising antibiotic resistance increases the need to search for new therapeutic strategies; this might include prevention in form of vaccination. Much work has been done on developing viable vaccines aimed at providing an alternative strategy to control *H. pylori* infection and related diseases, including stomach cancer (Selgrad and Malfertheiner, 2008). Researchers are studying different adjuvant, antigens, and routes of immunization to ascertain the most appropriate system of immune protection; however, most of the research only recently moved from animal to human trials. An economic evaluation of the use of a potential *H. pylori* vaccine in babies found its introduction could, at least in the Netherlands, prove cost-effective for the prevention of peptic ulcer and stomach cancer. A similar approach has also been studied for the United States (Blaser, 2006).

The presence of bacteria in the stomach may be beneficial, reducing the prevalence of gastroesophageal reflux disease and esophageal cancer by influencing systemic immune responses (Blaser, 2006).

# Chapter Three

# 3. Materials and Methods

## 3.1 Study design

This was a descriptive prospective cross sectional study

## 3.2 Study area

The study was conducted in different universities in Khartoum State( Sudan).

## 3.3 Study population

Samples had been collected from students with gastroenteritis in different universities in Khartoum State (Khartoum University, AlzahimAlazhari University and OmdrmanAlahliaUniversity).

## 3.4 Study duration

Study was carried out during 3month from April to June 2014.

## 3.5 Ethical consideration

Permission to conduct this study was obtained from College of Graduate Studies, Sudan University of Science and Technology. All patients examined were informed for the purpose of the study before collection of the samples and the verbal consent was taken from them.

## 3.6 Inclusion criteria

Students sufferfrom gastritis and they were*H. pylori* ICT positive.

## 3.7 Exclusion criteria

Students suffer from gastritis and they were*H. pylori* ICT negative.

## 3.8 Data collection

Data were collected using questionnaire (Appendix 1) with informed consent.

## 3.9. Sample size

A total of ninety subjects were enrolled in this study.

## 3.10. Sample collection

Under aseptic condition after the wearing the gloves, alcohol antiseptic (70%) was used to clean the skin. Venous blood (3ml) was obtained from students. Serum was collected in to the collection tubes and left to settle for 30 minutes in the rack to clot and then centrifuged at 3000 rpm for 5 minutes.Samples were kept at -20˚C until used.

## 3.11Laboratory methods

### 3.11.1 Immunochromatograpy test

#### 3.11.1.1 Principle of the test

Rapid *H. Pylori* antibody test employs chromatographic lateral flow test device in a cassette format.

Colloidal gold conjugated *H. pylori* antigens (Au-Ag) are dry-immobilized at the end of nitrocellulose membrane strip. *H. Pylori* antigens are bound at the Test Zone (T). When the sample was added; it was migrates by capillary diffusion rehydrating the gold conjugate. If anti- *H. Pylori* antibodies present in sample, antibodies will bind with the gold conjugated antigens forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by *H. pylori* antigens generating a visible red line. If there is no anti-*H. Pylori* antibodies in sample, no red line are formed in the Test Zone (T). A built-in control line will always appear in the Control Zone (C) when the test has performed properly, regardless of the presence or absence of anti- *H. pylori* antibodies in specimen.

#### 3.11.1.2 Storage and stability

The sealed pouches in the kit may be stored between 3-4˚Cfor the duration of the shelf life as indicated on the pouch.

#### 3.11.1.3 Procedure of the test

### For test strip

The strip and sample were allowed to reach room temperature if necessary.

The pouch was then opened, thestrip was dipped into the specimen with arrow marked end towards the sample was pipetted 80-100 µl (two drops) of serum to the arrow marked end of the strip.

The strip was placed on flat surface and the results were read at 10 minutes. Strong positive sample may show result earlier.

## For test card

The kit components were brought to reach room temperature if necessary.

The pouch and card were opened. Once opened, the test card must be used immediately.

The test card was labeled with patient's identity.

2-3 drops (80-120 µl) of serum were applied to the sample well marked as " S ".

At the end of 10 minutes the results were read. Strong positive sample may show result earlier.

### 3.11.1.4. Interpretation of the results

## Negative

Only control line appears

## Positive

Bothcontrol line and the test line appear. It indicates the antibodies to *H*. *pylori* have been detected.

## Invalid result

If after 10 minutes no line is visible within the control zone, the result is invalid. The test should be repeated with a new test card.

