# 1. Introduction

## 1.1. Background

The bacterium *Helicobacter pylori* is a fastidious, microaerophilic spiral Gram-negative microorganism, previously named *Campylobacter pyloridis* bacterium that infects various areas of the stomach and duodenum ( Johannes *et al* , 2006).

*H. pylori* remains the most medically important bacterial inhabitant of the human stomach. the bacterium *Helicobacter pylori* plays a significant role in the pathogenesis of chronic gastritis, peptic ulcer, and gastric cancer .*H. pylori's* helical shape (from which the genus name is derived) is thought to have evolved to penetrate and favor its motility in the mucus gel layer( Ketley, 2007) .

Its worldwide distribution, at least 50% of the world's human population are infected .However, most individuals infected with *H. pylori* do not experience symptoms or have signs of recognizable disease.

The high level of prevalence and the importance of associated pathologies make the elimination of *H. pylori* a very useful approach to treating and controlling these gastroduodenal diseases, since its eradication results in a marked reduction in the rate of recurrence of duodenal and gastric ulcer ( Genta , 2002 ) .

## 1.2. Rationale

Once *H.pylori* is responsible of about 85% gastroduodenitis , 95% gastric ulcer and duodenal ulcer , diagnosis of *H. pylori* in the right way is very important in infection control (kuıper *et al* , 1995 a) .

Since caltivation of *H.pylori* is very difficult and time consuming, other methods that used for diagnosis of *H. pylori* give variable results like rapid screen of antibodies, rapid urease test and urea breath test . Polymerase chain reaction (PCR) was used in this study due to high specificity and sensitivity for diagnosis of *H. pylori* infection ( Blaser and Atherton 2004) .

## 1.3. Objectives

### 1.3.1. General objective

To detect *Helicobacter pylori* in patients with gastroduodenitis and peptic ulcer in Khartoum State by employing PCR technique .

### 1.3.2 Specific objective

1. To detect urease gene of *Helicobacter pylori* in gastric and duodenal biopsies from patients with gastroduodenitis and peptic ulcer in Khartoum State by using specific primers .
2. To determine the possible risk factor associated with *H.pylori* infection among gastroduodenitis and peptic ulcer.

## 2. literature review

### 2.1 History

The bacterium *H.pylori* was initially named *Campylobacter pyloridis*, then *C. pylori* ( after a correction to the Latin grammar) and in 1989, after DNA sequencing and other data showed that the bacterium did not belong in the *Campylobacter* genus, it was placed in its own genus, *Helicobacter*. The name *pylori* means "of the pylorus" or pyloric valve (the circular opening leading from the stomach into the duodenum), and its Greek word means gatekeeper. In 1875, German scientists found helical shaped bacteria in the lining of the human Stomach. The bacteria could not be grown in culture and the results were eventually forgotten (Suerbaum and Josenhans ,2007).

In 1893, the Italian researcher Giulio Bizzozero described helical shaped bacteria living in the acidic environment of the stomach of dogs (De Groot *et al*, 2005) 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 helical shape, which he called *Vibrio rugula*. He was the first to suggest a possible role of this organism in the pathogencity of gastric diseases( Andersen , 2007) .

Then The bacterium was rediscovered in 1979 by Australian pathologist Robin Warren, who did further research on it with Barry Marshall beginning in 1981; they isolated the organisms from mucosal specimens from human stomachs and were the first to successfully culture them. 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 befor (Goodwin *et al.*,1989 )

## 2.2 Classification

The most important stage in the development of the taxonomy of gastric microorganisms was the proposal in 1989 to establish a new genus called *Helicobacter* to mean a spiral rod - and that *C. pylori* should be transferred to that genus as *H. pylori* (Table 2.1 ) .

**Table 2.1** Classification of *Helicobacter pylori* .

| | |
|---|---|
| *Kingdom* | *Bacteria* |
| *Phylum* | *Proteobacteria* |
| *Class* | *Epsilon Proteobacteria* |
| *Order* | *Campylobacterales* |
| *Family* | *Helicobacteraceae* |
| *Genus* | *Helicobacter* |
| *Species* | *H. pylori* |
| Binomial name | *Helicobacter pylori* |

(Goodwin *et al* , 1989 )

## 2.3 Cellular morphology

*H. pylori* is a Gram-negative, s-shaped or curved rod 0.5-0.9 mm wide by 2-4 mm long with 1 to 3 turns when observed in vivo. No spores are formed in blood agar cultures (*in vitro*), and spiral forms are less obvious with cells appearing more frequently as singly curved rods( Axon , 1996 ).

Cells of *H. pylori* typically have up to six polar flagella filaments.

The Cells are mostly actively motile although some cultures may appear to be non motile in hanging drop preparations. Other forms of *H. pylori* reported in culture and occasionally *in vivo* include spherical, V-shaped, U shape (ox-bow) and straightened forms . Infrastructure features Flagella of *H. pylori* are sheathed with a covering that is continuous with the outer membrane components of the body wall. Freeze-fracture `ultra structure studies suggest that the normal configuration of flagella is seven. Flagella are each about 30 nm in diameter with a filament of 12-15 nm. Some flagella have distinctive terminal bulbs but no function has been assigned to such structures ( Allen *et al* ,1997 ) . Electron microscopy also reveals the presence of a 40 nm thick glycocalyx or capsule-like polysaccharide rich layer external to the cell wall unit membrane, which is thicker in vivo than in cultured bacteria. General physiological properties of *H. pylori* is a microaerophilic , growing best in an atmosphere of 5% oxygen with 5-10% $CO_2$ on blood containing media such as Oxoid brain heart infusion agar (BHI) and 5% horse blood agar enriched with 1% IsoVitaleX, which is a chemically defined supplement containing vitamin B,2, L-glutamine, L-cysteine, and various other growth promoting compounds. It has a respiratory type of metabolism. The cultures grow optimally at 37°C after 3—5 days. All strains grow over a relatively narrow temperature range of 33—40°C, whereas some grow poorly at30°C and 42°C, none grow at 25°C. *H. pylori* will grow on a suitable culture medium over a wide pH range (5.5-8.5) with good growth between pH6.9 and 8.0. *H. pylori* does not tolerate low pH *in vitro (*Blaser and Atherton ,2004 *).*

**2.4. Virulence factors**

*Helicobacter pylori's* pathogenic properties are provided by its special ability to survive in a gastric acid milieu, it is able to move and multiply for decades in the mucus immediately adjacent to the apical pole of epithelial cells in spite of the local and cellular reaction that it causes with its host. The bacteria's virulence is

on the one side an effect of the direct action of its products, and on the other side of the induction and modulation of the associated inflammatory reaction .

