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Sudan University of Science and Technology

College of Graduate Studies

Detection of *Helicobacter pylori* in Stool Specimens using Immunochromatographic Assay and Polymerase Chain Reaction in Saad Rshwan Hospital

الكشف عن البكتريا الحلزونية البوابية في عينات البراز باستخدام الكشف المناعي و تقنية سلسلة تفاعلات البلمرة في مستشفى سعد رشوان

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الآيـــــة

قال تعالى :

(فَلِلَّهِ الْحَمْدُ رَبِّ السَّمَاوَاتِ وَرَبِّ الْأَرْضِ رَبِّ الْعَالَمِينَ)

صدق الله العظيم

سورة الجاثيه الايه (36).

DEDICATION

To my beloved mother,

For her wise, support,

You are always there for me

To my father

To my brothers

To my sisters

To my friends

AKNOWLEDGEMENT

Firstly, thanks to my LORD, ALMIGHTLY ALLAH to reconcile the study. After intensive work and patience of ten months. Today is the day writing this note of thanks is finshing touch of my dissertation. It has been a peroid of intense learing for me not only in the scientific area, but also on a personal level. Writing adissertation has a big impact on me. Iwould like to reflect on the people who have supported and helped me so much through the period. I would to thank my supervisor **Prof: Yousif Fadlalla Hamedelnil** for supervision and valuable advices, and all supports and motivations. My thanks also extend to **Dr**. **Hisham Alteyab Nor Aldaem** for his excellent cooperation an all opportunities and his patience. Also thanks extend to **Ustaza Muram** for continuous guiding and following. I am gratefull too many persons who shared memories and experiences. I thank very much every one who helps me.

ABSTRACT

Helicobacter pylori is a microaerophlic, Gram negative, motile, curved rod, which inhabits the gastric mucosa of the human stomach. The organism chronically infects billions of people world wide. Infections with organism potentially induces chronic gastritis and peptic ulcer disease. In a ddtion *H. pylori* a role in the etiology of gastric cancer and gastric MALT lymphoma. In this cross sectional study, the aim of present study to detect *H. pylori* in patients stool specimens using immunochromatography test and polymerase chain reaction among patients attending Saad Rshwan Hospital at time of specimens collections. Faecal samples were collected from 50 patients suffering from dyspeptics, consisting of 20 (40%) males, 30 (60%) females aged from 10 years to 85 years (mean = 32.24, SD = 17.6). *H. pylori* antigen rapid test immunochromatography were used to analyze the faecal samples for detection *H. pylori* antigen in stool. Among 50 faecal samples positive for *H. pylori* antigen, also feacal samples DNA were extracted by iNtron stool genomic DNA extraction mini kit, and by conventional polymerase chain reaction targeting the ure A gene in *H. pylori* was carried out to detect *H. pylori* DNA in feacal samples of already positive feacal samples by *H. pylori* ICT. Chisquare statistical analysis was used to determine *p.value* (0.05) significance range. Twenty nine (58%) of fifty feacal samples that had previously tested positive for the organism by ICT *H. pylori* antigen were confirmed positive by PCR, 10 (20%) males, 19(38%) females. But the associations between the ure A gene of *H. pylori*, age groups (*p. value*=0.8), gender (*p. value*=0.7), educations level (*p. value*=0.9), marital status (*p. value*=0.8), family history (*p. value*=0.4) and smoking behavier (*p. value*=0.5) of patients were not significant, not reach the significant range ≤ 0.05. The present study revealed ahigh frequency

of *H. pylori* in feacal samples, and the PCR may be more accurate in the diagnosis. Further work is needed to validate these results.

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المستخلص

البكتريا البوابيه هي سالبه الجرام ، اليفه الهواء القليل،متحركه،منحنيه، وعصوية. تعيش في الغشاء المخاطي في المعدة في الإنسان. هذا الكائن الحي يصيب بصورة مزمنة البلايين من سكان العالم. من المحتمل الإصابة بهذا الكائن الحي يحث التهاب المعده المزمن، التهاب القرحه الهضمية, بالإضافة الى سرطان المعدة وسرطان الغدد الليمفاوية. في هذة الدراسة الوصفية، الهدف منها الكشف عن الحلزونية البوابية في عينات البراز بواسطة الكشف المناعي وتفاعلات البلمره السلسلية لدى المرضى الذي حضروا مستشفى سعد رشوان في وقت جمع العينات. عينات البراز جمعت من خمسين مريض يعانون من أعراض التخم، يضموا 20 (40%) ذكور، و 30(60%) إناث،وأعمارهم تترواح من 10 سينين الى 85 سنة، متوسط أعمارهم 32.24 سنه،والإنحراف المعياري للاعمار هو17.6. مولد المضاد للبكتريا البوابية في عينات البراز حلل بواسطه الكشف المناعي.من بين 50 عينة براز إيجابية لمولد المضاد للبكتريا الحلزونية البوابية أيضا أستخرج الحمض النووي الرايبو سومي من عينات البراز بواسطه إنترون لجينوم الحمض الرايبوسومي منقوص الاكسجين من عينات البراز، وبواسطه تفاعلات البلمره السلسلية التقليدية، التي إستهدفت جين اليوريا للحلزونية البوابية. للكشف عن جين اليوريا للحلزونية البوابية في عينات البراز و التي هي حقيقه ايجابية للبكتريا الحلزونية البوابية بواسطه الكشف المناعي.وعن طريق التحليل الأحصائي تم تحديد *P.value* (58%)29 من 50 عينه براز والتي هي مسبقا والتي هي إيجابية لوجود الحلزونية البوابية بواسطة الكشف المناعي. أكدت بواسطة تفاعلات البلمرة السلسليه. من بين عينات المرضى10(20%) رجال، و19 (38%) نساء. وتوصل الى ان إنتشار الجين Ure A في هذة الدراسة ليس له دلالة مهمة مع الجنسين، وفئاتهم العمريه والحاله الزوجيه، للمرضى والسوابق العائلية للاصابة والمراحل التعليميه للمرضئ. وأختتمت هذة النتائج بتردد عالي لهذا الممرض، وأن تفاعلات البلمرة السلسلية قد يكون أكثر دقة في التشخيص وقد يكون هناك حاجة الى المزيد من العمل للتحقق من صحة هذة النتائج.

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ABBREVIATIONS

ATP Adenosine Triphosphate.

Bab A Blood group antigen binding adhesion.

Cag A Cytotoxin associated gene A.

ºC Centigrate.

Cag PAI Cytotoxin associated gene Pathogenicity Island.

DNTP Double nucleotide tri phophate**.**

Dup A Duodenal ulcer promoting gene A.

Fla Flagella.

GERD Gastric Eseophageal Reflux Disease.

GGT GammaGlutamyl Transpeptidase.

IDA Iron Deficiency Anemia.

IHD Ischemic Heart Disease**.**

IL Interlukien

ITP Idiopathic Thrombocytopathic Purpura.

IU International Unit.

Kat A Catalase A.

Le antigen Lewis antigen.

LPS Lipopolysaacharide.

MALT Mucosal Associated Lymphocytic Tissue.

MCV Mean Corpuscular Value.

MS Multiple Sclerosis**.**

µl Microliter.

NUD Non Ulcer Despsia.

OMP Outer Membrane Protien.

PCR Polymerase Chain Reaction

Rpm rotate Per Minute.

RUT Rapid Urease Test.

Sab A Sialic acid binding adhesion.

SAT Stool Antigen Test.

Sop Superoxidase dismutase.

UBA Urea Breath Test.

Vac A Vacuolating associated cytotoxin A.

