Sudan University of Sciences and Technology

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Diagnosis of *Helicobacter pylori* Infection Using Immune – chromatography Tests among Patients Attending Tamboul Hospital in Gazera State

تشخيص الإصابة بالملوية البابية بإستخدام الإختبارات المناعة اللونية لدى المرضى المترددين علي مستشفي تمبول بولاية الجزيرة

A Dissertation Submitted in Partial Fulfillment for the Requirements of M.Sc. Degree in Medical Laboratory Science (Microbiology)

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الآيـــــــــــــــــة

قال تعالى:

﴿إِنَّ اللَّهَ عِنْدَهُ عِلْمُ السَّاعَةِ وَيُنَزِّلُ الْغَيْثَ وَيَعْلَمُ مَا فِي الْأَرْحَامِ وَمَا تَدْرِي نَفْسٌ مَاذَا تَكْسِبُ غَدًا وَمَا تَدْرِي نَفْسٌ بِأَيِّ أَرْضٍ تَمُوتُ إِنَّ اللَّهَ عَلِيمٌ خَبِيرٌ﴾

صدق الله العظيم

سورة لقمان، الآية (34)

Dedication

To my Parents

To my teachers

To my sister and brothers

To my colleagues and friends

Acknowledgements

By the grace of **ALMIGHTY ALLAH**I completed this study. Special thanks to my supervisor **Dr. Wafaa Mohmmed Abdalla** for her guidance and advices through this study.

Extended thanks and appreciations to my colleagues and Tamboul Hospital staff for their supporting and helping me.

Great thanks to patients for their patience and understanding this study.

Abstract

Helicobacter pylori (*H.pylori*) causes a major health problem worldwide and more than half of the world population are infected with this pathogen. The diagnosis of infection was initially made through invasive methods but now non- invasive methods were develop to make diagnosis easier.

This is cross sectional study was conducted in Tamboul City, Gazira State, during the period from March 2016 to December 2019 to compare between antigens and antibodies tests results used for diagnosis of *H. pylori* infection among symptomatic and asymptomatic Sudanese patients.

The stool and blood samples were collected and analyzed for presence of antigen and antibodies by Immunochromatography (ICT) cards. One hundred (n=100) specimens serum and stool were collected, in which 50 were symptomatic and 50 were asymptomatic patients. In symptomatic patients 18/50 (36%) were males and 32/50 (64%) were females with mean age of 16.7±24.6 S.D, while in asymptomatic patients 19/50(38%) were males and 31/50(62%) were females with mean age of 16.7±20.4 S.D.

In symptomatic patients 35/50 (70%) showed positive results for stool antigen while 30/50(60%) for serum antibodies. In asymptomatic patients 18/50(36%) were positive for stool antigen and 25/50(50%) for serum antibodies. There were significant association between antigen results and patients groups (*P.value* = 0.001) but there was insignificant association between antibodies results and patients groups (*P. value* = 0.317).

Age groups, history of infected persons in the family, blood group and previous treatment were all not associated with *H. pylori* infection (P≥0.05).

In conclusion, the frequency of *H.pylori* antigen was higher than antibodies in symptomatic patients, while the frequency of *H.pylori* antibodies was higher than antigen in asymptomatic patients.

المستخلص

تسبب الملوية البابية مشكلة صحية أساسية في جميع أنحاء العالم، ويصاب بها أكثر من نصف سكان العالم. بدأ تشخيصها بالطرق الإجتياحية لكن الآن تطورت الطرق الغير إجتياحية لتسهيل التشخيص.

هذه الدراسة المقطعية أجريت بمستشفي تمبول، ولاية الجزيرة خلال الفترة من مارس2016 حتي يناير 2020، لمقارنة نتائج إختبارات كشف الأجسام المضادة والمستضدات المستخدمة في تشخيص الإصابة بالبكتريا الملوية البابية وسط المرضي السودانين مصحوبي وغير مصحوبي الأعراض.

جمعت عينات دم وبراز وحللت لوجود الأجسام المضادة و المستضدات بواسطة كروت إختبار المناعة اللوني.

تم جمع 100(ن=100) عينة دم وبراز، منهم 50 مريض مصحوب بأعراض المرض و50 مريض غيرمصحوب الأعراض. في المرضى مصحوبي الأعراض كان عدد الذكور18/50 (36%) والإناث 32/50 (64%) وكان متوسط أعمارهم16.7±20.4 إنحراف معياري، بينما في غير مصحوبي الأعراض كان عدد الذكور19/50 (38%) وعدد الإناث 31/50 (62%) بمتوسط أعمار 16.7±24.6 إنحراف معياري.

في المرضى مصحوبي الأعراض 35/50 (70%) أظهرت نتائج إيجابية للمستضد في البراز بينما 50/30 (60%) للأجسام المضادة في السيرم. في الأشخاص غير مصحوبي الأعراض 50/18(36%) أظهرت نتائج إيجابية للمستضد في البراز و25/50(50%) للأجسام المضادة في السيرم. هنالك علاقة معنوية بين نتائج المستضد ومجموعتي المرضى (القيمة الإحتمالية=0.001) ولكن ليس هنالك علاقة معنوية بين نتائج الأجسام المضادة ومجموعتي المرضى (القيمة الإحتمالية = 0.317).

مجموعات العمر، تاريخ لأشخاص مصابين في الأسرة، فصيلة الدم، والعلاج السابق من المرض أظهرو عدم وجود علاقة بينهم والإصابة بالبكتيريا الملوية البابية.

خلصت الدراسة إلى أن تكرار مستضد الملوية البابية أعلي من تكرار الأجسام المضادة في المرضي مصحوبي الأعراض بينما فى المرضي غير مصحوبي الأعراض تكرار الأجسام المضادة أعلي من تكرار المستضد.

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List of Abbreviations

Abbreviation	Full name
ABO	Blood group system
Abs	Antibodies
Ag	Antigen
BabA	blood group antigen-binding adhesion
BHI	Brain Heat Infusion Agar
Cag A	Cytotoxine- associated gene
C band	Control band
CO2	Carbon dioxide
C	Carbon
CD74	Cluster of Differentiation 74
DNA	Deoxyribonucleic Acid
ELIZA	Enzyme LinkeImmunosorbant Assay
H.pylori	Helicobacter pylori
ICT	Immune-Chromatography Test
IceA	Protein induced by contact with epithelium
Le Ags	Lewis Antigens
O2	Oxygen
OipA	outer inflammatory protein
PH	Power of Hydrogen
PPI	Proton-Pump Inhibitors
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
RNA	Ribonucleic Acid
RUT	Rabid Urease test
T band	Test band
Th1	T Helper 1
TMB	Tetra Methyl Benzaldehyde
UBT	Urea Breath Test
VacA	vaculatorycytotoxin

CHAPTER I

1. INTRODUCTION

1.1. Introduction

Helicobacter pylori (*H.pyloi*) is a Gram-negative, microaerophilic, spiral rods shaped bacteria that inhabit the antral gastric mucous layer, on the surface of epithelial cells (Basher *et al*.,2011).

Humans are considered the only reservoir of infection (Abdallah*et al*., 2014).

This pathogen usually are symptomless and without clinical manifestations, particularly in poor communities. However, signs and symptoms associated with the diseases are primary due to gastric or peptic ulcer illness or duodenal inflammation and other symptoms such as nausea, vomiting, and abdominal pain may be attributed to other gastrointestinal diseases (Gold *et al.*, 2014).

The attachment of *H.pylori* to gastric mucosa is mediated by the lewis (Le^6) antigen and that the availability of receptors might therefore be reduced in individual of blood group A and B compared to people with blood group O in addition to H antigen represent an important gastroduodenal mucosal cell- receptor for *H.pylori* attachment (Alcout*et al*.,2000).

The prevalence of *H.pylori* is still high in most countries; in north European and North America populations, about one-third of adults are still infected, whereas in south and east Europe, South America, and Asia, the prevalence is offer higher than 50% (Eusebi *et al*., 2014). In developing countries, up to 93.6% of adults are infected with *H.plori* (Olakoba *et al.*, 2013) and in Sudan; the prevalence of infection was estimated to be between 65.8%and 80% (Abdallah *et al*., 2014).

