Sudan University of Science and Technology

College of Graduate Studies

Immuno-Chromatography Tests for Diagnosis of *Helicobacter pylori* in Patients with Peptic Ulcer in Relation to Selected Factors in Elnehoud City

اختبار المناعة اللوني لتشخيص بكتريا الملوية البابية في المرضى المصابين بقرحة المعدة وعلاقتها ببعض عوامل الخطر المختارة في مدينة النهود

A dissertation submitted in partial fulfillment for the requirements of M.Sc in Medical Laboratory Science (Microbiology)

By

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الآيـــــــة

قال تعالى (قُل لَّوْ كَانَ الْبَحْرُ مِدَاداً لِّكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَن تَنفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَداً).

صدق الله العظيم

سورة الكهف الآية (109)

Dedication

To,,,

All patients who sustain the pain and their hopes to become well and good

To,,,

My parents who give me hope and help on the way of life

To,,,

My friends and teachers

To,,,

All persons care about health and well-being

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Thanks to Almighty Allah for the blessing of success and bringing this work to reality.

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Abstract

Helicobacter pylori are gram-negative, spiral rods that cause a major health problem worldwide. More than half of the world population is infected with this pathogen.

This study was conducted in Elnehoud city, Western Kordofan State, during the period from March to September 2016 to compare between the results of antigen and antibody tests used for the diagnosis of *Helicobacter pylori* infection.

One hundred (n = 100) symptomatic patients were included in this study. Fifty six were males and 44 were females. The age of patients ranging from 20 – 60 years. Data on smoking and other demographic information were obtained by questionnaire and ABO blood group phenotypes were determined. The stool and blood samples were collected and analyzed for antigen and antibody respectively by Immunochromatography test (ICT) cards. Fourty eight (48%) patients showed positive results for *H. pylori* antibody while 28 (28%) showed positive results for *H. pylori* stool antigen. Smoking has shown significant variation among both antibody and antigen detection methods of *H. pylori* ($P < 0.05$). Patients with blood group (O) were more prone to *H. pylori* infection compared with patients in other blood groups since 32 (67%) patients with blood group (O) were positive out of 48 (100%) for antibody and 24 (86%) positive out of 28 (100%) for stool antigen.

In conclusion, the frequency of *H. pylori* antibody was higher than the *H. pylori* antigen and patients with blood group (O) were more susceptible to infection by *H. pylori* compared to other blood groups.

Future studies should be conducted with larger sample size, wider area and advanced techniques to confirm the present results. Moreover, molecular techniques should be involved to determine the blood group gene responsible for increased susceptibility to *H. pylori* infection.

المستخلص

الملوية البابية هي بكتريا سالبة الجرام، قضيبة الشكل، وهي تسبب مشكلة صحية كبيرة في جميع أنحاء العالم. أكثر من نصف سكان العالم مصابين بهذا الممرض.

أجريت هذه الدراسة في مدينة النهود، ولاية غرب كردفان خلال الفترة من مارس حتى سبتمبر 2016م، للمقارنة بين نتائج المستضد في البراز والكشف عن الأجسام المضادة في الدم للمصابين ببكتريا الملوية البابية. تضمنت هذه الدراسة 100 مريض لديهم أعراض الإصابة ببكتريا الملوية البابية. وقد كان عدد الذكور 56 والإناث 44 وأعمارهم تتراوح بين 20 – 60 عاماً. وقد تم الحصول على معلومات عن التدخين والبيانات الديموغرافية عن طريق الاستبيان وتم الكشف عن فصائل الدم ABO في المرضى المختارين. تم جمع عينات البراز والدم وتحليل المستضد والأجسام المضادة بواسطة اختبار المناعة اللوني (ICT). خلُصت النتائج إلى أن 48 (48%) من المرضى أظهروا نتائج إيجابية للأجسام المضادة لبكتريا الملوية البابية وأظهرت أيضاً 28 (28%) نتائج إيجابية للمستضد في البراز. أفادت هذه الدراسة أن هناك علاقة بين التدخين ونتائج الأجسام المضادة والمستضد لبكتريا الملوية البابية حيث كانت القيمة الإحتمالية أقل من 0.05. كما أوضحت الدراسة أن المرضى ذوو فصيلة الدم (O) كانوا أكثر عرضة للإصابة بالبكتريا مقارنة مع المرضى ذوو فصائل الدم الأخرى، حيث كان 32 مريضاً ذو فصيلة (O positive) من إجمالي عدد النتائج الموجبة 44، و كذلك 24 نتائج إيجابية للمستضد من مجموع النتائج الإيجابية (28) لبكتريا الملوية البابية.