Positive ICT student samples were retested by (ELISA).

### 3.11.2Enzyme Linked Immuonosorbant Assay (ELISA)

### 3.11.2.1 Principle of ELISA

### IgA

The ELISA test kit (EUROIMMUN) provide semquantitative in vitro assay for human antibodies of the IgA class against *H. pylori* in serum. The test kit contains micro-titer strips each with 8 break-off reagent wells coated with *H. pylori*antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the case of positive samples, specific IgA antibodies will bind to the antigens. To detect the bound antibodies, second incubation is carried out using an Enzyme-labeled anti- human IgA(Enzyme conjugate) Catalysing a color reaction.

### IgG

The ELISA test kit (EUROIMMUN) provide quantitative in vitro assay for human antibodies of the IgG class against *H. pylori* in serum. The test kit contains micro-titer strips each with 8 break-off reagent wells coated with *H.pylori* antigens. In the first reaction step, diluted patient samples are incubated in the wells. In the cause of positive samples, specific IgG antibodies will bind to the antigens. To detect the bound antibodies, second incubation is carried out using an Enzyme-labeled anti- human IgG(Enzyme conjugate). Catalysing the color reaction.

### 3.11.2.2 Storage and stability

The test kit has to be stored at temperature between +2˚C to +8˚C.Do not freeze. Unopened, all test kit component are stable until he indicated expiry date.

### 3.11.2.3 Procedure of the test

Purified *H. pylori* antigen was coated on surface of micro wells. Diluted patient samples (appendix 2), was added to the well *H. pylori* IgG specific

antibody, if present,willbind to the antigen all unbound materials were washed away. Enzyme conjugate was added, which binds to the antibody – antigen complex. Excess enzyme conjugate was washed off and thechromogen (substrate reagent) was then added. The enzyme catalytic reaction was stopped at specific time by sulphuric acid.

According to manufactures protocol 100µl from positive and negative controls and diluted patient samples were transferred into the microplate wells then incubated for 30 minutes at room temperature. The wells were then emptied and subsequently manual washed 3 times using 300 µl of wash buffer (appendix 2).Then 100 µl of enzyme conjugate was added into each of micro plate wells and incubated for 30 minutes at room temperature. The wells were then emptied and subsequently washed 3 times using 300 µl of wash buffer (appendix 2).Then 100 µl of TMB chromogensubstrate solution was added into each of microplate wells and incubate for 30 minutes at room temperature. The reaction was stopped by adding 100 µl of stop solution(sulphuric acid) into each microplate wells in the same order and the same speed as the substrate solution was introduced. Photometric measurement of the colour intensity was done at wavelength of 450 nm and reference wavelength between 620nm within 30 minutes of adding stop solution. Prior to measuring the microplatewas slightly shake to ensure homogenous distribution of the solution.

The above mentioned procedure was carried out for both IgG and IgA.

### 3.11.3.4 Reading of the results

Results can be evaluated by calculating a ratio of the extinction value of the control or patient sample over the extinction value of calibrator.

EUROIMMUN recommends interpreting results as following**:**

Ratio < 0.8: negative

Ratio ≥ 0.8 to <1.1: borderline

Ratio≥ 1.1: positive

## 3.12. Data analysis

All the data were analyzed using statistical package for social science (SPSS). Computer soft ware was used for data analysis. Significant level were set at ($p<0.05$).

The obtained data analysis was analyzed in parameter of gender, family, coffee, smoking and alcohol.

Figures were performed by using Microsoft office and excel soft ware program.

# Chapter Four

## 4. Results

Total of ninety serum samples were collected from students with *H. pylori* positive by ICT (serum) from different universities in Khartoum State were tested for IgG and IgA using ELISA procedure.Age ranged from 18-28 years. Fourtythree (52.3%) of study population were males and fourtyseven (47.7%) were females(Table 1).

### 4.1 Table 1: Distributionof *H.Pylori* in males and females using ICT.

| Sex | Frequency | Percent |
|---|---|---|
| Male | 43 | 47.8% |
| Female | 47 | 52.2% |
| Total | 90 | 100.0% |

Serum specimen were collected from students with ICT positive and then retested by ELISA technique.

Eight nine (98.8%) were positive of IgG antibody and one (1.2%) were negative of IgG antibody(Table 2).