CHAPTER ONE
INTRODUCTION

1. Introduction

1.1. Introduction:

Helicobacter. *pylori* is Gram negative rods, microareophil, spiral bacterium (Chessbrough 2006). *Helicobacter Pylori* bacterium found in mucosa overlying the gastric mucosa (cells line the stomach) (Pagana and pagana 2011). Itis etiology of peptic ulcer disease, gastritis, gastric mucosa associated lymphoid tissue lymphoma and gastric adeno carcinoma (Fallahi and Maleknejad 2007). *H. pylori* inhabits stomach or lower gastro intestinal tract of humans, also can be present in saliva, dental plaque and feces (Syam *et al.,* 2015). Person to person transmission occur vial oral oral or fecal oral routes. More than half of world´s populations is known to be infected with *H. pylori,* (Ozbey and Hanafiah 2017). In developing countries, its infection rate over 60%. Anumber of studies found poor hygiene standards, crowded house hold, deficient sanitations are important acquistion of infection in childhood and spreading of these disease (Syam *et al.*, 2015). In developed countries the prevalance 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).The 20% prevalence of infection with *H. pylori* a mong adolscents in United States in comparison to infection rates exceeding 90% by five years of age in parts of developing countries (Fallahi and Maleknejad 2007). Lower socioeconomic, non filtered water and smoking to be risk factors for *Helicobacter pylori* infections.The role played by other factors including blood grouping type, alcohol consumption, tobacco use, dietry, nutritional influences and genetic predisposition to infection has also been studied, but results have been in consistent (Frederich *et al*., 2002). *Helicobacter pylori* is risk factor of gastric and duodenal ulcers, or even ulcerative esophagitis (Pagana and Pagana 2011), it also class one carcinogen to the gastro

intestinal tract by World Health Organization. It is in the same category as cigarette smoke is to lung cancer (Meena *et al*., 2017).Gastric colonization by this organism has been reported in about 90% to 95% of patients with duodenal ulcers, 60%to70% of patients with gastric ulcers, and in about 20% to25% of patients with gastric cancer(Pagana and Pagana 2011). Aproximately 10% of healthy persons younger than age of thirteen have gastric clonization with *H. pylori*. Gastric clonization increase with age, and most of people with gastric clonization remain asymptomatic and never develop ulceration (Pagana and Pagana 2011). *H. pylori* has been recognized as one most chronic infections in humans and a ssociated with peptic ulcer disease, gastric adenocarcinoma and primary gastric B cell lymphoma (Syam *et al*., 2015). Researchers have found that infection with *H. pylori* can result in cardio vascular diseases, hematological, hepatobilliary and metabolic diseases (Liu *et al*., 2017). Various diagnostic methods exist to detect infection, and the choice of one method or another depend on several factors such as accessibility, advantages and disadvantages of each method, cost and the age of patients (Gonzalez *et al*., 2014).Techniques utilized to detect *H. pylori* infection are grouped invasive and non invasive tests, the invasive include rapid urease test (RUT), microbiological culture (biopsy), histology, and polymerase chain reaction (esophago gastro duodenoscopy is applied), the non invasive methods consist of stool antigen, urea breath test (UBT) and blood test *H. pylori* antigen or anti *H. pylori* anti body, however single test is non reliable.

1.2 Rationale

More than half of world´s populations is 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 prevelance *of H. pylori* in 2015 was estimated to be 4.4 billion people (Bjorkma 2017). It is estimated that *H. pylori* positive patients have a 10-20% life time risk of developing distal gastric cancer.*H. pylori* lead to acute and chornic gastritis, peptic ulcer and duodenal ulcers. *H. pylori* approximately cause 95% of duodenal ulcers and 85% of gastric ulcers. According to WHO is classified as class 1 carcinogen and also cause Gastric MALT lymphoma. In Sudan many studies done on *H. pylori* which reflect the infections with *H. pylori.*

1.3. Objectives:

1.3.1. General objectives:

Detectionof ure A gene of *Helicobacter pylori* in stool specimens using immunochromatographic assay and polymerase chain reaction in Saad Rshwan Hospital.

13.2 Specific objectives:

1. To determine *H. pylori* antigen from stool samples of the patients by immunochromatography test.

2. To detect urease (ure A) gene of *H. pylori* of the patients by using Polymerase Chain Reaction technique.

3. To determine correlation of age, gender, smoking, faimly history, marital status and educations level and *H. pylori* ureA gene positive detections.

CHAPTER TWO
LITERATURE REVIEW

2. Literature Review

2.1 History

The presence of spiral shaped microorganism in human stomach was discovered hundered years ago by polish clinical reseacher, professor Warely Jaworski at Cracaw Jagiellonian University (Konturek 2003), who investigated sediment of gastric washing obtained by lavage from humans in 1899, among some rods like bacteria, found bacteria with characterstic spiral shape, which he called *vibrio rugula* (Konturek 2003), he was the first to suggest the possible role of this organism in the pathogensis of gastric diseases, his work was included in hand book of gastric diseases, but it had little impact because, was written in polish, the presence was then confirmed in animals by Giullio Bizzazero (Konturek 2003) but was not taken seriously until the late 1970s, when RobinWarren pathologist in Perth, Australia, noted the presence of spiral bacterium overlying gastric mucosa over inflamed tissue (Konturek 2003), who researched further with Barry Marshal from 1981(Marshall 1984), after unsuccesful attempts at culturing the bacteria from stomach, finally succeeded culturing these organism 1982 from eleven patients with gastritis, when un intentionally left their petridishes incubating for five days over Easter weekend, to demonsterate *H. pylori,* caused gastritis, Marshal drank the beaker of *H. pylori* culture, he became ill with nausea and vomiting, several days later, an endoscopy ten days after inoculation revealed signs of gastritis, and the presence of *H. pylori,* the sign of gastritis resolved after subsequential therapy with first doxycycline and bismith sub salicylate *(*Kusters 2006*).* These results suggested *H. pylori* was the causative agent (Marshal 1984). The discovery was made by Warren and Marshall, it revolutionized the treatment of duodenal and gastric ulcers (Greenwood *et al.,* 2003). This discovery result in awarding of 2005 Nobel Prize in physiology or

medicine to Robin Warren and Barry Marshall after discovery of this bacteria and its role in gastric and peptic ulcer disease(Kuster 2006). The Sydney gastroenterologist Thomas Borrody invented the first triple therapy for treatment of duodenal ulcers in 1994(Borody *et al.,* 1989). Originally called *Campylobacter pyloridis* the name changed to *campylobacter pylori* and then later to *Helicobacter pylori* as specific morphologic, structural and genetic features indicated that it should placed in to a new genus (Konturek 2003).

2.2. *Helicobacter*

To day the genus Helicobacter contain 20 recognized species, with many species a waiting formal recognition (Kusters 2006). Helicobacter species and specific host *Helicobacter pylori, Helicobacter cinadei, Helicobacter fennelliae, Helicobacter pullorum, Helicobacter heilmanii Helicobacter rappinii* thier host is human, while *Helicobacter heilmanii* also can infect dogs and cats *Helicobacter mustelae* infects dogs, cats, while *Helicobacter hepaticus* infect rodents (Chakraborty 2004., Kusters 2006).

2.3. General properities of Helico bacters

Helicobacters are motile usually with multiple bipolar sheathed flagella, *Helicobacter pylori* in contrast to other Helicobacter species posses multiple monopolar sheathed flagella (Chakraborty 2004). Members of genus Helicobacter species are all microaerophilic organisms and in most cases are catalase and oxidase positive and many but not all are also urease positive (Kusters 2006).

2.4. Habitat

Helicobacter species can be sub divided in two major lineage, gastric Helicobacters species which include: *Helicobacter pylori*, *Helicobacter*

mustelae and *Helicobacter heilmanii*, these adapted to live in conditions found in gastric mucosa, surface and entero hepatic (non gastric) Helico bacter species(Kusters *et al.,* 2006) include, *Helicobacter hepaticus* (Kusters 2006) *Helicobacter canis*, *Helicobacter cinaedi*, *Helicobacter fennelliae*, *Helicobacter pullorum* and *Helicobacter rapinii* these inhabiting the lower gastro intestinal tract of man (small intestine, colon, rectum, hepatobiliary tract, these entric Helico bacter isolated from rectal swabs and feaces (Chakraborty 2004).

