Sudan University for science and Technology

Collage of Graduate Studies

Detection of *Helicobacter Pylori* Immune Globulin G antibody Using Immune-chromatography Test in Asthmatic Patients in Khartoum State

الكشف عن الغلبيولينات المناعية (ج) للبكتيريا الحلزونية البوابية بإستخدام إختبار المناعة الكروماتوغرافي لدى مرضى الأزمة في ولاية الخرطوم

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

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الآية

قال تعالى:

بسم الله الرحمن الرحيم

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا
أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا)

سورة الإسراء – الآية (٨٥)

DEDICATION

To my

Dear parents Kawther and Ali, who were candles, lit me on my way

…

To my dear husband who supported me and to my lovely mother in law, who greatly helped me

…

To my beloved children and to my sweet brothers and sisters who are the joy of my life

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First of all, thanks to ALMIGHTY ALLAH who has been always helping me.

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ABSTRACT

The main objective of this study was to find out the prevalence of IgG antibodies of the bacteria *Helicobacter pylori* in patients who suffer from an asthma disease and compare them with those who do not suffer from it. And who were reviewing the various hospitals and clinics in Khartoum State in the period from April to December 2013.

This descriptive study included 86 patients of both sexes. Their age range 2-80 years.

Serum samples were collected from the study participants and were tested to detect IgG antibodies to the bacteria *H. pylori* using immune chromatographic test (ICT).

Of the total 43 asthmatic patients, 30 (70%) gave a positive result. Similarly, of the 43 non- asthmatic 30 (70%) were positive. The Results indicated there are no difference in the prevalence of antibodies to the bacteria *H. pylori* in asthmatic and non- asthmatic. It is concluded that insignificant association between *H. pylori* and asthma disease.

Volunteer range in 21-40 years and 41-60 years revealed 26 and 23 respectively. These between 2-20 years and 61-80 reported low *H. pylori* positive.

Males showed 26 positive while females reported 34 *H. pylori* positive.

ملخص الأطروحة

الهدف الرئيسي من هذه الدراسة هو معرفة نسبة انتشار الأجسام المضاده لبكتريا جرثومة المعده الحلزونيه في المرضى الذين يعانون من مرض الأزمة ومقارنتهم مع الذين لا يعانون منها والذين يراجعون المستشفيات والعيادات المختلفة في مدينة الخرطوم في الفترة من ابريل الي ديسمبر ٢٠١٣.

شملت هذه الدراسة الوصفية 86 مريض من كلا الجنسين وتراوحت أعمارهم بين 2-80 سنه.

تم جمع المصل من المشاركين بالدراسة وتم اختبارها لمعرفة احتوائها على الغلبيونات المناعيه (ج) المضادة لبكتيريا جرثومة المعدة باستخدام اختبار المناعة الكروماتوغرافي.

من مجموع 43 مريضا يعانون الأزمة 30أعطوا نتيجة ايجابية70% وكذلك من مجموع 43 مريضا لا يعانون الأزمة30 أعطوا نتيجة ايجابية% 70 للاجسام المضادة لبكتيريا جرثومة المعدةالحلزونيه.

أشارت هذه النتائج ان معدل انتشار الاجسام المضادة لبكتيريا جرثومة المعدة الحلزونيه في مرضى الأزمة يساوي نسبة وجودها للذين لا يعانون منها بمعنى أن جرثومة المعدة ليس لها علاقة بمرض الأزمة.

أعلى معدل انتشار لها في الاعمار مابين 21-40 سنة و 41-80 سنة 23,26 على التوالي .

العينات الموجبه في الذكور كانت 23 وفي الاناث 34.

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ABBREVIATIONS

CagA	Cytotoxin-Associated Gene A
CLO	*Compylobacter*-Like Organsim
CSO	Consensus Statement OnLine
ELISA	Enzyma-Linked Immunosorbent Assay
IRAC	International Agency for Research on Cancer
IgG	Immunoglobulin G
MALT	Mucose-Associated Lymphoid Tissue
NSAIDs	Non-steroidal Anti-infammatoryDurgs
PCR	Polymerase Chain Reaction
PG	Pepsinogen
PPIs	Proton Pump inhibtors
SPSS	Statistical Package for Social Science
UBT	Urea Breath Test
USA	United Stats of America
VacA	Vacillating Cytotoin A
DNA	Deoxyribo Nucleic Acid
ICT	Immune Chromatographic Test

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1. Introduction

Asthma is a common chronic inflammatory disease of the airways, characterized with symptoms of wheezing and shortness of breath (Finiasz *et al*., 2012). The etiology of asthma remains largely unclear. Smoking and environmental factors as well as genetic factors are thought to be its risk factors (Burke *et al*., 2012).