Fourty seven (52.2%) were positive of IgA and fourty three (47.8%) were negative of IgA antibody(Table 2)..

Fourty seven (52.8%) were positive for both IgG, IgAimmunoglobulin and fourty two (46.6%) were negative for one of bothIgG, IgA immunoglobulin(Table 2).

## 4.2 Table 2: Prevalence of anti *H. Pylori* antibodies using ELISA technique.

| Antibody | Positive | Negative |
|---|---|---|
| IgG | 89/90 (98.8%) | 1/90 (1.2%) |
| IgA | 47/90 (52.2%) | 43/90 (47.8%) |
| From both IgG, IgA | 47/90 (52.2%) | 43/90 (47.8%) |

From both seropositive (IgG, IgA) antibodies the study were found twenty three (48.9%) males and twenty four (51.1%) females (Table 3 andFig 1).

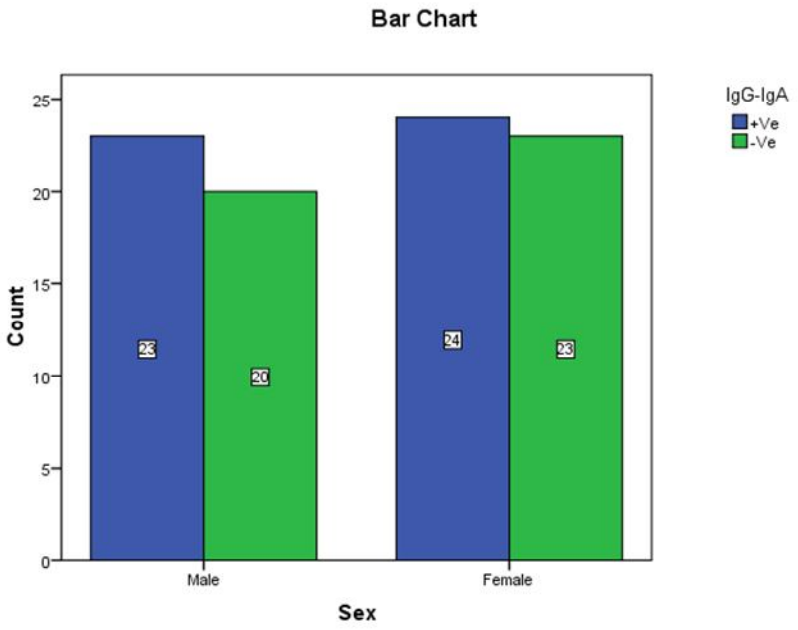
## 4.3Table 3: The relationship of *H. pylori*IgG , IgA ELISA positiveandgender

| Gender | Positive IgG, IgA | NegativeIgG, IgA |
|---|---|---|
| Male | 23/43 (53.5%) | 20/43 (46.5%) |
| Female | 24/47 (51.1%) | 23/47 (48.9%) |
| Total | 47/90 (52.2%) | 43/90 (47.8%) |

P.value: (.818). Not Significant different at the 0.05 level.

## Fig 1: Distribution of seropsitiveanti *H. pylori*antibodies

**according to gender.**



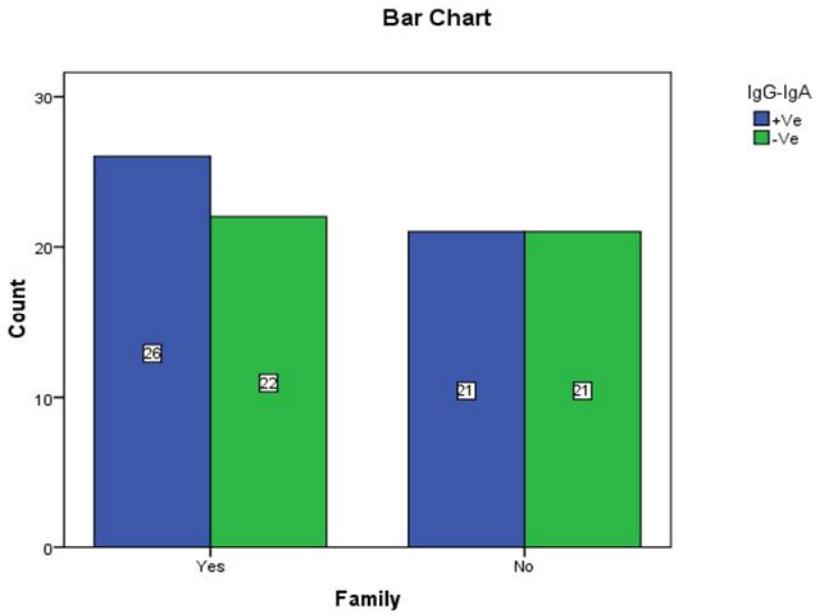

From both seropositive (IgG, IgA) antibodies the study were found other member of family infected by *H. pylori* 26(54.2%) and 21(50.0%) were non infected (Table 6 andFig 2).

