CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Helicobacter pylori (H. pylori) are Gram-negative spiral bacteria that are associated with chronic gastritis, a known precursor of gastric carcinoma (Nomura et al., 1991). Person at high risk for gastric carcinoma have been shown to have a high prevalence of H. pylori infection (Nomura et al., 1991). The discovery that H. pylori infection is the main cause of most gastroduodenal diseases has been a major breakthrough in gastroenterology (Megraud, 2004). The persistence infection with H. pylori is associated with recurrence of duodenal ulcer (Hentschel et al., 1993).

The organism was first isolated by Warren and Marshall in 1982 and soon after was linked with chronic antral gastritis and peptic ulceration (Marshall and Warren 1984). Initially, this bacterium was classified as Campylobacter pylori, but in 1989 was proposed as a new genus, Helicobacter, and was renamed as Helicobacter pylori (Goodwin et al., 1989).

Among patients with H. pylori infection, many of them had a history of upper gastrointestinal bleeding and during that period they were taking low-dose of aspirin and the eradication of H. pylori is always equivalent to treatment with omeprazole in preventing recurrent bleeding (Chan et al., 2001). The recognition of gastritis due to H. pylori as a factor of major importance has revolutionized the therapeutic approach to peptic ulcer disease (Wood et al., 1995). H. pylori infection is a risk factor for gastric adenocarcinoma (Parsonnet et al., 1994). Chronic H. pylori gastritis has been put forward as a risk factor for development of gastric mucosal atrophy and gastric cancer (Kuipers et al., 1995). H. pylori–infected gastric mucosa evolves through stages of chronic gastritis, intestinal metaplasia (IM), glandular atrophy (GA), and dysplasia before carcinoma develops. The studies show that if H. pylori eradication would alter the course of
premalignant histologic changes in the stomach (Sung et al., 2000). Infection with H. pylori is strongly associated with an increased risk of gastric carcinoma but not necessary H. pylori infection leads to gastric carcinoma (Nomura et al., 1991). A study conducted by Parsonnet et al., (1994) on 33 cases of gastric non-Hodgkin’s lymphoma occurred after a period of 14 years of infection showed that patients with gastric lymphoma were significantly more likely to have evidence of previous H. pylori infection. In the same study, Non-Hodgkin’s lymphoma affecting the stomach but no other sites is associated with previous H. pylori (Parsonnet et al., 1994). H. pylori infection increased the risk of peptic-ulcer disease in NSAID (Nonsteroidal anti-inflammatory drugs) takers 3•53-fold in addition to the risk associated with NSAID (Nonsteroidal anti-inflammatory drugs) use (odds ratio 19•4). Similarly, in the presence of risk of peptic-ulcer disease associated with H pylori infection (18•1), use of NSAID (Nonsteroidal anti-inflammatory drugs) increased the risk of peptic-ulcer disease 3•55-fold (Huang et al., 2002). H pylori infection and NSAID (Nonsteroidal anti-inflammatory drugs) use increased the risk of ulcer bleeding 1•79-fold and 4•85-fold, respectively. However, the risk of ulcer bleeding increased to 6•13 when both factors were present (Huang et al., 2002). The eradication of H. pylori is strongly recommended in all patients with peptic ulcer (Malfertheiner et al., 2002) to avoid any further.

1.2. Rationale

H. pylori are the principal cause of chronic active gastritis and peptic ulcer disease and a major contributor for gastric adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. Statistics shows that it infects around two-thirds of the people in the world and these patients are mostly children In Sudan, information about the prevalence of H. pylori infection is very patchy. and there is only one study which showed high prevalence (80%) of H. pylori infection among patients with symptoms of gastritis, 56% with duodenal ulcer, while 60% with duodenitis and 16% apparently look normal infection is very patchy. and there is only one study which showed
1.3. Objectives

1.3.1. General objective

To detect *H. Pylori* antibodies among University students in Khartoum State.

1.3.2. Specific objectives

A) To perform Immunochromatography test for detection of *H. pylori* infection among university students.

B) To determine the occurrence of *H. Pylori* infection among enrolled students.
CHAPTER TWO
LITERATURE REVIEW

2.1. Historical background

Helicobacter pylori (H. pylori) were first discovered in the stomachs of patients with gastritis and stomach ulcers in 1982 by Dr. Barry Marshall and Dr. Robin Warren of Perth, Western Australia. At the time, the conventional thinking was that no bacterium can live in the human stomach, as the stomach produced extensive amounts of acid of strength similar to the acid found in a car battery. Marshall and Warren rewrote the textbooks with reference to what causes gastritis and gastric ulcers. In recognition of their discovery, they were awarded the 2005 Nobel Prize in Physiology or Medicine (The Nobel Prize in Physiology or Medicine 2005.). German scientists found spiral-shaped bacteria in the lining of the human stomach in 1875, but they were unable to culture it, and the results were eventually forgotten (Blaser and Atherton 2004). The Italian researcher Giulio Bizzozero described similarly shaped bacteria living in the acidic environment of the stomach of dogs in 1893 (Bizzozero 1893). Professor Walery Jaworski of the Jagiellonian University in Kraków investigated sediments of gastric washings obtained from humans in 1899. Among some rod-like bacteria, he also found bacteria with a characteristic spiral shape, which he called Vibrio rugula. He was the first to suggest a possible role of this organism in the pathogenesis of gastric diseases (Konturek 2003). Several small studies conducted in the early 20th century demonstrated the presence of curved rods in the stomach of many patients with peptic ulcers and stomach cancer (Egan and O’Morain 2007). Interest in the bacteria waned, however, when an American study published in 1954 failed to observe the bacteria in 1180 stomach biopsies (Palmer 1954).

