CAPTER ONE

INTRODUCTION

1.1 Introduction

H.pylori is Gram negative, microaerophillic, spiral rod shaped, that live just beneath the antral gastric mucous layer, on the surface of epithelial cells (Bashir *et al*., 2011). *H.pylori* is a type of bacteria. These germs can enter your body and live in digestive tract. After many years, it can cause sores, called ulcer in the lining of stomach as more of the world gets access to clean water and sanitation, fewer people than before are getting the bacteria. With good health habits, you can protect yourself and children from *H.pylori* (Blahd, 2016) .Person to person transmission occur throuph oral or fecal oral routes. More than half of world´s populations are known to be infected with *H.pylori*, (Ozbey and Hanafiah, 2017). In developing countries, its infection rate over 60%. A number of studies found poor hygiene standards, crowded house hold; deficient sanitations are important acquisition of infection in childhood and spreading of these diseases (Syam *et al*., 2015). In developed countries the prevalence of children is low (1.2%- 12.2%) compared with developing countries *H. pylori* is frequently isolated bacteria in ten years (Ozeby and Hanafiah, 2017). Gastrointestinal manifestations include gastritis, atrophic gastritis and peptic ulcers (Herrero *et al*., 2014) *H.pylori* infections have been acknowledged to contribute to the majority of gastric carcinoma cases worldwide. Growth retardation and nutritional deficiencies particularly iron deficiency anaemia, resulting from impaired gut iron absorption in children, have also been associated with *H.pylori* infections in developing countries (Zullo *et al.*,2014).The diagnosis of *H. pylori* infection can be made through many laboratory tests. The techniques are divided into two groups, invasive and non-invasive tests. All invasive tests are based on endoscopic examination during which biopsy specimens are obtained for direct (histological analysis, isolation)

or indirect (urease test) diagnosis of *H. pylori* infection. The difficulties associated with this approach are risk and discomfort to the patient. Moreover, *H. pylori* tend to colonize in patches and may be missed by biopsy. Culturing of *H. pylori* from biopsy is difficult and time consuming. These difficulties may lead to false negative results. Non-invasive methods reveal the presence of *H. pylori* by measuring the activity of urease (urea breath test), then by confirming the presence of antibodies in the serum. The urea breath test is highly sensitive and specific; however, it requires ingestion of isotopic ally labelled urea as well as specialized instrumentation for detection of C14 or C13. Other non-invasive tests were also evaluated in several studies including, detection of *H. pylori* antigens in stool and presence of *H. pylori* in saliva. Stool antigen tests have recently been welcomed with great expectations as they are convenient to the patients and can be easily performed even in small laboratories. The antigen test is a rapid, non-invasive method which provides direct detection of an active infection. However, serological tests are reported to be unreliable for the diagnosis of *H. pylori* since they may return false negative results for up to 60 days after infection and remain positive for considerable time after eradication (Abdurrahman *et al*., 2014).

1.2 Rationale

More than half of world´s populations are infected, *H. pylori* is higher in developing countries and decline in United States. Incidence of new infections in developing countries is 3 to 10% of population each year compared to 0.5% present in developed countries (Rosenberg, 2010). The global prevalence of *H. pylori* in 2015 was estimated to be 4.4 billion people (Bjorkma, 2017). It is reported by washwatch that the total number of people in South Sudan lacking access to an "Improved" Water Supply in 2015 was 5,015,000 (Refugees ,2016).It is estimated that 58.726% of the population of Southern Sudan has access to an improved watersource, such as a hand pump, a protected well or for a small minority piped water supply. Even those with access to an improved water source often do not receive safe water (Refugees, 2016).There are limited studies on the prevalence of *H. pylori* in the source countries of refugees; Factors contributing to increased exposure to the organism include increasing age, large family size, low socioeconomic status, overcrowding and poor sanitation as well as having an infected sibling (Pandeya *et al.*, 2011). This study was carried out to diagnose *H.pylori* in symptomatic and asymptomatic southran sudan refugees using different laboratory methods.

1.3.Objectives

1.3.1 General Objective

To study *H.pylori* among southern Sudan refugees in Tasabih Medical complex in Khartoum state.

1.3.2 Specific Objectives

1. To detect stool antigens of *H. pylori* in symptomatic and asymptomatic feces.
2. To detect serum antibodies against *H. pylori* in symptomatic and asymptomatic sera.
3. To determine selected risk factors associated with *H.pylori* infection.
4. To compare between the different laboratory diagnostic methods.

CHAPTER TWO

LITERATURE REVIEW

2. Introduction

Helicobacter pylori are non-spore forming, curved, spiral or fusiform Gram negative bacteria, typically 0.2 to 1.2 um in diameter, and 1.5 to 10.0 um in length. In old cultures it appears coccoid. It commonly colonized in upper gastrointestinal tract, especially in the stomach. It has been estimated that 50 % of global population are infected with *H. pylori* due to its highly contagious nature. In 1982 it was classified as *Campylobacter pyloridis*, it resemble Campylobacter in many aspects but it differ in important feature such as flagellum morphology, fatty acid contents and 16s rRNA sequence. It named *Helicobacter pylori* in 1989 (Ibrahim, 2017). *H. pylori* are ubiquitous, found worldwide. *H. pylori* infection occurs more frequently in developing countries than in the developed countries (Parija, 2012). *H.pylori* is a capnophilic organism that requires an atmosphere with a high level of CO_2 (from 5% to 10%). It has been considered a microaerophile, but the concentration of O2 required for its growth is still a topic of discussion (Lopes *et al*., 2014).

2.1 Epidemiology

The prevalence of *H. pylori* shows large geographical variations. Rates of isolation range between 70-90% in developing countries and 25-50% in developed countries. In various regions of sub-Saharan Africa 61-100% of the population may harbor the pathogen. In Africa, the prevalence of infection is very high but the incidence of gastric carcinoma and other *H. pylori* associated morbidities are relatively low (Tanih *et al*., 2010). Epidemiological studies have suggested an association between *H. pylori* infection and certain other extra-gastric complications such as ischemic heart disease, neurodegenerative diseases, and hematological disorders (iron deficiency anemia, immune-thrombocytopenic purpura, and vitamin B_{12} deficiency (Jiang *et al.,* 2017).

recently found that *H. pylori* infection in pregnant women increases the risk of developing preeclampsia, which is a potent contributor to maternal and fetal morbidity and mortality (Bellos *et al*., 2018) Another complication, hyper emesis, gravid arum, can be found in up to 2.0% of women with early pregnancy and its onset has been associated with *H. pylori* infection (Ng QX *et al*., 2018).