Previously, respiratory infections by microbes such as bacteria and viruses might play complex roles in the development of asthma, either triggering asthma symptom or reducing the incidence of asthma (Papadopoulos and Konstantinou, 2007: Liu, 2002) *Helicobacter pylori* (*H. pylori*) , a helical shaped Gram-negative bacterium , has been shown reported to associate with gastric cancer risk (Sibony and Jones.,2012).

Approximately two thirds of world population are infected with *H. pylori*, any age can get infection and women are affected just as often as men .*H. pylori* is more prevalent among the elderly and more frequent in males than female (Yamaoka,2008) .The role for its infection in the disorders of respiratory system has been addressed for several years. *H. pylori* infection might have a role in the development of chronic bronchitis, bronchiectasis , lung cancer and tuberculosis (Zhuo *et al.*,2009) .However , the roles of *H. pylori* infection in the development of asthma remain controversial (Kanbay *et al*., 2006).

In general, asthma is believed to be caused by exaggerated immunologic responses to antigens in the environment, which are driven by a Th2-mediated immune response. The exogenous infection and microbial substances including *H. pylori* infection may elicit a Th1-mediated immune

response, which suppresses Th2 responses. The lack of adequate stimulation of the Th1 might result in an overactive Th2, which in turn lead to asthma (D'Eliosand Bernard., 2010). Moreover , the acquisition of *H. pylori* may be of importance the induction of regulatory T cells, which could effectively reduce the possibility of allergic asthma (Arnold *et al*.,2011, Strickland *et al*.,2011) Thus , one the *H. pylori* factors , the neutrophil-activating factor of *H. pylori* (HP- NAP)which might drive Th1 polarization and display a powerful inhibition of allergic Th2 response , is used as a potential antigen for treatment of asthma (D'Elios and Bernard., 2010: Amedei *et al*., 2010).

Specific immunoglobulin M (IgM) antibody can be detected shortly after has infection by *H. pylori*, but IgA and IgG antibody titers indicate chronic infection.

1.2 **Rationale**

H. pylori are human pathogen that causes several illnesses and it is the commonest bacterial infection worldwide. The annual incidence rate of it is 4-15% in developing countries (including Sudan) compared with 0.5% in industrial countries (according to Center for Disease Control and Prevention CDCP).

Asthma is a common chronic medical condition that affects both children and adults worldwide. Its prevalence is increasing among all ages, sexes and race (Finiasz *et al*., 2012). Asthma has a significant impact on an individual's quality of life, and places a huge economic burden on society due to missed work days and hospital admissions. From 2001-2009 the prevalence of asthma increased from 7.3% to 8.2%, a 12.3% increase (Finiasz *et al*., 2012). It has observed that asthma is can in patient with *H. pylori* on the other hand are of *H. pylori* may lead to improve of asthma.

1.3 Objectives

1.3.1 General Objective

To determine the prevalence of *H. pylori* IgG antibody in patients with asthma at Khartoum State.

1.3.2 Specific Objective

1- To determine the association between *H. pylori* and asthma.
2- To determine the possible risk factors including age and gender associated with *H. pylori* infection among asthmatic patients.

CHAPTER TWO

LITERATURE REVIEW

2. Literature review

2.1. History

H. pylori was identified in 1982 by Barry Marshall and Robin Warren, who found that it was present in patients with chronic gastritis and gastric ulcers, conditions that were not previously believed to have a microbial cause. It is also linked to the development of duodenal ulcers and stomach cancer (Blaser, 2006). However, over 80% of individuals infected with the bacterium are asymptomatic and it has been postulated that it may play an important role in the natural stomach ecology (Blaser, 2006). In 1893, the Italian researcher GiulioBizzozero described helical shaped bacteria living in the acidic environment of the stomach of dogs (Bizzozero, 1893).