Interest in understanding the role of bacteria in stomach diseases was rekindled in the 1970s, with the visualization of bacteria in the stomach of gastric ulcer patients (Steer
The bacterium had also been observed in 1979, by Australian pathologist Robin Warren, who did further research on it with Australian physician Barry Marshall beginning in 1981. After numerous unsuccessful attempts at culturing the bacteria from the stomach, they finally succeeded in visualizing colonies in 1982, when they unintentionally left their Petri dishes incubating for 5 days over the Easter weekend. In their original paper, Warren and Marshall contended that most stomach ulcers and gastritis were caused by infection by this bacterium and not by stress or spicy food, as had been assumed before (Marshall and Warren 1984). Although there was some skepticism initially, within several years numerous research groups verified the association of H. pylori with gastritis and, to a lesser extent, ulcers (Atwood 2004). To demonstrate H. pylori caused gastritis and was not merely a bystander, Marshall drank a beaker of H. pylori culture. He became ill with nausea and vomiting several days later. An endoscopy ten days after inoculation revealed signs of gastritis and the presence of H. pylori. These results suggested H. pylori was the causative agent of gastritis, Marshall and Warren went on to demonstrate that antibiotics are effective in the treatment of many cases of gastritis. In 1987, the Sydney gastroenterologist Thomas Borody invented the first triple therapy for the treatment of duodenal ulcers (Borody et al., 1989).

In 1994, the National Institutes of Health (USA) published an opinion stating most recurrent duodenal and gastric ulcers were caused by H. pylori, and recommended antibiotics be included in the treatment regimen (Liddell and Scott 2004).

The bacterium was initially named Campylobacter pyloridis, then renamed C. pylori (pylori being the genitive of pylorus) to correct a Latin grammar error. Later it was discovered that the organism did not belong to Campylobacter species (Marshal et al., 1984), because the cellular fatty acid profile of this organism lack of methylated menaquinone-6 that found in all campylobacters, also the flagella lack of distinct pit-like depression at each pole from which the single unsheathed non terminally bulbed flagellum of the campylobacters arises. So the new genus helicobacter was introduced and thus the name Helicobacter pylori (H.pylori) referring to its helical morphology.
(Cullen. 1997), the genus derived from the ancient Greek ἕλιξ/ἐλιξ "spiral" or "coil", the specific epithet pylōri means "of the pylorus" or pyloric valve (the circular opening leading from the stomach into the duodenum), from the Ancient Greek word πυλωρός, which means gate keeper (Liddell and Scott 1966).

2.2. Morphology

H. pylori is a helix-shaped (classified as a curved rod, not spirochetes), Gram-negative bacterium, about 3 micrometers long with a diameter of about 0.5 micrometers, it is microaerophilic, that is it 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 (H2) produced by intestinal bacteria (Olson and Maier 2002). It produces oxidase, catalase and urease, it is capable of forming biofilms and can convert from spiral to a possibly viable but nonculturable coccoid form, both likely to favor its survival and be factors in the epidemiology of the bacterium (Kusters et al., 2006).

H. pylori possesses five major outer membrane protein (OMP) families, the largest family includes known and putative adhesions, the other four families include porins, iron transporters, flagellum-associated proteins and proteins of unknown function (Kusters et al., 2006). Like other typical Gram-negative bacteria, the outer membrane of H. pylori consists of phospholipids and lipopolysaccharide (LPS), the outer membrane also contains cholesterol glucosides, which are found in few other bacteria (Kusters et al., 2006). H. pylori has four to six lophotrichous flagella; all gastric and enterohepatic Helicobacter species are highly motile due to flagella (Josenhans et al., 2000), the characteristic sheathed flagellar filaments of Helicobacter are composed of two copolymerized flagellins, FlaA and Fla B (Rust et al., 2008).

2.3. Genome

H. pylori consist of a large diversity of strains and the genomes of three have been completely sequenced. The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1,550 genes, the two sequenced strains show large genetic
differences, with up to 6% of the nucleotides differing (National Center for Biotechnology Information 2008).

2.4. Classification

The Domain is Bacteria, phylum Proteobacteria, class Epsilonproteobacteria; order Campylobacter ale, family Helicobacteraceales, Genus Helicobacter, species H.pylori ((Rust et al., 2008).

2.5. Antigenic structure

Lipopolysaccharide (LPS) is a prevalent macromolecule in the outer membrane of Gram negative bacteria and represents an important virulence factor. LPS is composed of three parts: lipid A which is embedded in the outer membrane, the core oligosaccharide, and the O antigen, Lipid A is also known as endotoxin, which refers to the induction of fatal reactions of the human immune system at very low LPS concentrations, bound to lipid A is the core oligosaccharide, which is relatively well conserved among closely related bacteria, the O antigen represents the outermost region of the LPS. The O antigen of Helicobacter pylori contributes in several respects to the virulence of this human gastric pathogen, which is recognized by the World Health Organization as a Type 1 carcinogen (Raetz. et al., 2007).

