Chapter one

1. Introduction

1.1. Introduction

Helicobacter pylori is a Gram negative, spiral, flagellated bacterium with the capability of abundant urease production. *H. pylori* bacterium is usually found under the mucus layer in the gastric pits and in close opposition to gastric epithelial cells (Malfertheiner *et al.*, 2007).

Since the discovery of *H. pylori* by Warren and Marshall, it has been evidently demonstrated that the organism plays a major role in several upper gastrointestinal diseases which present as dyspepsia (Arents *et al.*, 2002).

Helicobacter pylori infection causes chronic active gastritis in the antrum (antral gastritis), the corpus (corpus gastritis) or in both (pan gastritis). It is a major etiological factor in peptic ulcer disease, gastric carcinoma, and gastric mucosal associated lymphoid tissue (MALT) Lymphoma, Hemorrhage and perforation are the most frequent complications of peptic ulcer disease and are associated with substantial morbidity, mortality and health care costs (Van Keeken *et al.*, 2006). Peptic ulcer disease can be cured by eradicating *H. pylori* so that complications no longer occur (Malfertheiner *et al.*, 2007).

There are various diagnostic tests for *H. pylori* which can be broadly classified into invasive and noninvasive tests (Holcombe, 1999). Invasive tests utilize endoscopic gastro duodenal biopsy samples for histology, culture, rapid urease test (RUT), polymerase chain reaction and fluorescent in-situ hybridization. The non-invasive tests do not require endoscopy; they include urea breath test, immunoglobulin G, A and M serology, stool antigen test, saliva antibody test and urinary antibody test (Miwa and Sato, 2000).

1.2. Literature review

1.2.1. History

By the late 19th and early 20th centuries, several investigators had reported the presence of spiral microorganisms in the stomachs of animals (Bizzozero, 1893). Soon afterward, similar spiral bacteria were observed in humans, some of whom had peptic ulcer disease or gastric cancer. The etiological role of these bacteria in the development of peptic ulcer disease and gastric cancer was considered at the time, and patients were sometimes even treated with high doses of the antimicrobial compound bismuth (Pel, 1899). This possibility was later discarded as irrelevant, probably because of the high prevalence of these spiral bacteria in the stomachs of persons without any clinical signs. The bacteria observed in human stomachs were thus considered to be bacterial overgrowth or food contaminants until the early 1980s. At this time, Warren and Marshall performed their groundbreaking experiments, leading to the identification of a bacterium in 58 of 100 consecutive patients, with successful culture and later demonstration of eradication of the infection with bismuth and either amoxicillin or tinidazole (Marshall *et al.*, 1987). The organism was initially named "*Campylobacter*-like organism,""gastric *Campylobacter*-like organism," "*Campylobacter pyloridis*" and "*Campylobacter pylori*" but is now named *Helicobacter pylori* in recognition of the fact that this organism is distinct from members of the genus *Campylobacter* (Goodwin *et al.*, 1989). It soon became clear that this bacterium causes chronic active gastritis, which in a subset of subjects may progress to other conditions, in particular, peptic ulcer disease, distal gastric adenocarcinomas, and gastric lymphomas (Ernst and Gold, 2000).

1.2.2. Microbiology

H. pylori is a helix-shaped (classified as a curved rod, not spirochete), Gram-negative bacterium, about 3 micrometers long with a diameter of about 0.5 micrometers. It is microaerophilic; that is, it requires oxygen. It contains a hydrogenase which can be used to obtain energy by oxidizing molecular hydrogen (H_2) produced by intestinal bacteria (Olson and Maier, 2002). It produces Oxidase, catalase, and urease. It is capable of forming and can convert from spiral to a possibly viable but non-culturable coccoid form, both likely to favor its survival and be factors in the epidemiology of the bacterium (Chan *et al.*, 1994).

1.2.3. Genome

Study of the *H. pylori* genome is centered on attempts to understand pathogenesis, the ability of this organism to cause disease. Approximately 29% of the loci are in the "pathogenesis" category of the genome database.

This pathogenicity island is usually absent from *H. pylori* strains isolated from humans who are carriers of *H. pylori*, but remain asymptomatic (Baldwin *et al.*, 2007). The acytotoxin- associating gene A (CagA) gene codes for one of the major *H. pylori* virulence proteins. Bacterial strains that have the *cagA* gene are associated with an ability to cause ulcers (Broutet *et al.*, 2001).

The *cag* pathogenicity island (PAI) has about 30 genes, part of which code for a complex type IV secretion system. The low GC-content of the *cag* PAI relative to the rest of the *Helicobacter* genome suggests the island was acquired by horizontal transfer from another bacterial species (Tomb *et al.*, 1997).

1.2.4. Transmission

H. Pylori is contagious, although the exact route of transmission is not known. Person-to-person transmission by either the oral-oral or fecal-oral routes most likely (Cave, 1996). Transmission occurs mainly within families in developed nations yet can also be acquired from the community in developing countries (Delport and Vander Merwe, 2007). *H. pylori* may also be transmitted orally by means of fecal matter through the ingestion of waste-tainted water, so a hygienic environment could help decrease the risk of *H. pylori* infection (Brown, 2000).

1.2.5. Pathophysiology

H. pylori produce 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). Some strains of *H. pylori* produce a vacuolating cytotoxin A (VacA), and a cytotoxin- associating gene A (CagA). The CagA gene is a marker for strains that confer an increased risk of both peptic ulceration and gastric malignancy; although other factors play a role as strains lacking the toxin can still cause gastritis (Ketley, 2007).

Colonization of the stomach by *H. pylori* can result in chronic gastritis, an inflammation of the stomach lining, at the site of infection (Dumrese *et al.*, 2009).

Ulcers in the stomach and duodenum result when the consequences of inflammation allow stomach acid and the digestive enzyme pepsin to overwhelm the mechanisms that protect the stomach and duodenal mucous membranes (Dixon, 2000).

Two related mechanisms by which *H. pylori* could promote cancer are under investigation. 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 "per genetic pathway", and involves enhancement of the transformed host cell phenotype by means of alterations in cell proteins, such as adhesion proteins (Tsuji *et al.*, 2003).

1.2.6. Symptoms

Over 80% of people infected with *H. pylori* show no symptoms or copmlication (Bytzer *et al.*, 2011). Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea, where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: stomach pains, nausea, bloating, belching and sometimes vomiting (Ryan and Kenneth, 2010).

Individuals infected with *H. pylori* have a 10 to 20% lifetime risk of developing peptic ulcers and a 1 to 2% risk of acquiring stomach cancer (Kusters *et al.*, 2006). Inflammation of the pyloric antrum is more likely to lead to duodenal ulcers, while inflammation of the corpus (body of the stomach) is more likely to lead to gastric ulcers and gastric carcinoma (Suerbaum and Michetti, 2002).