2.5. *Helicobacter pylori*

2.5.1. Classifications

Kingdom Bacteria, phylum Proteobacteria, class Epsilonprotbacteria, order Campylobacterales, family Helico bacteraceae, genus Helicobacter, species *Helicobacter pylori* (Goodwin *et al.,* 1989).

2.5.2. Morphology

H. pylori is Gram negative spirally shaped bacterium, motile, 0.5-0.9 wide, 2-4 μm length,stirctly microaerophilic, it require carbon dioxide for growth, and has tuft of sheathed unipolar flagella (Greenwood *et al.,* 2003).

2.5.3. Growth requirements

H. pylori its microaerophilic, with optimal growth at oxgen level of 2% to 5%, additional need of 5% to10% and high humidity.There is no need for hydrogen which is no deterimental for growth. Many laborateries utlize standard microaerophilic condtions of 85% nitrogen, 10% carbon dioxide and 5% oxgen for *H. pylori* culture. Growth occur at 34 ºC to 40 ºC, with optimum at 37 ºC. *H. pylori* is neutralophile, growth occurs at narrow PH range 5.5 to 8.0 with optimal growth at neutral PH (Kuster *et al.,* 2006).

2.5.4. Specimens

Biobsy, fecal specimens and dental plaque (Goodwin *et al.,* 1989).

2.5.5. Culture

Helicobacter pylori can grow on different media containing blood or blood products (blood or lysed blood agar plates). Most studies used Brucella agar or Columbia agar as the agar base, an amount of 7% to10% blood compared to 5% blood, horse blood improve the growth of *H. pylori* compared to sheep blood. Egg yolk emulsion described as blood free medium for growth of *H. pylori*. Skirrow´s and Dent´s selective media the best commercial selective media available (Andersen *et al*., 2001). *H. pylori* grows slowly in liquid media with formation of high number of cocoid forms, selective media addtion to non selective media is usually recommended in routine culture (Andersen *et al*., 2001).

2.5.6. Identification

2.5.6.1. Clonial morphology

H. pylori colonies are small (0.5 to 2µm), translucent to yellowish colonies on 7% lysed horse blood agar and with transluscent to pale grayish colonies of (0.5-1.0)mm in size on blood agar (Goodwin *et al.,*1989). Invery young culture its appears almost straight rods on microscopy, after 3to 5 days of incubation the bacteria look pleomorphic with irregular curved rods (Goodwin *et al*., 1989).

2.5.6.2. Biochemical Tests

Catalase test, oxidase test, and urease test are positive. Glycine (1%) tolerance test, growth on nutrient agar, brucella agar, blood agar, brain heart inffusions, serum supplement agar are positive growth at 35 ºC and 40 growth at 1.5% sodium cholride growth at 0.5 and 0.75% sodium cholride (Thirunavukkarasu *et al.,* 2017).

2.5.7. Virulence factors

Inspite of major research efforts world wide there is still lack of knowledege on specific virulence factor of *H. pylori* which determine pathogensis of microbe and this due to:

Human being the only known host to *H. pylori* and thus animal model are mostly use less.The association of specific factors with gasteroduodenal disease of only regional value and being only assesed for small local population and might not of general value (Abdi and Kuster 2013). Many virulence factors involved in patho mechanisms of infection including various enzymes (urease, catalase, lipase, phospholipase, protease, produced by all described strains of *H. pylori* and toxins such as vacuolating cycotoxin gene, cycotoxin associated gene major *H. pylori* virulence factor include:

2.5.7.1 Adhesions

2.5.7.1.1 Outer membrane protien (OMP)

which encoded by OipA gene, outer membrane protien act as a dhesion (Argent 2008), one such OMP is OipA,act as adhesion, is reported to be involved in a ttachement of *H. pylori* to gastric epithelial cell in vitro, also play role in colonization of gastric mucosa and act as pro inflammatory response (Lui 2004).

25.71.2 Blood group antigen – binding adhesion (babA)

BabA, encoded by the b ab A2 gene is an adherence factor that binds to to Lewis B (Le^b) blood group antigens found on the gastric epithelial cells. Bab a mediate adherence of the *H. pylori* to gastric epithelium, play a critical role of the delivery of bacterial virulence factor that damage host tissue either directly or throught inflammatory or auto immune reaction that may lead to ulcer disease (Lui 2004).

2.5.7.1.3 Sialic acid binding adhesion (SabA)

SabA 70 kD protien that belong to hop family of *H. pylori* outer membrane protien genes including babA, sabAis found to be present into majority of *H. pylori* isolates. The Sab A bind sialy- Lewis x antigen in the membrane glycolipids (Lui 2004).

2.5.7.2 Flagella (Fla A, Fla B)

Flagella is crucal for rapid passage to the lumen and seqential entery of mucosal layer, the adhesion to epithelial is not enough for sucessful clonization (Abdi and Kuster 2013).

2.5.7.3 Phospholipase and alcohol dehydrogenase

Providing acess to the epithelium by altering, weaking mucosal barrier, and direct damage to epithelial cells (Lui 2004).

2.5.7.4. Catalase (KatA) and superOxidase dis mutase (SOD)

Both protect bacteria against damaging effect of hydrogen peroxide (Hazell *et al.,* 1991). KatA is homotetrameric protien, a typical catalase, lacks peroxidase activit, catalase appears to be cytosolic protien, however there are data indicating periplasmic and possible surface location.The gene

KatA has been sequenced, KatA is an excellent vaccine antigen against *H. pylori* (Harris et *al*., 2003).

2.5.7. 5. Phosphatase and adenosin tri phosphatase (ATP)

Are essential for generation of energy as well as the synthesis and transportation of ions and cell products (Lui 2004).

2.5.7.6. Urease enzyme

It comprise up to 10% of the total bacteria protien content, which reflect the important of this enzyme for this bacteria, the ammonia produced not only increase PH, but also has cytotoxic effect on gastric epithelial cells (Abdi and Kuster 2013). The low PH can be advantage of this bacteria and it has adverse effect on their competives colonize the stomach, in addtion to stability of the anti microbial used to eradicate the *H. pylori* infection (Abdi and Kuster 2013).

2.5.7.7. Gamma glutamyl transpeptidase (GGT)

Is bacterial virulence factor that convert the glutamine into glutamate and ammonia, and convrts glutathione into glutamate and cysteinlglycine. *H. pylori* cause glutamine and glutathione consumption in the host cells, ammonia and oxgen reactive species generation. These products induce cell arrest, appotosis and necrosis in gastric epithelial cells. GGT may also inhibit appoptoss and induce gastric epithelium proliferation by induction of epidermal growth factor related peptides, inducible nitric oxide synthase. *H. pylori* induces immunotolerance through of Tcell mediated immunity and dendritic cell differentiation (Ricci *et al*., 2017).

2.5.7.8. Vacuolating associated cytotoxin A (VacA)

It is highly immunogenic, 45KD (Rosler et *al*., 2014). It comprises of two variable part s region encode the signals peptide and the m region (middle), (Argent 2008). VacA is present in all *Helicobacter pylori* strains (Abdi and Kuster 2013), but is pleomorphic among strains (Argent 2008). Vacuolating cyotoxin bind to host cell and it induces cyto plasmic vacuolation in gastric cell, also induce massive vacuolation in epithelial cell in vitro, it induce distruption of endosomal and lysosomal activity, it interferes with cytosekleton-dependant cell function. It induces membrane channel formation, appoptosis due to release of cytochrome from mitochondria, immunomodulation. Vac A inhibit Tcell activation and proliferation, some studies show that the vacA inhibit some other signaling (Abdi and Kuster 2013).