Professor Walery Jaworski of the Jagiellonian University in Krakow investigated sediments of gastric washings obtained from humans in 1899. Among some rod-like bacteria, he also found bacteria with a characteristic helical shape, which he called *Vibrio rugula.* He was the first to suggest a possible role of this organism in the pathogenncity of gastric diseases. This work was included in the "Handbook of Gastric Diseases" but it did not have much impact as it was written in Polish (Konturek, 2003). Warren and Marshall contended that most stomach ulcers and gastritis were caused by infection with this bacterium and not by stress or spicy food as had been assumed before (Marshall and, Warren, 1984).

The medical community was slow to recognize the role of this bacterium in stomach ulcers and gastritis, believing that no microorganism could survive for long in the acidic environment of the stomach.

The community began to come around after further studies that were done, including one in which Marshall drank a Petri dish of *H. pylori,* developed gastritis, and the bacteria were recovered from his stomach lining, thereby

satisfying three out of the four of Koch's Postulates. The fourth was satisfied after a second endoscopy ten days after inoculation revealed signs of gastritis and the presence of *H. pylori.* Marshall was then able to treat himself using a fourteen day dual therapy with bismuth salts and metronidazole. Marshall and Warren went on to show that antibiotics are effective in the treatment of many cases of gastritis. In 1994, the National Institutes of Health (USA) published an opinion stating that most recurrent gastric ulcers were caused by *H. pylori,* and recommended that antibiotics be included in the treatment regimen (CSO, 2004).

2.2. Taxonomy and Classification

The presence of spiral-shaped bacteria on human gastric mucosa was first recognized nearly one hundred years ago (Pel, 1913). These bacteria were originally named *Campylobacter pylori (C. pylori)* (Warren, 1983). In 1989, a new genus, *Helicobacter,* was proposed, and *C. pylori* were renamed *Helicobacter pylori* (Goodwin *et al,*1989).

Recently the genus *Helicobacter* has been included with the genus *Wolinella*in the family *Helicobacteraceae,* which with the family *Campylobacteraceae,* constitutes the *Epsilonproteobactenia*. According to the usual site of colonization, *Helicobacter* species can be divided into gastric and enteric or enterohepatic *Helicobacter* types. (Goodwin *et al,* 1989)

2.3. Structure

H. pylori are a spiral or slightly curved Gram negative rod with 2—6 characteristic unipolar flagella. The bacterium has bluntly rounded ends and measures 2.5—4.0 µ.m in length and 0.5—1.0 µ.m in width. The cell wall is smooth and may be coated with a prominent glycocalyx with a thickness of up to 40 nm (Goodwin *et al,* 1989).It is covered with ring like subunits with a diameter of 12—15 nm. Occasionally, the bacterium may contain bacteriophages. The

flagella measure 2.5 µ.m in length and around 30 nm in thickness, and have a distinctive terminal bulb. (Goodwin and Worsley, 1993).

2.4. Transmission and Epidemiology

2.4.1. Transmission

2.4.1.1. Person to Person Route

Person to person contact is believed to be the primary route of transmission in developed countries, and is also important in developing countries (Escobar and Kawakami, 2004). Close personal contact, particularly within the family including parents to child, sibling to sibling and spouse to spouse, has been consistently demonstrated as a risk factor for transmission of infection (Escobar and Kawakami, 2004). Brenner *et al* determined current *H. pylori* infection in 670 spousal pairs by 13 C-Urea breath test and monoclonal antigen immunoassay for *H. pylori* in stool. The prevalence of infection was significantly greater in women with infected partners, compared to women whose partner was not infected (34.9% vs14.5%) (Brenner *et al*, 2006). Person to person transmission can occur in several ways (Parsonnet. *et al*, 1999).

2.4.1.2. Oral-Oral Route

H. pylori DNA has been detected in the saliva of *H pylori* positive subjects by PCR (Madinrer *et al*, 1997). *H. pylori* organisms have also been successfully detected from the dental plaque of infected persons (Nguyen *et al*, 1993).

2.4.1.3. Faecal-OraI Route

H. pylori has been detected in faeces by culture and it's DNA by PCR (Namavar *et al*, 1995*).*

2.4.1.4. Waterborne Transmission

Studies in the People's Republic of China and in Latin America found that the source of water used for consumption, bathing or swimming could possibly be associated with *H. pylori* infection (Goodman *et al,* 1996).