Laboratory testing for *H.pylori* infection is very important part of the diagnosis process for gastric and duodenal inflammatory disease and anumber of different diagnostic test methods invasive and non- invasive are available. Invasive methods have high specifity and sensitivity in detection of *H.pylori* infection. However, they are expensive and need special gastroenterologist to perform. In contrast, non-invasive methods such as antibodies(Ab) detection is cheaper, easy to perform but may give false positive result since antibodies level falls slowly after eradication in addition to low specificity (Quartero *et al*., 2000).

In addition, other non-invasive tests can be used include detection of *H.pylori* antigens in stool and presence of *H.pylori* in the saliva. Stool Ag test have recently been more acceptable with great expectations as they are convenient to the patients

and can easily performed even in small laboratories (logan and walker, 2001; Malfertheiner*etal*., 2007).

1.2. Rationale

Diagnosis of *H.pylori* using Immun-chromatographytest antibodies are not accurate because antibodies cause false positive results due to past infection with other disease (Naji*et al*., 2014).

The right diagnosis of*H.pylori* infection is one of the important issues that require special and specific tests, equipment and skilled personnel.

Due to increase, the recurrence request of *H.pylori* in stool and serum and increase the symptoms of *H.pylori* infection at Tamboul Hospital this study was conducted to be a base line in diagnosis of *H.pylori* infection by using immune-chromatography rapid antigen test, because antigen is more specific.

For knowledge, this is the first study applying rapid one-step immune-chromatography assay to screen the presence of *H.pylori* antibodies and antigen among patients at Tamboul City in Gazera State.

1.3. Objective

1.3.1. General Objective

To diagnose *Helicobacter pylori* infection by using immune-chromatography tests among patients attending Tamboul Hospital in Gazira State.

1.3.2. Specific Objectives

1. To detect *H.pylori* antibodies in symptomatic and asymptomatic patient's serum.
2. To detect *H.pylori* antigens in symptomatic and asymptomatic patient's stool.
3. To compare between antibodies and antigens results.
4. To determine the possible association between the possible risk factors (e.g.age, blood group, gender, infect person in family and previous treatment) and *H.pylori* infection.

CHAPTER II

2. LITERATURE REVIEW

2.1. *Helicobacter pylori*

2.1.1. History

The presence of gastric spiral bacterium was first reported in 1893 by Bizzozer an Italian Pathologist who described spiral bacteria in canine stomach (Marshall, 1989)and demonstrated for first time in human in 1906 (Warren and Marshal, 1983) Nine years later spiral microorganisms in the stomach of patients with gastric and duodenal ulcerations were reported (Rosenow and Saford, 1915).

2.1.2. Cellular morphology

H.pylori is gram negative bacterium, S–shaped or curved rod, about 2-4 micrometers long with diameter about 0.5-0.9micrometer, and other form of *H.pylori* reported in culture and occasionally *in–vivo* include spherical, U shape and straightened forms. It is non-spore forming bacterium and mostly active motile with up to six polar flagella filament (Owen *et al*., 2001; Black, 2004).

2.1.3. Colonial morphology

Colonies of *H.pylori* from primary on supplemented blood agar at 37 ^{0}C take 3-4 days to appear. They are circular 1-2mm, convex and translucent in appearance. There is slight Hemolysis on blood agar around colonies, which are grayish in color (Wood *et al*., 2003; Black, 2004).

2.1.4. Physiological properties

H.pylori is micro aerophilic and growing best in atmosphere of 5% oxygen with 5-10 CO_2 on blood containing medium such as Brain Heart Infusion Agar (BHI) and 5% Horse blood enriched with 1% isovialx (Wood *et al*.,2003).

Most strain grow over a relatively narrow temperature range of 33°-44^{0}C, whereas some poorly at 30^{0}C and 40^{0}C but not at 25^{0}C.*H.pylori* will grow in suitable culture medium over a wide PH range (5.5-8.5) with good growth between 6.9 and 8 and does not tolerate PH *in-vitro* (Owen *et al.*, 2000; Wood *et al*., 2003).

2.1.5. Biochemical characteristics

It is inactive in most of the conventional biochemical tests. Not fermented carbohydrate and oxidized. It produces catalase, cytochrome oxidase, DNAase, and strong level of uerase and alkaline phosphatase, it usually negative hippurate hydrolsis, niturate reduction and indole formation (Wood *et al*., 2003).

2.1.6. Macromolecular characteristics

Its homogenous species with respect to a number of important molecular chemo-taxonomical markers such as genomic DNA, fatty acid composition, extra chromosomal DNA and lipopolysaccharides (Owen *et al*., 2001).

2.1.7. Antigenic structure and serotypes

Lipopolysacharides of *H.pylori* contain lewis antigen that mimic human glycan structures (Hug *et al*., 2010).

Serotyping systems are very limited for *H.pylori d*ue to little known about the antigenic structure of it, have shown by antigenic analysis of lipopolysacharides (LPS) extract from *H.pylori* both a common antigen and strain-specific antigens that are sufficiently divers to be used in an O antigen serotyping system. With passive hemagglutination, six serotypes (O group1 through 6). Analysis of LPS O antigen of *H.pylori*ATCC 43504 contains fucosylated-acetyllactosamino-glycans with Lewis x (Le^x) determinants. The LPS of two other *H.pylori* strains, MO19 and P466 contain Lewis y (Le^y) andLe^x plus Le^y respectively. These antigens are known as blood group antigens (Hug *et al*., 2010).

2.1.8. Host range and ecology

Itis able to live in the acidity of the stomach and duodenum, living on the lining of the stomach, causing several health problems for the host. *H.pylori* can also be isolated from domestic cats and other animals including pigs, dogs and rhesus monkeys (Solnick*et al*., 2004).

2.1.9.*H.pylori* genome

It was originally classified in the genus Campylobacter but is now known to be differ from the Campylobacter in 16 S RNA sequence; it is unique fatty acid content and possess of multiple flagella which are located at polar side as shown by electron microscope, it is now believed to be phylogenetically closer to Welinella than Campylobacter and it is assignment to new genus *Helicobacter* (Pasteur, 2002; Arora, 2006).

The genetic diversity of *H.pylori* can be exploited by using molecular typing based on DNA analysis and PCR technique is able to isolate and replicate *H.pylori* DNA in biological material then detect the genetic variation between different strain of *H.pylori* (Pasteur, 2002; Arora, 2006).

It consists of large diversity of strains and genomes of three have been completely sequenced (Pasture, 2002).

Study of *H.pylori* genome is central on attempts to understand pathogenicity, ability of this organism to cause disease, approximately 29% of the loci are in the pathogenesis category of the genome database both sequenced strains have an approximately 40 Kb-long CagA pathogenicity island (a common gene sequenced believed to be responsible for pathogenesis) that contains over 40 gene and this pathogenicity island is usually absent from *H.pylori* strain isolated from human who are carriers of *H.pylori* but remain asymptomatic (Medis and Marshall, 2008).

The CagA gene code for one of the major *H.pylori* virulence protein, Bacterial strain that have Cag A gene one associated with an ability to cause ulcer (Bladwin *et al*., 2007).

2.1.10. Virulence factors

Several factors explain the virulence developed by *H,pylori*, including urease synthesis in order to neutralize the acid ambience and digestive enzyme in the gastric mucosa. Other virulence factors influence the severity of infection those factors include, cytotoxin associated gene A (CagA), vaculatorycytotoxin (VacA), Protein induced by contact with epithelium (IceA), blood group antigen-binding adhesion (BabA) and outer inflammatory protein(OipA) (Hug *et al*., 2010).

2.1.11. Epidemiology

H.pylori Infection is widespread through the world and is present in about 50% of the global human population: with 80% in developing countries and 20-50 in industrialized countries (Naj*et al*.,2014).

In Sudan, the prevalence of infection was estimated to be between 65% and 80% (Abdallah *et al*., 2014).