2.5.7.9. Cytotoxin associated gene A (Cag A)

The cytotoxin associated gene is the marker for agenomic pathgenicity Cag island of about 35 kb whose presence is associated with amore sever clinical outcome. All most all cag +ve strains produce the cagA protien, which in turn elicits detectable local and systemic antibody response.Cag A was described as an immuno dominant antigen with molecular weight of 120 to140kD (Lui 2004). In recent studies have shown that the translocationof cag A into host epithelial cells is accomplished, through type IV secretion system that is encoded in the cag pathogenicity island (Lui 2004).

2.5.7.10. Cytotoxin associated gene pathogenicity island (Cag PAI)

Approximately a 40Kb, clusters of genes in *H. pylori* chromosome.Gene encode eighteen protiens that serve as bulding bloks of syringe like structure (type IV secretion apparatus, which faciltate the translocation of

cagA into cystol of gastric epithelial cells. peptidoglycan and possibly the bacterial factors into host cells(Rosler *et al.,* 2011).

2.5.7.11. Duodenal ulcer promoting gene (DupA)

In 2005 first disease specific virulence factor that induce duodenal ulcer and had action on gastiric cancer was identified and was named Dup (Abdi and Kuster 2013).

2.5.7.12. Lewis antigen mimacry

The O antigen of lipo poly saccharide (LPS) in most *H. pylori* expresses the lewis blood group antigen (Le) which is similar to those of the host.The ability of *H. pylori* to produce lewis antigen resembling of those of the host appears favor survival of the bacteria in the stomach by evading the immuno response, in addtion this adaptation may also faciltate adherence of the bacteria to the gastric mucosa, another role of the presence of the Lewis determinant is the possible involvment in the pathogenicity. It was prposed that during an infection *H. pylori* induce anti Le antibodies which bind the bacteria and also the gastric epithelial cell, subsequently lead to tissue injury (Beeling 2004).

2.5.7.13. Interlukin -8

Is small peptide (chemokine), secreted by avaritey of cell types. IL-8 serves as apotent inflammatory mediator reccuriting activated neutrophils. Several studies show that *H. pylori* strain capable of inducing IL-8 secretion from gastric carcinoma invitro (Dunn *et al*., 1997).

2.5.8. Risk factors of *H. pylori* infection

Several socioeconomic has been associated with *H. pylori* infection, in particular subjects with low socioeconomic status,measured also as alow

family income had higher likehood of carring *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, occuptionally acquired infection is reported espcially when endoscopists who did not wear gloves during procedure increasing the risk of become infected (Eusebi *et a.,l* 2014).

2.5.9. Transmission

The way by which *H. pylori* transmited still unclear. Interpersonal transmission appears to be main route three routes were described, the least common is iatrogenic, in which tubes endoscopes or specimen in contact with gasteric or gasteric mucosa from one person are introduced to another improved disinfection of endoscopies reduce the incidince of transmission. Fecal oral transmission is perhaphs most important, fecally contaminated water may be source of infection, but the organism has not been isolated from water.Food borne transsmission has not been substained. Finally oral-oral transmission has been identified in the case of African women who pre masticate foods given to their infants. There is no identified association of infection with sexuall transsmission, therefore if it occur uncommon (Eusebi *et al*., 2014).

2.5.10. Signs and symptoms

If you have an ulcer, you may fell dull or buring pain in the belly, it may come and go, but you shall probably feel it most when stomach is empty, such as bettween the meals, or in the middle of night. It can last for few minutes, feel better after you eat, drink milk or take anti acid, other signs of an ulcer include: nausea, loss of appetite, vomiting, loss weight for no clear reason. Ulcer can be bleed in the stomach, if have any of these symptoms: Stool that is bloody, trouble breathing, feeling very tired for no reason and vomit that have blood or look like coffee ground (Blahd 2016).

2.5.11. Gastrointestinal diseaes associated with *Helicobacter pylori* infections

2.5.11.1 Gastritis

H. pylori is amjor cause of gastritis.This inference is based on the following observation: i) ingestion of *H. pylori* lead to acute gastritis in small number of case studies ii) *H. pylori* colonization of stomach virtually always accompained by inflammation of the mucosa iii) *H. pylori* can be detected in 95% of patients with inflammation of gastric mucosa iv) this inflammation dis appears completely with in two to three years after eradication of infection (Monograph, 1994).

2.5.11.2. Non ulcer dyspesia (NUD)

NUDor functional dyspesia is defined as persistent upper abdominal pain or discomfort in patients without detectable abnormalities in structural or biological examination (Beeling 2004). Many possible causes have been suggested for NUD including life style factors, stress, altered viscreal sensation, increased serotonin sensivity, alteration in gastric acid secretion, gastric empty and *H. pylori* infection (Beeling 2004). In supjects with

dyspepsia endoscopic studies have reported that 60% have NUD. Itwas observed that NUD occurs more frequently in younger patients 38% in patients younger that 25 years old compared with older patients 3-7% in patients older than 60 years old (Beeling 2004).

2.5.11.3 Peptic ulcer disease

The *H. pylori* is major cause, but not only of peptic ulcer disease world wide, *H. pylori* infection is present in 70% of patient with peptic ulcers, the mechanism by which *H. pylori* induce peptic ulcer disease is incompletely understood, but most likely involves combination of gentic predisposing of the host, virulence factor of the organism (eg, Vac A and CagAprotiens), mechanical damage to the mucosa, alteration of gastric secretions (Beeling 2004). Non steroidal anti inflammatory drugs and bile reflux. Eradication of infection prevents ulcer recurrence (Beeling 2004).

2.5.11.4. Duodenal ulcer disease

H. pylori is the most significant risk factor for duodenal ulcer disease, it has been estimated that up to 10% of infected people will devlop duodenal ulcer during life. Study had shown that *H. pylori* infection might cause the duodenal ulceration by stimulating the increaed releaseed of gastrin and increased acid secretion.Strong evidence that *H. pylori* causative agent of duodenal ulcer disease is based on improved rate of healing with *H. pylori* suppression, and the marked decreased recurrence rate after eradication of the bacteria (Monograph 1994).

2.5.11.5. Atrophic gastritis, intestinal metaplasia and gastric cancer

Chronic *H. pylori* induced inflammation can evenually lead to loss of of the normal mucosal architecture, with destruction of gastric glands and replacement by fiberous and intestinal type epithelium. This process of

atrophic gastritis and intestinal metaplasia occurs in approximatelly half of *H. pylori* colonized populations. The risk for a trophic gastritis depend on distribution and pattern of chronic active inflammation. As such subject with decreased acid out put show more rapid progression towards atrophy. Areas with glands loss and intestinal metaplasia extend with time multifocally, although they donot give arise for any specific symptoms, they increase the risk for gastric cancer by 5 to 90 fold depending on the extent and severity of a trophy (Khatun 2014). Evidence that *H. pylori* incrases the risk of gastric cancer development via the sequence of atrophy and metaplasia originate from various studies, in which was shown that *H. pylori* positive supject develop these condition more often than do uninfected controls.The risk of development of a trophy and cancer in the presence of *H. pylori* is a gain related to host and bacterial factors, which influence the severity of chronic inflammatory response. As such the risk is increased in supject colonized with Cag A positive strains, but also in those with a gentic predisposition to higher IL-1 production in response to colonization (Khatun 2014).

2.5.11.6. Gastric mucosal associated lymphoid tissue (MALT)

The association of *H. pylori* and MALT oma nearly is an established fact. The gastric mucosa does not normally contain lymphoid tissue, but MALT always a ppears in response to colonization with *H. pylori* in rare condition amonoclonal population of Bcells may arise from this tissue and slowly proliferate to form a MALT lymphoma, because rarity of this disorder, the exat incidince in *H. pylori* positive subjects is unknown, but MALT lymphomas occur in less than 1% of *H. pylori*- positive subjects (Khatun 2014).