1.2.7. Treatment

Once *H. pylori* is detected in a person, the standard first-line therapy is a one week "triple therapy" consisting of proton pump inhibitors such as omeprazole and the antibiotics clarithromycin and amoxicillin (Olczak *et al.*, 2002). Variations of the triple therapy have been developed over the years, such as using a different proton pump inhibitor, as with pantoprazole or rabeprazole, or replacing amoxicillin with metronidazole for people who are allergic to penicillin (Suerbaum and Michetti, 2002). Such a therapy has revolutionized the treatment of peptic ulcers and has made a cure to

the disease possible; previously, the only option was symptom control using antiacids, H_2-antagonists or proton pump inhibitors alone (Shiotani and Graham, 2002).

1.2.8. Prevention

H. pylori is a major cause of certain diseases of the upper gastrointestinal tract. Rising antibiotic resistance increases the need to search for new therapeutic strategies; this might include prevention in form of vaccination (Selgrad and Malfertheiner, 2008). Much work has been done on developing viable vaccines aimed at providing an alternative strategy to control *H. pylori* infection and related diseases, including stomach cancer (Blanchard and Nedrud, 2010). 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 (Tsuji *et al.*, 2003). An economic evaluation of the use of a potential *H. pylori* vaccine in babies found that its introduction could at least in the Netherlands prove cost-effective for the prevention of peptic ulcer and stomach cancer (De Vries *et al.*, 2009). A similar approach has also been studied for the United States (Rupnow *et al.*, 2009).

1.2.9. Prognosis

Approximately 10-20% of those colonized by *H. pylori* will ultimately develop gastric and duodenal ulcers. *H. pylori* infection is also associated with a 1-2% lifetime risk of stomach cancer and a less than 1% risk of gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Kusters *et al.*, 2006). In the absence of treatment, *H. pylori* infection—once established in its gastric niche is widely believed to persist for life (Brown, 2000).

1.2.10. Epidemiology

At least half the world's population is infected by the bacterium, making it the most widespread infection in the world. Actual infection rates vary from nation to nation; the developing world has much higher infection rates than the West, where rates are

estimated to be around 25%. Infections are usually acquired in early childhood in all countries. However, the infection rate of children in developing nations is higher than in industrialized nations, probably due to poor sanitary conditions. In developed nations it is currently uncommon to find infected children, but the percentage of infected people increases with age, with about 50% infected for those over the age of 60 compared with around 10% between 18 and 30 years (Pounder and Ng, 1995). The higher prevalence among the elderly reflects higher infection rates when they were children rather than infection at later ages (Kusters *et al.*, 2006).

However, antibiotic resistance is appearing in *H. pylori*; there are already many Metronidazole- and Clarithromycin-resistant strains in most parts of the world (Mégraud, 2004).

1.2.11. Laboratory Diagnosis

1.2.11.1 Invasive Testing Through Endoscopy

1.2.11.1.1 Biopsies and Histopathology

The definitive diagnosis of *H. pylori* and the evidence of the consequences of infection can be made reliably only by endoscopy with multiple biopsy specimens obtained in one or more regions of the stomach including antrum, body, and transition zones (i.e., cardia and incisura). Histology provides information regarding the presence of *H. pylori* and the severity and topographic distribution of gastritis including the presence of atrophic gastritis, intestinal metaplasia, and mucosa-associated lymphoid tissue (MALT) lymphoma (Dohil *et al.*, 1999).

H. pylori can be visualized at high magnification with conventional hematoxylin and eosin (H & E) - stained sections. Bacteria are located in the mucus adherent to the surface epithelium and are often found deep within the crypts. However, H & E staining may be unreliable when few bacteria are present. In addition, luminal debris on the surface

of the epithelium can be mistaken for *H. pylori* in H & E stained sections. Histological identification of bacteria is facilitated by using special stains such as the Warthin-Starry and modified Giemsa stains (El-Zimaity *et al.,* 1996).

1.2.11.1.2 Rapid Urease Testing of Biopsy Tissues

Urease testing provides indirect identification of *H. pylori* infection within a few hours of endoscopy (Elitsur and Neace, 1999). The basis of the test is the ability of *H. pylori* to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and bicarbonate in the presence of an indicator phenol red, the raises in pH changes the color of the specimen from yellow (Negative) to red (Positive). Urease testing is much more rapid and less costly (Marshall *et al.,* 1987).

1.2.11.1.3 Bacterial Culture

Culture of *H. pylori* from the gastric mucosa provides an opportunity to obtain a profile of antibiotic sensitivity that could identify potential treatment failure due to antibiotic resistance (Van der Hulst *et al.,* 1998).

Culture also provides a bacterial strain for use in epidemiologic studies to examine associations of virulence characteristics with disease outcome. However, bacterial culture for *H. pylori* is relatively expensive and success rates for recovery of the organism in many clinical laboratories are low (Holton, 1997). Avariety of selective and non selective media are available for culture of *H. pylori* (Hachem *et al.,* 1995).

H. pylori require a microaerobic environment, high humidity, and incubation at 35 to 37°C for a maximum of 7 to 10 days. Positive cultures are usually detected after 3 to 5 days of incubation. *H. pylori* is identified on the basis of colony morphology (translucent colonies varying in size from barely detectable with the naked eye to approximately 3 mm); colonies consist of Gram-negative, curved (not usually helical) rods that are urease, catalase, and oxidase positive (Queiroz *et al.,* 1987).

1.2.11.1.4. Polymerase Chain Reaction (PCR)

Polymerase chain reaction (PCR) is a highly sensitive technique that can be used to detect the presence of *H. pylori* in body fluids (e.g., gastric juice and stool), tissues (e.g., gastric mucosa), and water .Testing of *H. pylori* genomic DNA by PCR can be used to advance knowledge at the molecular level for example, by providing information about point mutations conferring resistance to antibiotics and about putative bacterial virulence factors. However, PCR is expensive, the assay is difficult to set up, specificity may be compromised by inadvertent contamination, and it is not widely available outside the research laboratory (Westblom, 1997).

The basis of the test is to amplify a single or a few copies of target deoxy ribonuclic acid (DNA) *H. Pylori* across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence (Hirschl and Makristathis, 2007).

It's a rapid and simple mean of detecting even a small quantity of target DNA molecule even when the source DNA is of poor quality, and thus regarded as golden standard method (Calvet *et al.*, 2009).

1.2.11.1.5 Real- time PCR (RT-PCR)

Rather than benefits of traditional PCR, Real – time PCR can detect the accumulation of amplicon during the reaction, which is much more precise than traditional PCR.

RT-PCR technologies for *H. pylori* detection and identification of antibiotic resistance have recently become available. *H. pylori* susceptibility to Clarithromycin can be assessed with the detection of mutations in the 23S ribosomal ribonuclic acid (rRNA) gene (Fontana *et al.*, 2003).