2.2 Transmission

The exact mode of transmission of *H. pylori* is not known. It has the potential to spread by faecal contamination, and from person to person by saliva. Majority of the people who are infected were infected as children and this may explain why the rate of infection is so high in poorer countries and in socio-economic groups characterized by crowded living conditions, poor sanity condition and lack of clean water. Personal hygiene is also very important since food handlers and cookers could be a source of infection (Hunt *et al*., 2010).

2.3 Signs and symptoms of *H. pylori*

Primary infection with *H. pylori* is either silent or causes an illness with nausea and upper abdominal pain lasting up to 2 weeks. Year later, the finding of gastritis and peptic ulcer disease include nausea ,anorexia , vomiting ,epigastric pain, and even less specific symptoms such as belching. Many patients are asymptomatic for decades, even up to perforation of an ulcer. Perforation can lead to extensive bleeding and peritonitis due to the leakage of gastric contents into the peritoneal cavity (Kenneth *et al*, 2010).

2.4 Pathogenesis

After entering the host stomach, *H. pylori* utilize its urease activity to neutralize the hostile acidic condition at the beginning of infection. Flagella mediated motility is then required for *H. pylori* to move toward host gastric epithelium cells, followed by specific interactions between bacterial adhesions with host cell receptors, which thus leads to successful colonization and persistent infection. Finally, *H. pylori* releases several effectors proteins/toxins, including

cytotoxin associated gene A (CagA) causing host tissue damage (Kao *et al*., 2016). In addition, the gastric epithelium layer, which forms the major interface between *H. pylori* and the host, secretes chemokines to initiate innate immunity and activate neutrophils, and further lead to the formation of clinical diseases such as gastritis and ulcer (Kao *et al*., 2016). *H. pylori* weaken the protective mucus coating of the stomach and duodenum which allows acid to go through to the sensitive lining beneath. Both acid and bacteria irritate the lining and cause a sore or ulcer (Rana *et al*., 2017).

2.5 *H. pylori* infections

2.5.1Gastritis

Gastritis refers to inflammation of the gastric mucosa. Gastritis can be grouped as acute or chronic gastritis. Acute gastritis refers to the transient inflammation of the gastric mucosa. It is most commonly associated with local irritants such as bacterial endotoxins, caffeine, alcohol and aspirin. Depending on the severity of the disorder the mucosal response may vary from moderate oedema and hyperaemia to haemorrhagic erosion of the gastric mucosa. The changes may become dysplastic and possibly transform into carcinoma. *H. pylori* and a number of factors such as chronic alcohol abuse, cigarette smoking and chronic use of non-steroid anti-inflammatory drugs (NSAIDs) may contribute to the development of the disease. *H. pylori* gastritis is a chronic inflammatory disease of the antrum and body of the stomach (Tanih *et al*., 2010).

2.5.2 Peptic ulcer disease

Helicobacter pylori is now has been documented that virtually all persons with duodenal ulcer and 70% of those with gastric ulcer have *H. pylori* infection. Duodenal ulcers occur five times more commonly than gastric ulcers. Peptic ulcer can affect one or all layers of the stomach or duodenum. Occasionally, an ulcer may penetrate the outer wall of the stomach or duodenum; with spontaneous remissions and exacerbations being common (Levinson, 2014).

2.5.3 Gastric cancer

Infection with *H. pylori* appears to serve as a co-factor in some types of gastric carcinoma. Gastric carcinoma is the major cause of cancer death worldwide. Among factors that increase the risk of gastric cancer is genetic predisposition, carcinogenic factors in diet (e.g., Nnitros compounds and benzopyrene found in smoked and preserved foods) and autoimmune gastritis. Virtually all tumors are adenocarcinoma arising from mucus secreting cells in the base of the gastric crypts. Stomach cancers are either „intestinal", arising from areas of intestinal metaplasia or „diffuse" arising from normal gastric mucosa. Carcinomas are more common and arise against background of chronic mucosal injury (Levinson, 2014).

2.5.4 Gastric MALT lymphoma

MALT lymphomas are B-cell tumors located typically in the stomach, but they occur elsewhere in the gastrointestinal tract as well. *H. pylori* are often found in the MALT lesion and the chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually a B-cell lymphoma. Antibiotic treatment directed against the organism often causes the tumor to regress (Levinson, 2014).

2.6 Risk factor of *H.pylori* infection

Several socioeconomic has been associated with *H. pylori* infection, in particular subjects with low socioeconomic status, measured also as allow family income had higher like hood of caring *H. pylori* infection, also most studies show that individuals with lower education levels had higher risk than those with higher education, several factors associated with residence had been found associated with infection. Living in rural area, in crowded homes and having contaminated source of water were risk factors for *H. pylori* , among life style habits smoking and alcohol consumptions showed discordant result, some authors reported that regular smoking and drinkers were higher risk in contrast to one study result showed that regular alcohol drinking was protective factor

for *H. pylori* infection, occupationally acquired infection is reported especially when endoscopists who did not wear gloves during procedure increasing the risk of become infected (Mnena *et al*., 2017).

2.7 Diagnosis of *H. pylori* infections

Several methods are currently available to detect the presence of *H. Pylori* according to whether or not an endoscopy is necessary. Invasive as biopsy based tests include histological evaluation, culture, polymerase chain reaction (PCR) and the rapid urease test (RUT) all of which are performed on tissue obtained during endoscopy, alternatively the urea breath test (UBT), serology and stool antigen test (SAT) can be performed as non-invasive tests (Segamwenge *et al*., 2014).