2.5.11.7. Gastro- oesophageal reflux disease (GERD)

Some studies suggest that *H. pylori* protect human supject from development of gastroesophageal reflux disease (GERD), where others postulate causative association bettween them. Some studies have identified an association bettween them. Some studies identified an association between the CagA-positive strains sand increased acid secretion that in turn leads to gastro-esophageal reflux. The casuative association between *H. pylori* ang GERD needs further research for confirmation (Khatun 2014).

2.5.12 Extra gastro intestinal diseases

2.5.12.1Cardiovascular diseases

Studies domenstrated that high serological IL-6 levels are significantely associated with *H. pylori* infection possibly playing role in schemic heart disease (SHD).Other study showed that ahigh circulating levels of IL-6 and B-type natriuretic peptide biomarker heart failture in patients with coronary artery disease infected with Cag A positive strain (Franceschi, *etal.,* 2014).

2.5.12.2 Diabetes Mellitus (DM)

Study from China reported that chronic *H. pylori* infection is significantely associated with a high risk level of glycated haemoglobin A1C and type 2 D.M in patients over 65 years old and decrease level of insulin and insulin sensivity in subject under 45 years old also reported that significant association bettwen *H. pylori* infection and DM(Franceschi *et al*., 2014).

2.5.12.3. Neurological Disease

Katan et al (2013) clearly showed that infection burden sustained by *C. pneumoniae*, cytomegalovirus, herpes simplex virus1, herpes simplex virus 2 and *H. pylori* significantely increase the risk of stroke. Two studies evaluated the role of *H. pylori* on dementia, Haung et al. (2014) (reported that *H. pylori* infection may increase the riskof devoloping non al zahaimer disease dementia by 1.6 fold. Similarly Chang et al. showed that *H. pylori* eradication in patients with alzaheimer disease is associated with decreased progression of dementia. Concering multiple sclerosis (MS) Mohebi et al.(2013) found alower prevelence of MS in patients with *H. pylori*, thus proposing a protective against neurological disease (Franceschi *et al.,* 2014).

2.5.12.4 Haematological disease

2.5.12.4.1 Iron deficiency anemia (IDA)

Study showed that while prevalence of *H. pylori* in patients with IDA is higher compared with that of general populations, 46-75% of the patients reported compelete disapearance of IDA after *H. pylori* eradications.Clearly identified *H. pylori* infections as predictor of low ferritin and haemoglobin in children from Latin America and itis associated with alower mean corpuscular value (MCV). Another study performed in patients with IDA showed that *H. pylori* may be considered as the cause of IDA in 38% of patiens espically in post menopausal womens (Franceschi *et al*., 2014).

2.5.12.4.2 Idiopathic thrombocytopathic purpura

The role of the *H. pylori* on idiopathic thrombocytopathic purpura (ITP) via modulation of Fcᵞ-receptor balance of monocytes/ macrophages or molecular mimacry mechanisms (Franceschi *et al.*, 2014).

2.5.13. *Helicobacter pylori* in pediaterics

2.5.13.1 Clinical manifestations

2.5.13.1.1 Gastro intestinal manifestations

Generally only small proportion of *H. pylori* infected children devolop symptoms. Symptoms of *H. pylori* related peptic ulcer disease are non specific and may include epigastric pain espically after meal, night time waking, unexplained nausea and or vomiting, a norexia, hematemesis and iron deficiency anemia. Study on patients aged from 5-15 years showed that recurrent abdominal pain was significantelly associated with *H. pylori* infection (Lawanczak and Francavailla 2014)

2.5.13.1.2 Iron deficiency anemia (IDA)

Harris, et al studied the links between *H. pylori* associated iron deficiency anemia in 123 children, they found that low serum iron in *H. pylori* infected children indicating that direct role of *H. pylori* infection in the etiology of IDA (Lawanczak and Francavailla 2014).

2.5.13.1.3 Growth retardation

Controversy exists Concering the relationship of *H.pylori* and growth retardation in children.In poor resource setting where malnutrition and *H. pylori* coexsits in young children. *H. pylori* might play potential role.The gastrointestinal ghrein enzyme regulates food intake in humans and decreased appetite in *H. pylori* infected children has been related to low

plasma ghrelin enzyme levels which return to normal after *H. pylori* eradication (Lwanczak and Francavailla 2014).

2.5.14 Epidemiology:

H. pylori infections is now recognized as world wide problem. It's the most common cause of chronic gastritis and it's strongly linked to peptic ulcer disease and gastric cancer, while the infection is usually acquired in childhood, there is typically long peroid of latency with disease, manifestations of disease not appearing untill adulthood. Gastric cancer doesnot usually manifest untill old age (Malaty 2007). The prevalence of infection varies but is falling in most developed countries. Primary a caquistion in adults or re infection after successful eradications does occur with annaual incidince of 0.3-0.7% in developed countries and 6-14% in developing countries (Logan and Walker 2001).The infection has a high morbidity rate but low mortatlity rate and is curable with antibiotics (Maltay 2007).

2.5.15. Diagnosis of *H. pylori* Infections

Several methods are curently available to detect the presence of *H. pylori* according to whether or not an endoscopy is necessary. Invasive as biobasy 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, alteranatively the urea breath test (UBT), serology and stool antigen test (SAT) can be performed as non invasive tests.

2.5.15.1 Invasive methods

2.5.15.1.1. Histology

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

`2.5.15.1.2. Rapid Urease Test (RUT)

Principle of test is the biobsy from gastric mucosa placed in medium containing urea, *H. pylori* urease breaks down urea to ammomnia and carbon dioxide, ammonia leads to increase in PH and colour shift by PH indicater (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 biobsy at least 1000cells are required.Low RUT sensitivity and specificity reported in the presence of blood, RUT specifity decrease with increase incubation time, false negative result from decreased urease activitivity by increase aciditity of stomach or as a result of recent intake of antibiotics, bismuth compound or proton pumb inhibitors(Gonzalez*, et al.*, 2014).

2.5.15.1.3. Culture and Antimicrobial Sensitivity:

Detectionof *H. pylori* by culturing from gastric biobsies testing for sensitivity to metronidazole and clarithromycine (Bytzer, 2010). Culture procedure is less invasive when use gastric juice compared to biobsy.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. Biobsies can be kept in atransport 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 microarobic conditions (85%N2, 10%CO_2, and 5% O_2) at 35to 37ºC for at least seven days before discarding culture as negative. Positive identifications based on morphological characterstics and positive catalase, oxidase and urease reaction.Culture is most specific method for detecting *H. pylori*, although the result depend on microbiologist experience, specimen quality and use of transport media. For many years the role of the role of culturing *H. pylori* was limited to research and epidemiological studies. In clinically practice culture has mainly been used to confirm antibiotics sensitivity of *H. pylori* after 2 treatment failure in patients. It is generally not considered aroutine diagnostic method and is not available in most medical instituations world wide.It would be desirable for more laboraties to able to perform for more laboraties to able to perform culture and susceptibility test before two treatment failtures have occurred (Gonzalez *et al.,* 2014).

2.5.15.1.4. Polymerase Chain Reaction

Allows researchers and clinicians to identify *H. pylori* in small samples that have few bacteria present. Can be performed on samples obtained by both invasive and non invasive method. Considerable drawback of PCR is that it can detect DNA segment of dead bacterium in gastric mucosa of patients after treatment, it can produce false positive results. Molecular detection of *H. pylori* using PCR is possible in materials obtained by non or minimally invasive procedure such as gastric juice, saliva and stool (Gonzalez *et al.*, 2014).

2.5.15.2. Non invasive methods

2.5.15.2.1. Urea Breath Test (UBT)

Is base on the ability of *H. pylori*, if present in the gastric enviroment, to break down orally absorbed ^{13}C labled 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 donot require endoscopy. ^{13}C is not radio active, and can be safely used in children and women of childbearing age, however the machine is expensive. The sensitivity and specifity of UBT exceed 90% in most studies. False positive results due to other urease- forming pathogens are rare.UBTmay produce false negative result s if performed after the use of the *H. pylori* and urease suppresive therapies, such as Proton Pump Inhibitors (PPI) and antibiotics (Gonzalez, *et al.*, 2014).