1.2.11.2. Non-invasive Tests

1.2.11.2. 1. Immunoassay Tests to Detect *H. pylori* Antibodies

Enzyme-linked immunosorbent assays (ELISAs) to detect *H. pylori* antibodies are relatively inexpensive and easy to implement in the clinical setting. Many tests are available for use to test whole blood, plasma, or serum. However, compared with histology, the sensitivity and specificity of serologic assays are poor in both adults and children unless used in the populations in which they were initially developed (Breslin and Morain, 1997).

In general, the accuracy of serum based immunoassays and whole blood tests for use in the physician's office in developed countries are poor, with a range of sensitivity of only 60% to 70% (Czinn, 1998). One immunoassay developed in a research center to detect *H. pylori* specific immunoglobulin G (IgG) was 91% sensitive compared with sensitivity of less than 70% in three commercially available assays (Khanna *et al.*, 1998).

1.2.11.2. 2. Saliva and Urine Tests for *H. pylori* Antibodies

Similar to serologic tests, saliva based tests also detect the presence of *H. pylori* specific (IgG) antibodies. The tests are easy to perform, painless, and inexpensive. Saliva tests are less sensitive than assays of serum or whole blood (Fallone *et al.*, 1997). The protein concentration of saliva appears to affect the accuracy of test results. Urine-based assays are easy to perform, require minimal labor for collection, and are painless. However, these assays are highly variable and are not yet commercially available. Therefore, saliva and urine assays for the detection of *H. pylori* antibodies cannot be recommended (Alemohammad *et al.*, 1993).

1.2.11.2. 3. Stool Test for *H. pylori* Antigens

Testing of *H. pylori* antigens in stools has shown promising results in adults for the non-invasive diagnosis of gastric infection using a commercially available kit (Vaira *et al.*, 1997). Testing for *H. pylori* antigens in feces also appears to be accurate for use in monitoring the success of eradication therapy. However, patients may be reluctant to

collect stool specimens. In addition, refrigerated stools are more difficult to test. Additional pediatric studies evaluating the accuracy of stool antigen testing for both initial diagnosis and post treatment follow-up are required before specific recommendations can be considered (Oderda *et al.*, 2000).

1.2.11.2. 4. Urea Breath Testing

Urea breath tests are non-invasive and have high sensitivity and specificity (>95%) both in adults and children (Rowland *et al.*, 1997). Urea is provided as a substrate which, in the case of the UBT, is ingested as either [13C] or [14C] urea. *H. pylori* urease hydrolyzes the ingested urea into labeled bicarbonate, which is exhaled as labeled CO_2, which is collected. The 14C isotope is detected with a scintillation counter, while the 13C isotope is detected by mass spectrometry most commonly (Koletzko *et al.*, 1995).

1.2.12. Previous studies

Study done by **(**Jemilohun *et al.*, 2011) in Ibadan, Nigeria to evaluate the diagnostic accuracy of rapid urease test in patient with dyspepsia.

Eighty-six consecutive adult patients with dyspeptic symptoms presenting for endoscopy were recruited after giving informed consent. Gastric antral biopsies were collected at endoscopy for Rapid Urease Test (RUT) and histology. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of RUT was calculated using histology as the reference standard.

Of the 86 subjects, there were 39 (45.3%) males and 47(54.7%) females. The mean age was 49.19±13.75 years. The age range was 23 to 85 years. The sensitivity, specificity, PPV, NPV of RUT was 93.33%, 75.6 %, 80.76 %, and 91.17 % respectively.

Rapid urease test was found accurate for the diagnosis of *H. pylori* infection. Its use will serve as a good alternative to histology in management of patients with dyspepsia in resource poor environments, except in patients who need histology for reasons other than *H. Pylori* diagnosis.

Other Study done by (Brooks *et al.*, 2004) in Dunedin, New Zealand to determine to what degree polymerase chain reaction (PCR) was superior to histology and culture, and whether a noncommercial urease tests was of value, in detecting *Helicobacter pylori* in gastric biopsy specimens. Gastric biopsy specimens from the antrum and corpus of 134 consenting patients were subjected to PCR, targeting the *glmM* (*ureC*) gene, histology, culture, and a rapid urease test. PCR detected *H. pylori* in the biopsy specimens from 59 patients. All methods showed a high degree of sensitivity and specificity, but histology gave 2 false-negatives, and culture and the urease test gave 1 false-negative compared with PCR. PCR of a *glmM* gene segment was superior to the other methods for the detection of *H. pylori* infection and was comparable to histology in terms of cost. Nevertheless, in this study, histology and culture were found to be relatively reliable methods for examining gastric biopsy specimens.

In study done by (Elhag and Omer Ali, 2014) in Khartoum, aimed to detect frequency of *Helicobacter pylori* IgM, IgG antibodies, and to determine the relationship between the presence of antibodies and certain factors such as (sex, age and genetic susceptibility). Results showed that 19 (21.1%), 57 (63.3%) were positive for IgM antibody and IgG respectively, while 15 (16.7%) were positive for both. Females were more affected than males, high frequency of positive results was observed among 15-30 age groups. Statistical analysis showed that there was insignificant correlation between age, gender, genetic susceptibility and presence of *Helicobacter pylori* antibodies ($P > 0.05$).

Other study done by (Azim et al., 1994) in sudan studied the prevalence of *Helicobacter pylori* in Sudanese subjects with gastroduodenal inflammation, H. pylori was looked for in biopsy specimens taken from the antrum by two methods: rapid urease test [Campylobacter-like organism (CLO) test] and culture using Skirrow's selective supplement. results of one hundred subjects showed that *H. pylori* was found in 80% of patients with gastritis, 56% of patients with duodenal ulcer, 60% of patients with duodenitis and 16% of normal control subjects. It was neither detected in patients with gastric ulcer, nor in patients with oesophagitis or in those with oesophageal varices due to schistosomiasis, when using culture. However, it was found in 50% of patients with oesophagitis, when using CLO test.

Also study done by (Abdalaziz *et al*., 2013) in Sudan aimed to determine the association between *H. pylori* infection and stomach cancer using PCR technique. Stomach samples were collected from 60 patients suffering from stomach tumors using endoscopes, each sample was divided into two specimens, one specimen was fixed in 10% formalin, prepared in paraffin block and stained with Heamatoxilin and Eosin (H&E) for general morphology; another specimen was fixed in 10% normal saline and prepared for Polymerase Chain Reaction (PCR) to detect *H. pylori*. The age of the patients ranged from 35-75 years. Their mean age was 54.9+11.6 year. Male to female ratio was about (1:1) histopathologically, 30(50%) cases were diagnosed as malignant tumor, 22 (73.3%) of cases were diagnosed as well differentiated adenocarcinomas, 6 (20.0%) were poorly differentiated adenocarcinomas, while 2 (6.6%) were carcinoid tumors. The other 30(50%) cases were diagnosed as polypoid (benign) tumors. *H. pylori* was detected in malignant cases only. This bacterium was seen in 9(30.0%) sections stained with H&E. While this bacterium was seen in 19 (63.3%) cases when using conventional PCR technique.