2.7.1 Invasive methods

Histological method to diagnose *H. pylori* infections (Gonzalez *et al*., 2014), the principle of test is detection of *H. pylori* using microscopy of gastric mucosal biopsies (Bytzer, 2010).In histological section *H. pylori* appears as curved or spiral on epithelial surface in mucus layer or within gastric gland (Gonzalez *et al*., 2014).The sensitivity and specificity varies from 53% to 90% depending on the size of biopsies, biopsy sites (Bytzerb, 2010) and density of colonization. Histopathological procedure is uncomfortable; time consuming and less practical on children's because needs endoscopy (Gonzalez *et al*., 2014).

2.7.2. Rapid Urease Test (RUT)

Principle of test is the biopsy from gastric mucosa placed in medium containing urea, *H. pylori* urease breaks down urea to ammonia and carbon dioxide, ammonia leads to increase in PH and colour shift by PH indicator (Bytzer, 2010).The RUT produce a result in average of minutes up to 24hours (Gonzalez *et al*., 2014).The sensitivity is 50-95% and specificity is >95% (Bytzer, 2014).The RUT sensitivity is affected by the amount of bacteria in biopsy at least 1000cells are required. Low RUT sensitivity and specificity reported in the presence of blood, RUT specificity decrease with increase incubation time, false

negative result from decreased urease activity by increase acidity of stomach or as a result of recent intake of antibiotics, bismuth compound or proton pump inhibitors (Gonzalez *et al*., 2014).

2.7.3 Culture

Culture procedure is less invasive when use gastric juice compared to biopsy. Culturing typically has sensitivity greater than 90% and specificity of 100% when performed under optimal conditions. *H.pylori is* very delicate and needs to become cultured as soon as possible after sampling. Biopsies can be kept in a transport medium (Stuart´s transport medium) for up to 24 hours at 4ºC.Once isolated can be stored frozen at -80ºc, preferably in broth with 15%to 20% glycerol. Several types of medium can be used for *H. pylori* including selective agar (Pylori agar, Skirrow agar Wang media and others) which contains specific antibiotics to inhibit commensal bacteria, and non selective agars (blood agar Columbia blood agar and others). Cultures should be incubated under microaerobic conditions (85% N, 10% CO_2, and 5% O2) at 35to 37ºC for at least seven days before discarding culture as negative. Positive identifications based on morphological characteristics and positive catalase, oxidase and urease reaction. Culture is most specific method for detecting *H. pylori*, although the result depends on microbiologist experience, specimen quality and use of transport media (Gonzalez *et al*., 2014).

2.7.4. Polymerase Chain Reaction

Since the application of polymerase chain reaction (PCR) to detect *H.Pylori* infection, PCR has been used extensively for the diagnosis of *H. pylori* from gastric biopsy specimens, saliva, stool, gastric juice and variable specimens. PCR provides excellent sensitivity and specificity, greater than 95%, as compared with other conventional tests and has more accurate results of detecting *H. pylori* in patients with bleeding.Several target genes including UreA,glmM,UreC,16S,rRNA,HSP60 and VacA genes ,had been used for detection of *H.Pylori*. (Wang , 2015).

2.8.2 Non-invasive methods

2.8.2.1 Urea Breath Test (UBT)

Is based on the ability of *H. pylori,* if present in the gastric environment, to break down orally absorbed ^{13}C ladled urea into carbon dioxide and ammonia, carbon dioxide diffuses in the blood, is exhaled via the lungs, and can be measured in the exhaled air. The test is easy to perform, and do not require endoscopy. ^{13}C is not radioactive, and can be safely used in children and women of childbearing age, however the machine is expensive. The sensitivity and specificity of UBT exceed 90% in most studies. False positive results due to other urease- forming pathogens are rare. UBT may produce false negative result s if performed after the use of the *H. pylori* and urease suppressive therapies, such as Proton Pump Inhibitors (PPI) and antibiotics (Gonzalez *et al*., 2014).

2.8.2.2. Serology

People infected with *H. pylori* generally have specific IgG and IgA antibodies circulating in their blood and these can be detected by serological tests. Tests for detection of antibodies to *H. pylori* circulating in blood, or found on saliva have excellent sensitivity and specificity of above 95% cheap and simple compared with invasive techniques. They can give very quick result even with minutes of first consultation, and is the only tests which are not to give false negative results in patients who taken antibiotics, or bismuth compounds or omperazole in recent past, because there are different strains of *H. pylori,* antigen for antibody manufacture is generally prepared by using preparations from several different strains. Antibody assays in blood have measured IgG and IgA antibodies which have been shown to be specific for *H. pylori* and not other Gram negative organisms. Where both IgG, IgA assays have been compared with other testing methods, like culture and histology, IgG assays tend to have slightly higher sensitivity and specificity, and so anti IgG method tend to be favoured. The commercially assays are of two sorts: either microtiter- plate

assays for use in the laboratory, both types of assay usually have acut-off value set with control sera so that differentiate patients with *H. pylori* infection from those who don't, rather than quantify the concentration of the of circulating anti- *H. pylori* immunoglobulin. Antibody tests have high sensitivity and specificity, they also have the advantages of being non invasive and less cost (Khatun, 2014).

2.8.2.3 Stool Antigen Test (SAT)

This is a rapid test based on monoclonal antibody immunochromatography of stool samples. This test has been reported to be very specific (98%) and sensitive (94%).The results are positive in the initial stages of infection and can be used to detect eradication after treatment (follow up). Stool antigen tests are appropriate where prevalence of *H. pylori* infection is less than 30%. Other test methods are based on a sandwich enzyme immunoassay with antigen detection. *H.pylori* stool antigen test is accurate (as urea breath test), simple, fast and relatively inexpensive. Therefore, physicians can trust this test and start patient's treatment (Ali and Muhammad, 2015).

2.9 Treatment and prevention of *H. pylori* infection

The therapy for *H. pylori* infection involves multiple steps. In addition to antibiotics to eradicate the bacteria, another goal is to decrease the amount of acid secretion in the stomach and remove risk factors from further stomach irritation. Antibiotic therapy: two week course of combination antibiotic therapy is recommended, Clarithromycin, Metronidazole, Amoxicillin, and Tetracycline. It is important to confirm eradication of *H. pylori* (Alesandro *et al*., 2014). Since 50% of world population has detectable *H. pylori* in their stomachs, it is very difficult to prevent infection with this bacterium; however, suggestions have been made to prevent ulcers. Certain measures such as non-smoking, no drinking for alcohol, substituting acetaminophen (Tylenol and others) for aspirin for pain control, avoidance of caffeine, check for GI symptoms and immediate treatment, identify and reduce or avoid stress, wash

hands with clean water to avoid contracting the bacterium, and if infected with *H. pylori*, antimicrobial treatment may avoid ulcer formation and extension of disease (Charles, 2015).