Many studies on persons infected with *Helicobacter pylori* have evidenced numerous mechanisms through which the bacterium perturbs the local equilibrium of the gastric mucosa ( Nedrud *et al,* 2002 ).

1. *Helicobacter pylori's* motility is also involved in the infection's persistence colonization is possible even in the case of Helicobacter pylori variants that have mutations of the flagellins, but the preservation of the bacterial reserve in the mucus layer (chronic infection) necessitates the normal expression of both A and B flagellins. *Helicobacter pylori* bacteria adhere to the surface of gastric epithelial cells at the level of several membrane segments that contain cadherins, integrines and antigens of blood type H1 and Lewis ( Zhong *et al* , 2008 ).

2. The ammonia production (caused by the action of the bacterial ureasis) determines the increase of intracellular pH for the mucosal gastric cells in the superficial epithelium; the ammonia rapidly spreads through the cell membrane and reacts with H+ ions, forming ammonia ions that produce consecutive alkalinisation of the intra- cellular environment. Modifications of the pH lead to an increase of intracellular Ca2+ concentration that activates calcium-dependent intracellular cascades and results in the final release of chemotactic factors (especially interleukin 8 – IL8) for inflammatory cells (Zhong *et al* , 2008 ).

*Helicobacter pylori* produces proteases that break down glycoprotein of the mucus layer and phospholipases that damage the epithelial cells by direct interfering with the protective factors of the mucosa. Moreover, the phospholipases are directly involved in leucotrines release, thus multiplying the noxious effect upon the mucosa .

Once the inflammatory alteration of the gastric mucosa appears, other mechanisms intervene and accelerate local lesions. The afflux of PMN (a characteristic event for active gastritis) leads to mieloperoxidase release into the interstitial tissue, which reacts with the hydrochloric acid thus determining the

formation of hypochloric acid; the latter, combined with local ammonia transforms into ammonia hypochlorite. Both the hypochloric acid and the ammonia hypochlorite are extremely aggressive towards human cells and tissue, determining tissue necrosis ( D'elios *et al* 2004 ).

Colonization of the corporeal gastric mucosa determines modifications of the local histology. Chronic antral gastritis leads to a decrease in number of D cells (somatostatine producing cell); consecutive hypergastrinemia stimulates the proliferation of parietal cells in fundic and corporeal areas and implicitly gastric acid hypersecretion. The increase of the production of gastric acid implies an increased chlorhydro-peptic aggression of the duodenal mucosa and the increased risk of duodenal ulcer. Chronic corporeal gastritis eventually associates atrophy with subsequent disappearance of parietal cells and hyposecretion of acid ( Antony , 1999 ).

3. The vacuolization cytotoxin gene (VacA) is present in all HP types; the VacA gene codifies a protoxin of a molecular mass of approximately 140 KDa with a signal sequence in the antino- terminal position. Recent spectrometric analyses have proved that the real dimension of the cytotoxin is of 124 KDa, as it contains a peptide signal (3KDa), the secreted toxin (82.2 Kda) and the carboxyl-terminal domain (33 KDa) (4,9); only 45% of these produce an active cytotoxin, VacAs1, which can determine vacuolization of the epithelial cells; VacA is involved in the emergence of the gastric ulcer and gastric cancer; the infection with Type I HP (VacA+) leads to more serious gastric damage than the infection with Type II HP (VacA-) in both animals and humans; moreover, intragastric administration of VacA produces ulcerations of the gastric mucosa. Due to the action of VacA, intra- cytoplasmatic vacuoles in the gastric cells appear through the expansion of terminal endosomes; in the vacuoles' membranes one can identify vacuolar ATP- asis and rab7 (endocytic vesicles markers) or rab9 (lysosomes markers) (Konturk *et al* , 2003).

In vitro Vac A is associated to different phenotypes:

cytotoxicity causing vacuolization when VacA is accumulated in the endosome membranes of epithelial cells by apoptosis .

permeability of the cell membrane by the decrease of the trans-epithelial electric resistance of the cells and the increase of permeability for Fe3+ and Ni2+ ions.

the formation of a pore in the lipid membrane: the VacA monomers activate the acid pH and, once inserted in the lipid membrane, re- associate themselves at a membrane level to form a hexameric pore involved in the circulation of anions ( $HCO^{-}3$) .

In vivo the role of VacA cytotoxin is controversial. Studies done on animals did not confirm the role of VacA in the occurrence of epithelial lesions. The current opinion about in vivo activity of VacA cytotoxin is based on studies regarding the distribution of different alleles of the VacA gene in the strains isolated in clinical practice: two alleles from the cytotoxin's central area (m1 and m2) and two alleles from the area that codifies the signal sequence (s1 and s2) the alleles (s1 and s2, m1 and m2) was correlated with the level of VacA cytotoxicity; that is the *Helicobacter pylori* strains carrying types s1 and m1 present an intense cytotoxic activity while the strains containing the alleles s2 and m2 have no cytotoxic activity. Numerous studies have demonstrated that gastric infections with *Helicobacter pylori* strains containing VacA types1 alleles are associated to a much higher risk of developing an ulcerous disease than those containing s2 alleles. In conclusion, recent studies suggest that in the Occident the VacA s1, m1 strains are often associated to a severe pathology . The vacuolizant cytotoxin is involved in the development of gastric ulcer and cancer. Infection by HPT I (VacA+) produces a much more serious gastric affliction than the infection by HP Type II (VacA-) in both animals and humans ( Zhong *et al* , 2008 ).