2.5.15.2.2. Serology:

People infected with *H. pylori* generally have specific IgG and IgAantibodies 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 specifity 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 preperations from several different strains. Antibody assays in blood have measured IgG and IgA antibodies which has 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 specificty, 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 specifcity, they also have the advantages of being non invasive and less cost (Khatun 2014).

2.5.15.2.3. Stool Antigen Test (SAT):

The *H. pylori* Stool Antigen Test (HpSA) is an enzymatic immunoassay that detect the presence of *H. pylori* antigen in the stool specimen.These antigens are captured by the polyclonal anti *H. pylori* antibodies that are absorbed on the microwells plate (Lui 2004). It is a reliable method to diagnose an active infection and to confirm an effective treatment of infection.Stool sample may be stored for 24 hours at room temperature or 72 hours at 4 ºC. Result of SAT may be affected by disorder of digestive tract, proton pumb inhibitor or presence of bleeding ulcer. The diagnostic

accuracy is detecting eradication of *H. pylori* infection has been evaluated (Gonzalez *et al*., 2014).The initially devoloped stool antigen test was polyclonal antibody test and found to have variable sensitivity and specifity for diagnosis of *H. pylori* infection. The development of new ELISA test using monocolonal antibodies give new dimension and greater precision for stool antigen testing. *H. pylori* antigen detection in stool is a rapid, non invasive, easy to perform test that can be used to detect active infection, monitor effectivness during therapy, and to confirm cure after antibiotic use.The collection of specimen, espically in children, in whom endoscopy would be difficult, and the non requirement of espically trained staff to collect and perform test, also prior preperation of the patients is not necessary unlike in upper gastrointestinal endoscopy. The senstivity and specifity of this test to other detection of *H. pylori* have been confirmed and documented.*H. pylori* stool antigen has a 95% correlationwith reference methods such as endoscopy, urea breath test. It is more developed as alteranative to urea breath test (Khatun 2014).

2.5.16. Treatment

2.5.16.1. Traditional treatment of *H. pylori*

Doctors typically describe a combination of two antibiotics and an acid reducing drug to treat *H. pylori*, this know as triple therapy, if the patient, resistant to antibiotic increase , the doctor may add another medication to patient treatment plan.Treatment usually lasts no more than two weeks. Using two antibiotics instead of one may reduce the risk of antibiotic resistence. Antibiotics used to treat *H. pylori* include: amoxicillin, tetracycline, metronidazole and clarithromycin. Acid reducing medications help stomach lines to heal. Some of these are proton pumb inhibitors such as omperazole and lansoprazole, which stop acid production in the

stomach. Histamine blockers, such as cimetidine which block acid triggering histamine and bismuth subsalicylate, which coat and protect the lining of stomach (McDermoth 2016).

2.5.16. 2. Natural treatment of *H. pylori*

Many invivo and invitro studies on natural *H. pylori* treatments have been done. Most treatments reduced the number of bacteria in the stomach but failed to permanently eradicate them.

2.5.16.2.1 Probiotics

Probiotics help maintain the balance between good and bad gut bacteria. According to a 2012 study, taking probiotics before and after standard *H. pylori* treatment may improve eradication rates. Antibiotics kill both good and bad bacteria in patient stomach. Probiotic help to replenish good bacteria. They may also reduce the yeast over growth. Researchers found evidence that the bacteria *Lactobacillus acidophilus* delivers the best results (McDermoth 2016).

2.5.16.2.2 Honey

Honey has shown antibacterial abilities against *H. pylori.* Researchers suggest that using honey with standard treatments may shorten treatment time (McDermoth 2016).

2.5.16.2.3 Green tea

A 2009 study on mice showed that green tea may help kill and slow the growth of *H. pylori*. The study found that consuming green tea before an infection prevent stomach inflammation (McDermoth, 2016).

2.5.16.2.4 Olive oil:

Olive oil may also treat *H. pylori*, many studies showed that olive oil has strong anti bacterial a bilities against *H. pylori* (McDermoth 2016).

2.5.18. Prevention and control:

H. pylori presents in contaminated food and water, therefore, it is important to avoid these resources e.g flow water, raw sewage. Washing the hands throughly with warm soapy water after using the restroom and before eating also may help to prevent infection. Eating utensilis and drinking glasses should never be shared, since the bacteria can be spread through saliva (Swiezewski, 2008).Avoid accidental transmission throgh the fecal oral route or oral oral route, don't eating any food that is not cooked throughly and avoid food served by people who have not washed their hands (Blahd 2016).

CHAPTER THREE
MATERIALS AND METHODS

3. Materials and Methods

3.1. Study design

This is a descriptive, cross sectional study.

3.2. Study area and duration

The study was carried out at Saad Rshwan Medical Centre, and Research Laboratory of Sudan University of Science and Technology. The study was carried out during the period from January 2018 to February 2018.

3.3. Study populations

Patients having gastrointestinal complains, mainly dyspeptic symptoms (nausea, vomiting, and epigastric pain) and who ICT *H. pylori* stool antigen test were positive.

3.4. Inclusion criteria

Patients suffering from gastrointestinal symptoms and who were positive ICT *H. pylori* stool antigen test.

3.5. Exclusion criteria

Patients who were negative *H. pylori* stool antigen test.

3.6. Sample size:

Fifty (n=50) stool samples were collected from patients suffering from dyspeptic symptoms and whose I CT *H. pylori* stool antigen test were positive.

3.7. Sampling technique:

This study is based on non probability convience sampling technique.

3.8. Data collection

Method of data collection through a self limited administered questionnaire. Questionnaire desgined to record demographical clinical data.

3.9. Ethical consideration

Permission to conduct this study was obtained from College of Medical Laboratory Science, Sudan University of Science and Technology, Saad Rshwan Hospital and verbal consent from patients.

3.10. Collection and transport of the specimens

Stool specimen was collected from each patient in clean, dry, water proof container containg no detergent, preservaties or transport media. After collection stool specimenswere examined for *H. pylori* antigen within 1 hour. *H. pylori* stool antigen specimens were preserved in ice bag, and transported to laboratory in SUST for DNA extraction and PCR.

3.11. Laboratory procedures

3.11.1. Stool antigen detection

3.11.1.1 Processing of stool

For solid specimen, the cap of specimen collection tube was unscrewed, and then specimen collection applicater will be stabbed randomly into fecal specimen at least in three different sites to collect approximately 50 mg of feces, transferred into specimen collection tube containing extraction buffer. For liquid specimen, the droper was held vertically, the specimen was spirated and then transfered 2 drops into specimen collection tube containing extraction buffer.

3.11.1.2. *H. pylori* Stool antigen assay (HpSA)

Diluted stool samples were analyzed using *H. pylori* Antigen Rapid Test Cassette All Test Biotech company device immunochromatography used for detection for *H. pylori* antigen.The specimen collction tube was held up.Tip of the collection tube was broken off and inverted to transfer 2 drops of extracted specimen to specimens of test device.Then timer was started.The result was read at 10 minutes after dispensing specimen. Appearance of two distinct lines indicated positive result and one line in the control region indicated negative. Disappearance of control line indicated invalid result.