خلصت الدراسة إلى أن معدل الإصابة ببكتريا الملوية البابية عن طريق الكشف عن الأجسام المضادة كان مرتفع مقارنة بالكشف عن المستضد، كما أوضحت الدراسة أن المرضى ذوو فصيلة الدم (O) كانوا أكثر عرضة للإصابة بالمرض.

أوصت الدراسة بإجراء مزيد من الدراسات تتضمن أكبر حجماً للعينة، وتتضمن مساحة أوسع وتقنيات أحدث. علاوة على ذلك، ينبغي أن تستخدم تقنيات حديثة مثل تقنيات الأحياء الجزيئية (PCR) لتحديد جينات فصيلة الدم المسئولة عن زيادة القابلية للإصابة ببكتريا الملوية البابية.

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CHAPTER ONE

INTRODUCTION

1 INTRODUCTION

1.1 Background

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

Infection with *H. pylori* is a major health problem worldwide. More than half of the world's population is infected with this pathogen and humans are considered the only reservoir of infection. Gastritis, peptic ulcer, gastric carcinoma and mucus associated lymphoid tissue (MALT) lymphoma are recognized complications of *H. pylori* infection (Abdallah *et al*., 2014).

The bacteria establish a great adaptive relation in the human stomach, in a co-evolutionary environment. Several factors explain the virulence developed by *H. pylori*, including urease synthesis in order to neutralize the acid ambience and digestive enzymes in the gastric mucosa (Macrolopez *et al*., 2013).

In other hand, other virulence factors influence the severity of infection. Those virulence factors include cytotoxin associate gene A (CagA), vacuolating cytotoxin (VacA), blood group antigen-binding adhesion (BabA), protein induced by contact with epithelium (IceA) and outer inflammatory protein (oipA) (Macrolopez *et al*., 2013).

In response to these virulence factors, the immune system typically mounts a response through production of immunoglobulins to organism-specific antigens. These antibodies can be detected in serum or whole-blood samples easily. The presence of IgG antibodies to *H. pylori* can be detected by use of a biochemical assay (Lerang *et al*., 1998).

Other noninvasive tests can also be used include, detection of *H. pylori* antigens in stool and presence of *H. pylori* in the saliva. Stool antigen tests have recently been welcomed with great expectations as they are

convenient to the patients and can be easily performed even in small laboratories (Malfertheiner, *et al*., 2002; Logan and Walker, 2001).

In Sudan, the prevalence of *H. pylori* inf ection was estimated to be 80% among patients with gastritis (Abdallah *et al*., 2014). However, there is few published data on the reliable and convenient method for earlier diagnosis of the disease. Therefore, the aim of this study is to compare antibody detection method with stool antigen detection for diagnosis of *H. pylori* in Western Kordofan, Sudan, since there is no published studies conducted there and to determine selected risk factors associated with the infection.

1.2 Rationale

Infection with *H. pylori* is a world-wide health problem especially in developing countries (Abdallah *et al*., 2014). Although, in Sudan, the prevalence of the disease is estimated to be 80%, they are very few published data on the reliable and ideal method for early diagnosis of the infection (Mirghani *et al*., 1994). Laboratory testing for *H. Pylori* infection is a very important part of the diagnosis process for gastric and duodenal inflammatory disease. A number of different diagnostic test methods both invasive and non-invasive are available. Invasive methods have high specifity and sensitivity in the detection of *H. pylori* infection, however, they are expensive, include invasiveness and need special gastroenterologist to be performed. In contrast, non-invasive methods such as antibody (Ab) detection is cheap, easy to perform and can be performed in small laboratories, despite of that, antibody detection is not practical for the diagnosis of ongoing *H. pylori* infection, as the antibody level falls slowly after eradication, yielding false positive results and thus low specificity (Feldman and Evans, 1995; Quartero *et al*.,2000). A new non-invasive diagnostic tool is now available; detection of *H. pylori* antigen (Ag) in stool samples which indicate the current presence of the infection and thus increase the specifity. This study is aimed to

compare between antibody and stool antigen detection for diagnosis of current *H. pylori* infection.