4. The flagellin's genes – flaA, flaB. The *Helicobacter* mobility is an indispensable factor for bacterial colonization of the gastric mucosa. The researchers estimate that 80% of bacterial populations multiply in the mucus, the remaining 20% colonizing the entire surface of gastric epithelial cells . Among

the proteins involved in the biogenesis of the machine that ensures the mobility of the HP FlagE (the hook protein) and FlbA and FlgR were studied .

5. The ureasis genes – ure A and ure B, ure C and ure D, ure E, ure F, ure G, ure H and ure I. The ureasis is an essential determinant of the bacterial virulence. The nonureolitic mutants are incapable to colonize the gastric mucosa .The ureasis is codified by an operon that contains structural genes of the enzyme (ureA and ureB) and five other genes (ure IEFGH) whose products determine the enzyme's activation by incorporation of the nickel ions; an extremely compact ferric complex is created, thus offering the special acid resistivity to the enzyme. One of the unique characteristics of the ureasic operon in *Helicobacter pylori* is related to the presence of the gene ure I; it codifies a membrane protein involved in a membrane pore formation; this pore opens at low levels of pH and therefore allows the efficient transport of ure I when the bacterium is found in an acid milieu. Ure I is crucial for the bacteria and constitutes a sort of acidity sensor.

6. Adhesion factors. Several types of adhezines have been identified to date. Third adhezines permit HP to stick to the surface of epithelial cells. They are coded by the bab A gene, the bab P gene, the alp A and alp B and later by the gene sab A . The genome's analysis has revealed that all these genes belong to a family of 32 genes that codify the external membrane's proteins.

7. Pic B has the capacity to induce the production of interleukin IL8 (Labigne and Reuse) by the gastric epithelium.

8. Superoxide dismutase (SOD) – catalyses the transformation of superoxide and peroxide into hydrogen and oxygen ions; these are the enzymes that allow *H. pylori* to resist to the oxidative stress generated by phagocytes.

9. CagA, the gene of an associated cytotoxin codifies a protein with a high molecular weight(120–140 kda); the pathogenicity's island – the cag region, formed by zones cag I and cag II, is similar to those discovered in *Salmonella* or *Escherichia* coli. The *H. pylori* strains were classified in two categories – some

containing a complete and functional pathogenicity island (cag PAI) and others without any pathogenicity island or with a less active one. The island codifies a secretion system able to translocate a protein into cells, the immune-dominant protein CagA with a variable molecular mass (120–140kda) through which the bacterium is put in connection with the epithelial or macrophage cells. This contact between the CagA pathogen island and the epithelial cell has the following effects:

it induces the secretion of interleukins IL8, IL 10 and Il 12 by activating the nuclear factor kappa B; this effect supports the idea that the strains that contain this pathogenicity island determine an inflammatory response by far more superior to the others.

it produces the secretion and translocation of the CagA protein into cells, followed by the protein's phosphorylation.

it produces the re-arrangement of the cytoskeleton of cells that come in contact with it, followed by the creation of a pedestal-like structure and of the "humming bird"-type phenomenon (the elongation of the cells' shape).

it produces a cellular signal associated to the induction of transcriptional factors.

it determines the expression of the pr s the expression of the proto- oncogenes f. fos and c-jun .Many studies have correlated the presence of CagA to a severe inflammatory pathology, and a diverse pathology varying from duodenal ulcer to gastric adenocarcinoma, situation applied especially to the occidental population .

**2.5Transmition**

It is not known how *H. pylori* is transmitted or why some patients become symptomatic while others do not. The bacteria are most likely spread from person to person through fecal-oral or oral-oral routes. Possible environmental reservoirs include contaminated water sources. Iatrogenic spread through contaminated endoscopes has been documented but can be prevented by proper cleaning of equipment ( Nedrud j *et al* , 2002 ).

## 2.6 Pathophysiology

Adaptation to the stomach's acidic environment to avoid the acidic environmet of the interior of the stomach (lumen), *H. pylori* uses its flagella to burrow into the mucus lining the stomach to reach the epithelial cells underneath, where there is a more neutral pH. *H. pylori* is able to sense the pH gradient in the mucus and move towards the less acidic region (a process called 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. *H. pylori* is 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. One such adhesion is BabA, which binds to the Lewis b antigen displayed on the surface of stomach epithelial cells. In addition to using chemotaxis to avoid areas of low pH, *H. pylori* also neutralizes the acid in its environment. It does this by producing large amounts of urease, which breaks down the urea present in the stomach to carbon dioxide and ammonia. The ammonia, which is basic, then neutralizes stomach acid. by inducing Inflammation, gastritis, and ulcer *H. pylori* harms the stomach and duodenal linium (Guarner *et al* , 2003 ) .

## 2.7 Pathology

More than 50% of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries.

*H. pylori* helix shape (from which the generic name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach (Yamaoka ,2008).

Colonization and long-term persistence of *H. pylori* can induce a complex immune response that can potentiate severe mucosal damage, including atrophy , intestinal metaplasia and dysplasia . This makes *H. pylori* the etiologic agent of

acute and chronic gastritis, peptic ulcer disease (75% of gastric ulcers and 90% of duodenal ulcers),and two forms of gastric cancer (mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma)( Ernst *and Gold* ;2000). The association with the development of two forms of cancer led to the classification of *H. pylori* by the World Health Organization as the only bacterial class I carcinogen (Yamaoka ,2008).

### 2.7.1 Acute and Chronic Gastritis

Colonization with *H. pylori* virtually always leads to infiltration of the gastric mucosa in both antrum and corpus with neutrophilic and mononuclear cells . This chronic active gastritis is the primary condition related to *H. pylori* colonization, and other *H. pylori*-associated disorders in particular result from this chronic inflammatory process ( Perez-perez *et al* , 2005 ).