2.4.1.5. latrogenic Transmission

Endoscopes used routinely in upper gastrointestinal procedures may be the source of iatrogenic infection as a result of improper disinfection between procedures (Tytgat, 1995*).*

2.4.2. Epidemiology

The prevalence of *H. pylori* shows large geographical variations. In various developing countries, more than 80% of the population is *H. pylori* positive, even at young ages (Perez-Perez *et al*, 2004). The prevalence of *H. pylori* in industrialized countries generally remains under 40% and is considerably lower in children and adolescents than in adults and elderly people (Pounder and Ng, 1995) Within geographical areas, the prevalence of *H. pylori* inversely correlates with g socioeconomic status, in particular in relation to living conditions during childhood(Malaty and Graham, 1994). In Western countries, the prevalence of this bacterium is often considerably higher among first- and second generation immigrants from the developing world (Perez- Perez *et al,* 2005).

While the prevalence of *H. pylori* infection in developing countries remains relatively constant, it is rapidly declining in the industrialized world (Genta, 2002).

2.5. Pathology and Clinical Manifestations

More than 50%of the world's population harbor *H. pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in Western countries. *H. pylori* helix shape (from which the generic name is derived) is thought to have evolved to penetrate the mucoid lining of the stomach (Yamaoka, 2008).

Colonization and long-term persistence of *H. pylori* can induce a complex immune response that can potentiate severe mucosal damage, including atrophy, intestinal metaplasia and dysplasia. This makes *H. pylori* the etiologic agent of acute and chronic gastritis, peptic ulcer disease *(*75%of gastric ulcers and 90% of duodenal ulcers), and two forms of gastric cancer (mucosa-associated lymphoid tissue lymphoma and gastric adenocarcinoma) (Ernst and Gold, 2000).

The association with the development of two forms of cancer led to the classification of *H. pylori* by the World Health Organization as the only bacterial class I carcinogen (IARC, 1994).

2.5.1. Acute and Chronic Gastritis

Colonization with *H. pylori* virtually always leads to infiltration of the gastric mucosa in both antrum and corpus with neutrophilic and mononuclear cell. This chronic active gastritis is the primary condition related to *H. pylori* colonization, and other *H. pylori-associated* disorders in particular result from this chronic inflammatory process (Perez-Perez *et al*, 2003)

2.5.1.1. Acute Gastritis

The acute phase of colonization with *H. pylori* may be associated with transient non-specific dyspeptic symptoms, such as fullness, nausea, and vomiting, and with considerable inflammation of both the proximal and distal stomach mucosa, and pangastritis. This phase is often associated with hypochlorhydria, which can last for months. It is unclear whether this initial colonization can be followed by spontaneous clearance and resolution of gastritis and, if so, how often this occurs. Follow-up studies of young children with serology or breath tests suggest that infection may spontaneously disappear in some patients in this age group. This has not been observed in adults other than under specific circumstances, such as development of atrophic gastritis (Perez-Perez *et al*, 2003).

2.5.1.2. Chronic Gastritis

When colonization does become persistent, a close correlation exists between the level of acid secretion and the distribution of gastritis. This correlation results from the counteractive effects of acid on bacterial growth versus those of bacterial growth and associated mucosal inflammation on acid secretion and regulation. This interaction is crucial in the determination of outcomes of *H. pylori* infection. In subjects with intact acid secretion, *H. pylori* in particular colonize the gastric antrum, where few acid secretory parietal cells are present. This colonization pattern is associated with an antrum predominant gastritis. Histological evaluation of gastric corpus specimens in these cases reveals limited chronic inactive inflammation and low numbers of superficially colonizing *H. pylori* bacteria.

Subjects in whom acid secretion is impaired, due to whatever mechanism, have a more even distribution of bacteria in antrum and corpus, and bacteria in the corpus are in closer contact with the mucosa, leading to a corpus predominant pangastritis (Kuipers*et al* ,1995a). The reduction in acid secretion can be due to a loss of parietal cells as a result of atrophic gastritis, but it can also occur when acid secretory capacity is intact but parietal cell function is inhibited by acid suppressive drugs, in particular, proton pump inhibitors (PPIs) (Kuipers *et al* ,1995a).