1.3 Objectives

1.3.1 General objective

To diagnose *Helicobacter pylori* using Immuno-Chromatography Tests in patients with peptic ulcer in relation to selected risk factors in Elnehoud city.

1.3.2 Specific objectives

1. To detect serum antibodies in patients suspected to be infected by *H. pylori*.
2. To detect stool antigens in patients suspected to be infected by *H. pylori* .
3. To determine selected risk factors associated with *H. pylori* infection.

CHAPTER TWO

LITERATURE REVIEW

2. LITERATURE REVIEW

2.1 Historical background

The presence of spiral microorganisms in the stomachs of animals was observed in the late 19th and early 20th. Soon afterward, similar spiral bacteria were observed in humans, some of whom had peptic ulcer or gastric cancer. The bacteria observed in human stomachs were considered to be overgrowth or food contamination until the early 1980s. At this time Warren and Marshall performed their groundbreaking experiments, leading to the identification of a bacterium in 58 of 100 consecutive patients, with successful culture. This organism was initially named Compylobacter-like organism but is now named *Helicobacter pylori* in recognition of the fact that this organism is distinct from members of the genus Campylobacter (Kusters *et al*., 2006).

2.2 General characteristics

Helicobacter pylori (*H. pylori*) is a Gram-negative microaerophilic fastidious bacterium which over centuries has successfully infected around 50 percent of human individuals throughout the world. This human pathogen is known to induce several gastric disorders, but may also be associated with extragastric diseases like anaemia, dyspepsia, and some immunological disorders. Almost all infected patientss develop chronic gastritis, and a considerable percentage of patients further develop ulcer disease or gastric cancer (Kalali *et al*., 2015). It is a neutrophilic, motile bacterium which is unique in its ability to colonize the normal human stomach, spiral-shaped bacillus, grows well on non-selective media such as blood agar or heated blood (chocolate) agar, giving small colonies in 3 - 7 days at 35 - 37°C. They are oxidase positive and catalase positive and exhibit strong urease activity (Collee *et al*., 1996).

2.3 Morphology

Helicobacter pylori is a Gram-negative bacterium. Although usually spiral-shaped, the bacterium can appear as a rod, while coccoid shapes appear after prolonged in vitro culture or antibiotic treatment. These coccoids cannot be cultured in vitro and are thought to represent dead cells. The organism has 2 to 6 unipolar, sheathed flagella, which often carry a distinctive bulb at the end. The flagella confer motility and allow rapid movement in viscous solutions such as the mucus layer overlying the gastric epithelial cells (Kusters *et al*., 2006).

2.4 Growth requirements

A key feature of *H. pylori* is its microaerophilicity, with optimal growth at O_2 levels of 2 to 5% and the additional need of 5 to 10% CO_2 and high humidity. Growth occurs at 34 to 40°C, with an optimum of 37°C. Although its natural habitat is the acidic gastric mucosa, *H. pylori* is considered to be a neutralophile. The bacterium will survive brief exposure to pH of 4, but growth occurs only at the relatively narrow pH range of 5.5 to 8.0, with optimal growth at neutral pH. *H. pylori* is a fastidious microorganism and requires complex growth media. Often these media are supplemented with blood or serum. These supplements may act as additional sources of nutrients and possibly also protect against the toxic effects of long-chain fatty acids. Commonly used solid media for routine isolation and culture of *H. pylori* consist of columbia or brucella agar supplemented with either (lysed) horse or sheep blood or, alternatively, newborn or fetal calf serum. For primary isolation but also routine culture, selective antibiotic mixtures are available, liquid media usually consist of either brucella, Mueller-Hinton, or brain heart infusion broth supplemented with 2 to 10% calf serum or 0.2 to 1.0%cyclo-dextrins, often together with either Dent or Skirrow supplement. Most of the commercially available synthetic media, such as tissue culture media,

do not support the growth of *H. pylori* without the addition of serum (*Kusters et al*., 2006). Isolation of *H. pylori* from gastric biopsy samples are difficult and not always successful. Cultures should be inspected from day 3 to day 14. *H. pylori* forms small, translucent, smooth colonies. It should be noted that once a culture reaches the stationary phase, the growth rate rapidly declines, accompanied by the morphological change to a coccoid form (Kusters *et al*., 2006).