### 2.7.2 Acute Gastritis

The acute phase of colonization with *H. pylori* may be associated with transient nonspecific dyspeptic symptoms, such as fullness, nausea, and vomiting, and with considerable inflammation of both the proximal and distal stomach mucosa, or pangastritis (Kajkawah *et al* , 2007 ). This phase is often associated with hypochlorhydria, which can last for months. It is unclear whether this initial colonization can be followed by spontaneous clearance and resolution of gastritis and, if so, how often this occurs. Follow up studies of young children with serology or breath tests suggested that infection may spontaneously disappear in some patients in this age group , this has not been observed in adults other than under specific circumstances, such as development of atrophic gastritis( Perez-Perez *et al* , 2005 ) .

### 2.7.3 Chronic Gastritis

When colonization does become persistent, a close correlation exists between the level of acid secretion and the distribution of gastritis . This correlation results from the counteractive effects of acid on bacterial growth versus those of bacterial growth and associated mucosal inflammation on acid secretion and

regulation. This interaction is crucial in the determination of outcomes of *H. pylori* infection. In subjects with intact acid secretion, *H. pylori* in particular colonizes the gastric antrum, where few acid secretory parietal cells are present. This colonization pattern is associated with an antrum predominant gastritis. Histological evaluation of gastric corpus specimens in these cases reveals limited chronic inactive inflammation and low numbers of superficially colonizing *H. pylori* bacteria. Subjects in whom acid secretion is impaired, due to whatever mechanism, have a more even distribution of bacteria in antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus predominant pangastritis. The reduction in acid secretion can be due to a loss of parietal cells as a result of atrophic gastritis, but it can also occur when acid secretory capacity is intact but parietal cell function is inhibited by acid suppressive drugs, in particular, proton pump inhibitors (PPIs) (Kuipers *et al*,1995 b ). .

**2.7.4 Peptic Ulcer**

Gastric or duodenal ulcers (commonly referred to as peptic ulcers) are defined as mucosal defects with a diameter of at least 0.5 cm penetrating through the mucus mucosa ( Engel *et al* , 1995 ).Both gastric and duodenal ulcer diseases are strongly related to *H. pylori*. In initial reports from all over the world in the first decade after the discovery of *H. pylori*, approximately 95% of duodenal ulcers and 85% of gastric ulcers occurred in the presence of *H. pylori* infection (Fox *et al,* 2002) .

**2.7.5 *Helicobacter* and cancer**

Two related mechanisms by which *H. pylori* could promote cancer are under investigation (Axon , 2007).

One mechanism involves the enhanced production of free radicals near *H. pylori* and an increased rate of host cell mutation. The other proposed mechanism has been called a "perigenetic pathway" and involves enhancement of the transformed host cell phenotype by means of alterations in cell proteins such as

adhesion proteins. It has been proposed that *H. pylori* induces Inflammation and locally high levels of TNF-alpha and/or interleukin 6. According to the proposed perigenetic mechanism, inflammation-associated signaling molecules such as TNF-alpha can alter gastric epithelial cell adhesion and lead to the dispersion and migration of mutated epithelial cells without the need for additional mutations in tumor suppressor genes such as genes that code for cell adhesion proteins (Kuipers *et al* ,1995a) .

## 2.8 Diagnosis of *H. pylori* Infection

Several diagnostic tests are used to detect *Helicobacter pylori* infection (Table 2.1 ). These tests, including invasive and noninvasive techniques, have high sensitivity and specificity. The advantages of the various techniques are described below (Benjamin *et al* , 2000 ).

### 2.8.1 Invasive Techniques

#### 2.8.1.1 Culture

Because of fastidious nature of *H. pylori*, culturing the bacterium is often tedious and is no more sensitive or specific than simple histologic analyses. Culturing *H. pylori* also involves the cost of endoscopy, making the method even less practical.

#### 2.8.1.2 Histologic analysis of biopsy.

Routine histologic analysis of biopsy samples is common and practical. This technique is helpful, because one can visualize the mucosa, permitting detection of histologic gastritis and lesions such as MALT-type lymphomas, which are tumors of lymphoid tissues. There are, however, clear drawbacks that should be considered. First, the organism may have a patchy distribution, especially at the base of the stomach, so more than two biopsy specimens are necessary for accurate results. Also, standard staining techniques (i.e., eosin staining) are usually unreliable for detection of *H. pylori* by microscopy. Adding to the impracticality of this method is that it requires endoscopy and diagnosis cannot be obtained until several days after the procedure **(** Chev and Lai , 2009) .

### 2.8.1.3 *Camplyobacter*-like organism (CLO) test

This test is based on the fact that mucosal biopsy specimens can be inoculated into a medium containing urea and phenol red, a dye that turns pink in a pH of 6.0 or greater (Axon, 1996 ). The pH will rise above 6.0 when *H. pylori*, the *Campylobacter*-like organism, metabolizes urea to ammonia by way of its urease activity. This test is commercially available and therefore quite inexpensive. Only one-half hour is required for diagnosis of infection, and the test has shown 98% sensitivity and [100% specificity. These qualities have made the CLO test the invasive technique of choice for diagnosing *H. pylori* infection (Benjamin *et al* , 2000 ).

### 2.8.2 Noninvasive Technique

### 2.8.2.Breath test

Although *H. pylori* itself cant be detected noninvasively, its urease activity can be detected by way of a breath test. In this test, urea that is radioactively labeled with carbon 13 and carbon 14 is ingested(Benjamin *et al* , 2000 ). Bacterial urease splits off labeled carbon dioxide, which can be detected in the breath. Accuracy is not a problem for either of these breath tests, since both elicit 100% sensitivity and specificity. The breath test technique reflects only current infection with *H. pylori* but can demonstrate very rapidly the existence of infection. A disadvantage of this technique is that it may involve a small amount of exposure to radiation. Although carbon 13 is a stable isotope and does not emit radiation, its detection requires a mass spectrometer, which may not readily available. The breath test is not yet commercially available(Benjamin *et al* , 2000) .