2.5.2. Peptic Ulcer Disease

Gastric or duodenal ulcers (commonly referred to as peptic ulcers) are defined as mucosal defects with a diameter of at least 0.5cm penetrating through the muscularismucosa. Both gastric and duodenal ulcer diseases are strongly related to *H. pylori.* In initial reports from all over the world in the first decade after the discovery of *H. pylori,* approximately 95% of duodenal ulcers and 85% of gastric ulcers occurred in the presence of *H. pylori* infection (Kuipers *et al*, 1995 b).

2.5.3. Ulcer Complications

Complications of ulcer disease include bleeding, perforation, and stricture formation. Bleeding is the most common complication of ulcer disease and is estimated to occur in 15 to 20% of ulcers. Approximately, 40% of patients presenting with upper gastrointestinal bleeding have a bleeding ulcer. The treatment of bleeding ulcer by endoscopic therapy can be performed by several methods, including injection of adrenalin, coagulation with a heater probe, or clipping of the bleeding vessel (Liu *et al*, 2003 :Gisbert *et al*, 2004).

2.5.4. Gastric Cancer

Evidence that *H. pylori* increase the risk of gastric cancer development via the sequence of atrophy and metaplasia originates from various studies, in which it was shown that *H. pylori* positive subjects develop these conditions more often than do uninfected controls (Kuipers, 1998).

The risk of development of atrophy and cancer in the presence of *H. pylori* is again related to host and bacterial factors, which influence the severity of the chronic inflammatory response. As such, the risk is increased in subjects colonized with cagA positive strains (Parsonnet *et al*, 1997), but also in those with a genetic predisposition to higher IL-1 production in response to colonization (El-Omar *et al*, 2000)

2. Pathogenesis and Virulence factors of *H. pylori*

2.6.1. Pathogenesis of *H. pylori*

To colonize the stomach, *H. pylori* must survive the acidic pH of the lumen and use its flagella to burrow into the mucus to reach its niche, close to the stomach's epithelial cell layer (Amieva and El Omer, 2008). Many bacteria can be found deep in the mucus, which is continuously secreted by mucus-secreting cells and removed on the luminal side. To avoid being carried into the lumen, *H. pylori* senses the pH gradient within the mucus layer by chemotaxis and swims away from the acidic contents of the lumen towards the more neutral pH environment of the epithelial cell surface (Schreiber *et al*, 2004).

H. pylori are also found on the inner surface of the stomach epithelial cells and occasionally inside epithelial cells .It produces adhesins which bind to membrane-associated lipids and carbohydrates and help it adhere to epithelial cells. For example, the adhesion BabA binds to the Lewis b antigen displayed on the surface of stomach epithelial cells (Ilver *et al,* 1998).

H. pylori produce large amounts of the enzyme urease, molecules of which are localized inside and outside of the bacterium. Urease breaks down urea (which is normally secreted into the stomach) to carbon dioxide and ammonia. The ammonia is converted to ammonium by accepting a proton (H), which neutralizes gastric acid. The survival of *H. pylori* in the acidic stomach is dependent on urease. The ammonia produced is toxic to the epithelial cells, and, along with the other products of *H. pylori* including proteases, vacuolating cytotoxin A (VacA), and certain phospholipases, damages those cells (Smoot , 1997).

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

pathogenicity island, which also encodes a secretion system capable of injecting bacterial macromolecules, including CagA, into host cells. The injecting CagA protein is phosphorylated by a host kinase and subsequently interacts with various signal transducation pathways to affect epithelial cell morphology and behavior. An anti apoptotic effect may aid bacterial persistence on the gastric epithelium (Ketley, 2007).

2.6.2. Virulence Factors *of H. pylori*

2.6.2.1. Cytotoxin-Associated Gene A (CagA)

The CagA protein is a highly immunogenic protein encoded by the *CagA*gene, this gene is present in approximately 50 to 70% of *H. pylori* strains (Ching *et al*, 1996).

2.6.2.2. Vacuolating Cytotoxin A (VacA)

Approximately *50%* of all *H. pylori* strains secrete VacA, a highly immunogenic 95-kDa protein that induces massive vacuolization in epithelial cells *in vitro* (Cover and Blaser, 1992). Although VacA is not essential for *in vitro* growth of *H. pylori,* it was reported to significantly contribute to murine gastric colonization by *H. pylori* (Salama *et al*, 2001).