## 4.4 Table 4: The relationship of *H. pylori*IgG, IgA ELISA positive and Family history.

| **Family history** | **Positive IgG, IgA** | **NegativeIgG, IgA** |
|---|---|---|
| Yes | 26/48 (54.2%) | 22/48 (45.8%) |
| No | 21/42 (50.0%) | 23/47 (50.0%) |
| Total | 47/90 (52.2%) | 43/90 (47.8%) |

P.value: (.693). Not Significant different at the 0.05 level.

**Fig 2: Relationship between family history and *H. pylori* infection.**



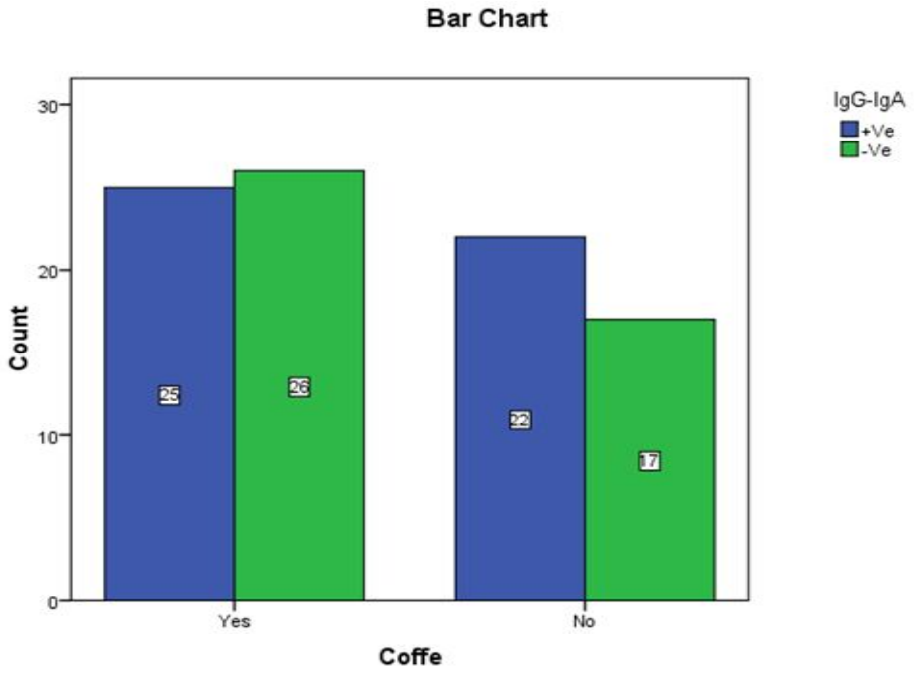
From both seropositive (IgG, IgA) antibodies the study were found twenty fife (49.1%) were drinking coffee and twenty two (56.4%) were not drinking coffee (Table 5 andFig 4).

## 4.5 Table 5: The relationship of *H. pylori*IgG, IgA ELISA positive and coffee consumption.

| Coffee | Positive IgG, IgA | NegativeIgG, IgA |
|---|---|---|
| Yes | 25/51 (49.1%) | 26/51 (50.9%) |
| No | 22/39 (56.4%) | 17/39 (43.6 %) |
| Total | 47/90 (52.2%) | 43/90 (47.8%) |

P.value: (.487). Not Significant different at the 0.05 level.

## Fig 3: Relationship between drinking coffee and *H. pylori* infection



From both seropositive (IgG, IgA) antibodies the study were found three (100%) were drinking alcohol and fourty four (93.6%) were not drinking alcohol (Table 8 andFig 4).

| **Alcohol** | **Positive IgG, IgA** | **NegativeIgG, IgA** |
|---|---|---|
| Yes | 3/3 (100%) | 0/3 (0.0%) |
| No | 44/87 (50.6%) | 43/87 (49.4 %) |
| Total | 47/90 (52.2%) | 43/90 (47.8%) |

P.value: (.092). Not Significant different at the 0.05 level.