### 2.8.2.2 Detection of IgG antibody

When a host recognizes *H. pylori* an immune response immediately stimulates IgG and secretory antibody IgA. Therefore, serologic testing for antibodies to H. pylori using the enzyme-linked immunosorbent assay (ELISA) has become widely accepted diagnostic test. The test is simple, inexpensive, and readily available. ELISA detects IgG with a sensitivity of up to 99% and is 100% specific. Since spontaneous clearing of *H. pylori* by lgG or IgA is rare, an elevated antibody titer indicates current infection. This test also detects the decline in antibody titer after removal of the organism; however, the rate of decline of IgG after eradication is still not known. This technique, although useful and accurate, still has certain limitations. In order to determine a clear decline in antibody titer, the patient must be monitored for at least six months, and the cutoff for a significant decline is unclear. In addition, in order to control the inherent variability of the test, the base and follow-up titer must be measured simultaneously. Still, the outstanding accuracy and low cost makes this test an attractive choice for detecting *H. pylori* infection Benjamin (*et al* , 2000 ) .

In current practice, endoscopy is still required for diagnosis of infection by *Helicobacter pylori.* The full range of noninvasive techniques is expected to be more readily available soon, with the antibody tests ideal for assessing current infection, and the carbon 13-urea breath test the method of choice for determining the response to infection (Benjamin *et al* , 2000 ).

**Table2.1** DiagnosticTests for *Helicobacter pylori*

| Test | Sensitivity (%) | Specificity (%) | Endoscopy needed? | Relative cost |
|---|---|---|---|---|
| Culture | 77–92 | 100 | Yes | High |
| Histologic study | 93–99 | 95–99 | Yes | High |
| Rapid urease test (*Campylobacter*-like organism test) | 89–98 | 93–100 | Yes | Low |
| Serologic study | 88–99 | 86–100 | No | Low |
| Carbon 13 urea breath test | 90–100 | 98–100 | No | Moderate |
| Carbon 14 urea breath test | 90–97 | 89–100 | No | Moderate |

## 2.9 Treatment of Peptic Ulcer Disease

Many excellent treatments for peptic ulcer disease exist. In the case of duodenal ulcers and gastric ulcers, histamine H2-receptors can be blocked to cause healing in about 90% of cases within 8 weeks. However, both duodenal and gastric ulcers rapidly recur after successful anti-secretory therapy. Relapses can be prevented by long-term low-dose therapy with any histamine H2-receptor blockers. The National Institute of Health recommends that all patients infected with *H. pylori* be treated with an antibiotic. However, although the bacterium is sensitive to most antimicrobial therapy in vitro, in vivo results have been disappointing. Researchers have attributed this discrepancy to the locale of *H. pylori* infection, under the mucus gel layer in the stomach. Environments which are this acidic often decrease the antimicrobial activity of most antibiotics ( Axon, , 1996) .

### 2.9.1 Triple Therapy

Eradication of *Helicobacter pylori* is defined as the absence of the organism four or more weeks after eradication therapy. Since the eradication rate for single-drug therapy is only 19% and that for double-drug therapy is still only 48%, researchers have found that combining three antibiotics offers a better chance for eliminating the bacterium. The highest eradication rate, 82%, was achieved by combining bismuth, metronidazole, and tetracycline (Fig.1). There are obvious drawbacks to this type of treatment. First of all, it is inconvenient for the patient, so it is difficult for doctors to convince their patients to comply with the therapy. Second, such multidrug therapy is almost always associated with many adverse side effects, namely diarrhea, nausea, and vomiting, which occur in approximately 20% of all patients ( Logan and Walker , 2001).

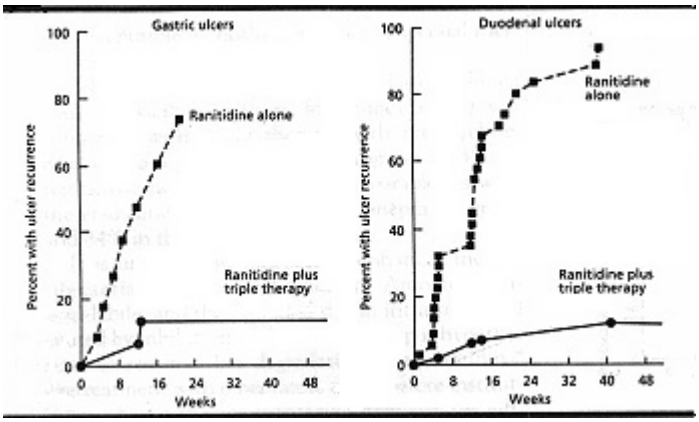


**Fig . 1** Recurrence rate of gastric ulcers and duodenal ulcers after successful he aling with triple antimicrobial therapy

### 2.10 Immune Response

The human immune response to *H. pylori* involves the activation of neutrophils, monocytes and macrophages, and the production of serum antibody IgG and secretory antibody IgA. In addition, T cells proliferate as in a cell mediated response. However, as stated earlier,*Helicobacter pylori* infection, once acquired, persists indefinitely.,(Wyat and Rathbone, 1988 ). Therefore, although there is a definite and immediate immune response to *H. pylori*, the host is still unable to eliminate the parasite.

The intensity of the host immune responses can culminate in one of several ways:

The most common result is chronic superficial gastritis, which is an inflammation of the stomach lining due to the infiltration of lymphocytes, plasma cells, eosinophils, and monocytes into the mucosal lining of the stomach, which causes injury to the gastric glands.

The immune response can actually benefit *H.pylori* by releasing nutrients locally for the organism.

The host could be harmed by the immune response due to the direct damage of epithelial cells, which affects their function and vitality.

The host, in order to avoid this type of cell damage, will often down-regulate its immune response, making it even more difficult to completely eliminate *H.*

*pylori* from the affected area .

The immune response can also cause inflammation of the duodenum, leading to duodenal ulcers.