3.11.2. DNA extraction:

About 180-220 mg stool transferred in a 2ml micro centrifuge tube and placed the tube on ice. When the sample in liquid phase about 200µl was pipeted in the tube. A 200µl SPL (pre lysis buffer) added to each stool sample, vortexed, continously for 1min untill the stool sample was thoroughly homogenized, the suspension was heated for 5 min at 70ºC.The i-genomic stool IR spin column was placed into anew 1.5ml tube, then the supernatant was transferred promptly into i-genomic stool IR (inhibitor absorption column) spin column and it was centrifuged at 13,000 rpm for 1min, the spin column was removed from the 1.5ml tube. A 200µl SL (lysis buffer). A 10µl of protienase k and 5µl of RNAase solutions were added to 1.5 ml tube and mix by vortexing, and incubated at 65ºC for 30 min and homogenized suspension was obtained. After lysis completely 20µl of SB (binding buffer) was added to lysate and was mixed by inverting. 250µl of 80% ethanol was added to lysate and was mixed thoroughly by pipetting. 750µl of mixture was pipeted into spin column inserted in to 2ml collection tube, and it was centrifuged into at 13,000 rpm at RT, for 1 min, then

discarded the flow through, and collection tube altogether. The spin column was placed into a new 2ml collection tube, centrifuged at 13000 rpm for 1min. 700µl SWA(washing buffer) was added to spin column and it was centrifuged at 13,000 rpm for 1min, then the flow through was discarded, the collection tube was reused and the spin column was placed into collection tube. 700µl SWB (washing buffer) was added to spin column, and it was centrifuged at 13,000 rpm for 1 min to dry membrane then the flow through, collection tube was discarded altogether.The spin column was placed into a new 1.5 ml tube not supplied, and 50µl SE buffer directly added onto membrane, it was incubated for 1min at RT, and it was centrifuged at 13,000 rpm to elute 30µl DNAextract.

3.11.3. DNAStorage:

The eluted DNA aliquots was stored as stock at -20 ºC up to date of using.

3. 11.4. Table Primer sequencing:

Primers	**Primer sequencing**	**Product base pair**
F(Forward)	5´AACCGGATGATGTGATGGAT 3´	217 bp
R(Reverse)	5´GGTCTGTCGCCAACATTTTT 3´	

3.11.5. Preparation of PCR premix:

PCR reaction mixture include master mix 7µl, forward primer 0.5 µl, reverase primer 0.5 µl, Extracted DNA 2µl D.W 10µl.

3.11.6. PCR protocol:

Amplification was carried out into a DNA thermal cycler (Analytikjena, Germany). Intiatial denaturating of targeting DNA at 94ºC for 3minutes, then steps of denaturation at 94ºC for 30 seconds, annealing at 53 ºC for 30 minutes, and extension steps at 72ºC for 45 minutes atotal of 35cycles, and final cycle including extension at 72ºC for 5 minutes

3.11.7 Agrose gel and gel electrophoresis technique:

1.5 gm of agrose powder was weighted using sensitive balance, followed by addition to clean and dry flask.100 ml of 1X tris borate EDTA (Tris Borate EDTA) added to powder. Then the flask was put on microwave for 1:30 seconds seperated by agitations, after complete disolving of agarose, cooled, and poured on chamber at which comb fixed, an agrose gel is placed in 1X TBE filled box, an electrical field was applied via the power supply. The negative terminal is at far end (black wire), so DNA migrates toward the positively charged anode (red wire).

3.11.8. PCR product detection:

5 µl amount of each PCR mixture separated in a 1.5% agarose stained with ethidium bromide result was considered positive when aband of size 217 was visible in the stained gel examined using a transilluminator (Ultra violet gel documentations system), in parallel with 100 bp ladder considered as molecular size marker.

3.12. Data analysis

Collected data were analyzed using the statistical package of social science (SPSS) program. Chi square statistical analysis was used to determine *P.value* (0.05).

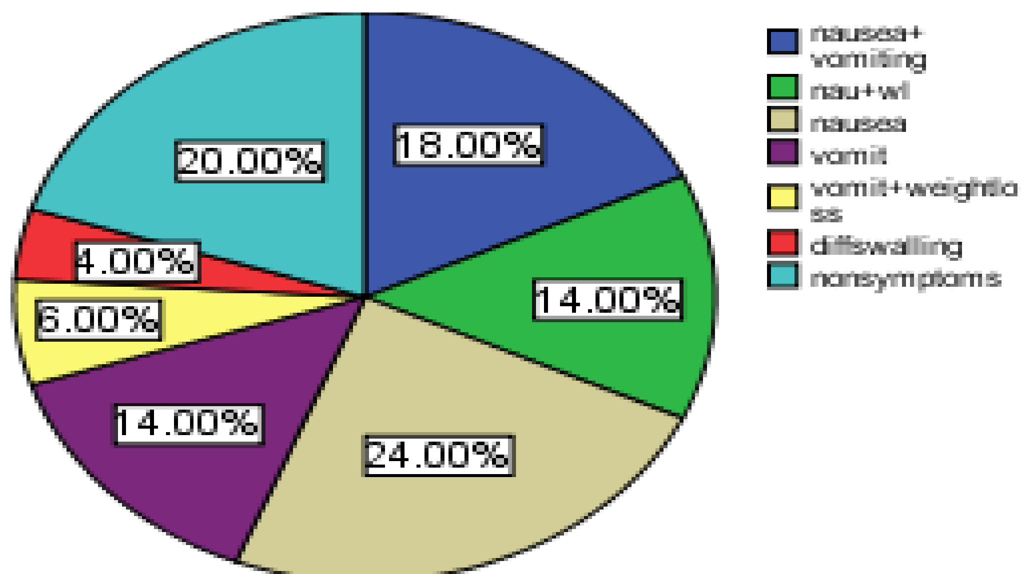
CHAPTER FOUR
RESULTS

4. RESULTS

Atotal of 50 stool samples were collected from patients suffered from gastrointestinal symptoms and their *H. pylori* positive by ICT (stool) from Saad Rshwan Medical in Khartoum State. Among 50 extracted stool 29(58%) positive for ureA gene presence using polymerase chain reaction. Age ranged from 10-85years, with age mean (32.24) and age standard deviations (17.6). Twenty (40%) of study populations were males, and thirty (60%) were females.

4.1 Table show frequency of infected patients with *H. pylori* ICT a ccording to patient's age groups which showed the highest percentage (62%) of the infected patients were in the age group 10-30 followed by age group 30-50.

Age groups	*H. pylori* positive by ICT	Percentage %
10-30	31	62
31-50	13	26
51-70	4	8
71-90	2	4
Total	50	100



4.1 Figure showed the frequency of symptoms among the infected patients who show nausea most frequent symptoms associated by *H. pylori* infections.

4.2 Table show frequency and percentge of educations levels among study groups a ccording to ICT. The level of education has shown little role in frequency of disease and it was higher in males with higher education and in females with primary education.

Educations levels	Males	Percentge%	Females	Percentge%
Primary	0	0	15	30
Secondry	9	18	4	8
Higher	11	22	0	0
Illiterate	0	0	11	22
Total	20	40	30	60

4.3 Table frequency and percentge of antibiotics treatment among study groups a ccording to ICT, which of there was no difference in the frequency of the disease under treatment or not between both genders.

Treatment	Males	Females
Yes	5	13
No	15	17
Total	20	30

4.4 Table of relationship between the detection of the ure A gene and tients age groups which showed no significant statistical variations between ureA gene of *H. pylori* detection and patient's age groups.

			Age groups				Total
			10-30	31-50	51-70	71-90	
UreA gene	**Yes**		19	7	2	1	29
		% of Total	38.0%	14.0%	4.0%	2.0%	58.0%
	No		11	7	2	1	21
		% of Total	22.0%	14.0%	4.0%	2.0%	42.0%

P. value = 0.8

4.5 Table of association and percentge of ure A in patients according to gender which showed no significant statistical association between ureA gene of *H. pylori* and patient's gender.

			Gender		Total
			Male	**Femle**	
UreA gene	**Yes**		10	19	29
		% of Total	20.0%	38.0%	58.0%
	No		8	13	21
		% of Total	16.0%	26.0%	42.0%

$P.value = 0.7$

4.4 Table of association of ure A in patients according to their educations levels which showed no significant statistical associations between education level and ureA positive detection

			Education levels				Total
			Primary	Secondary	Higher	Illiterate	
ureA gene	**Yes**		9	7	6	7	29
		% of Total	18.0%	14.0%	12.0%	14.0%	58.0%
	No		5	6	5	5	21
		% of Total	10.0%	12.0%	10.0%	10.0%	42.0%

P.value=0.9

4.6. Table of associations between urea A gene and smoking behavier of patients, which showed all the patients showing Ure A were found between non smoker's patients.