1.3. Rationale

Helicobacter pylori, is the main cause of chronic active gastritis, and has major role in development of duodenal ulcer, also associated with but not necessary the cause of gastric carcinoma ASM. There are 80% of chronic gastritis caused by *H. pylori* in addition to another form of chronic gastritis are seen, which include eosinophilic gastric associated with food allergy and reflux gastric (Holstonk and Calom, 1997)., therefore the right diagnosis of *Helicobacter pylori* infections is one of the important issues that require special and specific tests, equipments and skilled personnel (Saad and Chey, 2005).

1.4. Objectives

1.4.1. General objective

To evaluate the diagnostic accuracy of biopsy urease test in the diagnosis of *H. pylori* infections in comparison to polymerase chain reaction (PCR) in patients with gasteritis attending some hospitals in Khartoum State during April to June 2014.

1.4.2. Specific objectives

1- To determine the frequency of. *H .pylori* infection in patients with gastritis.

2- To determine if there is significant differences between urease test results and PCR or not.

3- To co-relate between, gender, symptoms and presence of *H. pylori 16S rRNA* gene.

4- To determine if there is differenece between different age groups in detection of *H.pylori* 16S rRNA gene

Chapter two

2. Materials and Methods

2.1. Material

2.1.1. Study approach: It is aqualitative, prospective study.

2.1.2. Study Type: comparitive, cross sectional study.

2.1.3. **Study design:** hospital- based analytical study.

2.1.4. Study Area:

This study was conducted at Omdurman teaching hospital, Omdurman military hospital and Alshurta hospital.

2.1.5. Study population:

Patients with gasteritis symptoms presenting for gastroscopy in the hospitals Endoscopy unit.

2.1.6. Inclusion criteria:

Patients observed by physician during endoscopy with gastritis signs were included.

2.1.7. Exclution criteria;

Patients under antibiotic treatment against *H. pylori* and patients not fully diagnosed with gasteritis were excluded.

2.1.8. Study Variable

Results of PCR consider as independent variable and the following variables as dependent variables: Age, sex, symptoms and urease.

2.1.9. Sampling type: non-probability purposive sampling.

2.1.10. Sampling frame: gasteritis patients in Khartoum State.

2.1.11. Sampling strategy: convenience sampling where patients were investigated on the bases of accessibility

2.1.12. Sample size: Fifty (n=50) endoscopy biopsies were collected randomly from patients.

2.1.13. Ethical consideration

Ethical approval was taken from Ethical Review Board of Sudan University of Science

and Technology, Fuculty of Medical Laboratory Sciences. Also written consents were taken from hospital management and patients to take samples, and the results of investigations were delivered to them maintaining confidentiality of the results.

2.2. Method

2.2.1. Data collection method and tool

Data was collected using a structured interviewing questionnaire which is designed to collect and maintain all valuable information concern each case examined. (Appendix (1))

2.2.2. Specimens collection

An upper endoscopy was performed by physician, and two gastric biopsies were obtained. (Appendix (2)).

One specemin was placed directly into urea broth media and the other sample in sterile normal saline and kept at -80 ^{o}C (Snijders scientific / model: UFE 120-86E).

2.2.3. Procedures

2.2.3.1 Urease test:

Biopsy specimens were placed in 3mL of 2% urea and 0.03% phenol red in phosphate buffered saline in screw-capped tubes. Incubated at 37°C (Scott Science UK (LIB-030M), aerobically for 8-12 hours. Prepared from a urea broth base, available in dehydrated form, prepared according to manufacture instructions as follow:

About 0.9 gram powder was added to 95ml of de-ionized water, mixed by swirl then sterilized by autoclave(121^{o} C for 15 minutes), after that 2% of urea crystals were prepared and added aseptically to urea broth base, then 3ml was dispensed aseptically into each sterile tube, and stored at 4^{o} C till used.

Changing color to red-pink indicates positive results while when no change in color that indicate negative results.

Control of urease test was done as followed:

Positive urease control: *Proteus vulgaris*.

Negative urease control: *Escherichia coli*.

2.2.3.2 DNA extraction from biopsy specimens

Extraction was done by using vivantis kit “Vivantis, Malaysia” (cataloge (GF-TD-100).

according to manufactories instruction 5-10 mg of frozen tissue was transferred to 1.5 ml micro tube,then 250 µL of tissue lysis buffer and 20 µL of proteinase k solution was added to the sample, mixed by pulsed vortexing (Lab Tech (LVM-202). until homogenus solution was obtained. after that 12 µL of lysis enhancer was added and mixed immediately, then incubated at 65 ^{o} ***C*** for 3 hours in a shaking water bath (Scott science U.K, model LWB-122D), then 650 µL of tissue genomic DNA binding buffer added and mixed throuhly by pulsed vortexing until homogenus solution was obtained and

incubated for 10 minutes at 65^{o} ***C***, 200 µL of absolute ethanol was added and mixed immediately ,650 µL of sample transferd into a column assembled in clean collection tube, centrifuged at 5000 x g (Hettich zentrifugen (EBA 20)) for 1 minute and flow was discarded then column was washed two times with 650 µL of washing buffer and centrifugation for 5000 x g for 1 minute. Column centrifuged at 10000 x g for 1 minute to remove all traces of ethanol and placed into clean microcentrifuge tube and 200 µL of preheated elution buffer added directly into column membrane and standed at room tempreture for 2 minutes before it was centrifuged at 5000 x g for 1 minute to elute DNA and stored at 4^{o} ***C***.

2.2.3.3. Polymerase chain reaction (PCR)

2.2.3.3.1. Primers

Primers designated 16s rRNA-f (forward 5GCGCAATCAGCGTCAGGTAATG3) and16S rRNA-r (reverse: 5GCTAAGAGATCAGCCTATGTCC3) were used. The primer produced 470 bp band sizes in case of *H. pylori* infection.

2.2.3.3.2 Preparation of 20 µL PCR master mix

This was done by adding 1 µL of forward and 1 µL of reverse primer to 14 µL of reaction mixture which contain 7 µL distilled water, 3 µL buffer, 3 µL $MgCl_2$, 1 µL dNTPs then 4 µL of sample DNA was added to complete the 20 µL total reaction volume . The mixture was mixed and vortexed.