2.1.12. Transmissions of *H.pylori* infection

The exact mode and route of transmission of *H.pylori* infection remain unproven. It can spread directly from person to person mainly by oral-oral or fecal-oral while indirectly from an infected person to the environment. Interpersonal pathways are more probable than environmental exposure rout. In addition, vector transmission has been suggested (Maldenova and Durazzo, 2018).

2.1.13. Signs and symptoms of *H.pylori* infection

Up to 85% of infected with *H.pylori* never experience symptoms or complications (Bytzer *et al.,* 2011)

Individuals infected with *H.pylori* have a 10to20lifetime risk for developing peptic ulcer, acute infection may appear as an acute gastritis with abdominal pain or nausea,

where these develops into chronic gastritis, the symptoms are often those of non-ulcer dspepsia: stomach pains, nausea and vomiting that occur when the stomach is empty and is relieved by eating (Ryan and Kenneth, 2010).

2.1.14. Complications of *H.pylori* infection

Ulcer; can damage the protective lining of the stomach and small intestine. This can allow stomach acid to create an open sore, inflammation of the stomach lining and stomach cancer. *H.pylori* infection is strongly risk for certain type of stomach cancer (Chey *et al*., 2007).

2.1.15. Immune response to *H.pylori* infection:

H.pylori express lipopolysccharides and flagellin that do not activate efficiently Toll-like receptors and express dedicated effectors, such as γ-glutamyltrsanspeptidas, vacuolatingcytotoxin(vacA), arginase, tat actively induce tolerogenicsingles.Some of these factors are stimulate via the engagement of toll-like *H.pylori* can be considered as a commensal bacteria belonging to the stomach microbiota. However, when present in the stomach, it reduces the overall diversity of the gastric microbiota and promotes gastric inflammation by inducing Nod1-dependent pro-inflammatory program by activating neutrophils through the production of a neutrophil activating protein. The maintenance of a chronic inflammation in the gastric mucosa and the direct action of virulence factors (vag A and cytoxin-to associated gene A) present pro-carcinogenic activates to *H.pylori*. Hence, it cannot be considered symbiotic bacteria but rather as part of the pathological organism.(Moyat and Velin, 2014).

2.1.16. Diagnosis of *H.pylori* infection

Accurate diagnosis of *H.pylori* infection is a crucial part in the effective management of many gastroduodenal diseases. Several invasive and non-invasive diagnostic tests are available and each test has its usefulness and limitations in different clinical situations. Although none can be considered as a single gold standard in clinical practice, several techniques have been developed to give the more reliable results. Invasive tests are performed *via* endoscopic biopsy specimens and include culture, histology, rapid urease test as well as molecular methods. Urea breathing test and stool antigen test are most widely non-invasive tests, whereas serology is useful in screening and epidemiological studies (Wang *et al*., 2015).

2.1.16.1. Invasive tests

2.1.16.1.1. Culture

Culturing of *H.pylori* from gastric biopsy specimen is a highly specific but less sensitive method. Because of delicate and fastidious nature of it, the cultivation in vitro requires particular transport medium, growth medium and incubation environment. Biopsy specimens can be kept in a transport medium, like portagerm pylori or stuart's transport medium for up to 25 h at 4 C. Several types of agar can be used for culture as *H.pylori* are isolated, the commonly used media include pylori agar, Skirrow agar Columbia blood agar, Brucella agar, Brain heart infusion agar or Trypicase soy agar supplemented with sheep or horse blood. The agar plates are incubated in a microaerobic environment (80%-90%N_2, 5%-10%Co_2, and 5%-10% O_2) at 35to36°C for at least 5-7 days (Wang *et al*., 2015). Although culture is a time-consuming, expensive and laborious test for *H.pylori* diagnosis, the antibiotic sensitivity test of *H.pylori* provided by culture is a particular advantage in clinical practice (Malfertheiner *et al*., 2012).

2.1.16.1.2. Rabid Urease test (RUT)

For routine clinical practice, rapid urease test is the most useful invasive test for the diagnosis of *H.pylori* infection because it is rapid, inexpensive, easy to perform, highly specific and widely available. Based on the activity of *H.pylori* urease enzyme, the presence of *H.pylori* in biobsy specimen convert the urea test reagent to ammonia, leading to an increase in the pHand a color change on the pH monitor. Several commercial urease tests including gel-based tests, paper-based tests and liquid-based tests are available now and those have different reaction time to provide results, gel-based tests take 24 h to obtain accurate result, whereas paper-based tests takes 1 h and liquid- based tests takes 5 min to provide more rapid result (Vaira *et al*., 2010).

2.1.16.1.3. Histopathology

Histology is usually considered the gold standard in the direct detection of *H.pylori* infection and it is the first method used for the diagnosis. However, several factors influence the accuracy of histology, such as site, size and number of biopsies, staining methods, proton pump inhibitor, antibiotics and experience of the examining pathologist(Malfertheiner *et al*., 2012).

Staining is the critical part of histological method and several stains like routine HE staining, Giemsa,Warthin-strray and immunohistochemical.Giemsa stain is the

preferred method in clinical practice because it is simple, highly sensitive and less expensive(Hartman and Owen, 2012).

2.1.16.2. Non-Invasive tests

2.1.16.2.1. Urea Breath test

The urea breath test (UBT) the most popular and accurate non-invasive test used, by the urease activity of *H.pylori* the 13C-or 14C-labeled urea ingested by patients is hydrolyzed to labeled CO_2 in stomach, then labeled CO_2 is absorbed in the blood and exhaled by breathing in which labeled CO_2 can be measured. It is a highly sensitivity and specificity and it most useful for epidemiological studies and is method used for diagnosis of infection in pediatric. UBT distinguish an ongoing from past infection; hence, it is able to detect the eradication process after treatment. (Ferwana *et al*., 2015)

2.1.16.2.2. Molecular tests

Polymerase Chain Reaction (PCR) is based on detection of *H.pylori*could be categorized under invasive as well as non-invasive methods. It remains the best molecular techniques because it provides a wide range of clinical applications such as specific or broad-spectrum pathogen detection, early detection of bio threat agents and antimicrobial resistance, it could more applied for the detection of *H.pylori* in biopsies and more qualified for its use in sample taken from the oral cavity or from stool. If it applied as non-invasive approach (i.e., from stool samples) tend to be more cost effective than other method. In addition to high sensitivity, specifity, simplicity and automated procedures, there are several other advantages to be considered. Practically, regardless of genome size, any genomic material could be used as a template sample for PCR, Since antibiotic resistance is currently the major challenge in microbiology, it has to be pointed out that the fast acquirement of results of not only the diagnosis of *H.pylori* but also of its susceptibility to the right antibiotics is extremely important (Kalati *et al*., 2015).

2.1.16.2.3. Fecal antigen test

It detects antigens in stool samples. ELISA formats comprising monoclonal antibodies against *H.pylori* proteins showed improved result compared to polyclonal approaches. The current guideline evaluates the use of stool antigen test as equivalent to the UBT if validated laboratory-based monoclonal antibodies wre used. Degradation antigens in the intestine and consequent disintegration of epitopes might lead to false negative result. Moreover, the process of sample handling could be fastidious for patients.

False positive result may occur when the bacterial load is low, due to proton-pump inhibitors and the recent use of antibiotics (Kalati *et al*., 2015).

2.1.16.2.4. Serological tests

2.1.16.2.4.1. Enzyme Linked Immune Sorbent Assay (ELISA)

It is the most extensively used in serological detection of *H. pylori* and had shown to produce consistent and reliable results (Abusiyanik *et al*., 2004).

This test based on purified *H. pylori* antigen is coated on the service of mirowells. Diluted patients serum is added to the well and the *H. pylori* immunoglobulin specific antibodies, if present, binds to the antigens and all unbound material is washed away (Graham, 2004).

Enzyme conjugate is added, which bind to the antibodies antigen complex. Excess enzyme, conjugate is washed off and a solution of tetra methyl benzaldehyde (TMB) substrate is added. The enzyme conjugate catalytic reaction is stopped at specific time (15 minutes). The intensity of the color generated is proportional to the amount of Igs-specific antibodies in the sample.