2.5 Pathology

Immediately following infection, *H. pylori* causes acute gastritis characterized by neutrophil infiltration. *H. pylori* causes a persistent infection in the majority of infected individuals. The acute phase lasts 1 to 4 weeks and is replaced gradually by a chronic, mononuclear infiltrate in the lamina propria. Active gastritis refers to the presence of neutrophils mixed with mononuclear cells in the gastric mucosa. Chronic active gastritis occurs in the majority of infected individuals and consists of surface epithelial degeneration, persistent neutrophil infiltration of the epithelium and lamina propria, and mononuclear infiltration (lymphocytes and plasma cells) of the lamina propria. Lymphoid hyperplasia in the gastric mucosa is suggestive of *H. pylori* infection. Generally, gastritis is most prominent in the corpus and antrum, with evidence of inflammation of the cardia in most infected individuals. Long-term infection by *H. pylori* results in chronic gastritis, a condition manifest as multiple pathologic entities. Chronic gastritis due to *H. pylori* infection may be separated into distinct, clinically relevant phenotypes (Versalovic, 2016). Nonatrophic pangastritis occurs in the majority of *H. pylori*-infected individuals with no predisposition to peptic ulcer disease or gastric atrophy. Prominent mucosal inflammation in chronic active gastritis often is evident in the antrum (antral-predominant gastritis),

predisposing to hyperacidity and duodenal ulcer disease (Versalovic, 2016).

2.6. Signs and symptoms

Up to 85% of people infected with *H. pylori* have no symptoms or complications (Bytzer *et al*., 2011). Acute infection may appear as an acute gastritis with abdominal pain (stomach ache) or nausea (Butcher and Graham, 2003). It usually occurs two to three hours after a meal or in the middle of the night (when the stomach is empty) and is relieved by eating, drinking milk or taking anti-acid medications. Where this develops into chronic gastritis, the symptoms, if present, are often those of non-ulcer dyspepsia: stomach pains, nausea, and bloating, belching, and sometimes vomiting or black stool (Ryan, 2010). Symptoms of *H. pylori* infection include the following: abdominal pain or a burning sensation in the abdomen ,bad breath, blood in the stool or vomit (may appear black in color), excessive burping (belching), flatulence (passing gas from the rectum), loss of appetite, nausea, vomiting and weight loss (Butcher and Graham, 2003).

2.7 Laboratory diagnosis

Testing for *H. Pylori* infection has become a very important part of the diagnostic process for gastric and duodenal inflammatory disease, since the presence or absence of infection determines the type of treatment to be applied. A number of different diagnostic test methods, both invasive and non-invasive are available (Stenstrom *et al*., 2008).

2.7.1. Invasive Methods

2.7.1.1. Histology

The presence of typical spiral motile bacteria accompanied by inflammatory reaction in the histopathological sections of stomach was the first described method used for the diagnosis of the *H. pylori*. Along

with routinely applied stains there are some more specific staining procedures which facilitate the diagnosis of *H. pylori* infection. However, the accuracy of the histo-pathological diagnosis of *H. pylori* always depends on the number and the location of collected biopsy materials. While *H. pylori* can be detected in even a single biopsy taken from the correct site, to achieve a higher sensitivity, multiple biopsies are recommended. Moreover, the possible presence of other bacterial species with a similar morphology to *H. pylori* in the stomach can be another source of error which negatively affects the accuracy of the test. In addition, treatment with proton pump inhibitors (PPI) or antibiotics prior to sampling may transform the shape of *H. pylori* to a coccoid form (Kalati *et al*., 2015).

2.7.1.2. Culture

Although it should be stated that *H. pylori* culture is not a routine procedure in initial diagnosis, in many bacteriology laboratories *H. pylori* isolation via the culture of biopsy samples is a routine second line approach. Because of the demanding character of this bacterium, this method remains challenging. This technique, although highly specific, is not as sensitive as other tests like histology and the rapid urease test. As well as for purposes of scientific research, cultured live *H. pylori* is used for diagnostic approaches and for the detection of antibiotic resistance if treatment failure is suspected (Kalati *et al*., 2015).

2.7.1.3. Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction based detection of *H. pylori* could be categorized under invasive as well as non-invasive methods. Molecular diagnostics have dramatically changed the clinical management of many infectious diseases in the past decades. PCR currently remains the best developed molecular technique as it provides a wide range of clinical applications, including specific or broad spectrum pathogen detection,

evaluation of emerging novel infections, surveillance, early detection of bio threat agents, and antimicrobial resistance profiling. While PCR could be applied for the detection of *H. pylori* in biopsies, this technique is more qualified for its use in samples taken from the oral cavity or from stool. PCR-based techniques, if applied as a non-invasive approach (i.e., from stool samples) tend to be more cost effective than other traditional methods. In addition to the improved specifications of this technology like high sensitivity and specificity, 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, which allows sampling from multiple origins. The high efficiency of this method also achieves fast results. 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).