2.10. Previous studies

A study conducted by Nur *et al* (2016) in South Australia aimed to detect the prevalence of *H.pylori* infection in newly arrived refugees attending the migrant health service found that out of 922 refugees 198 (21.5%) were *H.pylori* positive. Other study by Sarah *et al* (2008) in Australia aimed to detect the epidemiology of *H.pylori* infection in African refugee children resettled in Australia found that out of 183 children 149(182%) had *H.pylori* infection.

A study conducted by Mohammed hasosah (2019) in Saudi Arabia aimed to detect Accuracy of invasive and non invasive methods of *H.pylori* infection diagnosis in Saudi Arabia found that out of 303 children (49.8%) were *H.pylori* positive. all diagnostic test (serology(73%) and stool antigen test (78%))showed moderate to high specificites but Biopsy and histology showed the highest specificity. Other study by Yuli *et al* (2009) in Colombia amid to Comparison between ELISA and UBT which found the UBT presents a Sensitivity and Specificity in post treatment equal or greater than 95% comparing to ELISA with monoclonal antibodies.

CAPTER THREE

3. MATERIALS AND METHODS

3.1 Study design

This is a descriptive, cross- sectional study.

3.2 Study area duration

The study was conducted at Tasabih Medical Complex in Khartoum State Sudan from period between March and October 2019.

3.3 Study population

Patients from both genders of southern Sudan refugees attending Tasabih Medical Complex were enrolled in this study.

3.4 Inclusion criteria

All Southern Sudan refugees that attending Tasabih Medical Complex from March and October 2019 with different ages and genders.

3.5 Exclusion criteria

Non Southern Sudan refugees whom attending Tasabih Medical Complex between March and October 2019.

3.6 Ethical consideration

This study was approved by college of Medical Laboratory Science Ethical Board, SUST. Permission from Tasabih Medical Complex was applied and verbal consent was taken from participants involved in the study.

3.7 Sample size

A total of two hundred (n=200) of southern Sudan refugees were participated in this study.

3.8 Sampling technique

This study based on non probability, convenience sampling technique.

3.9 Data collection

Structured questionnaire was used for collection of the data.

3.10 Specimen collection

3.10.1 Blood sample

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

3.10.2 Stool sample

Fresh Stool samples were collected into wide mouth container outer labelled for detection of *H. pylori* antigen.

3.11 Laboratory methods

3.11.1 Immunochromatograpy test

The kit components were brought to reach room temperature if necessary. The pouch and card were opened. Once opened, the test card must be used immediately. The test card was labelled with patient's identity. 2-3 drops (80-120 µl) of serum were applied to the sample well marked as "S ". At the end of 10 minutes the results were read. Strong positive sample may show result earlier. For stool sample using wood stick small portion of the stool sample was transferred into the buffer, incubated for 2 minutes and then two to three drops of the mixture were poured in the hole of the ICT of *H. Pylori* stool antigen detection. (*H. pylori* Antigen Rapid Test Cassette All Test Biotech Company).

3.11.1.1Interpretation of the results

Negative: only control line appears.

Positive: both control line and the test line appear.

3.11.2 Enzyme Linked Immuonosorbant Assay (ELISA)

Purified H. pylori antigen was coated on surface of micro wells. Diluted patient serum was added to the well *H.pylori* IgG specific antibody, if present, will bind

to the antigen all unbound materials were washed away. Enzyme conjugate was added, which binds to the antibody–antigen complex. Excess enzyme conjugate was washed off and the chromogen (substrate reagent) was then added. The enzyme catalytic reaction was stopped at specific time by sulphuric acid.According to manufactures protocol 100µl from positive and negative controls and diluted patient samples were transferred into the micro plate wells then incubated for 30 minutes at room temperature. The wells were then emptied and subsequently manual washed 3 times using 300 µl of wash buffer (appendix 1).Then 100 µl of enzyme conjugate was added into each of micro plate wells and incubated for 30 minutes at room temperature. The wells were then emptied and subsequently washed 3 times using 300 µl of wash buffer (appendix 1).Then 100 µl of TMB chromogen substrate solution was added into each of micro plate wells and incubate for 30 minutes at room temperature. The reaction was stopped by adding 100 µl of stop solution (sulphuric acid) into each micro plate wells in the same order and the same speed as the substrate solution was introduced. Photometric measurement of the colour intensity was done at wavelength of 450 nm and reference wavelength between 620nm within 30 minutes of adding stop solution. Prior to measuring the microplate was slightly shake to ensure homogenous distribution of the solution. The above mentioned procedure was carried out for both IgG and IgM.

3.11.2.1Interpretation of the results

Result evaluated by calculating a ratio of the extinction value of the patient serum sample over the extinction value of the calibrator.

Ratio <0.8: negative

Ratio ≥0.8 to <1.1: borderline

Ratio ≥1.1: positive

3.11.3 Urea breath test

The test kit was open, two paper cups were removed and filled with water, Patient has swallowed the capsule with one cup of water, Wait for 3 minutes,

and then asked the patient to drink the second cup of water, Wait for 7 minutes and insert straw into balloon in the kit through the slit in the neck of the balloon. After this 7 minute period, asked patient to hold their breath for 10 seconds and then blow into the balloon with one breath. Once the balloons was filled, remove the straw and tie a knot in the neck of the balloon, Placed the balloon in the box provided and return to the laboratory for analysis.

3.11.3.1 Interpretation of the results

If Co2 more than 50 that mean *H.pylori* positive

If Co2 less than 40 that mean *H.pylori* negative

3.10 Data analysis:

The data that collected from questionnaire and laboratory results were analyzed by Statistical Package for Social Sciences (SPSS) version 11.5 computerized program Significant level were set at ($p<0.05$).