The results are read by a micro well reader compared in a parallel manner with calibrator and controls (Graham, 2004).

2.1.16.2.4.2.Immunoblotting technique

It is sensitive serological test that enables the detection of antibodies against specific *H. pylori* antigens (Abusiyanik *et al.,* 2004)

Western immunoblotting technique, electrophoretically separated components are transferred from a gel to solid support and probed with reagent that is specific for particular sequences of amino acid. The immunoblotting is therefore extremely useful for the identification and quantification of specific protein in complex mixtures of protein that are not radio labeled. Because electrophoretic separation of proteins is usually carried under denaturing condition, any problems of solubilization, aggregation co-precipitation of the target protein with adventitious protein are eliminated (Abusiyanik *et al.,* 2004).

2.1.16.4.3.Immunochromatography test (ICT)

Because of the emerging need for rapid diagnosis and treatment of virulent strains of different viral and bacterial infections, discussions, regarding routine applications of RDT are increasing wit in current medical circles. Results of ICT become available within coupled minutes to a few hours. A clinical specimens is processed in a few steps (preferably in a single step) at the site where it collected (point of care) the

quality and value of the ICT is determined by its sensitivity and specificity, the time required for the results, its cost and availability, it appear that those antigen with either high or low molecular weight are more specific. There are currently many of *H.plori* ICT kits commercially available. However, how far these tests fit in the standard clinical practice is still undetermined (Kalati *et al*., 2015).

2.1.16.5. Gold standard technique

The choice of diagnostic tests to determine *H.pylori* infection status depends on sensitivity, specifity, reproducibility, availability cost and rapidity of the results. There a need for a reference method to be used as gold standard to find patients truly infected. Unfortunately, none of the currently used methods is able to further this criterion. One solution is to combine the results of two or more techniques, and compare with results of each method being evaluated. PCR may be slightly superior as compared to other diagnostic methods for detection of *H.pylori* infection and to verify eradication after treatment, PCR is regarded as a highly sensitive method to detect DNA of *H.pylori* from different clinical samples, both sensitivity and specifity of nested PCR have been reported 100% so it may be used as gold standard technique in diagnosis of *H.pylori* infection(Singh *et al*.,2008).

2.1.17. Treatment of *H. pylori* infections

Although *H.pylori*is sensitive to a wide range of antibiotics *in-vitro*, they all fail as monotherapy *in-vivo*. Infected patients, the most effective single drug is clarithromycine, which lead to an approximate eradication rate of 40% when given twice daily for 10-14 days. The lack of efficacy of monotherapy is related to the niche of *H.pylori*, residing at lower PH in a viscous mucus layer. Dual therapies, combining twice daily dosed PPI with, in particular amoxicillin, one still use in some countries, but dual the therapies have mostly been reported by triple therapies. These combine two antibiotics with either a bismuth compound or a PPI but now further alternative is provided by quadruple therapies, which combine the bismuth compound and PPJ with two antibiotics. The exact mode of action of bismuth compound is unknown, but *H.pylori*is susceptible to these compound both *in-vivo* and *in-vitro*. Tetracycline, amoxicilline, imidazoles (predominantly metronidizole and tinidazole), and a few selected macrolides (in particular clarithromycin) are probably the drugs most widely used for *H.pylori* eradication therapy (Kalati *et al*., 2015).

2.1.18. Vaccination

H.pylori is a major cause of certain disease of the upper gastrointestinal tract, rising antibiotic resistance increases the need to search for new therapeutic strategies; this might include prevention in the form of vaccination which aim to providing alternative strategy to control *H.pylori* infection and related disease as stomach cancer. Researchers are studying different adjutants, antigens and routes of immunization to ascertain the most appropriate system of immune protection; however, most of research only recently moved from animal to human trails(Selgrad and malfertheiner, 2008). An economic evaluation of the use of a potential *H.pylori* vaccine in babies found its introduction could, at least in Netherlands, prove cost-effective for prevention of peptic ulcer and stomach cancer (De Vries *et al*., 2009).

2.1.19. Prevention

To reduce transmission of *H.pylori* bacteria mustpractice good hygiene and hand washing especially with food preparation and also all patients with chronic gastrointestinal symptoms that may be associated with infection *H.pylori* infection should be tested and treated to prevent exposure to family member and must complete therapy to maximize the potential for a cure(Cheung and Wong, 2008).

2.2. Previous Studies

Many studies found relation between ABO and susceptibility to infection by the pathogen one of these studies worked in Turkey by Kanbay and his colleague at 2005 and found that blood group A represent 38.2%, O blood group represent 29.2% and AB blood group 14.8% (Kanbay *et al*., 2005).

A study done in Sao Paulo by Palanduz *et al*. (2018) and found that, the parents who were infected by *H.pylori* their children showing positive for *H.pylori* stool antigen, while parents negative their children showing negative antigen.

The reinfection by *H.pylori* after eradication commonly occurs, study done in Vietnam by Wheeldon and observed that reinfection after one year following successful eradication was 89.9% (Wheeldon *et al*., 2005) while reinfection after treatment were rare in study performed in Korea (Nam, 2019).

There were study about comparison between stool antigen and serum antibodies performed in Yemen by Naji *et al.* 2014 and demonstrated that antibodies was detected in 72 out of 100 samples whereas stool antigen was positive in 49 out of 100 samples tested (Naji *et al*., 2014).

Relation between age and gender with *H.pylori* infection performed in Sudan, Khartoum at 2012 by Elhaj *et al.*(2012) and they found that the highest rate of infection was observed among age group 15-30years and the infection is more common in females than in males (Elhaj *et al*., 2012).

Another study performed in Sudan, Gazera center 2015 and found O blood group represent (66.7%) while AB blood group shown less prone (Mohammed *et al*., 2015)

Study performed in Japan by Osaki *et al*.(2015) to demonstrate family members infection confirmed that mother to child transmission found in four out of five families (Osaki *et al*., 2015).

CHAPTER III

3. MATERAILS AND METHODS

3.1. Study design

This is a descriptive, cross sectional, hospital-based study.

3.2. Study area

The study was conducted at Tamboul Hospital in Geziera State.

3.3. Study duration

The study carried out during the period from March 2016to January 2020.

3.4. Study population

Patients with and without symptoms of *H.plori* infection.

3.4.1. Inclusion criteria

Patients with and other without symptoms of *H.plori* infection with different age and gender were included in this study.

3.4.2. Exclusion criteria

Symptomaticpatients undergoing treatment of *H.pylori* infection and patients with other diseases.

3.5.Ethical considerations

Ethicalapproval was taken from the Scientific Research Committee of College of Medical Laboratory Science, Sudan University of Science and Technology and Tamboul Hospital. Verbal consent was taken from all participants.

3.6. Data collection

Direct interview through structured questionnaire was used.

3.7. Sampling technique

Samples were collected by non-probability, convenience technique.

3.8. Sample size

Sample size was calculated according to the below formula. However, due to the high cost; sample size was one hundred (n=100) sera and stool samples were collected from patients in Tamboul Hosbital, Gazera State. In which50 patients were symptomatic and other (50) without symptoms of infection.

$n=t^2*p\ (1-p)/\mu^2$

t=1.96 (t^2=3.8416)

p=prevalence (0.80) (80%)

μ=0.05 (μ^2=0.0025).

30841*0.8(0.92)/0.0025=113

3.9. Specimen collection and processing

3.9.1. Blood Sample

Five mls of blood samples were collected into plain blood container. Then the serum was separated by centrifugation (3000 rpm for five minutes) to subsequent detection of *H.pylori* antibodies by using commercial kits ICT.

3.9.2. Stool Sample

Fresh stool samples were collected into spoon- cover and outer-labeled stool container for antigen detection. 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 tie ICT of *H.pylori* stool antigen detection.

3.10. Laboratory tests

ICT was used to detect the antigen and antibodies of *H.pylori*.

3.10.1.*H. pylori* antibodies rapid test

Chemoux bioscience Kits were used for qualitative detection of antibodies (IgGand IgM) anti-*H.Pylori* in human serum and plasma.