2.7.2 Non-Invasive Methods

2.7.2.1 Urease Breath Test

The urease breath test (UBT) is one of the most common non-invasive tests used. This non-invasive test, available in different versions, has been evaluated in different studies, showing high sensitivity, specificity and accuracy. The test is able to detect the infection indirectly by measuring the existence of bacterial urease produced by *H. pylori* in the stomach. There are different types of this test comprising 13C- or 14Cisotope labelled urea. If *H. pylori* is present, the urease hydrolyses the labelled urea and the exhaled isotope containing ammonia can be detected applying the samples to a measuring device. It has been shown that UBT can distinguish an ongoing from a past infection; hence, it is able to detect the eradication progress after treatment (Kalati *et al*., 2015).

2.7.2.2. Fecal Antigen Test

Fecal antigen tests detect antigens in stools samples. ELISA formats comprising monoclonal antibodies against *H. pylori* proteins showed improved results compared to polyclonal approaches. The current guideline evaluates the use of the stool antigen test as equivalent to the UBT if a validated laboratory-based monoclonal antibody is used. Degradation of antigens in the intestine and consequent disintegration of epitopes might lead to false negative results. Moreover, the process of sample handling could be fastidious for patients. False negative results may occur when the bacterial load is low, due to proton-pump inhibitors or the recent use of antibiotics or bismuth (Kalati *et al*., 2015).

2.7.2.3 Serological Test

Immune responses against *H. pylori* are utilized to detect infection by analyzing patients' blood or serum for IgG and IgA antibodies. Serology is the only test which is not affected by those local changes in the stomach that could lead to a low bacterial load and to false negative results. Only IgG detection is considered and the favoured method is ELISA. Currently, different formats of serological tests are available, including simple ELISAs that use whole lysates or recombinant produced *H. pylori* proteins as antigens. More recently, immunoblots, luminex-based bead assays and line assays were developed; these allow a more specific evaluation of the infecting *H. pylori* strain in terms of bacterial virulence factors and host immune responses towards the human pathogen. Moreover, they show improved sensitivity and specificity because additional and highly purified antigens are included. These non-invasive tests are easy and cheap to perform. The potential for developing a rapid diagnostic test makes serology an interesting option for testing populations in areas with little or no access to medical facilities (Kalati *et al*., 2015).

Despite numerous publications on rapid diagnostic testing (RDT) methodologies for infectious diseases, such testing has become neither commonplace nor an integral component of services offered by clinical microbiology laboratories. However, because of the emerging need for rapid diagnosis and treatment of virulent strains of different viral and bacterial infections, discussions regarding routine application of RDT are increasing within current medical circles. Results of RDT testing become available within a couple of minutes to a few hours. A clinical specimen is processed in a few steps (preferably in a single step) at the site where it is collected (point of care). The quality and value of the RDT is determined by its sensitivity and specificity, the time required for results, and its cost and availability. It appears that those antigens with either high or low molecular weight are more specific. There are currently many *H. pylori* RDT kits commercially available. However, how far these tests fit in with standard clinical practice is still undetermined (Kalati *et al*., 2015). Serologic tests should therefore include antigens for sensitivity, specificity testing, and antigens that identify infections by more pathogenic *H. pylori* strains, which might provide the basis for decisions about further treatment (Kalati *et al*., 2015).

2.8 Treatment

Although *H. pylori* is sensitive to a wide range of antibiotics in vitro, they all fail as monotherapy in vivo. In infected patients, the most effective single drug is clarithrornycin, which leads to an approximate eradication rate of 40% when given twice daily for 10 to 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, are still in use in some countries, but dual therapies have mostly been replaced by triple therapies. These combine two antibiotics with either a bismuth compound

or a PPI. A further alternative is provided by quadruple therapies, which combine the bismuth compound and PPJ with two antibiotics. The exact mode of action of bismuth compounds is unknown, but *H. pylori* is susceptible to these compounds both in vivo and in vitro. Tetracycline, amoxicillin, imidazoles (predominantly metronidazole and tinidazole), and a few selected macrolides (in particular clarithromycin, sometimes azithromycin) are probably the drugs most widely used for *H. pylori* eradication therapy (Kalati *et al*., 2015).