CAPTER FOUR

RESULTS

A total of two hundred Southern Sudan refugees (n=200) were included in this study , their age between 1-80 years with mean of age of (36.9±16.9SD). The age of participated refugees were grouped as following: from 0 to20 years were 34(17%), 21-40 years were 87(43.5%) ,41-60 years were 59(29.5%) and from 61-80 years were 20(10%) as shown in table (4.1). Males were 106(53%) and female were 94(47%) as shown in table (4.1).

The source of water were divided into tap water, service water and compound water which found 102(51%), 41(40.5%) and 47(23.5%) of them positive for *H.pylori* respectively as shown in table (4.1).

According to status of coming were divided into new coming refugees 120 (60%) from which 81(67.5) were positive for *H.pylori* and old coming refugees were 80(40%) of them 26(32.5%) were positive for *H.pylori* as shown in table (4.2).

According to Symptoms they divided into symptomatic 126(63%) which found 98(49.5%) of them positive for *H.pylori* and asymptomatic 84(37%) which found 9(4.5%) of them positive for *H.pylori* as shown in table (4.2).

All specimens were examined for the presence of *H.pylori* using ICT antigen and antibody, ELISA (IgM and IgG) and urea breath test(UBT) ,The result showed that out of 200 specimen 107(53.5%),123(61.5),102(51%), 116(58%) and105(52.2%) samples were found positive for *Helicobacter pylori* using UBT,ICT antigen, ICT antibody, ELISA IgG and ELISA IgM respectively as shown in table (4.3). Sensitivity and Specificity of ICT antibody, ICT antigen ELISA IgG ,ELISA IgM and UBT is 71%/49%,77.5%/79.5,78.5%/65, 83%/82.7% and 84.5%/84.2% respectively.

Table (4.1): Demographic data of Study Population

Variables		Frequency	Percentage
Age group /year	0-20	34	17%
	21-40	87	43.5%
	41-60	59	29.5%
	61-80	20	10%
	Total	200	100%
Sex	Male	106	53%
	Female	94	47%
	Total	200	100%
Status of coming	New coming	120	60%
	Old coming	80	40%
	Total	200	100%
Water sources	Tap water	102	51%
	Service water	51	25.5%
	Compound water	47	23.5%
	Total	200	100%
Symptoms	Yes	126	63%
	No	84	37%
	Total	200	100%

Table (4.2) :Associations between *H.pylori* and Risk factors

Variables		Positive	Negative	*P value*
Age group /year	0-20	17(8.5%)	17(8.5%)	0.474
	21-40	52(26%)	35(15.5%)	
	41-60	28(14%)	31(15.5%)	
	61-80	10(5%)	10(10%)	
	Total	107(53.5%)	93(46.5)%	
Sex	Male	62(31%)	44(22%)	0.133
	Female	45(22.5%)	49(24.5%)	
	Total	107(53.5%)	93(46.5)%	
Status of coming	New coming	81(67.5%)	39(32.5%)	0.00
	Old coming	26(32.5%)	54(67.5%)	
	Total	107(53.5%)	93(46.5)%	
Water sources	Tap water	41(40.2%)	61(59.5%)	0.00
	Service water	41(80.4%)	10(19.6%)	
	Compound water	25(53.2%)	22(46.8%)	
	Total	107(53.5%)	93(46.5)%	
Symptoms	Yes	98(49%)	28(14%)	0.00
	No	9(4.5%)	65(32.5%)	
	Total	107(53.5%)	93(46.5)%	

Table (4.3): Comparison between UBT and other methods

Methods		UBT		Total	P. value
		Positive	**Negative**		
ICT*H. Pylori* Ab	Positive	76 (38%)	47(23.5%)	123(61.5%)	0.003
	Negative	31 (15.5%)	46(23%)	77(38.5%)	
	Total	107 (53.5%)	93(46.5%)	200 (100 %)	
ICT *H.Pylori* Ag	Positive	83 (41.5%)	19 (9.5%)	102(51%)	0.00
	Negative	24 (12%)	74 (37%)	98(49%)	
	Total	107 (53.5%)	93 (46.5%)	200 (100%)	
ELISA IgG	Positive	84 (42%)	32 (16%)	116 (58%)	0.00
	Negative	23 (11.5%)	61(30.5%)	84 (42%)	
	Total	107(53.5%)	93(46.5%)	200(100 %)	
ELISA IgM	Positive	89 (44.5%)	16 (8%)	105(52.5%)	0.00
	Negative	18 (9%)	77(38.5%)	95(47.5%)	
	Total	107 (53.5%)	93(46.5%)	200(100%)	

CHAPTER FIVE

DISCUSSION

Among the 200 refugees 107(53.5%) were positively infected using the method urea breathing test while 93(46.5%) were negative.This result in accordance with result carried by Chaves (2009)inAustralia and his colleagues among Burmese Refugees in Australia which found 31\44 (80.5%) positive from Burmese refugees (Chaves *et al*., 2009).The present study revealed that there was no significant association between the prevalence of *H. pylori* infection and age, which was in agreement with study carried out in Eastern Sudan Which found 148(65.8%) tested positive for *H.pylori* (Tajeldin *et al.*,2014) by other researchers however, some other studies have shown different results in Mexico Which found in the age groups of 41-50(100%) and 61-70(100%) years old had significantly p=0.00 (Alvarado, 2013).

In this study there was no significant association between *H. pylori* infection and gender this was disagree with the finding reported in by Valliani Pakistan who found that *H. pylori* sero-positivity occurred more commonly in male than in female patients from 96 patient 36(38.7%) turned out to be *H.pylori* positive with a significant male (p =0.04) (Valliani *et al*., 2013).

Comparing the infection in newly coming refugees and old resident refugees , it was found that newly coming positively infected were 81/120(67.5%) while old were 26/80(32.5%) *H.pylori* is highy in new coming refugees significantly *p value* =0.00.

This study compared between the ICT and ELISA techniques in detection of *H.pylori* with sensitivity 71% and 83.% respectively our finding was similar to the findings of Khalifehgholi and his colleagues which found in a total of 91patient 46(50.5%) positive of *H.pylori* using serology and ELISA with sensitivity 91.3% and 73.9% respectively. (Khalifehgholi *et al.*, 2013).