3.10.1.1. Procedure of the test

Brought the specimens and test device to room temperature, removed the test device from the foil pouch, and placed it on a flat, dry surface, firstly added 2 drops sample (about 100µl) into the sample well (S) of the test device and then added 1 drop buffer into the sample well (S) of the test device.

As the test begun to work, purple color moved across the test window in the center of the device.

Interpreted test results at 10 minutes and did not interpret test result after 20 minutes (Perea *et al*., 1991).

3.10.1.2. Interpretation of results

A color band was appeared in the control section of the result window which that the test is working properly. This band is the Control Band.

The test section of the result window indicated the test results. If another color band appeared in the test section of the result window, this band is the Test Band.

Negative result: The presence of only control band (C) within the result window indicated a negative result.

Positive result: The presence of two color bands as test band (T) and control band (C) within the result window, no matter which band appeared first, indicated a positive result.

Invalid result: If the control band (C) is not visible within the result window after performed the test, the result was considered invalid. Some causes of invalid results are because of not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the specimen be re-tested using a new test kit (Ansorg *et al*., 1991).

3.10.2.*H.pylori* antigen rapid test

Chemoux bioscience kits were used for qualitative detection of *H.pylori* antigens.

3.10.2.1. Procedure of the test

Using a wood stick, a small portion of the stool sample was transferred into 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. The color migrated from the well containing the tested sample in the ICT device (Ansorg *et al*., 1991).

3.10.2.2. Explanation of results

A color band was appeared in the control section of the result window to show that the test is working properly. This band is the Control Band.

The test section of the result window indicated the test results. If another color band appeared in the test section of the result window, this band is the Test Band.

Negative result: The presence of only control band (C) within the result window indicated a negative result.

Positive result: The presence of two color bands as test band (T) and control band (C) within the result window, no matter which band appeared first, indicated a positive result.

Invalid result: If the control band (C) is not visible within the result window after performed the test, the result was considered invalid. Some causes of invalid results are because of not following the directions correctly or the test may have deteriorated beyond the expiration date. It is recommended that the specimen be re-tested using a new test kit (Ansorg*et al*., 1991).

3.11. Statistical analysis

Statistical Package for Social Science program (SPSS) version 20 was used and frequencies were presented in form of tables and figures. Chi-square test was used to compare between variables and *P values* were set as$\leq$ 0.05statistically significant.

CHAPTER IV

4. RESULTS

A total of 100specimens (100 stool and 100 sera) were collected from asymptomatic and symptomatic patients, with different gender, age groups and blood groups.

Age was classified into groups as following; <15 years, 15-50 years and >50years.

In asymptomatic patients, the means of age was 16.7 ± 24.6 S.D, most of them were females and the most frequent blood group was O+ve (figure 4.1).

While in symptomatic patients, the means of age were 16.7± 20.4 S.D,most of them were females and the most frequent blood group was O+ve (figure 4.2).

Patients with previous treatment had low frequency of infection in both asymptomatic and symptomatic patients than who had no previous treatment.

In both asymptomatic and symptomatic patients, 30 (60%) of their family members had history of infection; while 20 (40%) had not history of infection as illustrated in Table 4.1.

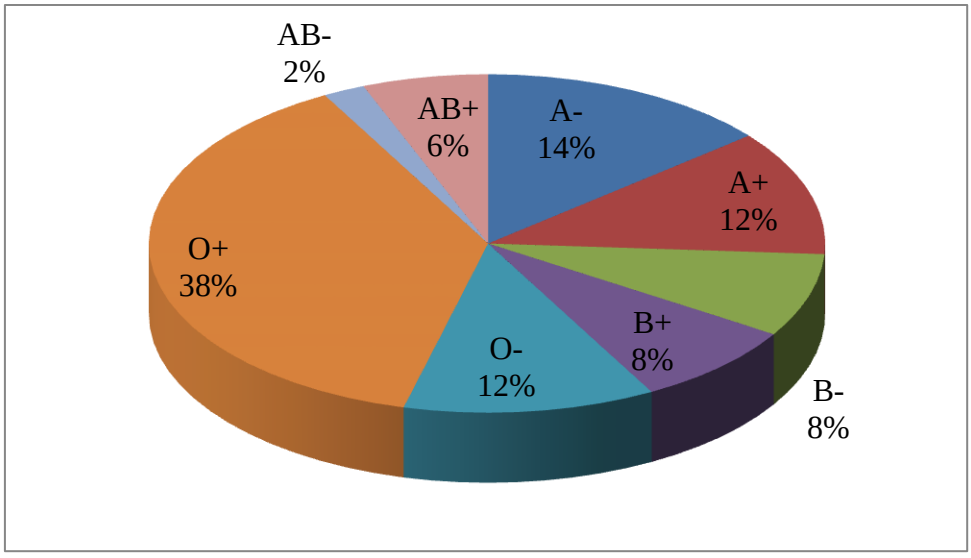

Figure 4.1: Frequency of blood groups among asymptomatic patients

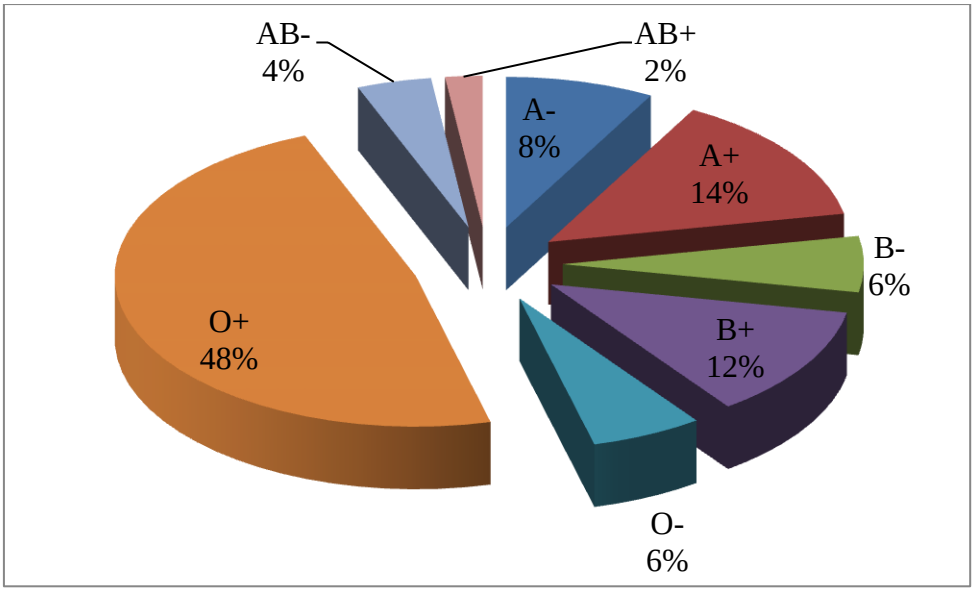

Figure 4.2: Frequency of blood groups among symptomatic patients

Table4.1.Demographic variables for patients (symptomatic and asymptomatic) with *H.* pylori infection

Variable	Symptomatic patients, n (%)	Asymptomatic patients, n (%)
Total	50	50
Gender		
Male	18(34%)	19(38%)
Female	32(64%)	31(62%)
History of infection in the Family		
Yes	30(60%)	30(60%)
No	20(40%)	20(40%)
Previous Treatment		
Yes	14(28%)	13(26%)
No	36(72%)	37(74%)
Age groups/ years		
<15 years	2(4%)	0(0.0%)
15-50 years	40(80%)	45(90%)
>50 years	8(16%)	5(10%)

There was a higher number of symptomatic patients with a positive antigen result (35/50; 70%) compared with asymptomatic patients (18/50; 36%). There was a significant association (*P-value* $\leq$ 0.05) between antigen result and patient groups (symptomatic and asymptomatic).