Smoking behavier	Number	UreA gene positive	Percentge
Smokers	4	0	0%
Non smokers	46	29	58%
Total	50	29	58%

P. value = 0.5

4.7. Table of associations between ure A gene and patients who had antibiotics treatment or not. There is no statistical significant associationbetween them majority of patients showing ure A having no treatments (38%).

Treatment	Total number	Ure A gene positive	Percentge
Yes	17	10	20%
NO	33	19	38%
Total	50	19	58%

P.value =0.9

4.8. Table of associations between ure A gene and patients marital status which showed no statistical significant association between presence of ure A gene and marital statatues is but the majoritity of urea A occurred among married patients according to frequency.

Marital status	Total number	UreAgene positive	Percentge
Yes	26	16	32%
No	24	13	26%
Total	50	19	58%

P. value =0.8

4.9 Table of associations between ure A gene and patients faimly history which showed family history for the presence of infections with *H. pylori* showed that the highest number of infected patients were a mong those with the disease had no family history of *H. pylori* infections.

Family history	Total number	UreA gene positive	Percentge
Yes	23	11	22%
NO	27	18	36%
Total	50	19	58%

P. value = 0.4

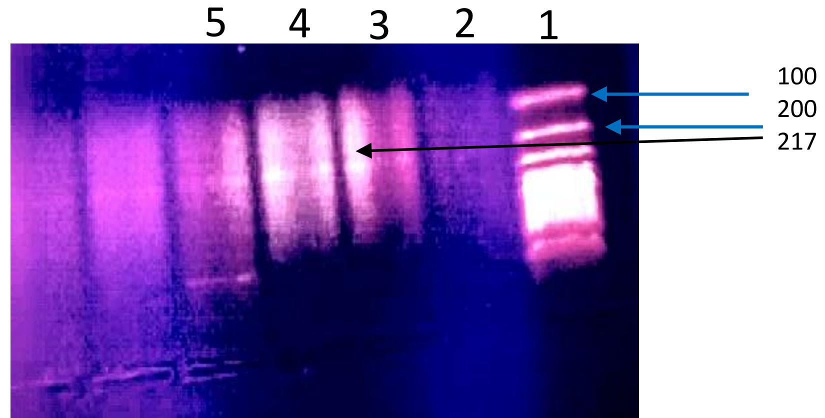


Figure (4): Gel electrophopresis of ureA gene of *H. pylori* PCR product, lane 1, ladder (100 bp), lane 2, negative control, 3, 4,5 stool DNA ure A (217 bp) gene.

CHAPTER FIVE

DISCUSSION

Disscussion

In *H. pylori* stool antigen (HpSA), and polymerase chain reaction test were used as diagnostic tools. HpSA was used to detect *H. pylori* antigen. The non invasive *H. pylori* stool antigen test is aquick and cost effective method. It does not require specialized expertise. The test can be perfomed in conjunction with other diagnostic procedures. Feces can be obtained easily, even in new born children, also *H. pylori* stool antigen can be used for monitring treatment success, for this reason, this method is more suitable for screening and epidemiology studies (Khatun, 2014). While PCR technique is also non invasive, it can be used in children, espcially under 10 years, as most hospital donot have child endoscopies. In the present study screened 50 stool samples from patients, males 20(40%), females 30(60%), with mean age 32.24 years suffering gastrointestinal symptoms, examined for *H. pylori* infection by use of *H. pylori* stool antigen ICT, 29 positive for ureA gene of *H. pylori* (58%). In the Sudan published studies on ureA gene detection from stool were few. The result of ureA gene positive *H. pylori* agreed with Sen, *et al.,* (2005), his study based on detection of *H. pylori* in stool by PCR, and found that ureAgene postive stool PCR was (59.3%), also agreed with Deenonpoe*, et al.,* (2017) study on prevelance of Helicobacter species and virulence factors in Thailand found that, ureA gene positive stool PCR (64.6%). Inaddtion to study of Queralt, *et al*., (2005) in North East Spain, those found that ureAstool DNA positive was (33.3%) ,and agreed also with Kelly *et al* .,(1994), in London found that ureAgene positive stool PCR (8%). The prevelance of ureA stool positive of *H. pylori* in men (20%), and women (38%), suggesting that there was insignificant difference between sex (*P.value*=0.4), as aresult obtained by Zhang, 2008, founded that there was no relationship between and gender and sex in adult. women had high

infection rate than men. Age group (10-30) years had a high rate of ureA stool positive of *H. pylori* than other age groups, but in generally, in present study their is no significant association between *H. pylori* gene detection and age groups, as such a result obtained by Dore *et al.,* 2000 on their study on Diabetes Mellitus and *H. pylori* infection they found that there was insignificant association between *H. pylori* infection and age groups. As for the education levels ureA gene positive stool PCR *H. pylori* (*P.value*= 0.9) with no significance, as a results obtained Agumen *et al* (2005), Fich *et al* (1993), their studies found that there was no association between *H. pylori* and educations levels. There was no association between ureA gene positive stool PCR and social factor such as marriage (*p.value*= 0.8), incontrast to Ozaydin *et al* (2013), who found that marital status playing critical role as co factor for *H. pylori* infection and family history (*p.value*= 0.4). In this study there was no association between smoking and *H. pylori* (*p.value*= 0.5), In this study all the patients infected with *H. pylori*were found among non smokers, but not reach the significant range, as a result obtained by Ozaydin *et al* (2013) that *H. pylori* was found very high in non smokers individuals.There is no significant associations between ureA stool DNA positive of *H. pylori* and patients had treatment (*p.value*= 0.9).

Conclusions:

The frequency of ure A gene in patients attending Saad Rshwan Medical Centre in duration of sample collection.The prevalence of ure A gene in this study was not associated with sex, age, age group of patients, patient's marriage status, family history of *H. pylori* infection, treatment of patients, patient's tap water source, also there is no association of ureA gene detections and illiterate patients.

Recommendations:

1. Gastrointestinal suffering patients should routinely be for *H. pylori* and it should be treated in any patient who tests positive.
2. Patients should be asked about previous antibiotics exposure to help guide of treatment regimen and avoid failture because the resistence.
3. Urea breath test, fecal antigen testing or biopsy based test should be used to determine treatment success.
4. Large sample size is needed to accurately determine the rate of infection.
5. Sequencing and phylogenetic analysis of *H. pylori* DNA should be done among *H. pylori* suffering patients.

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بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

APPENDIX

Qusetionnare

Date…………………………………………………………..

Saad Rshwan Hospital………………………………………………

Participant name……………………………………………………

ID…………………………………………………………………..

Age ……….years.

Sex: Male ( ). Female ( ).

Individual education: Primary ( ) Secondary ( ) Higher ( ).

Smoking: Never ( ) Former ( ) current ( ).

Marrital status Yes ( ) No ( ).

Signs and Symptoms:

Diffculty swallowing ( ) Unexplained weight loss (Nausea ( ) Recurrent vomiting ( )

Treatment: Yes ( ) No ( ).

H. pylori stool antigen test: positive ( ).

Preparation of 1X Tris borate EDTA:

TBE prepared by weight 48.45gm of Tris HCL, 55.5 gm of Boric acid and 7.44 gm of EDTA, dissolved in 1litter DW, this result in 10X TBE, 1Xprepared by dilution of 10ml of 10Xand 90ml DW.

Stool DNA extraction solutions .

Sensitive Balance

Microwave

Gel electrophoresis apparatus

microcentrifuge

Centrifuge

Thermal Cycler