2.7. Laboratory diagnosis

2.7.1. Methods of Diagnosis of *H .pylori* Infection

There are two general ways in which diagnosis of *H .pylori* infection can be made by using either an invasive and non-invasive procedures. The invasive procedures involve an endoscopy and biopsy. A biopsy is essential because the mucosa may appear macroscopically normal but nevertheless be inflamed.

The non- invasive tests include serological, urea breath technique and detection of *H. pylori* antigen in stool (Vaira *et al*, 1999).

2.7.1.1. Invasive Testing Through Endoscopy

2.7.1.1.1. Biopsies and Histopathology

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

2.7.1.1.2. Rapid Urease Testing of Biopsy Tissues

Urease testing provides indirect identification of *H. pylori* infection within a few hours of endoscopy (Elitsur and Neace, 1999) .The test is similar to urease test by using urea agar in which the ureas enzyme produced by *H. pylori* hydrolyses urea to produce CO_2 and ammonia. The release of ammonia alters pH to alkaline which is detected by color change of indicator. Rapid urease test, pyloritek read at 1 hr (Dohil *et al*, 1999).

2.7.1.1.3. Bacterial Culture

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

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

Several selective and differential media have been proposed and the media are usually based upon either Columbia or Brain Heart infusion agar base which

contains either blood or blood products or additive such as starch or charcoal. Sensitivity testing of *H. pylori* is important to give effectiveness of treatment and resistance of drugs (Dohil *et al*, 1999).

2.7.1.1.4. Polymerase Chain Reaction

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

2.7.2. Noninvasive Tests

2.7.2.1. Immunoassay Tests to Detect *H. pylori* Antibodies

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

In general, the accuracy of serum based immunoassays and whole blood tests for use in the physician's office in symptomatic children in developed countries is poor, with a range of sensitivity of only 60% to 70% (Khanna *et al*, 1998: De Oliveira *et al*, 1999). Furthermore, age related cutoff values for commercial immunologic tests have not been established for children. One immunoassay developed in a research center to detect *H. pylori* specific immunoglobulin (IgG)

in children was 91% sensitive compared with sensitivity of less than 70% in three commercially available assays (Khanna *et al,* 1998*).*

2.7.2.2. Saliva and Urine Tests for *H. pylori* Antibodies

Similar to serologic tests, saliva based tests also detect the presence of *H. pylori* specific IgG antibodies. The tests are easy to perform, painless, and inexpensive. Saliva tests are less sensitive than assays of serum or whole blood (Fallone *et al,* 1996). The protein concentration of saliva appears to affect the accuracy of test results. Urine-based assays are easy to perform, require minimal labor for collection, and are painless (Almohammad *et al,* 1993). However, these assays are highly variable and are not yet commercially available. Therefore, saliva and urine assays for the detection of *H. pylori* antibodies cannot be recommended.

2.7. 2.3. Stool Test for *H. pylori* Antigens

Testing of *H. pylori* antigens in stools has shown promising results in adults for the noninvasive diagnosis of gastric infection using a commercially available kit (Vaira *et al,*1999). Testing for *H. pylori* antigens in feces also appears to be accurate for use in. monitoring the success of eradication therapy. However, patients may be reluctant to collect stool specimens. In addition, refrigerated stools are more difficult to test. Additional pediatric studies evaluating the accuracy of stool antigen testing for both initial diagnosis and post treatment follow-up are required before specific recommendations can be considered (Oderda *et al,* 2000).

2.7.2.4. Urea Breath Testing

Urea breath tests are non-invasive and have high sensitivity and specificity (>95%) both in adults (Cutler, *et al,* 1995) and children (Bode *et al,* 1998). The test requires the ingestion of either radio labeled 14C-urea or urea tagged with the stable isotope 13C. Test results may be influenced by concurrent use of antibiotics and acid-suppressing medications and by the presence of other urease-producing

organisms present in the oral cavity. Test parameters are currently laboratory-specific (e.g., dosages for differing ages of children, cutoff values, duration of fasting, use of a test meal, times of sampling, and timing of post therapy testing) and have not been well standardized for children (Jones *et al* ,1997). In addition, urea breath testing is technically more difficult to perform in small children and infants, with failure rates in collection up to 10%, especially outside the clinical research setting (Rowland *et al* 1997).