Table 4.2: Association between *H. pylori* antigen results and study groups

Patient groups	Ag results		Total	*P.value*
	+ve	-ve		
Asymptomatic	18(36%)	32(64%)	50	0.001
Symptomatic	35(70%)	15(30%)	50	
Total	53 (53%)	47 (47%)	100 (100%)	

Similarly, there was a higher number of symptomatic patients with a positive antibody result (30/50(60%) compared to asymptomatic patients (25/50(40%). There was no significant association (*P-value* $\geq$ 0.05) between antibody results and patient groups (symptomatic and asymptomatic).

Table 4.3: Association between *H. pylori* antibody results and study groups

Patient groups	Abs results		Total	*P.value*
	+ve	-ve		
Asymptomatic	25(50%)	25(50%)	50	0.317
Symptomatic	30(60%)	20(40%)	50	
Total	55	45	100 (100%)	

Symptomatic patients in the 15–50 years age group had the highest number of positive results for antigen and antibodies (antigen, 27 (54%); antibody, 24 (48%)). In asymptomatic patients for this age group, a positive antigen and antibody result was seen in 17 (34%) and 23 (46%) patients, respectively.

However, there was no association between age groups and positive result for antigen and antibodies *to H. pylori* in both groups of patient (*p value*= 0.920, 0.331,0.480 and 0.766 respectively).

Women showed higher positive results than men for both antigen and antibodies in both asymptomatic and symptomatic groups (asymptomatic women, Ag 12 (24%), Abs 14 (28%); asymptomatic men, Ag 9 (18%), Abs 11 (22%); symptomatic women, Ag 21 (42%), Abs 19 (38%); symptomatic men, Ag 9 (18%), Abs 11 (22%)). This association between gender and antigen/antibodies presence was not significant in both groups of patients (*p value*= 0.192, 0.797 , 0.382 and 0.904 respectively)

Patients with O positive blood group showed higher positive results than other blood groups for both antigen and antibodies in both asymptomatic and symptomatic groups (asymptomatic O positive, Abs 14 (28%), Ag 17 (34%); symptomatic O positive, Abs 10 (20%), Ag 7 (14%)). The association between blood group and antigen/antibodies presence was not significant (*p value*= 0.341, 0.725, 0.925 and 0.694 respectively) in both groups of patients.

Patients in both groups with family members who also had *H. pylori* infection showed higher positive antigen results than those without family members who had the infection (symptomatic patients with family infection, 21 (42%), without family infection 14 (48%); asymptomatic patients with family infection, 12 (21%), without family infection 6 (12%). There was no significant association (*p value*= 0.470, 1.000, 0.740 and 0.239 respectively)between antigen results and history of infection in the family.

Asymptomatic patients who had no previous treatment for *H. pylori* showed higher antigen positivity (12/50; 24%) than those with previous treatment. This was similar in symptomatic patients (no previous treatment Ag, 25/50; 50%). There was no significant association (*p value*= 0.891, 0.314, 0.235 and 0.315 respectively) between previous treatment and antigen result in asymptomatic and symptomatic patients.

CHAPTER V

5. DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1. Discussion

H.pylori infection is a co-factor in the development of three important upper gastrointestinal diseases, duodenal or gastric ulcers, gastric cancer and gastric mucosa-associated lymphoid tissue (MALT) lymphoma (Kenneth and McColl, 2010).

The results of *H.pylori* antibodies in asymptomatic patients was 25(50%) while in symptomatic patients was 30 (60%), these result was similar to study carried by Naji *et al*. (2014) in Yemen which found antibodies was 28(72%).

This result was higher than Douraghi (2013) in Iran who found antibodies was 4(4.6%) and lower than Alfadil (2016) in Sudan 48(48%).

In asymptomatic patients, *H.pylori* antigen was detected in 18(36%) while in symptomatic patients was 35 (70%), these results were matched to study carried out in Nigeria by Omosor *et al*. (2018) (28; 56%).

The above results were higher than those reported by Douraghi (2013)in Iran (12.4%) and lower than Naji *et al*. (2014) in Yemen (49%).The variation may be due to difference in socioeconomic status.

Age group 15-50 years shown highest rate of infection in both symptomatic and asymptomatic patients for antigen and antibodies. That may be due to vast majority of individual acquired infection during childhood and the infection associated with poor hygiene and overcrowding.

This study results showed insignificant association between age groups and *H.pylori* infection, this was similar to a study done in Iran by Alavi and other in 2010, but it differed from Kabir, 2007 in Sweden who reported that the percentage of infection increase with age.

Also found that; women were more affected than men both in the symptomatic group (62% vs 38%.) and asymptomatic groups (64% vs 36%). This was a similar result to that found in Turkey by Kanbay *et al.* (2010) (60.6% vs 42.9%), but a dissimilar results to those found in Iran by Metanant *et al.* (2010) (32.8% vs 36.4%). The differences between studies could be due to random selection of patients regardless of their gender in this study.

In the current study, blood group O have fair frequent to *H.pyori* infection, inboth symptomatic and asymptomatic patients and represent 48% and 38% respectively. This results were harmonized with study done at Gazira State, Sudan by Mohammad

et al.(2015) who found blood group O represent 58.1%, but mismatched with study done in Turkey by Kandby *et al*.(2005) who reported that the blood group A had more frequent in *H.pyori* infection than other blood group(38.2%).Thisdissimilarity may be due differences in ethnic background and blood group O is most frequent blood group in Sudan (Hassan, 2010).

In this study, 12/50 (24%) of asymptomatic patients who had infection in their family were positive for antigen and/or antibodies. These results were similar to Gravina *et al*. (2016) in Italy, who found *H. pylori* infection in 28.4% household contacts. However, this study was higher than Mabeku *et al*. (2018) in Cameroon (80.39%).

In symptomatic patients, the frequency of antigen and antibodies with history of infection in the family were 21(42%) and 20(40%) correspondingly, that were similar to result of Gravina *et al*. (2016) in Italy who found *H. pylori* infection in 43.2% household contacts. This finding was higher than Manfredi *et al*. (2016) in Italy (81.7%), and variation may be due to personal hygiene.

In the current study, the frequency of antigen in asymptomatic patients who had previous treatment for *H. pylori* were 12% (6/50) while in symptomatic patients this was 20% (10/50). This agreed with a study carried out by Gisbert *et al*. (2005)in Spain, but was higher than the results reported by Nanivadekar *et al*. (1990) in India (2.4%), and lower than the results found by Bapat *et al*. (2000) in India (60%). This discrepancy may be due to resistance of standard triple therapy.

In this study, the association between previous treatment and *H. pylori* reinfection was not statistically significant.

In this study, there was statistically insignificant relationship between previous treatment and *H.pylori* reinfection.

5.2. Conclusions

Immune-Chromatography Tests (ICT) used for detection of antigen in stool specimens showed 70% positive in symptomatic patients and 36% in asymptomatic patients, while detected antibodies in serum was 60%in symptomatic patients and 50% in asymptomatic patients.

5.3. Recommendation

-ICT for detection of antigens and antibodies are better used according to phase of disease.

-Using more accurate techniques such as PCR and urea breath test for preliminary diagnosis and confirmation of *H.pylori* infection.

-Further studies to confirm eradication of bacteria after treatment and detect resistant strains must be done.

REFERENCES

Abdallah, T.M., Mohammed, H.B. and Ali A.A. (2014). Sero-prevalence and factors associated with *Helicobacter pylori* in Eastern Sudan. *Asian Pac J Trop Dis*, **4**(2):115.

Abusiyanik, M.F., Meral, T. and Barik, A.S. (2004). Enzyme Immunoblotting analysis of *Helicobacter pylori* infection in Turkish a symptomatic subjects. *J Diagon Microbiol infect Dis*, **50**(3):173-177.

Alcout, A., Blackwell, C. and Weir, D. (2000). Increase inflammatory responses of persons of blood groups to *Helicobacter pylori. J infect Dis*, **181**:1364-1569.

Alfadil, A. (2016).Compression between antigen and antibodies detection test in diagnosis of *Helicobacter pylori* infection. M.Sc. thesis. Sudan University for Science and Technolog. P24.

Alvali, S.M., Adel, S.H. and Raja, A.R. (2010). Seroprevalence study of *Helicobacter pylori* infection among visitors of cardiac patients in Razi Hosbital in Ahvaz, Iran, Jundishapur, *J microbial*, **3**(1):28-31.