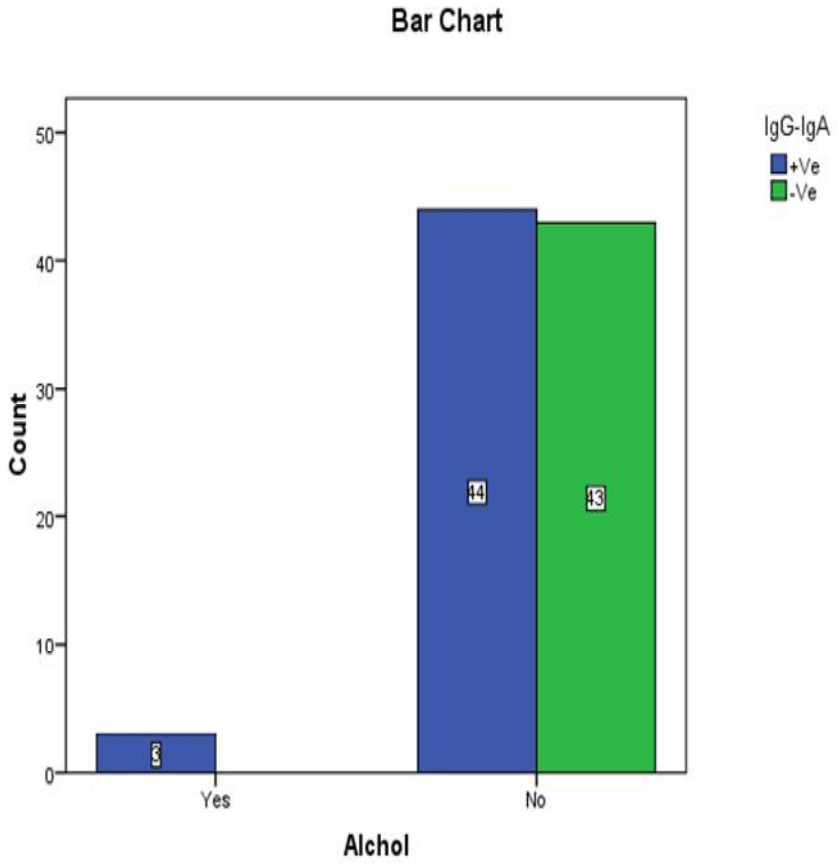



**Fig 4: Relationship between alcohol drinking and *H. pylori* infection**

From both seropositive (IgG, IgA) antibodies the study were foundseventeen (36.2%) were smoking and thirty (63.8%) were not smoking (Table 7 andFig 5).

| **Smoking** | **Positive IgG, IgA** | **NegativeIgG, IgA** |
|---|---|---|
| Yes | 17/31 (54.8%) | 14/31( 45.2%) |
| No | 30/59 (50.8%) | 29/59 (49.2 %) |
| Total | 47/90 (52.2%) | 43/90 (47.8%) |