2.2.3.3.3 PCR program

The amplification reaction was carried out in thermo cycler techne PCR system (UK, model FTC41F2D) (appendix (3)). with program system consisting of an initial denaturation step at 94^{o} ***C*** for 10 minutes and 30 cycle of denaturation at 94^{o} ***C*** for 1 minute, annealing at 53^{o} ***C*** for 2 minutes, extension at 72^{o} ***C*** for 3 minutes and final extension step at 72^{o} ***C*** for 5 minutes.

2.2.3.3.4. Gel Electrophoresis

2.2.3.3.5. Preparation of buffer

30 ml of 10x tris borate ethyl diamine tetra acetic acid buffer (TBE) was added to 270 ml of distilled water.

2.2.3.3.6. Preparation of gel

Thirty five gram of agar dissolved in 35 ml of prepared TBE buffer. Heated in hot plade starrier (Scott science, UK, model (LMS-1003)) until boiled, followed by adding 3µL of ethidium bromide and mixed well, then it was poured into electrophoresis tray and then combs were placed. After that gel was melted at room temperature for 30 minutes, and then combs were taken.

2.2.3.3.7 Loading of sample and Electrophoresis

Five µL of DNA was added to 2 µL of loading dye (promiga, USA, Blue range 6x loading dye) and mixed well. The mixture was loaded in the wells of the gel, and then gel tray was put in the electrophoresis tank and filled with buffer. Then electrophoresis was applied at 100 volt for 30 minutes. Ampair, 100 volt and remained for 30 minutes.

The product was examined with molecular size marker 100bp DNA ladder (solisbiodyne, cat No.07-11-00005) by using Gel documentation system (UK, model (Ingeniusl)).

Positive result gave clear band at 470 bp, while negative result gave no band (appendix (4).

2.2.4. Data analysis

The result obtained was analyzed by computer software statistical package for social science program (SPSS) vertion 11.5, and presented in form of graphs. Chi-square was used to determine correlations between *H.pylori* infection, sex, and symptoms (table (1)), oneway anova test to compaire between age groups, and paired sample T test was used to determin if there is significant differeces between urease test results and PCR test results or not.

Table (1) 2x2 cross tabulation of diagnostic test (urease test) & reference test (PCR)

True test	Test result		Total
	Positive	Negative	
Positive	A	B	A+B
Negative	C	D	C+D
Total	A+C	B+D	N

A = Number of true positive.

B = Number of false positive.

C = Number of false positive.

D = Number of true negative.

Sensitivity

It is a conditional probability that the test result is positive, given that the individual is in fact positive.

According to table (1)

$$\text{Sensitivity} = \frac{\text{Number of true positive (A)}}{\text{Number of True positive (A)} + \text{Number of False negative(B)}} * 100$$

Specificity

It is conditional probability that the test result is negative, given that the individual is in fact negative.

$$\text{Specificity} = \frac{\text{Number of true negative (D)}}{\text{Number of True negative (D)} + \text{Number of False positive(C)}} * 100$$

Chapter three

3. Results

A total number of 50 biopsies were collected from patients with gastritis, attending some hosoitals in KhartoumState during April to June 2014, were screened by urease test for the presence of *Helicobacter pylori* in samples and confirmed by PCR.

The sensitivity and specificity of urease test as diagnostic tool were 84.6%, 87.5% respectively.

Results of urease test in the study group were positive in 25 (50%) out of 50 and negative in 25 (50%), while it is positive in 26 (52%) out of 50 and negative in 24(48%) by PCR.

The age distribution among study group ranged from 20-79 as shown in fig (1).

Most of positive results 9 out of 26 observed ammong 30-39 years ammong them 7 were negative with PCR but there was no differenece between different age groups in detection of *H.pylori* 16S rRNA gene (*P* value = 0.115) as shown in table (2)

Most of study population were males 39 (78%), while 11 (22%) females (*P* value = 0.623) as shown in table (3).

From total PCR positive patients 21 (80.8%) complained epigastric pain and 5 (19.2%) with no epigastric pain, from total PCR negative 20 (83.3%) complained epigastric pain and 4 (16.7%) with no epigastric pain (*P* value = 0.814) as shown in table (4).

From total PCR positive 14 (53.8%) complained heart burn and 12 (46.2 %) with no heart burn, from total PCR negative 10 (41.7%) complained heartburn and 14(58.3%) with no heartburn (*P* value = 0.389) as shown in table (5).

There were 22 positive by both PCR and urease test (true positive), and 21 were negative by both PCR and urease test (true negative), 3 specimens was positive by urease test and

negative by PCR (false positive), 4 others were negative by urease and positive by PCR (false negative) as shown in table (6).

There was no association between, gender, and symptoms with *H.pylori 16S rRNA* gene detection as shown in table (3, 4 and 5).

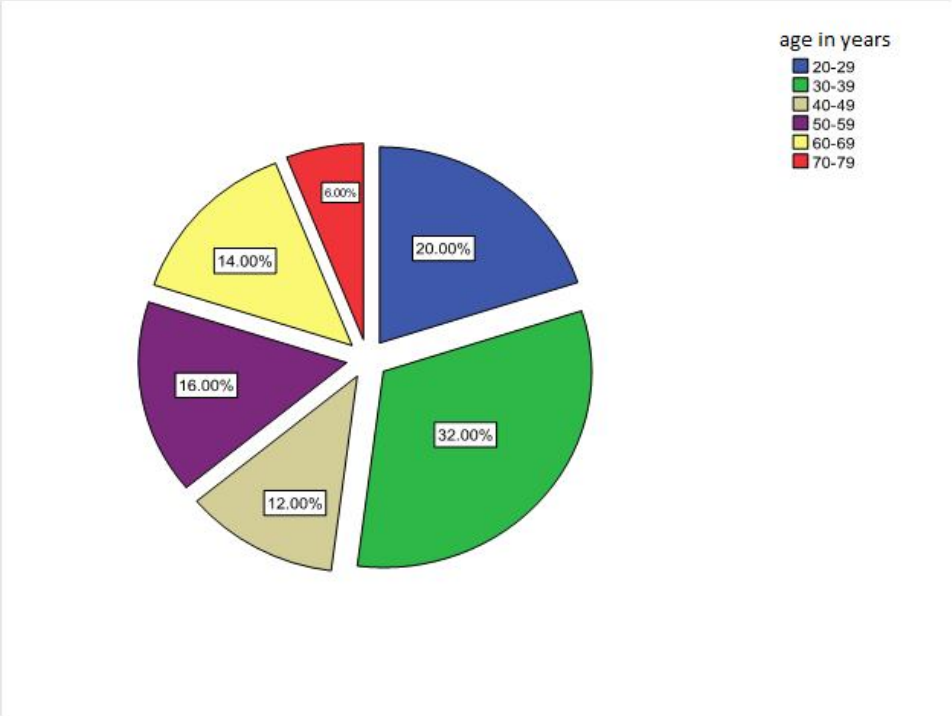


Fig (1): Age distribution of study group.