Ansorg,R.Von, G. Pomarius, R. *et al* (1991).Evaluation of techniques for isolation, subcultivation and preservation of *H.pylori J ClinMicrobiol*,29:51-53.

Arora, D.R.(2006).Textbook of Medical Microbiology.2nd edition, New Delhi-Bangalore: CBS Publisher and Distributors, p.p.302-312.

Bapat, M., 0 Abraham, P., Bhandarkar, P., Phadke, A. and Joshi, A. (2000). Acquisition of *Helicobacter pylori* infection and reinfection after its eradication are uncommon in Indian adults. *Indian J Gasteroentrol*, 19:172-4.

Bashir, A.,Yousife, S. and Mahmoud, M. (2011). Prevalence and effect of eradicative triple therapy on extra digestive *Helicobacter pylori* skin manifestation, EdHpSm.*Clinical Reviews and opinion*, **3**(2):14.

Bazillou, M.C., Feendri, O., Castel, P., Ingrand, J. and Fauchere, L. (1994).Serum antibodies response to the superficial and released components of *Helicobacter pylori*.*ClinDiagn Lab Immunol*, **2:**156-159.

Bladwin, D.N., Shephered, B. and Kraemer, P. (2007).Identification of *Helicobacter pylori* genes that contribute to stomach colonization. *Infect Immune*, **75**(2):1005.

Black, J.G. (2004).Microbiology principles and exploration., Oxford Black well science 6thed **3:648.**

Brook, G., Butel, J. and Morse, S. (2004). Jawetz, Melnickand Adelberg Medical Microbiology.23ed edition, New York: McGraw Hill international Edition, p.p. 275-6.

Butcher, A and Graham, P. (2003). Gastroenterology, AnIllustrated Color Text. Edinburgh: Charchill living stone, p.p.128- 129.

Bytzer, P., Dahlerup, J.F., Eriksen, J.R., Jabol, D.E., Rosenstock, S. Wildt, S. (2011). Diagnosis and Treatment of *Helicobacter pylori* infection. *Dan Med Bul*, **58**(4): C4271.

Chey,W.D and Wong, C.Y. (2007). Management of *Helicobacter pylori* infection *American j of Gastroenterol*, 102:1808-1825.

Cheung, T.K and Wong, B.C. (2008). Treatment of Helicobacter pylori and prevention of gastric cancer *J Dig Dis*, **291**(2): 187.

Chong, V.H., LimK, C. and Rajendran, N. (2008). Prevalence of active *Helicobacter pylori* infection among patients referrer for endoscopy in Brunei Darussalam.*SingaporemedJ*, **49(**1**):**42-46.

De Vries, R., Klok, R., Brouwers, J. and Postma, M. (2009).Cost-effectiveness of a potential future *Helicobacter pylori* vaccine in the Netherlands: the impact of varying the discount rate for health.*Vac*, **27** (6): 840-52.

Douraghi, M., Nateghi, M., Goudarzi, H and Ghalavand, Z. (2013). Comparison of stool antigen immunoassay and serology for screening for *Helicobacter pylori* infection in intellectually disabled children. *J Microbiol immunol*, **57**(11):772-7.

Elhag, W.I and Omer, A.L. (2014). Frequancyofh.pyloriantibodies among patients with gastrointestinal symptoms Attending Khartoum Teaching Hospital Sudan.*SOJ Microbial infect Dis*, **2**(1):5.

Elmahadi, A.M., Patchetl, S.E., Domizio, P and Fedial, S.S.(2003). Acid response to *H.pylori* infection in an African population. *J Clinmictobiol*, **39**(4):1323-1327.

Elomar, E., Owen, K., Murrcy, L.S., ELNujumi, A., Gillen, D., Williams, C., Fullarton, G. and McColl, K. (2001). Increased prevalence of precancerous change relatives of gastric cancer patients: critical role of *H.pylori.Gastroenterol*, **118**:22-30.

Eusebi, L.H., Zagari, R.M and Bazzoli, F. (2014).Epidemiology of *H.pylori* infection.*Helicobacter*,**19**: 1-5.

Ferwana, M., Abdulmajeed, I., Alhajiahmed, A., Madani, W., Firwana, B., Hasan, R., Altayar, O., Limurg, J., Murad, M and Knway, B. (2015). Accuracy of urea breath test in *Helicobacter pylori* infection: meta-analysis. *World J Gastroenterol*, **21**:1305-1314.

Figueiredo, C., Machado, J. and Pharoaah, P *el al*. (2002).*Helicobacterpylori* and interleukin 1 genotyping an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl CancerInst*, **94**(22):1680-1687.

Gold, B., Gilger, M. and Czinn, S. (2014). New diagnostic strategies for detection of *Helicobacter pylori* infection in pediatric patients, *J Gatroenterol Hepatol*, **10**(7): 1-19.

Graham, K. S. (2004). Contemporary diagnosis and management of *H.pylori* associated gastrointestinal disease. Hand Book in Health care Co., *Newtown* 39-67.

Gisbert, J., Trapero, M and Pajares, J. (2005). Evaluation of 3 different test for the detection of stool antigen to confirm *Helicobacter pylori* eradication after treatment, **10**: 615-618.

Gravina, A.G., Federico, A., Dallio, M., Sgammbato, D. and Miranda, A. *et al.* (2016). Intrafamilial Spread of *Helicobacter pylori* Infection. *J Gatroenterol Hepatol Endosc*, **1**(1): 1003.

Hartman, J and Owen, R. (2012). Are routine ancillary stains required to diagnosis Helicobacter infection in gastric biopsy specimen? An institutional quality assurance review.*Am J ClinPathol*, **137**:255-260.

Hassan, M. F. (2010). Frequancy of ABO, subgroup ABO and Rh (D) Blood Group in Major Sudanese Ethnic Groups. Pak *J Med Pes*, **49**(1):21.

Huge, I., Rooker, M., Taylor, D. and Feldman, M. (2010).*Helicobacter pylori* lipopolysacharideis synthesized via a Novel pathway with an Evolutionary Connection to protein N-Glycosylation. *Plospathog*, **6**(3):e1000819.

Kabir, S. (2007). Thestatus of *Helicobacter pylori* vaccines. *A review Helicobacter*, **12**(2):89-202

Kalati, B., Formichella, L. and Gerhard, D. (2015).Diagnosis of *Helicobacter pylor*i: Changes towards the future. *Dieseas*, **15** (3):125.

Kanby, M., Gur, G., Arslan, H. and Yilmaz, U. (2005).The relation between ABO blood group, age, gender, smoking and *Helicobacter pylori* infection. *Dig Dis Sci*, **50** (450):14.

Kenneth, E.L and McColl, M.D. (2010).*Helicobacter pylori* infection. N *Engl J med*, 362:1597-1604.

Lerang, F., Hung, J. and Mourn, B. (1998).Accuracy of IgG serology other test in confirming *Helicobacter pylori* eradication. *Scand J Gastroenterol*, **33**(7):710.

Logan, R and Walker, M. (2001). Epidemiology and Diagnosis of *Helicobacter pylori* infection *BMJ*, **323** (7310):920.

Mabeku, L., Ngama, M and Leundji, H. (2018).Potential risk factors and prevalence of *Helicobacter pylori* infection among adult patients with dyspepsia symptoms in Cameroon.*BMC infect Dis*,18:278.

Malfertheiner, P., Megrand, E., Morain, C.,Bazzoli, F and Elomar, G.D. (2012).Current concepts in the management of *Helicobacter pylori* infection. *J GatroenterolHepatol*,**28**(16):167-180.

Manfredi, M., Iuliano, S., Gismondi, P., Bizzarri, B andGaiani, F *et al*.(2016).*Helicobacter pylori* infection: We Should Always Verfiy the Interfamilial Transmission. *Biol Med*, 9:366.

Marshall, B.J. (1989).History of the discovery of *campylobacter pylori* in gastritis and peptic ulcer disease. *Blaser. MJ*, 5:7-24.