P.value: (.719). Not Significant different at the 0.05 level

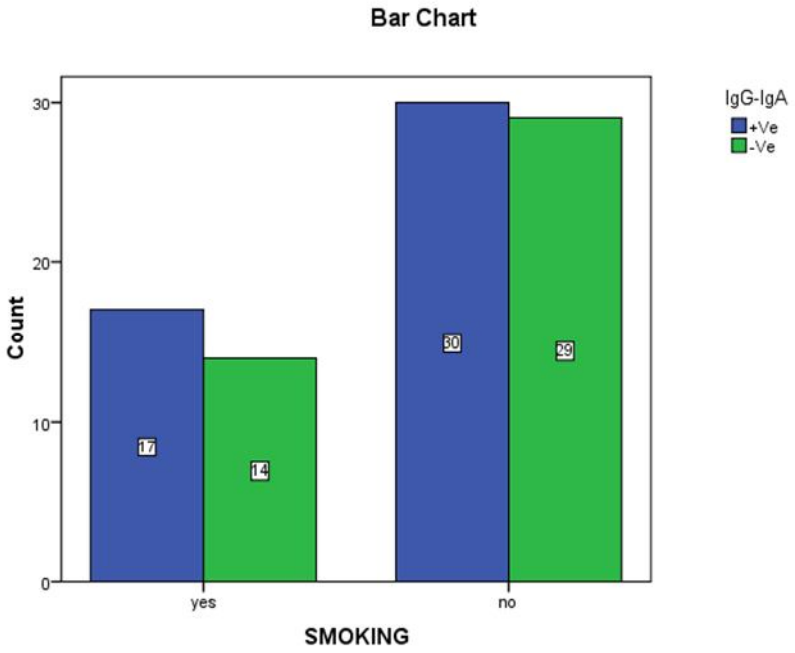



**Fig 5: Relationship between smoking and *H. pylori* infection**

# Chapter Five

## 5.1 Discussion

The prevalence of *H. pylori* infection show large geographical distribution especially in the developing countries. 80% of people were infected in the world wide. Detection of *H. pylori* infection using serological tests varies in sensitivity and specifity to give reliable and confirmed results.

The present study investigated the IgG and IgA antibodies and risk factors associated with *H. pylori*among students were ICT (antibody) positive.43(47.7%) of samples from females while 47(52.3%) from males.

The samples were tested by ELIZA for IgG, IgA because IgG for detection of the persist and the chronic infection andIgA for the detection of activity of *H. pylori.* While the IgM antibody not tested because it is short duration fell down in few days after infection and infection of *H. pylori* is a symptomatic the patient comes to clinic after symptoms appear in the chronic stage.

In this study the seropositive of *H. pylori* were found to be thatIgG 89/ 90 (98.8%), IgA 47/90(52.2%), both (IgG, IgA) were 47/90(52.2%).

The result ofIgG (98.8%) and IgA 47/90(52.2%) was disagree withOzlem*et al* (2006) foundIgG (50%) and IgA (30 %).The different of results may be duetothe difference in the stage of infection and the differ in the behavior of take medications in patients.

The study revealed that the females were more affected than males. But there was no significant correlation between *H. pylori* antibodies *(*IgG, IgA) and gender. This was an agreement with the result obtained in Egypt by Manal *et al* (2007) and other studies by Huang*et al* (2004)in Malysia, KikuchiaandDore(2005) in Iran and Mukherjee *et al* (2005) in Newther land.

The result was agree to Mirghani *et al* (2007) and his workers in Sudan; they found no significant difference between gender and *H. pylori* antibodies(IgG and IgA).

Also Versalovic and Fox (2003). reported that *H. pylori* was morefrequent in malesthan females and this was disagreed with present results.The difference of results may be duetothediffer in the life style.

In the present study there was no significant correlation between family infected and *H. pylori* seropositive which was dissimilar in study done in United State by Malatey*et al* (2001) which relevated that they was significant. Difference in the results may be dueto different in socioeconomic status.

In the present study showed no significant correlation between alcohol consumption, drinking coffee, smoking and *H. pylori* infection .these agreed withLizhang *et al* (2009) and Gikes*et al* (2004). However these results disagreed with Hermann (1997) showed that significant correlation between *H. pylori* infection and coffee. Different in the results may bedueto different in water supply and behaviors.

Also these result disagreed with Hermann (1997) and Wood ward*et al* (2000) whoever found significant correlation between*H. pylori* infection and smoking.Different in the results may be dueto different in the behavior.

Rosentock *et al*(2000) disagreed with the present results revealed were found the significant relation between*H. pylori* infection and alcohol consumption. Different in the results may be due to different in behavior and education. But agree with Ogihar (2000) andMoayyedi*et al*(2002) and were found notsignificant relation between*H. pylori* infection and alcohol consumption.

Different in the results may be due to variation in education,different in behaviors and socioeconomic status.

## 5.2 Conclusion

This study concluded that the frequency of *H. pylori* positive IgG98.8%, IgA 52.2% and both 52.8%. And frequency of *H. pylori* negative IgG1.2%, IgA47.8%. Female were more affected than males.

The results showed insignificant correlation between gender, coffee, smoking and alcohol consumption and presence of *H. pylori* antibodies

## 5.3 Recommendations

Based on this study results the following recommendations are to be considered:

1. More studies at Khartoum State using large sample size and advanced techniques to ensure more accurate result.

2.Detectionof IgM antibody to know recent infection among the students.

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