CHAPTER THREE

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Study design

This was a cross-sectional study.

3.2. Study duration

This study carried out during the period of March to September 2016.

3.3. Study setting

The study was carried out at Dr. Suliman Mohamed Ahmed Dispensary, Elnehoud city in West Kordofan state, Sudan.

3.4. Study population

Randomly one hundred symptomatic patients (n = 100), from both genders attending Dr. Suliman dispensary, were enrolled in this study.

3.5. Ethical consideration

Approval was taken from the faculty and hospital administration. A verbal consent was taken from each patient.

3.6. Data collection

A trained medical officer interviewed each patient and obtained the demographic data as well as the symptoms and the history of the disease.

3.7. Sample collection and processing

3.7.1. Blood sample

Five mls blood sample was collected into plain blood container, then the serum was separated by centrifugation to subsequent detection of *H. pylori* antibody by using commercial kits of Xiamen Boson Biotechn and Healgen Immunochromatography test card (ICT).

3.7.2. Stool sample

Fresh stool samples were collected into spoon-cover and outer labeled stool container for antigen detection. Using wood stick a small portion of the stool sample was transferred into the buffer provided by Biotech and Healgen, 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.

3.8. Statistical analysis

The data was analyzed using Statistical Package of Social Science (SPSS) version 20.0. Frequencies, crosstabulation and P. values were calculated.

CHAPTER FOUR

RESULTS

4. RESULTS

4.1 Distribution of patients according to gender

Out of 100 patients, 56 (56%) were males and 44 (44%) were females.

4.2 Distribution of patients according to residence

Sixty four (64%) were from rural areas, while the rest 36 (36%) were from urban areas.

4.3 Distribution of patients according to blood group

The frequency of the blood group were (68, 12, 12, 4 and 4) for blood groups O+ve, B+ve, A+ve, A-ve, and AB+ve respectively (figure 4.1)

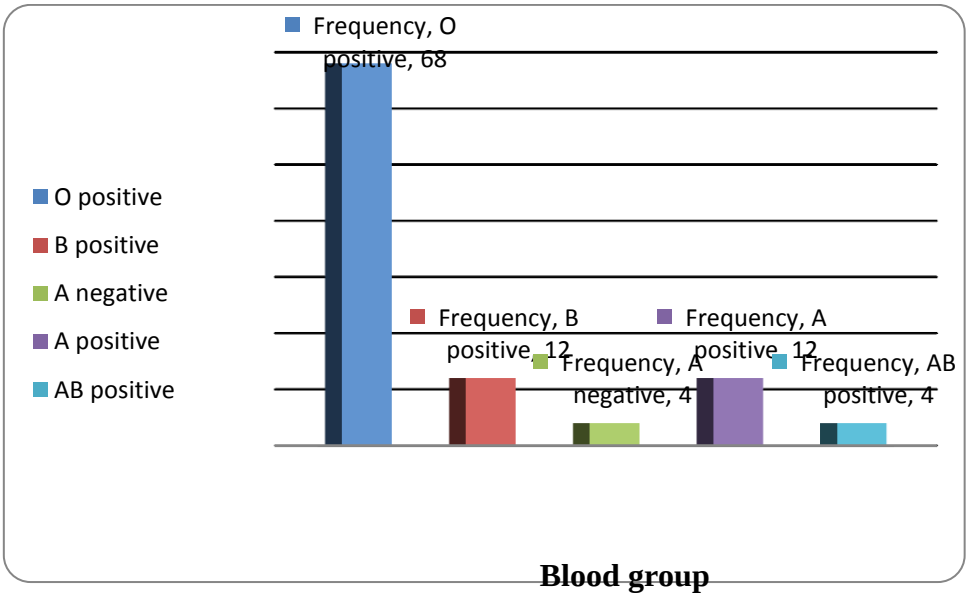

Figure 4.1: Distribution of patients according to blood group

4.4 Distribution of patients according to age group

The results of figure 4.2 illustrates that 32 were within age group 20 – 30 years, 28 were within 31 – 40 years, 16 were within 41 – 50 years, and 24 were within 51 – 60 years.