Table (2): The correlation between age and *H.pylori* 16S rRNA gene detection

Age groups (years)	***H.pylori* 16S rRNA gene detection by PCR**		Total
	Positive	Negative	
20-29	8(16%)	2(4%)	10
30-39	9(18%)	7(14%)	16
40-49	4(8%)	2(4%)	6
50-59	3(6%)	5(10%)	8
60-69	1(2%)	6(12%)	7
70-79	1(2%)	2(4%)	3
Total	26(52%)	24(48%)	50

(*P* value = 0.115): insignificant

There was no differenece between different age groups in detection of *H.pylori* 16S rRNA gene

Table (3): The frequancy of *H.pylori* 16S rRNA gene detection in relation to gender

			GENDER		Total
			Male	female	
PCR	Positive	Count	21	5	26
		% of Total	42.0%	10.0%	52.0%
	Negative	Count	18	6	24
		% of Total	36.0%	12.0%	48.0%
Total		Count	39	11	50
		% of Total	78.0%	22.0%	100.0%

(*P* value = 0.623): insignificant

There was no association between gender and detection of *H.pylori* 16S rRNA gene

Table (4): The relation between Epigastric pain & PCR test result

Epigastric pain	PCR test		Total
	Positive	Negative	
Yes	21 (80.8%)	20 (83.3%)	41
No	5 (19.2%)	4 (16.4%)	9
Total	26(100%)	24(100%)	50

(*P* value = 0.814): insignificant

There was no association between epigastric pain and detection of *H.pylori* 16S rRNA gene detection

Table (5): The relation between heartburn & PCR test result

Heartburn	PCR test		Total
	Positive	Negative	
Yes	14 (53.8%)	10 (41.7%)	41
No	12 (46.2%)	14 (58.3%)	9
Total	26(100%)	24(100%)	50

(*P* value = 0.389): insignificant

There was no association between heartburn and detection of *H.pylori* 16S rRNA gene detection

Table (6): The correlation between results of PCR (Polymerase Chain Reaction) and urease test

	urease test (diagnostic test)		
PCR (reference test)	Positive	Negative	Total
Positive	22(True+ve)	4(false –ve)	26
Negative	3(False+ve)	21(true –ve)	24
Total	25	25	50

(*P* value = 0.709): insignificant

There was no significant difference between urease test results and PCR results

Sensitivity: 22/ (22 + 4) = 84.6%

Specificity: 21/ (21 + 3) = 87.5%

Chapter four

4. Discussion

Helicobacter pylori infection causes chronic active gastritis in the antrum (antral gastritis), the corpus (corpus gastritis) or in both (pan gastritis). It is a major etiological factor in peptic ulcer disease, gastric carcinoma, and gastric mucosal associated lymphoid tissue (MALT) Lymphoma, Hemorrhage and perforation are the most frequent complications of peptic ulcer disease and are associated with substantial morbidity, mortality and health care costs (Van Keeken *et al*, 2006). Peptic ulcer disease can be cured by eradicating *H. pylori* so that complications no longer occur (Malfertheiner *et al*, 2007).

This study was done in patients with gastritis to evaluate the diagnostic accuracy of urease test in the diagnosis of *H. pylori* infection, using PCR as reference test.

Our study was in agreement with previous study done by (Jemilohun *et al*, 2011) in Nigeria, they detect (45%) positive biopsy specimen by urease test, with mild difference in sensitivity of test, they reported (93%) and present study detect (84.6%).

Our study was also in agreement with previous study done by (Brooks *et al*, 2004) in New Zealand, they detect (43%) positive biopsy specimen by urease test, with mild difference in sensitivity and specificity, they were more than (90%).

Our study was also in agreement with previous study done by (Elhag and Omer Ali, 2014) in Khartoum, they detect insignificant correlation between age, gender, genetic susceptibility and presence of *Helicobacter pylori* antibodies (P >0.05) and present study detect insignificant correlation between age, gender and symptoms with presence of *Helicobacter pylori* 16S rRNA gene (P >0.05)

But our study was disagreed with previous study done by (Azim *et al., 1994)* in Sudan, theire results showed that *H. pylori* was found in 80% of patients with gastritis while we

found 52% of patients with gastritis; which is explained by improvement of population hygen scince 1994 and also there is differences in techniqes used lead to this variations.

4.2. Conclusion

The sensitivity & specificity of Urease test as diagnostic tools for *Helicobacter pylori* was high 84.6% & 87.5% respectively.

Fifty patients with gastritis symptoms were screened for *H. pylori* using urease test, the frequency of infection was 52%.

The frequency of infection was high among younger patients and decrease with an increase of age.

There was no significace difference between urease test and PCR test results

There was no association between, gender, or symptoms with *H.pylori* 16S rRNA gene detection.

There was no differenece between different age groups in detection of *H.pylori* 16S rRNA gene

4.3. Recommendations

Rapid urease test is usuful tool for diagnosis of *H.pylori* infection due to it is sensitivity and specificity

Larger sample size needed to confirm our results

References

1. Abdalaziz M S, Munsoor M Mr, Siyam WA (2013). Association between *Helicobacter pylori* Infection and Stomach Tumors in Sudan Using Polymerase Chain Reaction. *Australian Journal of Basic and Applied Sciences,* 7(4): 769-773, ISSN 1991-8178

2. Alemohammad MM, Foley TJ, Cohen H (1993). Detection of immunoglobulin G antibodies to *Helicobacter pylori* in urine by an enzymeimmunoassay method. *J Clin Microbiol.*31:2174–7.

3. Arents NL, Thijs JC, and Kleibeuker JH. A (**2002)**. Rational approach to uninvestigated dyspepsia in primary care: review of the literature. *Postgrad Med J.;* 78:707-716.

4.Azim Mirghani YA, Ahmed S, Ahmed M, Ismail MO, Fedail SS, Kamel M, Saidia H (1994). Detection of *Helicobacter pylori* in endoscopic biopsies in Sudan: Trop Doct; 24(4):161-3.

5. Baldwin DN, Shepherd B, Kraemer P et al. (2007). "Identification of *Helicobacter pylori* Genes That Contribute to Stomach Colonization". *Infect Immun* **75** (2): 1005–16.

6. Bizzozero, G. (1893). Ueber die schlauchförmigen Drüsen des Magendarmkanals und die Beziehungen ihres Epithels zu dem Oberflächenepithel der Schleimhaut. Dritte mitteilung. Archiv Mikroskopische Anat. 43**:**82-152

7. Blanchard, T G; Nedrud, J G (2010). 9. *Helicobacter pylori* Vaccines". In Sutton, Philip; Mitchell, Hazel. *Helicobacter pylori in the 21st Century*. Mitchell, Hazel. CABI. pp. 167–189. ISBN 978-1-84593-594-8.