Atrophic gastritis, which is a nonspecific inflammation of the entire lining of the stomach, may be the result of the infiltration of lymphocytes into the stomach.

MALT-type and other lymphomas, which are tumors of the mucosal and lymphoid tissues, can also result from *H. pylori* infection.

The effects of infection by *Helicobacter pylori* represent a delicate equilibrium between the host's inability to remove the organism and its ability to contain the damage caused by the pathogen. It is the integrity of this equilibrium that allows *H. pylori* to persist in most cases for a lifetime in their hosts ( Wyatt and Rathbone 1988 ) .

**2.11 Other body site *H.pylori* may found**

stomach was supposed to be the only reservoir of infection in humans. Nevertheless *H. pylori* infection was detected in other sites recently. It was found in dental plaque and saliva and also in oropharyngeal lymphatic tissue. This finding is of great importance because of kno,iown carcinogenic potential of *H. pylori*. It was declared type I carcinogen by IARC . The question of direct contribution of *H. pylori* to oral and oropharyngeal diseases was not resolved yet.

**2.12 Prevention**

Since the source of *H. pylori* is not yet known,recommendations for avoiding infection have not been made. In general, it is always wise for persons to wash hands thoroughly, to eat food that has been properly prepared, and to drink water from a safe,clean source (Broutet *et al* ,2001).

## 2.13 Control and Prevention

CDC, with partners in other government agencies, academic institutions, and industry, is conducting anational education campaign to inform health care providers and consumers of the link between *H. pylori* and stomach and duodenal ulcers. CDC is also working with partners to study routes of transmission and possible prevention measures, and to establish an antimicrobial resistance surveillance system to monitor the changes in resistance among *H. pylori* strains in the United States (Broutet *et al* ,2001).

## 3. Materials and Methods

### 3.1. Study design

A descriptive , cross sectional study .

### 3.2. Study area and duration

The Study was carried out at the Military Teaching Hospital (MTH) and Omdurman Teaching Hospital (OTH) . The study was carried out during the period from April 2013 to January 2014 .

### 3.3. Study population

Samples had been collected from patients with gastroduodenitis and peptic ulcer attending to MTH and OTH .

### 3.4. Sampling technique

Patients with gastroduodenitis and peptic ulcer were randomly based on non-probability convenience sampling technique .

### 3.5. Sample size

Fifty-Seven (n =57) endoscopy biopsies were collected randomly from patients .

### 3.6.Gender

Male 34 .

Female 23 .

### 3.7.Inclusion criteria

Patients with gastroduodenitis and peptic ulcer attending the MTH and OTH were included.

### 3.8. Exclusion criteria

Patients under antibiotic treatment against *H . pylori* were excluded .

### 3.9. Data collection

Data were collected through a self administered questionnaire. Questionnaire was designed to record demographical clinical data .

### 3.10. Ethical consideration

Permission to conduct this study was obtained from College of Graduate Studies,

Sudan University of Science and Technology and verbal consent were obtained from patients and heads of endoscopies units at MTH and OTH .

### 3.11. Data analysis

Collected data were analyzed using the statistical package of social science (SPSS) program . Chi-square statistical analysis were used to determine *P. value* significance range .

### 3.12. Laboratory work

Polymerase chain reaction (PCR ) was used to detect Urease C (ure C) gene of *H.pylori* .

#### 3.12.1. Collection and Transport of Specimens

Upper endoscopy was performed and multiple gastric biopsy specimens were taken from the stomach antrum and the corpus and the duodenum . Transport of specimens were preserved and transported in normal saline (Boulos *et al* , 2002 ).

#### 3.12.2. DNA extraction

DNA was extracted using Vivantis GF-1 Nucleic acid extraction kit (Vivantis, MALAYSIA). This kit applies the principle of a spin mini-column technology and the use of optimized buffers ensure that only DNA and/or RNA is isolated while cellular proteins, metabolites, salts and other impurities are removed during subsequent washing steps. First, endoscopy biopsy samples were collected and extracted in plain container , followed by a lysis buffer and proteinase K . The DNA was extracted according to the manufacturer's instructions. Finally, the DNA was eluted in 200 µl elution buffer provided with the kit (Fig.3.1).