H. pylori mimics carbohydrate structures present on human epithelial cells, blood cells, and in secretions, by incorporating Lewis antigens on its O chains (Misra et al., 1999).

Lipopolysaccharide (LPS) of many Helicobacter pylori strains expresses Lewis antigens (Lex, Ley, Lea, Leb) which are similar to those expressed by gastric epithelial cells (“molecular mimicry”) (Appelmelk et al 1996), In addition H pylori LPS displays phase variation in these antigens—that is the high frequency, reversible switching of phenotype, for instance, a strain that expresses Lex may yield variants that express Ley, as yet, no definite role has been assigned to these Lewis antigens, nor to phase variation, in the pathogenesis of gastric disease (Appelmelk et al 1999).
The amounts of Lewis antigens and their location on the H. pylori O polysaccharide are variable, differing between strains and also between cells from the same isolate (Nilsson et al., 2006). This is due to the phase variable expression of the H. pylori fucosyltransferases, enzymes required for the synthesis of Lewis antigens (Appelmelk BJ. et al., 1999).

Unlike most bacteria, the genes involved in LPS biosynthesis in H. pylori are not arranged in a single cluster, but rather found in various locations distributed throughout the chromosome, nevertheless, many enzymes required for H. pylori LPS biosynthesis have been identified and characterized, these include glycosyltransferases responsible for the addition of the monosaccharide building blocks in the assembly of the O polysaccharide (Misra. et al., 1999), as well as several proteins involved in the synthesis and modification of the lipid A-core (Raetz et al. 2007).

2.6. Mode of transmission

Numerous epidemiological studies have been conducted to identify the factors influencing transmission of this pathogen, socioeconomic status is clearly the most important determinant for the development of H. pylori infection, with lower social classes exhibiting much higher prevalence, this factor encompasses conditions such as levels of hygiene, density of living, sanitation, and educational opportunities, which have all been individually identified as markers of the bacterium presence, largely based on epidemiological and microbiological evidence, several routes of transmission have been conjectured. Person-to-person transmission is widely seen as the most probable route of infection, mainly because of the apparent failure to consistently isolate H. pylori in places other than the human GI tract and of the perception that lower transit time between different hosts would certainly be favorable for the bacterium. Furthermore, numerous epidemiological studies have consistently identified domestic overcrowding and infection of family members as a risk factor for H. pylori transmission (Mitchell 2001).
Roma-Giannakos and colleagues (2003) found a strong homology of the H. pylori genome in infected members of the same family, and clustering of H. pylori infection in families has been widely reported in other studies, although these studies support the hypothesis of person-to-person transmission, exposure of a family to an alternative common source still remains a possibility (Roma-Giannakos et al., 2003).

The most relevant pathways of person-to-person transmission encompass the gastro-oral, oral-oral, and fecal-oral routes, Breastfeeding and iatrogenic transmission are also included as alternative ways for the dissemination of the pathogen (Megraud. 2003).

In addition, there are at least three possible vectors that have been suggested to sustain the bacterium in viable form: water, food, and animals, most authors agree that the relative importance of these routes in the transmission of the bacterium is likely to vary between developing and developed countries (Perez-Perez. et al., 2004).

2.6.1. Gastro-Oral Transmission

It has been suggested that exposure to microscopic droplets of gastric juice during endoscope manipulation could explain an higher prevalence of infection in gastrointestinal endoscopists, but the gastro-oral transmission has been postulated mainly for young children, among whom vomiting and gastro-esophageal reflux are common(Hildebrand et al., 2000).

2.6.2. Oral-Oral Transmission

The oral cavity has been considered to be a suitable reservoir for H. pylori subsistence, and oral-oral transmission has therefore been suggested to occur with kissing or other contact with infected saliva, the use of chopsticks by Chinese immigrants or, as it happens in some ethnic backgrounds, from mothers to their babies as they pre-masticate their food. The role of the oral cavity has been extensively reviewed by others (Dowsett and Kowolik. 2003; Luman. 2002).

2.6.3. Fecal-Oral Transmission
It has been suggested that the fecal-oral route for H. pylori transmission is very unlikely due to the contact with human bile, to which it is very sensitive, during the passage through the intestine. However; one epidemiological study appears to support the view that this transmission mode is less common than gastro-oral or oral-oral, by showing that exposure to an infected household member with diarrhea elevated, but not significantly, the risk for new infection (Perry et al., 2006).

2.6.4. Breastfeeding

The detection by PCR of H. pylori in breast milk has also raised the possibility of breastfeeding as a route of transmission (Kitagawa et al., 2001).

2.6.5. Zoonotic Transmission

Including contact with animals as a possible transmission mode is an obvious reasoning, as Zoonotic transmission represents one of the leading causes of illness and death from infectious disease worldwide (Boomkens et al., 2004).