Comparing tap, service and compound water it was found that refugees who are drink service water are more susceptible to infection by *H.pylori* than other *p value* =0.00 , which was in agreement with study carried out in Bhutan by Dorji Which found water and crowded living contribute to the prevelance of *H.pylori* (Dorji *et al.*, 2014).

5.2 Conclusion

Frequency of *H.pylori* is highy in Southern Sudan refugees especially in new coming refugees. There is no association between *H.pylori* of both age and gender, also the infection is higher in those whom drink service water. The UBT is more sensitive and specific than other methods.The antibody testing is for detection of *H. pylori* would continue to improve and play an ever increasing role in the primary clinician's diagnostic evaluation**,** but a positive serological test does not necessarily mean an active infection. ELISA testing is important in detection of *H. pylori* infection because results are given in a quantitative manner on contrast the ICT give only a qualitative result.

5.3 Recommendations

Urea Breath technique is recommended for use as a quick and easy method and standard for diagnosis and follow up of *H. pylori* infection.

ELISA technique is recommended for use as a quick and easy method to screen for *H. pylori* infection.

ELISA IgM technique is recommended for use as a specific and a reliable method for diagnosis of *H. pylori* recent infection.

ICT method should not be used as the only method of screening for *H. pylori* infection.

Further studies with large sample size should be done to diagnose *H. pylori* infections

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Appendices 1

Questionnaire

1. Patient no: ……..

2. Patient name: …….

3**. Age**: 0—20 ☐ 21—40 ☐ 41—60 ☐ 61—80 ☐

4. Gender: Male ☐ Female ☐

5. Water sources

A.Tap water ☐ B. Service water ☐ C. Compound water ☐

6. Symptoms: Symptomatic ☐ Asymptomatic ☐

7. Status of coming to Sudan

New coming refugees ☐ Old coming refugees ☐

8. Tests performed:

- ICT Test…… ☐
- ELISA Test……. ☐
- Urea Breath Test (UBT)…… ☐

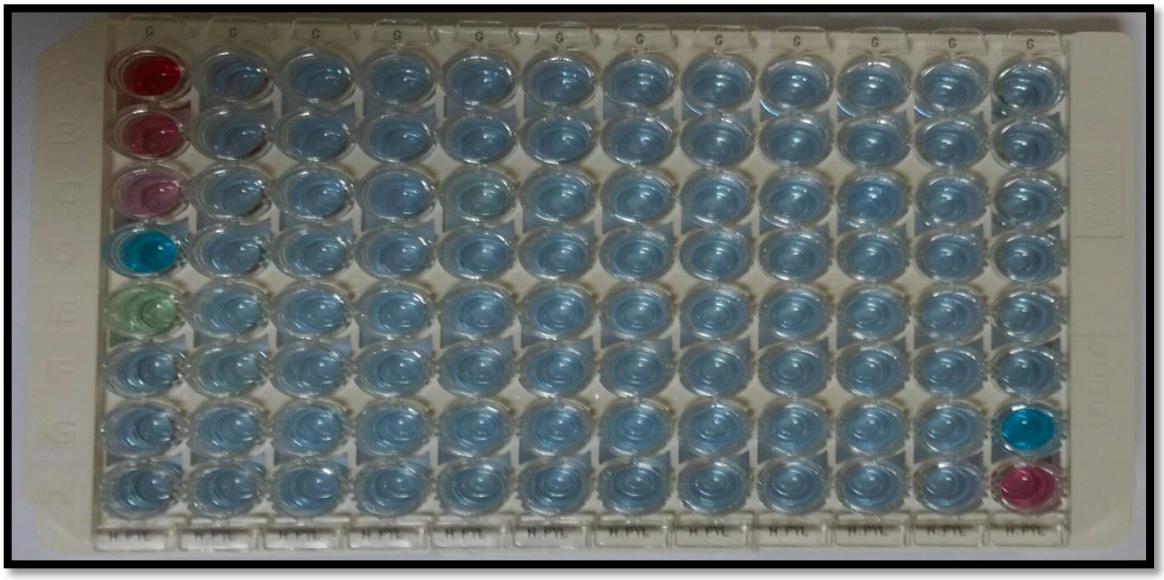
Laboratory results:

- ICT Test……
- ELISA Test…….
- Urea Breath Test (UBT)……

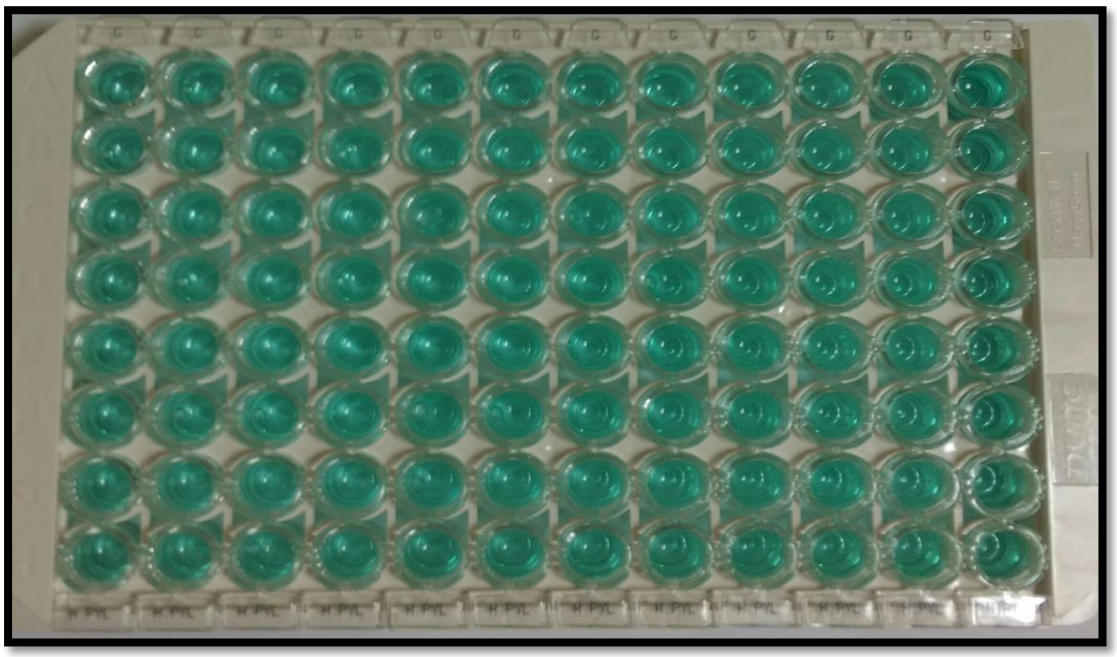
Appendices2

Image for ELISA, ICT practical work and UBT

Step 1: Dilution of sample in Crown tubes



Step 2: Addition of Samples, Calibrators and Control



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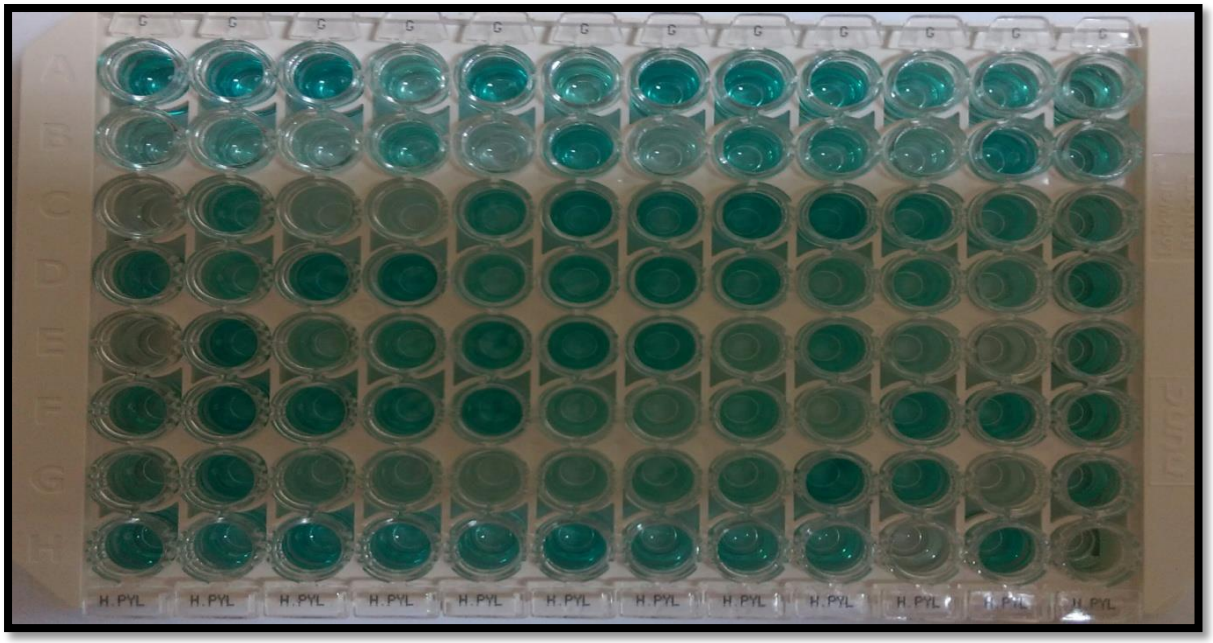
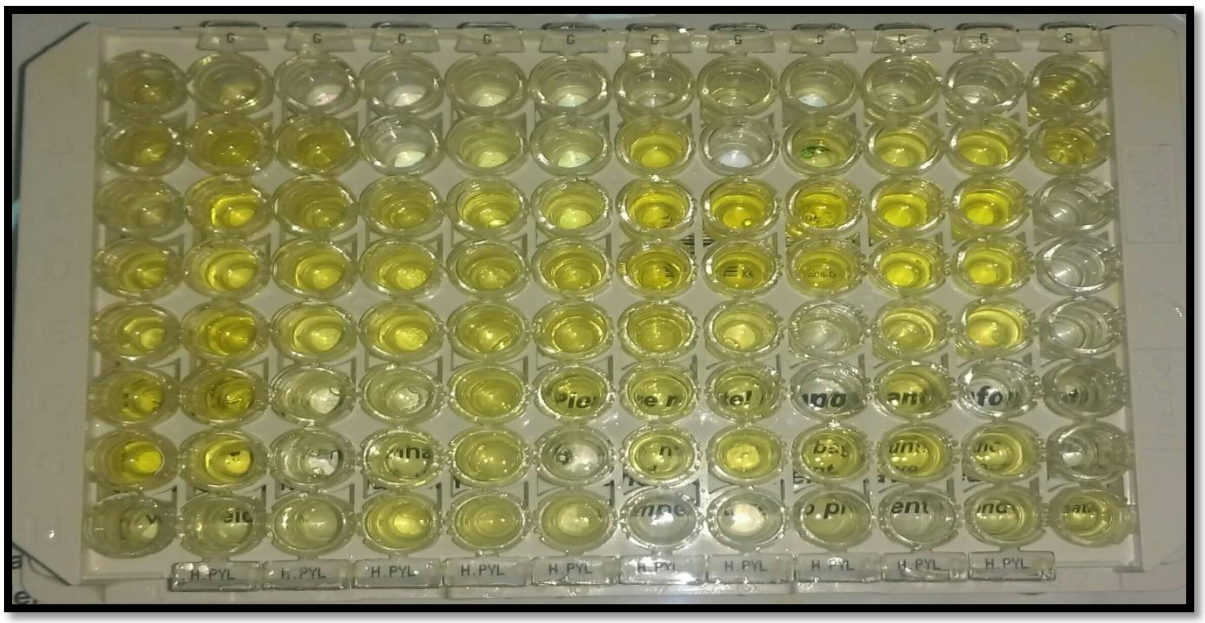
Step 3: Addition of enzyme conjugate