8. Breslin NP, O'Morain CA (1997). Noninvasive diagnosis of *Helicobacter pylori*: a review. *Helicobacter*. 2:111–7.

9. Brooksa, and D. Ahmeda, *et al.* (2004) Diagnosis of *Helicobacter pylori* infection by polymerase chain reaction: is it worth it? , *Diagnostic Microbiology and Infectious Disease* 50 (2004) 1–5.

10. Broutet N, Marais A, and Lamouliatte H *et al* (2001). cag A Status and Eradication Treatment Outcome of Anti-*Helicobacter pylori* Triple Therapies in Patients with Nonulcer Dyspepsia". *J Clin Microbiol* **39** (4): 1319–22.

11. Brown LM (2000). *Helicobacter pylori*: epidemiology and routes of transmission. *Epidemiol Rev* **22** (2): 283–97.

12. Bytzer P, Dahlerup JF, Eriksen JR, Jarbøl DE, Rosenstock S, Wildt S (2011). "Diagnosis and treatment of Helicobacter pylori infection". *Dan Med Bull* **58** (4): C4271.

13. Calvet X, Sanchez-Delgado J, and Montserrat A, *et al*. (2009) Accuracy of diagnostic tests for *Helicobacter pylori*: a reappraisal. *Clin Infect Dis*; 48:1385–1391.

14. Cave DR (1996). Transmission and epidemiology of *Helicobacter pylori*. *Am. J. Med.* **100** (5A): 12S–17S.

15. Chan WY, Hui PK, Leung KM, Chow J, Kwok F, and Ng CS (1994). Coccoid forms of *Helicobacter pylori* in the human stomach". *Am J Clin Pathol* **102** (4): 503–7.

16. Czinn SJ (1998). Serodiagnosis of *Helicobacter pylori* in pediatric patients. *J Pediatr Gastroenterol Nutr.* 28:132–4.

17. Delport W, and van der Merwe SW (2007). The transmission of *Helicobacter pylori*: the effects of analysis method and study population on inference. *Best Pract Res Clin Gastroenterol* : 215–36.

18. De Vries R, Klok RM, Brouwers JR, Postma MJ (2009). Cost-effectiveness of a potential future *Helicobacter pylori* vaccine in the Netherlands: the impact of varying the discount rate for health". *Vaccine* **27** (6): 846–52.

19. Dixon MF (2000). "Patterns of inflammation linked to ulcer disease. *Baillieres Best Pract Res Clin Gastroenterol* **14** (1): 27–40.

20. Dohil R, Hassall E, Jevon G (1999). Gastritis and gastropathy of childhood. *JPediatr Gastroenterol Nutr.* ; 29:378–94.

21. Dumrese C, Slomianka L, Ziegler U et al. (2009). The secreted Helicobacter cysteine-rich protein A causes adherence of human monocytes and differentiation into a macrophage-like phenotype. *FEBS Letters* **583** (10): 1637–43.

22. Elhag WI, Omer Ali LE (2014*).* Frequency of *H. pylori* Antibodies among Patients with Gastrointestinal Symptoms Attending Khartoum Teaching Hospital- Sudan. SOJ Microbiol Infect Dis 2(1):

23. Elitsur Y, Neace C (1999). Detection of *Helicobacter pylori* organisms by Hp-fast in children. Dig Dis Sci.; 44:1169–72.

24. El-Zimaity, H. M., D. Y. Graham, M. T. al-Assi, H. Malaty, T. J. Karttunen, D. P. Graham, R. M. Huberman, and R. M. Genta (1996). Interobserver variation in the histopathological assessment of *Helicobacter pylori* gastritis. *Hum. Pathol*. **27:**35–41.

25. Ernst, P. B., and B. D. Gold. (**2000).** The disease spectrum of *Helicobacter pylori*: the immunopathogenesis of gastroduodenal ulcer and gastric cancer. *Annu. Rev. Microbiol*. **54:**615-640.

26. Fallone CA, Elizov M, Cleland P (1996). Detection of *Helicobacter pylori* infection by saliva IgG testing. *Am J Gastroenterol.* 91:1145–9.

27. Fontana C, Favaro M, and Pietroiusti A (2003). Detection of clarithromycin-resistant *Helicobacter pylori* in stool samples. *J Clin Microbiol*; 41:3636–3640.

28. Goodwin, C. S., J. A. Armstrong, T. Chilvers, M. Peters, M. D. Colins, L. Sly, W. McConnell, and W. E. S. Harper. (**1989)**. Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., respectively. *Int. J. Syst*. Bacteriol. **39:**397-405.

29. Hachem, C. Y., J. E. Clarridge, D. G. Evans, and D. Y. Graham. (1995). Comparison of agar based media for primary isolation of *Helicobacter pylori*. J. *Clin. Pathol*. 48:714–716

30. Hirschl AM, and Makristathis A. **(2007)** Methods to detect *Helicobacter pylori*: from culture to molecular biology. *Helicobacter*; 12(*Suppl* 2):6–11.

31. Holcombe C. **(1999).** *Helicobacter pylori*: the African enigma. Gut. 1992; 33:429-431

32. Holstonk and Calom. J. (1997). *Helicobacter*. An engineering pathogen *European J*. of clinical investigation.24:501-510.

33. Holton J. (1997). Clinical relevance of culture: why, how, and when. *Helicobacter.* **1997**; 2 (suppl 1):S25–33.

34. Jemilohun1, A.C., Otegbayo2, and J.A., *et al.* (2011) diagnostic accuracy of rapid urease test in diagnosis of *H. pylori* infection in patients with dyspepsia. *AFR. J. CLN. EXPER. MICROBIOL*. 12(2):62-66.

***35.* Ketley J M**. (**2007)**. *Campylobacter* and *Helicobacter*. In: Medical Microbiology, 17 TH EDITIONS. *Elsevier limited, USA*, pp.300-306.

36. Khanna B, Cutler A, Israel NR (1998). Use caution with serologic testing for *Helicobacter pylori* infection in children. *J Infect Dis.*178:460–5.

37. Koletzko, S., M. Haisch, I. Seeboth, B. Braden, K. Hengels, B. Koletzko, and P. Hering (1995). Isotope-selective non-dispersive infrared spectrometry for detection of *Helicobacter pylori* infection with 13C-urea breathe test. Lancet **345:**961–962.

38. Kusters JG, van Vliet AH, Kuipers EJ (2006). "Pathogenesis of *Helicobacter pylori* Infection". *Clin Microbiol Rev* **19** (3): 449–90.

39. Malfertheiner P, Megraud F, O'MorainC, Bazzoli F, El-Omar E, and Graham D, ***et al*. (2007).** Current concepts in the management of *Helicobacter pylori* infection: *the Maastricht III Consensus Report*. Gut. 56:772-781.