2.7. Nutritional requirements

All Helicobacter species are characterized as fastidious, and most are associated with gastric or extra gastric diseases; Nutritional requirements of the human gastric pathogen, H. pylori, are not completely understood, and less is known about nutritional requirements of other Helicobacter species (Harper 2002). Putative genes for metabolic enzymes predicted a requirement for 8 to 9 amino acids, oxygen, phosphate, and thiamine but did not make any predictions on metal requirements. Furthermore; an aforementioned study predicted that purines (adenine, guanine, and hypoxanthine) should not be required for the growth of H. pylori, in contrast to previously published reports, and did not provide insight into the relative concentrations of nutrients required for optimal growth (Schilling et al., 2002). Additional studies revealed that H. pylori prefers to use amino acids rather than sugars as carbon sources, a commonly used tissue culture medium, supports the growth of H. pylori in the absence of serum or protein, opened up
many possibilities for H. pylori research but also raised new questions about the nutritional requirements of the organism (Tester man et al., 2001).

2.8. Pathophysiology

To colonize the stomach, H. pylori must survive the acidic pH of the lumen and use its flagella to burrow into the mucus to reach its niche, close to the stomach's epithelial cell layer (Amieva and El-Omar 2008).

Many bacteria can be found deep in the mucus, which is continuously secreted by mucus-secreting cells and removed on the luminal side. To avoid being carried into the lumen, H. pylori senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface (Schreiber et al., 2004).

H. pylori is also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells, it produces adhesins which bind to membrane-associated lipids and carbohydrates and help it adhere to epithelial cells, e.g., the adhesion BabA binds to the Lewis b antigen displayed on the surface of stomach epithelial cells (Petersen et al., 2002).

H. pylori produces large amounts of the enzyme urease, molecules of which are localized inside and outside of the bacterium, Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia, the ammonia is converted to ammonium by accepting a proton (H+), which neutralizes gastric acid, the survival of H. pylori in the acidic stomach is dependent on urease, the ammonia produced is toxic to the epithelial cells, and along with the other products of H. pylori including proteases, vacuolating cytotoxin A (VacA), and certain phospholipases, damages those cells (Smoot. 1997).

Inflammatory processes of H. pylori infections are also mediated by highly disulfide-bridged proteins, Helicobacter cysteine-rich proteins (Hcp), particularly HcpA (hp0211),
triggers an immune response through the differentiation of human myeloid Thp1 monocytes into macrophages. In analogy to eukaryotic cytokines, they interfere with host cell functions and change the morphology of monocytes, inducing the expression of the surface marker protein CD11b, phagocytic activity, as well as cell adherence, which are indicative of monocyte differentiation into macrophages (Dumrese et al., 2009).

Colonization of the stomach by H. pylori results in chronic gastritis, an inflammation of the stomach lining, the severity of the inflammation is likely to underlie H. pylori-related diseases (Shiotani and Graham 2002).

Duodenal and stomach ulcers result when the consequences of inflammation allow the acid and pepsin in the stomach lumen to overwhelm the mechanisms that protect the stomach and duodenal mucosa from these caustic substances, the type of ulcer that develops depends on the location of chronic gastritis, which occurs at the site of H. pylori colonization, the acidity within the stomach lumen affects the colonization pattern of H. pylori, and therefore ultimately determines whether a duodenal or gastric ulcer will form (Dixon 2000).

In people producing large amounts of acid, H. pylori colonizes the antrum of the stomach to avoid the acid-secreting parietal cells located in the corpus (main body) of the stomach (Kusters et al., 2006), the inflammatory response to the bacteria induces G cells in the antrum to secrete the hormone gastrin, which travels through the bloodstream to the corpus. Gastrin stimulates the parietal cells in the corpus to secrete even more acid into the stomach lumen (Blaser and Atherton 2004), Chronically increased gastrin levels eventually cause the number of parietal cells to also increase, further escalating the amount of acid secreted (Schubert and Peura 2008).

The increased acid load damages the duodenum and ulceration may eventually result, in contrast, gastric ulcers are often associated with normal or reduced gastric acid production, suggesting the mechanisms that protect the gastric mucosa are defective, in
these patients; *H. pylori* can also colonize the corpus of the stomach, where the acid-secreting parietal cells are located (Schubert and Peura 2008).

However chronic inflammation induced by the bacteria causes’ further reduction of acid production and eventually, atrophy of the stomach lining, which may lead to gastric ulcer and increases the risk for stomach cancer (Suerbaum and Michetti 2002).

About 50-70% of *H. pylori* strains in western countries carry the cag pathogenicity island (cag PAI) (Peek, Crabtree. 2006), western patients infected with strains carrying the cag PAI have a stronger inflammatory response in the stomach and are at a greater risk of developing peptic ulcers or stomach cancer than those infected with strains lacking the island (Kusters et al., 2006).

Following attachment of *H. pylori* to stomach epithelial cells, the type IV secretion system expressed by the cag PAI "injects" the inflammation-inducing agent, peptidoglycan, from their own cell wall into the epithelial cells, the injected peptidoglycan is recognized by the cytoplasmic pattern recognition receptor (immune sensor), which then stimulates expression of cytokines that promote inflammation (Viala et al., 2004).