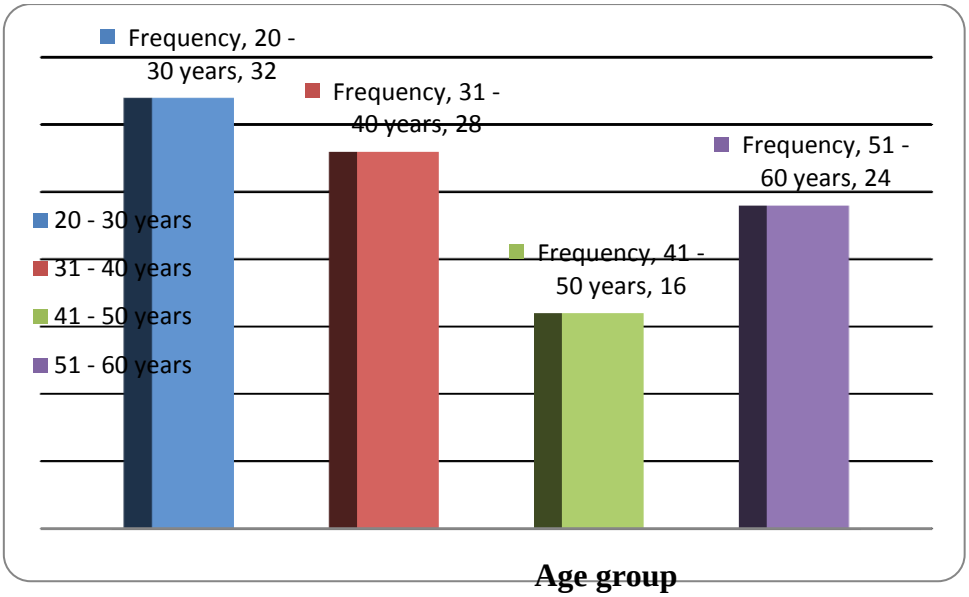

Figure 4.2: Distribution of patients according to age group

4.5 The relationship between the results of stool antigen and serum antibody detection of *H. pylori*

Table 4.1 exhibits that 48 patients were positive for antibody of *H. pylori* while 28 were positive for stool antigen.

These findings showed significant variation between the results of stool antigen and serum antibody ($p < 0.05$).

Table 4.1: The relationship between the results of stool antigen and serum antibody of *H. pylori*

The result	ICT		P. value
	Antigen	Antibody	
Positive	28 (28%)	48 (48%)	0.000
Negative	72 (72%)	52 (52%)	
Total	100 (100%)	100 (100%)	

4.6 The relationship between the results of serum antibody and stool antigen detection of *H. pylori* and blood group

Table 4.2 reveals that 32 were (O) positive show positive results for serum antibody of *H. pylori* and 24 positive for stool antigen, (B) positive blood group patients were all positive for serum antibody, and four were positive for stool antigen. Four (A) positive blood group patients show positive results using serum antibody.

These results showed significant variation between serum antibody and stool antigen results and blood group ($P < 0.05$).

Table 4.2: The relationship between the results of serum antibody and stool antigen of *H. pylori* and blood group

Blood group	Antibody		Antigen	
	+ve	-ve	+ve	-ve
O positive	32 (66%)	36 (68%)	24 (86%)	44 (61%)
B positive	12 (25%)	0 (0%)	4 (14%)	8 (11%)
A negative	0 (0%)	4 (8%)	0 (0%)	4 (6%)
A positive	4 (9%)	8 (16%)	0 (0%)	12 (16%)
AB positive	0 (0%)	4 (8%)	0 (0%)	4 (6%)
Total	48 (48%)	52 (52%)	28 (28%)	72 (72%)
P. value	0.000		0.045	

4.7 The relationship between the results of serum antibody and stool antigen detection of *H. pylori* and smoking

According to smoking 12 smokers show positive results for *H. pylori* antibody and antigen while 36 non-smokers also show positive results for *H. pylori* antibody and 16 non-smokers were positive by stool antigen.

These findings showed significant variation between serum antibody and stool antigen of *H. pylori* and smoking ($P < 0.05$) (table 4.3).