40. Marshall, B. J., J. A. Armstrong, G. J. Francis, N. T. Nokes, and S. H. Wee. (1987). Antibacterial action of bismuth in relation to *Campylobacter pyloridis* colonization and gastritis. Digestion **37:**16-30.

41. Mégraud F (2004). *H.pylori* antibiotic resistance: prevalence, importance, and advances in testing. *Gut* **53** (9): 1374–84.

42. Miwa H, and Sato N. (**2000).** Functional dyspepsia and *Helicobacter pylori* infection: a recent consensus up to. *J Gastroenterol Hepatol*.; 15:60-65.

43. Oderda G, Rapa A, Ronchi B (2000). Detection of *Helicobacterpylori* in stool specimens by non-invasive antigen enzyme immunoassayin children: multicentre Italian study. *BMJ.* 320:347–8.

44. Olczak AA, Olson JW, Maier RJ. (**2002)**. Oxidative-stress resistance mutants of *Helicobacter pylori*. *J. Bacteriol.* 184 (12): 3186–93.

45. Olson JW and Maier RJ (2002). Molecular hydrogen as an energy source for *Helicobacter pylori*. *Science* **298** (5599) 1788–90.

46. Pel, P. K. (**1899)**. Diseases of the stomach. De Erven Bohn, Amsterdam, *Neth J Med*.

47. Pounder RE, and Ng D (1995). The prevalence of *Helicobacter pylori* infection in different countries. *Aliment. Pharmacol. Ther.* **9** Suppl 2: 33–9.

48. Queiroz, D. M., E. N. Mendes, and G. A. Rocha. (1987). Indicator medium for isolation of *Campylobacter pylori*. J. Clin. Microbiol. **25:**2378–2379.

49. Rowland M, Lambert I, Gormally S (1997). Carbon 13-labeled ureabreath test for the diagnosis of *Helicobacter pylori* infection inchildren. J Pediatr. 131:815–20.

50. Rupnow MF, Chang AH, Shachter RD, Owens DK, Parsonnet J (2009). Cost-effectiveness of a potential prophylactic *Helicobacter pylori* vaccine in the United States. *J. Infect. Dis.* **200** (8): 1311–7.

51. Ryan, Kenneth (2010). *Sherris Medical Microbiology*. McGraw-Hill. pp. 573 and 576. ISBN 978-0-07-160402-4

52. **Saad R, Chey WD (2005).** A clinician's guide to the managing *Helicobacter pylori* infection. Cleve Clin J Med 72(2): 109-126.

53. Selgrad M, Malfertheiner P (2008). New strategies for Helicobacter pylori eradication. *Curr Opin Pharmacol* **8** (5): 593–7

54. Shiotani A, Graham DY. **(2002)** . Pathogenesis and therapy of gastric and duodenal ulcer disease. *Med. Clin. North Am.* 86 (6): 1447–66 .

55. Smoot DT. **(1997)** . How does *Helicobacter pylori* cause mucosal damage? Direct mechanisms. *Gastroenterology* 113 (6): S31–4.

56. Suerbaum S, and Michetti P (2002). *Helicobacter pylori* infection". *N. Engl. J. Med.* **347** (15): 1175–86.

57. Tomb JF, White O, and Kerlavage AR *et al* (1997). The complete genome sequence of the gastric pathogen *Helicobacter pylori*. *Nature* **388** (6642): 539–47.

58. Tsuji S, Kawai N, Tsujii M, Kawano S, Hori M (2003). Review article: inflammation-related promotion of gastrointestinal carcinogenesis--a perigenetic pathway. *Aliment. Pharmacol. Ther.* **18** (Suppl 1): 82–9.

59. Vaira D, Malfertheiner P, Megraud F (1999). Diagnosis of *Helicobacter pylori* with a new non-invasive antigen-based assay. *Lancet.*354:30–3.

60. Van der Hulst RW, van der Ende A, Homan A (1998). Influence of metronidazole resistance on efficacy of quadruple therapy for *Helicobacter pylori* eradication. *Gut; 42:166*–9.

61. Van Keeken N, van Hattum E, and de Boer WA (**2006).** Validation of a new, commercially available dry rapid urease test for the diagnosis of *Helicobacter pylori* infection in gastric biopsies. *Neth J Med*.; 64:329-333.

62. Westblom TU (1997). Molecular diagnosis of *Helicobacter pylori*. Immunol Invest. ; 26:163–74.

Appendix

Appendix (1)

Al-Sudan University

Faculty of Medical Laboratory Sciences

Department of Microbiology

Questionnaire

Diagnostic Accuracy of Biopsy Urease Test in Diagnosis of Gastritis Among Patients Attending Gastroenterology Units in Khartoum State

By: Mohanad Altahir Ismail Musa

Supervised by: Wafa Ibrahim Elhag

Patient No ( ) Date: / / 2014.

Name……

Age ……..... years.

Gender…….....

Epigastric pain Yes ( ) No ( )

Hard burn Yes ( ) No ( )

Previous diagnosis Yes ( ) No ( )

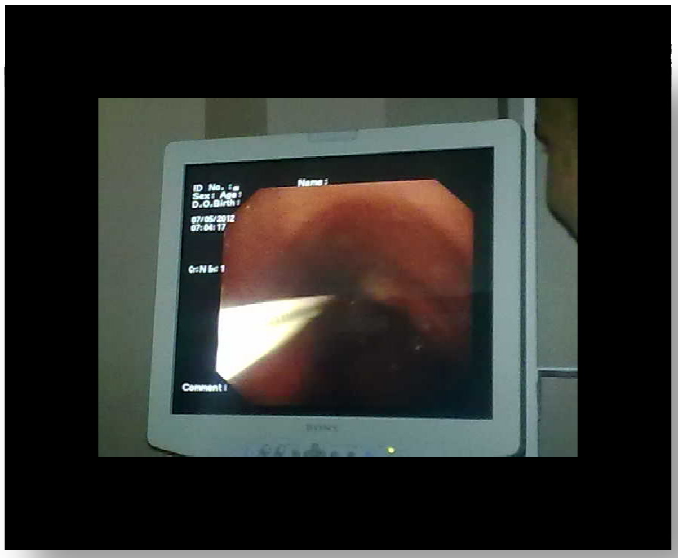
Treatment Yes ( ) No ( )

Urease result……

PCR result……

Note……

Appendix (2)



Collection of biopsy specimen during endoscopy.

(3) PCR machine: UK, model (FTC41F2D)

Thermocycler, PCR machine or DNA amplifiers

4. PCR results of *H. pylori* showed: Lane (1&3) negative samples, and Lane (2, 4, 5, and 6) positive samples with band size 470bp, lane (7) positive control, Lane (8) is MWt marker.