The type IV secretion apparatus also injects the cag PAI-encoded protein CagA into the stomach's epithelial cells, where it disrupts the cytoskeleton, adherence to adjacent cells, intracellular signaling, cell polarity and other cellular activities (Backert and Selbach 2008). Once inside the cell, the CagA protein is phosphorylated on tyrosine residues by a host cell membrane-associated tyrosine kinase (TK). CagA then allosterically activates protein tyrosine phosphatase/protooncogene Shp2 (Hatakeyama 2004).

Pathogenic strains of *H. pylori* have been shown to activate the epidermal growth factor receptor (EGFR), a membrane protein with a tyrosine kinase domain, activation of the EGFR by *H. pylori* is associated with altered signal transduction and gene expression in host epithelial cells that may contribute to pathogenesis, it has also been suggested that a C-terminal region of the CagA protein (amino acids 873–1002) can regulate host cell
gene transcription, independent of protein tyrosine phosphorylation (Baldwin et al., 2007).

2.9. Peptic ulcer

Peptic ulcer disease embraces both gastric and duodenal ulcers and has been a major threat to the world’s population. Helicobacter pylori causes inflammation of the gastric mucosa (gastritis) that may predispose infected individuals to develop ulcers or gastric tumors (Kitahara et al., 1998; Prinz et al., 2003), most colonized individuals remain asymptomatic, presenting with mild but diffuse inflammation of the stomach and show little or no atrophy throughout their lifetimes (Moayyedi et al., 2000; Kapadia, 2003).

A small percentage (0.1–4%) of chronically infected individuals, however, develop severe disease (gastritis) involving both corpus and antrum regions of the stomach. This leads to a diminished acid secretory capacity, the disease may eventually progress to adenocarcinoma requiring treatment (Uemura et al., 2001).

Upon infection, three clinical outcomes are possible:

1. Bacterial eradication or clearance, usually after treatment (Ulmer et al., 2003).

2. Chronic or persistent, low level, asymptomatic gastritis during which bacterial population sizes remain unchanged or in steady state (Prinz et al., 2003; Uemura et al., 2001).

3. Progression to severe disease such as atrophy of gastric glands, gastric ulcers or carcinomas (Kitahara et al., 1998; Prinz et al., 2003; Uemura et al., 2001).

2.10. Signs and symptoms

1. Abdominal pain, classically epigastric with severity relating to meal times, after around three hours of taking a meal (duodenal ulcers are classically relieved by food, while gastric ulcers are exacerbated by it).
2. Bloating and abdominal fullness.

3. Water brash (rush of saliva after an episode of regurgitation to dilute the acid in esophagus - although this is more associated with gastro esophagea reflux disease).


5. Loss of appetite and weight loss.

6. Hematemesis (vomiting of blood); this can occur due to bleeding directly from a gastric ulcer, or from damage to the esophagus from severe/continuing vomiting.

7. Melina (tarry, foul-smelling feces due to oxidized iron from hemoglobin).

8. Rarely, an ulcer can lead to a gastric or duodenal perforation, which leads to acute peritonitis. This is extremely painful and requires immediate surgery.

2.11. Complication

1. Gastrointestinal bleeding is the most common complication. Sudden large bleeding can be life-threatening. It occurs when the ulcer erodes one of the blood vessels, such as the gastro duodenal artery (Cullen et al., 1997).

2. Perforation of a hole in the wall often leads to catastrophic consequences. Erosion of the gastro-intestinal wall by the ulcer leads to spillage of stomach or intestinal content into the abdominal cavity. Perforation at the anterior surface of the stomach leads to acute peritonitis, initially chemical and later bacterial peritonitis. The first sign is often sudden intense abdominal pain. Posterior wall perforation leads to bleeding due to involvement of gastro duodenal artery that lies posterior to the first part of the duodenum.

3. Penetration occurs when the ulcer continues into the adjacent organs such as the liver and pancreas. Scarring and swelling due to ulcers causes narrowing in the duodenum and gastric outlet obstruction. Patient often presents with severe vomiting.
4. Cancer is included in the differential diagnosis (elucidated by biopsy), Helicobacter pylori as the etiological factor making it 3 to 6 times more likely to develop stomach cancer from the ulcer (Merck 2006).

2.12. Prevention

H. pylori is a major cause of certain diseases of the UGIT, rising antibiotic resistance increases the need to search for new therapeutic strategies, this might include prevention in the form of vaccination (Selgrad and Malfertheiner 2008). Extensive vaccine studies in mouse models have shown promising results (Hoffelner et al., 2008).

Researchers are studying different adjuvants, 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 (Kabir 2007), in popular culture, a number of foods may be useful to prevent colonization with H. pylori including: green tea, red wine, broccoli sprouts, and garlic (Lee 2008).

2.13. Treatment

Since the discovery of Helicobacter pylori in the early 1980s many treatment regimens have been developed to effectively treat this infection. International guidelines have allowed consensus on the best management and improved eradication rates. In recent years, increasing antimicrobial resistance has resulted in falling eradication rates with standard therapies. Currently, the first-line treatment remains clarithromycin, amoxicillin or metronidazole and proton pump inhibitor twice daily, but a number of recent studies have shown low eradication rates with this treatment, increasing antimicrobial resistance and falling eradication rates are the result of the widespread use of antibiotics (Malfertheiner, et al., 2003).