Table 4.3: The relationship between the results of serum antibody and stool antigen of *H. pylori* and smoking

Smoking	Antibody		Antigen	
	+ve	-ve	+ve	-ve
Yes	12 (25%)	0	12 (43%)	0
No	36 (75%)	52 (100%)	16 (57%)	72 (100%)
Total	48 (48%)	52 (52%)	28 (28%)	72 (100%)
P. value	0.000		0.000	

4.8 The relationship between the results of serum antibody and stool antigen detection of *H. pylori* and age group

Table (4.5) shows that the higher positive result of serum antibody of *H. pylori* was among age group 20 – 30 years 20 patients (62.5%), followed by 12 patients in age group 31 – 40 and 41 – 50 years each. For stool antigen of *H. pylori* positive results was distributed equally among blood group (20 – 30 years, 31 – 40 years and 41 – 50 years) which was 8 patients for each, and 4 positive results for age group 51 – 60 years.

These findings showed significant variation between the results of serum Ab detection and age group ($P < 0.05$) and insignificant for stool antigen ($P > 0.05$).

Table 4.4: The relationship between the results of serum antibody and stool antigen detection of *H. pylori* and age group

Age group (years)	Serum Ab		Stool antigen	
	+ve	-ve	+ve	-ve
20 – 30	20 (42%)	12 (23%)	8 (28%)	24 (33%)
31 – 40	12 (25%)	16 (31%)	8 (28%)	20 (28%)
41 – 50	12 (25%)	4 (8%)	8 (28%)	8 (11%)
51 – 60	4 (8%)	20 (38%)	4 (16%)	20 (28%)
Total	48 (48%)	52 (52%)	28 (28%)	72 (72%)
P. value	0.000		0.140	

CHAPTER FIVE

DISCUSSION

5. DISCUSSION

5.1 Discussion

The current study included 100 patients with different age groups and both genders (56 males and 44 females). The results of *H. pylori* antibody detection in patients serum was 48 (48%) positive and in stool antigen detection was 28 (28%) positive. These results were higher than those reported by (Naji *et al*., 2014) in Yemen. However, they were lower than those reported in Eastern Sudan by (Abdallah *et al*., 2014) where a seroprevalence of 65.8% was reported. These differences might be due to differences in ethnic background and age groups of the target populations.The significant association between blood group phenotypes and the results of *H. pylori* antibody and antigen ($P < 0.05$) reinforce the results of previous studies in Figueria and Kurdistan reported by (Jaff, 2011) and (Macrolopez *et al*., 2013) respectively. Findings of the current study support the epidemiological view of greater susceptibility of blood group (O) to *H. pylori* infection in which H antigen represents an important receptor for *H. pylori* adherence (Dickey *et al*., 1993).

Smoking was assessed as a risk factor. The positivity of *H. pylori* was 12 (42.9%) for stool antigen detection and 12 (25%) for serum antibody detection. Significant variation has been shown between the two methods and smoking ($P < 0.05$). These results were similar to those reported by (Kanby *et al*., 2005), and different from those in Japan which were reported by (Ogihara *et al*., 2000) in Japan which showed that smoking has negative association with *H. pylori* infection. These variations could be attributed to the fact that the previous investigator used fairly large sample size (8837) patients.

According to age groups, 20 (62.5%) patients showed positive results for antibody detection method in age group 20 – 30 years where as 8 (25%)

showed positive results for stool antigen, this age group was the highest positive group in this study. The study showed that *H. pylori* positivity decreased with age. These findings were in total disagreement with the results of (Kanby *et al*., 2005). Differences could be due to the random selection of patients regardless to their age in this study.

5.2 Conclusions

- The study showed that the serofrequancy of *H. pylori* was highly using serum antibody technique and low using stool antigen detection method.
- Findings of positive results in both serum antibody and stool antigen method were fairly highly among (O) positive blood group individuals.
- Smoking was shown to be an important risk factor for *Helicobacter pylori* colonization.

5.3 Recommendations

- Future studies should include larger sample size and cover wider area.
- Future studies should include other techniques including invasive techniques as well as Urea Breath Test.
- Future studies might involve culture and sensitivity before and after treatment to confirm the eradication of the bacteria.
- Molecular techniques such as PCR might be included to determine the specific blood group gene responsible for increasing susceptibility to *H. pylori* infection.

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APPENDICES

Sudan University of Science and Technology

College of Graduate Studies

Questionnaire

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1) Age: …….

2) Gender: Male ☐ Female ☐

3) Smoking: Yes ☐ No ☐

4) Blood group:

		Rhesus factor	
		Positive	**Negative**
ABO group	A		
	B		
	O		
	BO		

5) Serum Ab result: Positive ☐ Negative ☐

6) Stool Ag result: Positive ☐ Negative ☐