Medis, A and Marshall, B. (2008). TNF- α including protein carcinogenic factor secreted from *Helicobacter pylori. J. Clin Pathol*, **37**(8):608-612.

Megraud, F. (1996).Advantages and disadvantages of current diagnostic test for the detection of *Helicobacter pylori*. *scand J Gastroenterol suppl*, **215:** 57-62.

Metanant, M., Sharifi, B and Izadi, S. (2010). Prevalence of *Helicobacter pylori* in healthcare worker. *TurK J Med Sci*, **40** (6):965-969.

Mirghani, A., Salah, M. and Fedial, S.(2003).Detection biochemical and immunological characterization of *H.pylori* in Sudanese patients with gastro duodenal inflammation. Thesis 92-U Khartoum.

Mladenova, I and Durazzo, M. (2018).Transmission of *H.pylori*. *Minerva gastroentrolDietol*, **64**(3):251-254.

Mohammed, M., Suliman, O. and Khalfalla, O. (2015). Association between *Helicobacter pylori* infection, ABO Blood Groups and Rhesus factor in Peptic Ulcer Disease patients, in Gezira, Central Sudan. *BJMM*, 7(1): 11-16.

Monno, R., Volpe, A. Basho, M. Fumarula, L and Kondili, P. (2008). *Helicpbacter pylori* seroprevelance in selected groups of Albanian volunteers. *Infection*, 345-350.

Moyat, M and Velin, D. (2014). Immune responses to of *H.pylori* infection *world J gastroenrol*, **20** (19):5583-5593.

Naji, S.A., Ameri, A.G., Alkadasi, N.M., Hanash, S., Ali, M.A. and Zaid, A.A. (2014). Comparison of stool antigen and blood antibodies test methods for detection

of *Helicobacter pylori* infection and risk factors. *Int J microbiolAppSci*, **3** (12):118-127.

Nam, J., Choi, I., KooK, M., Lee, J and Cho, S. *et al*. (2014). OLGA and OLGIM stage distribution according to age and *Helicobacter pylori* status in the Korean population. *Helicobacter*, **19**:81-9.

Nanivadekar, S., Sawant, P., Patel, H., Shroff, C., Popat, U and Bhatt, P. (1990). Association or peptic ulcer with *H. pylori* and the recurrence rate .A three year follow up study. *J Assoc Physician India*, **38**(1): 703-6.

Olokoba, A., Gashu,W., Bwala,S., Adamu, A. and Salawu, F.U. (2013).*Helicobacter pylori* infection in Nigerians with dyspepsia. *Ghana Med J*, **47**:97-81.

Omosor, K.I., Omosor, O.H., Adejumo, B.G., Ibeh, I.N. and DImkpa, U. (2018). Comparative evaluation of stool antigen and blood antibodiestest methods for the screening of *Helicobacter pylori* infection in asymptomatic adult population in Delta State, Nigeria. *J MolMicrobiol*, **2**(1):3.

Osaki, T., Konno, M.,Yonezawa, H., Hojo, F. and Kamiya, S. (2015). Analysis of intra-familial transmission of *Helicobacter pylori* in Japanese families *J Med Microbol*, **64** (10):67-73.

Owen, R.J., Peters,T.M., Varea, R., Teare,E.L. and Savery, S.(2001).Molecular epidemiology of *Helicobacter pylori* in England: prevalence of cag pathogenicity island markers and IS605presence in relation to patient age and severity of gastric disease. *Immunol Med Microbial*, **30**(1):65-71.

Palanduz, A., Ederm, L., Cetin, B and Ozcan, N. (2018).*Helicobacter pylori* infection in family member patients with gastroduodenal symptoms. *Sao PauloMed. J*, **136(**3):222-227.

Pasteure, P. (2002).Genome inflammation for *Helicobacter pylori* and 199 strains.*Institute pasture*, 9-11.

Perea, P.G., Wilkin, M.D., Decker, S.S. and Blaswer, M.J. (1991). Seropervalence of *Helicobacter pylori* infection in couples. *J clin Microbiol*, 29:642-644.

Perez, P.G.I. (2004).Accurate diagnosis of *Helicobacter pylori*. Culture, including transport.*GastroenterolClin Nam*, **29**:879-884.

Peter, M. (2001).Diagnosis of *H. pylori-H.pylor*i it is role in gastrointestinal disease. *NEngI J Med*, 325:1127-1131.

Quartero, A., Numans, M., de Melker, R. anddeWit, N. (2000). Impractice evaluation of whole-blood *Helicobacter pylori* test: its usefulness in detecting peptic ulcer disease. *Br J Genpract*, **50** (450): 13.

Rosenow, E.C and Sanford, A.H. (1915).The bacteriology of ulcer of the stomach and duodenal in man. *Infect Dis*, 17:219-226.

Ryan, M.C and Kenneth, L.E. (2010). Reinfection rate and endoscopic changes after successful eradication of *Helicobacter pylori*. *World J Gastroentrol*, 16:251-255.

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

Selgrad, M and Malfethereiner, P. (2008). New strategies for *Helicobacter pylori* eradication. *CurrOpin Pharmacol*, **8**(5):593-7

Singh, V., Mishra, S., Rao, G., Jain, A., Mahajan, D., McClelland, M and Nath, G. (2008).Evaluation of nested PCR in detection of *Helicobacter pylori* targeting a highly conserved gene.*HSP60*, **34:** 2728-2730.

Sonlnick, J.,RouKe, P and VanDamme, A. (2004).The Prokayrotes: An Evolving electronic Resource for the Microbiology Community.3thed edition, New York:Springer, p.p. 155-157.

Stenstram, B., Menis, A. and Marshall, B. (2008).*Helicobacter pylori*- the latest in diagnosis and treatment.*AustFamPhysician*, **37** (8):608.

Vaira, D., Vakil, N., Gatta, L., Picci, C., Perna, F., Saracino, I., Fiorini, G and Holton, J. (2010). Accuracy of a new ultrafast rapid urease test to diagnosis Helicobacter pylori infection in 1000 consecutive dyspeptic patients. *Aliment Pharmacol Ther*, **31**:331-338.

Versalvic, J and Fox, J.G. (2003). Manual of Clinical Microbiology. 8th ed, Washinton, p.p.915-928.

Wang, K.Y., Kuo, C.F., Liu, J.C., Shin, Y.H., Kuo, H.C., Huang, K.Y. and Wu, C.D. (2015). Diagnosis of *H.pylori* infection: current options and developments. *world J gastroenrol*, **21** (40):11221-11235.

Walsh, J.H and Peterson, W.L. (2005).The treatment of *H.pylori* infection in the treatment of peptic ulcer disease *NEgl J Med*, **333**(15):984-991.

Warren, J.R and Marshall, B.J. (1983). Unidentified curved bacilli on gastric epithelium in active gastritis. *JClinpathol*, **25**:1273.

Wheeldon, T.U., Hong ,T.T., Phung, D.C., Bjorkman, A., Granstrom, M. and Sorberg, M. (2005). Long-term follow-up of Helicobacter pylori eradication therapy in Vietnam: Reinfection and clinical outcome. *Alimetpharmacol*, 21:1047-53.

Wood, D., Richard, C.B., Slack, J. and Peuther, F. (2003). Medical Microbiology A Guide to microbial infection: pathogensis, immunity. Laboratory diagnosis and control, Fifteen editions, Hong Kong*:* Elsevier, p.p:293-295

Appendix-1

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

Diagnosis of *Helicobacter pylori* Infection Using Immune–Chromatography Tests among Patients Attending Tamboul Hospital in Gazera State

Patient's No. (……..) **Age:**……

Gender: Male ☐ Female ☐

Blood group:

A+ve	A-ve	B+ve	B-ve	O+ve	O-ve	AB+ve	AB-ve

Medical history

Symptoms: Yes ☐ No ☐

Previous of treatment: Yes ☐ No ☐

Infected person in family: Yes ☐ No ☐

Investigation Results

Antigens result: +ve ☐ -ve ☐

Antibodies result: +ve ☐ -ve ☐