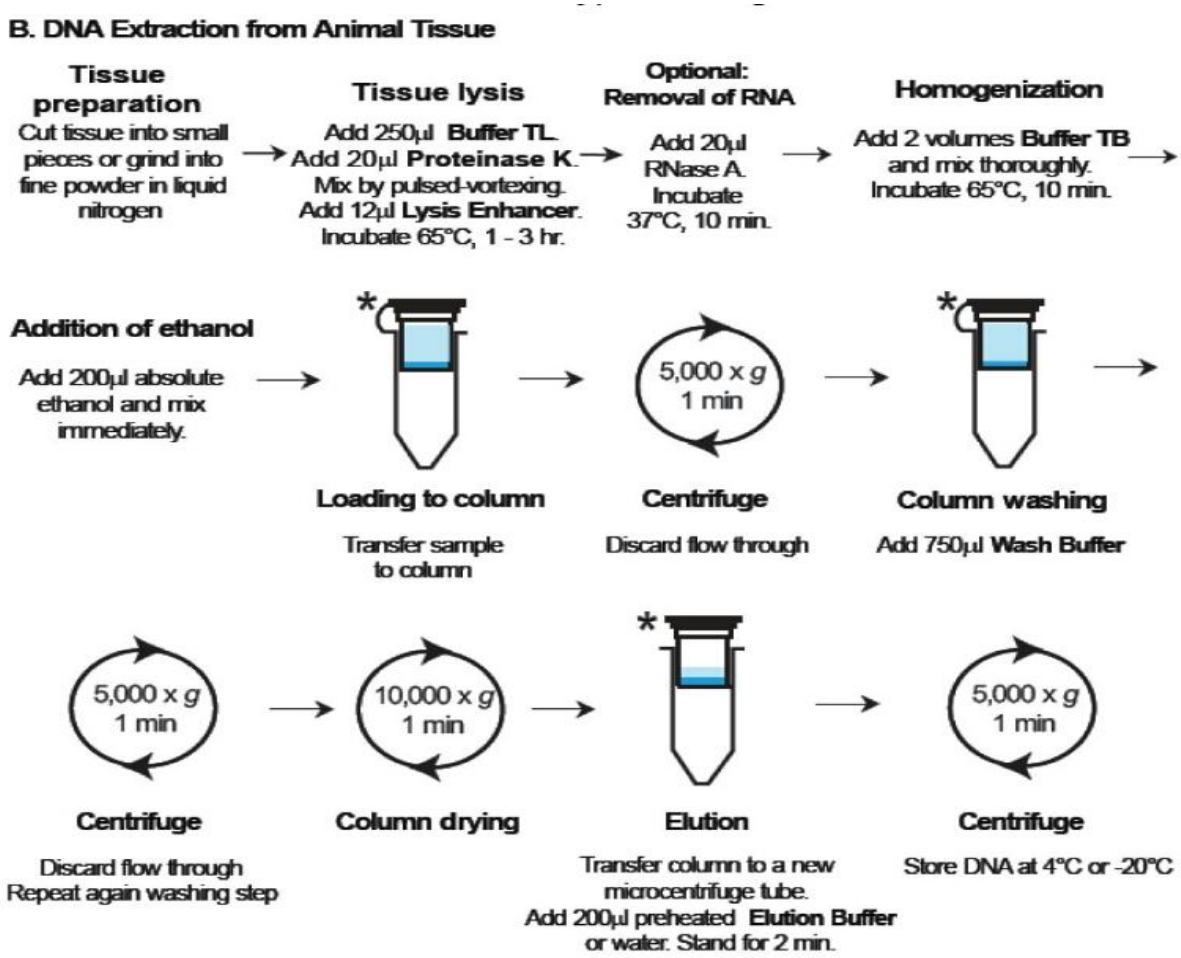


**Fig 3.1** DNA extraction procedure

### 3.12.3. Target amplification by Polymerase Chain Reaction (PCR)

The amplification and detection of Urease C gene of *H . pylori* was carried out by PCR method in a thermal cycler. For amplification, 5 µL of Urease C gene of H . pylori was added to a 15-µL reaction mixture. *H . pylori* primers (*H.pylori*-F:5'-AAGCTTTTAGGGGTGTTAGGGGTTT -3', *H.pylori* -R: 5'-AAGCTTACTTTCTAACACTAACGC -3') were used for the PCR reaction. The PCR reaction mixture contained in addition to DNA, 1µl of each primer, and Go Taq ready-to-use master mix (iNtRON Biotechnology) in a 20 µL total reaction volume . Thermocycled for 37 cycles (2 min denaturing step at 94 °C , 2 min annealing step at 55°C , and 2 min elongation step at 72 °C), and final extension was done at 72°C for 5 min.

### 3.12.4 PCR Products Detection

10 -μL amounts of each PCR mixture separated in a 1.5% agarose gel, then stained with ethidium bromide and viewed under gel documentation system . A result was considered positive when a band of the size 295 bp was visible in the gel. Standard procedures for reducing contamination were strictly followed.

## 4. Results

### 4.1. Frequences of Urease C Gene of *H. pylori* in patients with gastroduodenitis and peptic ulcer

Out of the 57 patients examined by conventional PCR, 13 were found positive (22.8 %) for Urease C Gene of *H. pylori*.

### 4.2. The effect of age of patients on detection of Urease C Gene of *H. pylori*

Table 4.1 Displays that, high rate of Urease C Gene of *H.pylori* was detected in the age group 61-80 year . However , 9/34 (26.4%), 1/17 (5.9%), and 3/6 (50%) were found *H.pylori*-positive among age groups 21-40 year, 41-60 and 61-80 years, respectively, with no significant difference (P value = 0.062 ) between them.

### 4.3. The effect of gender of the study populations infected with *H. pylori*

**The results in table 4.2** Showed that 10 out of 34 males (29.4 %) were found Urease C Gene of *H.pylori* - positive and 3 out of 23 females (13 %) were shown positive for Urease C Gene of *H.pylori*. There was no significant difference between the two genders ( *P value* = 0.148 ).

**Table 4.1** The effect of age of patients on detection of *H.pylori* .

| Age groups | *H. pylori* | | | | Total |
|---|---|---|---|---|---|
| | Positive | | Negative | | |
| | No. | % | No. | % | |
| 21-40 | 9/34 | 26.4 | 25/34 | 73.5 | 34 |
| 41-60 | 1/17 | 5.9 | 16/17 | 94.1 | 17 |
| 61-80 | 3/6 | 50 | 3/6 | 50 | 6 |
| Total | 13/57 | 22.8 | 44/57 | 77.2 | 57 |

**Table 4.2** The effect of gender of patients on detection of *H.pylori*

| | | Sex | | Total |
|---|---|---|---|---|
| | | Males | Females | |
| *H. pylori* DNA | No | 10/34 | 3/23 | 13/57 |
| | % | 29.4% | 13% | 22.8% |

( *P* value = 0.060 )