Clarithromycin resistance has a greater effect on treatment efficacy than nitro imidazole resistance (Fischbach and Evans 2007). The prevalence of secondary clarithromycin
resistance, i.e. after failure of a treatment including this drug, is extremely high, up to 60% (Megraud and Lehours 2007).

However, metronidazole resistance in vitro does not always predict treatment failure. There is poor correlation between different methods of metronidazole resistance detection and this may explain differing resistance rates between institutions (Gerrits et al., 2006).

2.12.1. Factors related to eradication failure

A number of other factors have been studied in H. pylori eradication. Smoking is an independent risk factor for H. pylori treatment failure (Suzuki et al., 2006); In a Finnish study, smoking and coffee drinking reduced the efficacy of therapy (Koivisto et al., 2005), in contrast, alcohol consumption may facilitate elimination of H. pylori infection among adults (Murray et al., 2005).

A number of studies have shown a positive effect of alcohol consumption on the success of eradication therapy (Baena et al., 2002). In a Polish study nonsmokers who drink alcohol had the highest eradication rate of 92% with standard triple therapy (Suzuki et al., 2007).

2.13. Diagnosis

2.13.1. Invasive method

Several factors guide the site and the number of biopsy specimens for detection of H. pylori infection, the number of biopsies required depends on the diagnostic method used. The best location to detect the organism is the antrum; especially mid-antrum at the lesser curvature in the children and the distal antrum (2 cm from the pylorus) in adult (Elistsur et al., 2002).
2.13.1.1. Rapid urease tests (RUTs)

Detection of H. pylori in antral or duodenal biopsy specimens usually entails histological or microbiological methods. Culture takes 7 days, and the histologically at least 24 h. The need for simple, fast and reliable test that the endoscopist could perform to diagnose the infection in the endoscopy room is very important (Europeon diagnostic manufacturers association, 2004).

The large amount of performed urease enzyme produced by H. pylori afforded a means of detecting the organism without culture langenberg described the unusual characteristic of rapid urea hydrolysis by H. pylori that indicated the presence of performed urease.

The one minute ultra –rapid urease test (URUT) is freshly prepared urea solution (10%) with phenol red indicator as described by Thillaininayagam (2002). Positive result is indicated by change in the color of the solution from orange to pink within the first minute of the addition of the biopsy specimen, URUT is useful test for detection of H. pylori with 92% sensitivity and 74% specificity ( Rogge et al., 1995), the advantage of one minute URUT is that the result become available before the patient even leaves the endoscopy room and reduce the number of patient lost to follow- up. It also allows the physician to be more confident that the patient has received the appropriate therapy and instructions necessary to ensure better compliance (Misra 1999).

2.13.1.2. Histological identification

The histological identification of H. pylori infection is now widely used as a mean of diagnosis to achieve this, several staining methods are in use, these include Genta, Gram’s, modified giemsa, Warthinstarry (HPSS) and Haematoxylin and eosin (H&E), acridine orange, H. pylori silver stain and immunohisto-chemistry H. pylori antibodies. The pathology laboratories must aspire to use the most effective method on routine practice on the basis of availability, reproducibility, rapidity, sensitivity and cost (Rotimi 2001). Genta stain is used in many laboratories because it allows simultaneous visualization of bacteria, the histological feature of the gastritis and any other
pathological lesions such as intestinal metaplasia can also be visualized (El-zimaity et al., 1999).

2.13.1.3. Flagellar staining method

The flagella are many times the length of the bacterial cells, averaging 0.01 to 0.05 micrometer; they are beyond the resolving power of light microscopes and can be seen only with an electron microscope.

2.13.1.4. Polymerase chain reaction (PCR)

Molecular methods, in particular polymerase chain reaction (PCR), have the potential to detect more cases of infection because of their greater sensitivity but are technically demanding (Ho and Windsor, 2000).

2.13.2. Non-invasive tests for diagnosis of H.pylori

During recent years, noninvasive diagnostic test have gained in significance and became part of the management strategies for assessment of H.pylori status in patients with dyspepsia, this is of particular importance for children, especially in post-treatment control, which, according to the Canadian and European consensus conference reports, should preferentially be performed by noninvasive tests (Sherman et al., 1999).

Noninvasive tests has the advantage of avoiding the sampling errors inherent in biopsy due to the patchy distribution of the organism (the distribution of the H.pylori and the associated inflammation is often patchy), also each biopsy specimen represents only approximately 0.001% of the surface of the stomach, noninvasive tests provide a more rapid, less expensive and offer similar accuracy to invasive test, so the availability of reliable and safe noninvasive diagnostic techniques coupled with the development of effective and tolerable treatment has enabled primary health care personnel to manage this infection (Mcnamara and O morain 2000).
Serological tests are widely used but they cannot differentiate a current infection from a past exposure. Performance of serological tests depends on the antigen preparation used since, H pylori strains differ among geographic locations, local validation of the test is necessary (Suerbaum et al., 2002, Makristathis et al., 2004)

2.13.2.1. Urea breath test

The 13C-urea breath test (13C-UBT) has been recognized as an excellent test because of its accuracy as well as of its robustness. The specimens can be transported without special conditions, and the result is independent of human interpretation. This urea breath test indirectly detects the presence of H pylori-associated urease by measuring CO2 in the patient's breath. A baseline breath sample is collected before the patient ingests 13C-urea, i.e., urea labeled with a naturally occurring, non-radioactive carbon isotope, a second sample is collected shortly after the ingestion. H pylori-associated urease degrades the urea, producing ammonia and CO2, the resultant CO2 is absorbed in the blood and then exhaled, an increase in the ratio of 13CO2 to 12CO2 between the pre- and post-ingestion samples indicates the presence of H pylori-associated urease (Peterson et al., 2000).