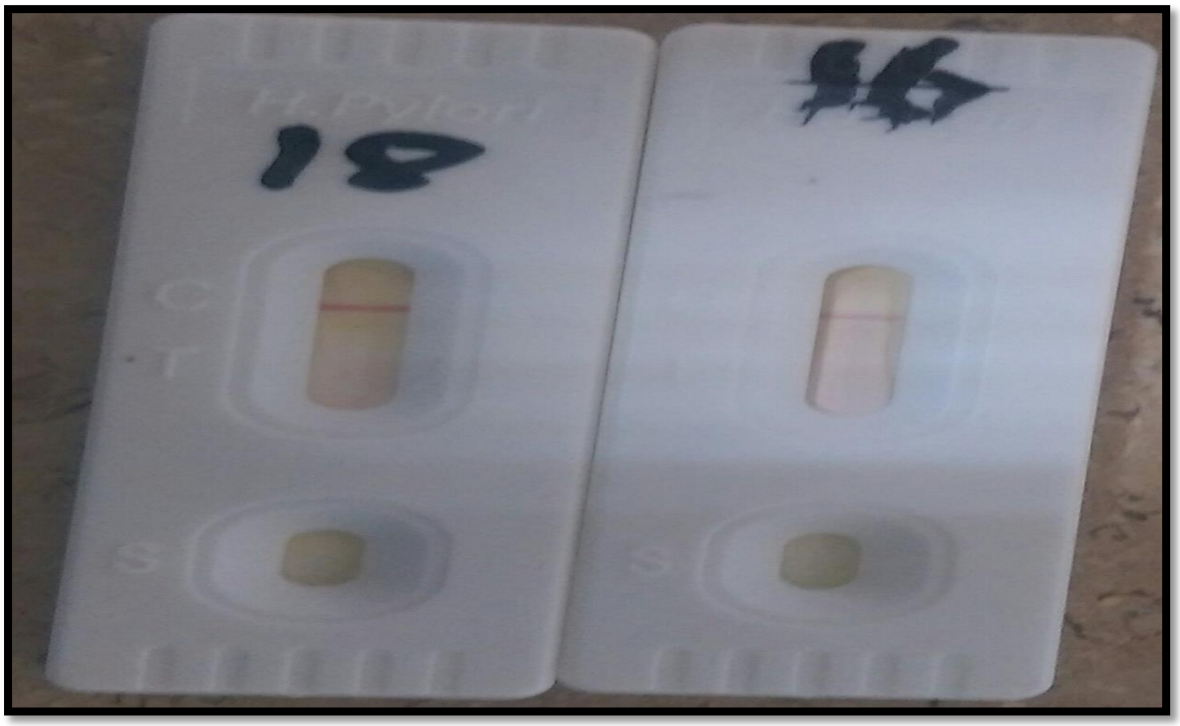


Step 4: Substrate addition

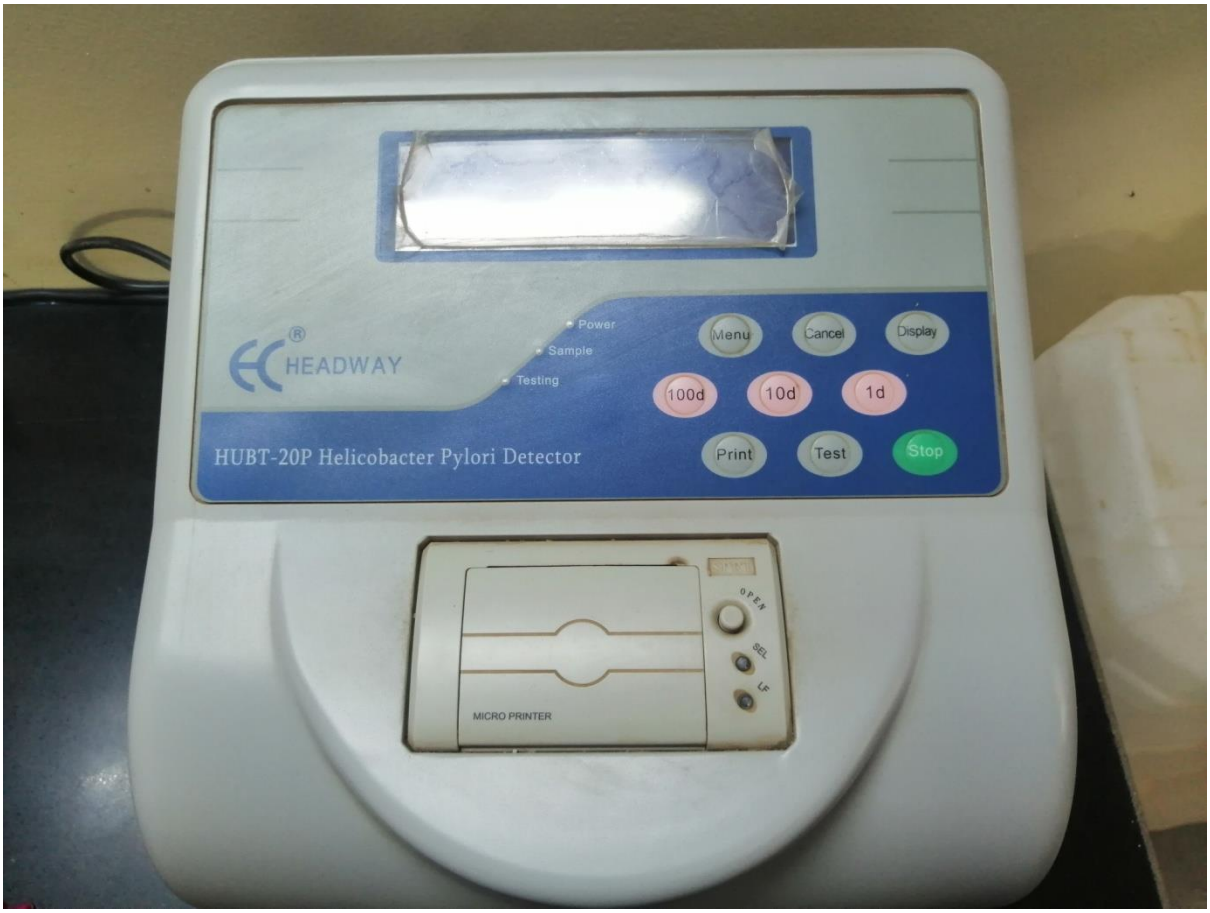


Step 5: Addition of stopping solution

ICT positive result



ICT negative results



Urea Breath test detector

Capsule of Urea