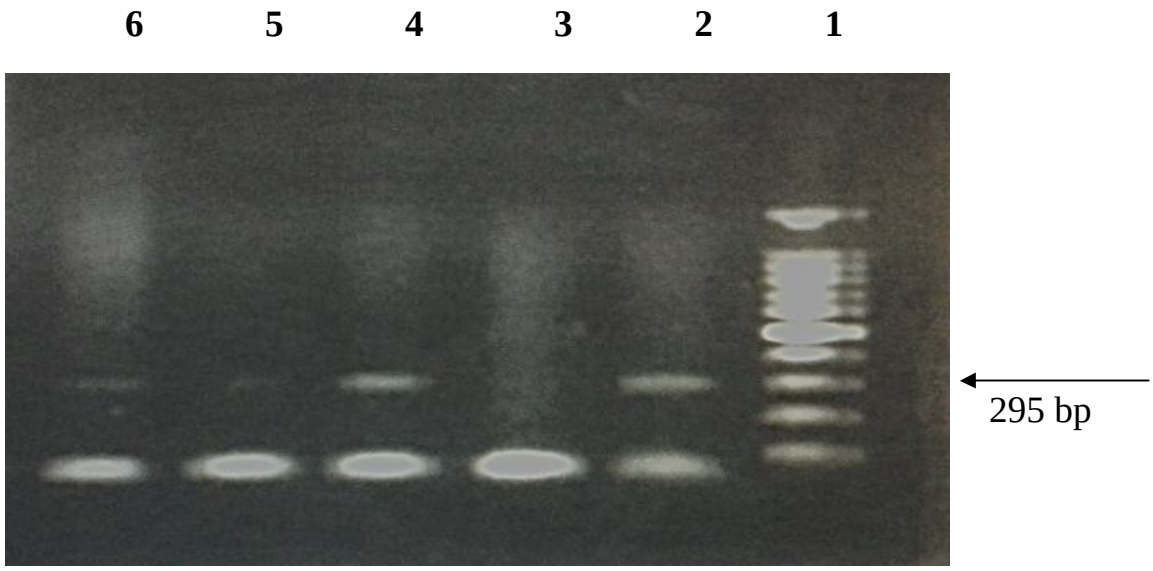


**Fig 4.3** Gel electrophoresis of *H. pylori* DNA PCR product.
Lane no. 1 contains 100-bp DNA ladder. Lane no. 2 contains control positive, other lanes 4,6 contains positive samples (band appear at 295 bp ) .

## 5. Discussion

In this study it was observed that *H. pylori* in gastric biobsy of 22.8% gastrodoudunitis patients, and this findings showed that there is apossible an association between *H.pylori* and stomach gastritis .

Fifty seven patints were randomly tested for the present study, 34 of them were males (59.6%), and 23 were females (40.4%), with mean age of 43 year.

In sudan , published studies related to our study were many few . However, Abbas Bakhit (2013) obtained result agreed this study , where the rate of infection (21.1%) , also Esfahani *et al* (2008) in Iran , where the rate of (22.8%). The results obtained in the study weren't agreed to Annika *et al* ( 1997 ) in Britain, where the rate of *H. pylori* infection was reported as 90%. Also high rate of *H. pylori* infection were reported by Richard *et al* (1994) in USA , Ousman *et al* (2011) in West Africa and Weiss *et al* (1994) in USA .

Although PCR is a very sensitive and specific method capable of detecting scarce bacterial amounts, its results depends on the primer's specificity and sensitivity. For example in a PCR test for detection of *H. pylori* in the gastiric biobsy the used primers related to the bacterial urease activity will be confounding factor. Last but not the least, (Nguyen *et al* 1995)

This variation in results can be due to ethnic variation, feeding habitats and the PCR related methodology.

## 5.2Conclusion

The findings of this study indicate that there is an association between gastroduodenitis and peptic ulcer with *H.pylori* infection .

## 5.3Recommendations

1- Gastroduodenitis patients should routinely be screened for *H. pylori* .

2-*H. pylori* DNA detection should be carried out in gastroduodenitis patients who tested negative for *H. pylori* antibodies to confirm the result .

3- Larger sample size is needed to accurately determine the rate of infection.

4-Sequencing and phylogenetic analysis of *H. pylori* DNA should be done among gastroduodenitis patients in Sudan.

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# Appendix 1

**GF-1 Tissue DNA Extraction Kit**

The GF-1 Tissue DNA Extraction Kit is designed for rapid and efficient purification of genomic DNA from up to 5 x 106 cultured animal cells and various organs such as kidney, heart, lungs, brain, muscles, liver, spleen, etc without the need for precipitation or organic extractions. This kit uses a specially-treated glass filter membrane fixed into a column to efficiently bind DNA in the presence of high salt. This kit applies the principle of a minicolumn spin technology and the use of optimized buffers to ensure that only DNA is isolated while cellular proteins, metabolites, salts and other low molecular weight impurities are removed during the subsequent washing steps. High-purity genomic DNA is eluted in water or low salt buffers and has a A260/280 ratio between 1.7 and 1.9 making it ready to use in many routine molecular biology applications such as restriction enzyme digestion, Southern blotting, PCR,DNA fingerprinting and other manipulations.


**GF-1 Tissue DNA Extraction Kit**

**Additional Materials to be Supplied by User**

Absolute Ethanol (>95%)

Phosphate Buffered Saline (PBS)

**Reconstitution of Solutions**

The bottle labeled **Wash Buffer** contains concentrated buffer which must be diluted with

absolute ethanol (>95%) before use.

For **GF-TD-100 (100 preps)**

Add **56ml** of absolute ethanol into one of the bottles labeled **Wash Buffer**.

Add **56ml** of absolute ethanol into the other bottle labeled **Wash Buffer** only prior to use.

Store **Wash Buffer** at room temperature with bottle capped tight after use.

**Storage and Stability**

1. Store solutions at 20°C - 30°C.
2. Store Proteinase K at -20°C.
3. Kit components are guaranteed to be stable for 12 months from the date of manufacture.

Thermocycler,  PCR machine or DNA amplifiers

Gel documentation system


Taq ready-to-use master mix (iNtRON Biotechnology)

## Appendix 2

### Sudan University of Science and Technology

### Questionnaire

**Molecular Detection of *Helicobacter pylori* in Patients with Gastroduodenitis and Peptic Ulcer from in Khartoum State .**

Name :…… NO (……..)

Age :…….. Sex:……

residence :……

Job :…….

**Medical history :**

Epigastric pain ☐ Hunger pain ☐

Using of antibiotic ☐ Using of proton pump inhibitor ☐

**Eating habits:**

……

……

…….....

**Endoscopy result :**

Gastritis ☐ Duodenitis ☐

Gastric ulcer ☐ Duodenal ulcer ☐

**PCR**

**result** :……

……..