2.13.2.2. Rapid urea test

This test was designed to assess the presence of H. pylori in biopsy specimens within 5 minutes. Two gastric biopsy specimens, one from the antrum and one from the corpus, were taken up in Christensen’s urea agar media (pH adjusted at 7.0) in screw-capped bottles, a change of color from yellow to pink by any specimen within 2 h was considered as positive. A urease test based on an immunological detection of urease was proposed for the first time in Japan. Its sensitivity was 96% but its specificity only 90% (Isomoto et al., 2006).

2.13.2.3. Immunochromatography test (ICT)

This calls for a simple, reliable and non-invasive diagnostic test for H pylori infection in clinical practice. At present there is no single test for H pylori that can be used as the
‘gold standard (Vaira et al., 2002). Culture, rapid urease test, and histology require endoscopic biopsy of gastric mucosal tissue that is expensive, inconvenient for the patient and available only at specialized centers. Moreover, because of a patchy distribution of H pylori in the gastric mucosa, biopsy tissue examination may yield false negative results (Vaira et al., 2002).

A recent development in H. pylori diagnosis is a commercial Immunochromatography test (ICT). It is homologous to a conserved secreted protein of H pylori. According to its manufacturer (Gene labs Diagnostics, Singapore), presence of IgG antibody is highly predictive for active H pylori infection. If so, it should be helpful for diagnosis of H.pylori infection where facility for endoscopy is not available (Rocha et al., 2000).

ICT test was performed by the commercial test kit according to the instructions of the manufacturer. When control (C) and test line (T) were visible the ICT test was regarded positive, when only control line (C) was visible, the test was regarded negative. When both control line (C) and test line (T) was absent the test was regarded to be invalid (Park et al., 2002).

2.13.2.4. Antigenic stool test

Non-invasive tests on faecal samples play an important role in the diagnosis of H.pylori infection. Fecal testing is particularly simple as feces can be obtained easily, as compared with samples collected by endoscopy, and the patients can supply the material in the privacy of their homes (Kabir. 2002).

Very few investigations have isolated H.pylori from feces, this because of the presence of massive number of diverse micro-organism in feces that may make it very difficult for fastidious bacteria such as H.pylori to grow, also the mechanism by which viable H.pylori can be extracted in the feces is not properly understood. H.pylori colonizes areas of the stomach that are not in contact with bile, and as bile is present in the duodenum and colon, so the organism may not survive transited through the alimentary tract in
association with feces, PCR and ELISA assay for antigen detection of Pylori in the stool have been developed (Dore. 2000).

2.13.2.5. Enzyme-linked immunosorbent assay (ELISA)

Many serological kits for H. pylori detection are commercially available in clinical practice, the sensitivity of ELISA based serological tests ranges between 90% and 97%, and the specificity ranges between 50% and 96%. The sensitivity and specificity of the serological tests mainly depend on the nature of the antigenic materials used. In addition to the antigens used, the presence of atrophic gastritis is also one of the important factors that influence the test’s accuracy (Hung et al., 2010).
CHAPTER THREE
MATERIALS AND METHODS

3.1. Study Design

3.1.1 Study type

This is a cross-sectional descriptive study.

3.1.2. Study Area

The study was conducted in Sudan University of Science and Technology.

3.1.3. Study Population

Students of Sudan University of Science and Technology were the target population this study.

3.1.4. Sample Size

One hundred students were selected randomly to participate in this study.

3.2. Ethical consideration

Approval to perform this research was obtained from College Ethical Committee, Sudan University of Science and Technology.

3.3. Collection of socio-demographic data

The socio-demographic data were collected by structured questionnaire (Appendix 1).

These including sex, age, symptoms and previous infection.

3.4. Data analysis

Data were analyzed by computer using Statistical Package of Social Science (S P S S) software program version 17.

3.5. Experimental Work
3.5.1. Collection of blood

The blood was collected by vein puncture in Heparin container as follows; a clear vein was determined, then tourniquet was tied on the above arm. The area was disinfected by 70% alcohol and then 5ml blood was drawn in vacationer tube.

3.5.2. Preparation of plasma

The plasma was prepared by centrifugation. The blood was centrifuged at 3000rpm for 2 minutes. The plasma was separated in plan container, labeled and stored at -20c until used.

3.5.3. Adoption of ICT for detection of H. Pylori antibodies

Immunochromatography test ICT (one step) for H. pylori (serum) was used. The test is a rapid chromatographic immunoassay for qualitative detection of antibodies to H. pylori in serum or plasma to aid in diagnosis of H. pylori infection.

3. 5.1. Principle

The H. pylori one step test device (serum/plasma) is a qualitative membrane strip based Immunoassay for the detection of H.pylori antibodies in the serum or plasma. In this test procedure, anti-human IgG is immobilized in the test line region of the device. After a serum or plasma is placed in the specimen well, it reacts with H.pylori antigen coated particles in the test. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized anti-human IgG. If the specimen contains H.pylori antibodies, a colored line will appear in the test line region indicating a positive test if the specimen does not contain H.pylori antibodies, a colored line will not appear in this region indicating a negative result.

3.5.3.2. Assay procedure

1. The test device was removed from the foil pouch and left on a clean leveled surface to reach room temperature.

2. The dropper was held vertically and three drops of plasma (approximately 100 µl) were transferred to the test well in the test device.

3. The result was read within 10 minutes.
3.5.3.3. Interpretation of the results

The results were interpreted according to manufacturer instruction as follows;

4. Positive results showed double lines.

5. Negative result showed one line
CHAPTER FOUR

RESULTS

In this study 100 participants were enrolled. Of them 50(50%) were males and 50(50%) were females. Their ages ranged from 21 to 25 years old (table 1). 12 of total participants were symptomatic while the rest (n-88) were asymptomatic (fig 1).

Study on the detection of H pylori antibodies revealed that 28(28%) were positive and 72 (72%) were negative (table 2)

Of the positive participants 10 were males and 18 were females (table3)

17 of the participants were known as previously infected and 11were detected during this study (fig 2)

Of the 17 previously infected four (no 4) only under treatment, while the rest (no 13) not treated before (fig 3)

Table 1. Distribution of participants according to their age and gender

<table>
<thead>
<tr>
<th>Gender</th>
<th>No</th>
<th>%</th>
<th>Age(Year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>50</td>
<td>50</td>
<td>Range(21- 25)</td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>50</td>
<td>Range (21- 25)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Fig 1. Distribution of the participants according to symptoms

Table 2. Frequency and percentage of H pylori antibodies positive and negative cases using I C T technique

<table>
<thead>
<tr>
<th>Results</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>Negative</td>
<td>72</td>
<td>72</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
Table 3. Distribution of H pylori anti bodies positive cases according to sex

<table>
<thead>
<tr>
<th>Result</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Male</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Total positive</td>
<td>28</td>
<td>28</td>
</tr>
</tbody>
</table>

Fig 2

History of H pylori infection among participants
Fig 3. DISTRIBUTION OF PARTICIPANTS ACCORDING TO MEDICATION
5.1. Discussion

Helicobacter pylori (H. Pylori) infection is diagnosed via invasive or noninvasive testing. Invasive tests generally include biopsy of the gastric mucosal tissue; noninvasive tests can be performed on serum, plasma or stool (Ramakrishna and Salinas, 2007).

This study was carried out in Sudan University of Science and Technology. The study utilizes a rapid technique namely Immune chromatographic test (ICT) recently introduced to diagnose H. pylori infection. The ICT is used worldwide to detect H. pylori infection in patients with upper gastrointestinal symptoms; though a small number of false-positive tests would result in some overtreatment (Tarun et al., 1998).

In the present study, out of the 100 participants diagnosed for presence of H. Pylori antibodies by ICT technique, 28(28%) were positive. This result is higher than that reported (5.2%) by Akamatsu et al., (2011) among 1224 Japanese students. However, it is less than that obtained (71.3%) by Nares-Cisneros et al., (2007) among Mexican children.

On the other hand, different studies evaluated the accuracy and diagnosed value of the immune chromatographic technique. Different results in this context were reported. Hu et al., (2007) evaluated this technique by testing 129 patients in Taiwan. They found 82 (64%) were H. pylori infected. Furthermore, Lang horst et al., (2002) conducted a prospective clinical study to evaluate a new whole blood antibody test (i.e. ICT) in Germany. Among 132 patients enrolled, they found 43.4% were infected. They concluded that the ICT showed sufficient sensitivity and satisfying specificity for H. Pylori diagnosis similar to or better than those of rapid urease tests or ELISA.
5.2. Conclusion

The study concluded that:

1. Immunochromatography test (ICT) may be useful for non-invasive diagnosis of Helicobacter pylori.
2. The prevalence of H. Pylori among enrolled group are in line with other studies performed elsewhere (28%)

5.3. Recommendations

1. The use the novel immune chromatographic stool test which is fast, easy to perform and provides good differentiation between positive and negative results.

2. Culture and histology are the gold standard tests for diagnosis of H. Pylori are recommended to be included in any rapid serological test as control.

3. Further research is needed to study the effectiveness of Immunochromatography test diagnosis H. Pylori.
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APPENDICES

Appendix 1. Questionnaire

بسم الله الرحمن الرحيم

Sudan University of Science and Technology
College of Graduate Studies

Name (optional) ………………………………………………………………………...
Sex ………………………………………………………………………………………
Age (year) ………………………………………………………………………………
Nationality ……………………………………………………………………………
Symptoms ……………………………………………………………………………
Medication ……………………………………………………………………………
Appendix 2. Immunochromatographic test (ICT) positive result.

Appendix 3. Immunochromatographic test (ICT) negative result.
Appendix 4. ICT manufacturer instruction