2.8. Treatment

Once *H. pylori* are detected in a person with a peptic ulcer, the normal procedure is to eradicate it and allow the ulcer to heal. The standard first-line therapy is a one week "triple therapy" consisting of proton pump inhibitors such as omeprazole and the antibiotics clarithromycin and amoxicillin (Olczak *et al*, 2002). Variations of the triple therapy have been developed over the years, such as using a different proton pump inhibitor, as with pantoprazole or rabeprazole, or replacing amoxicillin with metronidazole for people who are allergic to penicillin (Suerbaum and Michetti, 2002). Such a therapy has revolutionized the treatment of peptic ulcers and has made a cure to the disease possible; prcviously, the only option was symptom control using antacids, H_2-antagonists or proton pump inhibitors alone (Shiotani and Graham, 2002).

2.9. Prevention

H. pylori are a major cause of certain diseases of the upper gastrointestinal tract. Rising antibiotic resistance increases the need to search for new therapeutic strategies; this might include prevention in form of vaccination (Josenhans *et al*, 2000). Researchers are studying different adjuvants, antigens, and routes of immunization to ascertain the most appropriate system of immune protection; however, most of the research only recently moved from animal to human trials (Broutet *et al*, 2001).

Vaccines against *H. pylori* could be used as prophylactic vaccines to prevent the infection or as therapeutic vaccines to cure the infection, to improve the eradication success of standard regimens or to reduce the bacterial density in the gastric mucosa and the risk for emergence of antibiotic resistant strains.

In recent years, many attempts, using various *H. pylori* antigens such as urease, CagA, or combinations, many adjuvants and different routes of immunisation have been made to create vaccines against *H. pylori* infection. Although some attempts are promising, no effective and safe vaccine against *H. pylori* is currently available for humans.

New directions for immunization with the use of DNA, living vectors, microspheres etc. are currently under evaluation. The vaccination plan and the groups who should receive vaccination are still to be determined, but the vaccination will be useful, especially in developing countries. An intramuscular vaccine against *H. pylori* infection is undergoing Phase I clinical trials, and has shown an antibody response against the bacterium. Its clinical usefulness requires further study (Logan ad Walker, 2001). The study was done to evaluate the effect, on *H. pylori* infection, of IgY prepared from egg yolk of hens immunized with *H. pylori* urease (antiHpUIgY). Seventeen asymptomatic volunteers diagnosed as *H. pylori* positive by the 13C-urea breath test (UBT) were orally administered

antiHpUIgY for 4 weeks. Four weeks later, UBT values were significantly decreased although no case showed *H. pylori* eradication. An *H. pylori* positive 53-year-old female gastritis patient administered anti-HpUIgY plus lansoprazole for 8 weeks showed a decrease in serum pepsinogen (PG) I and UBT values as well as an increase in the PG I/Il ratio.

In conclusion, anti-HpUIgY may mitigate *H. pylori* associated gastritis and partially attenuate gastric urease activity. Furthermore, anti-HpUIgY combined with antacids appears to ameliorate gastric inflammation. These encouraging results may represent a novel approach to the management of *H. pylori* associated gastroduodenal disease (Suzuki *et al*, 2004).

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods

3.1. Study design

Descriptive cross sectional study was carried out.

3.2. Study area and duration

The study was carried out at different hospitals and clinics in Khartoum State. The study was carried out from April to December 2013.

3.3. Study Population

Samples were collected from asthmatic and non- asthmatic patients.

3.4. Sampling Technique

Samples were randomly selected based on non -probability convenience sampling technique.

3.5. Sample Size

Eighty six (n = 86) serum samples collected randomly.43 samples were taken from asthmatic patients and 43 samples were from non-asthmatic.

3.6. Inclusion Criteria

Any asthmatic patients at different age's group.

3.7. Exclusion Criteria

Non asthmatic.

3.8. Data Collection

Data were collected by using direct interviewing questionnaire. (Appendix).

3.9. Ethical Consideration

Permission of this study was obtained from College of Graduated Studies SUST and Verbal patients consent.

3.10. Data Analysis

Collected data were analyzed using the application of Statistical Package of Social Science (SPSS) and Chi – square statistical analysis.

3.11. Laboratory Work

Immune chromatography test (ICT) was used to detect anti-*H. Pylori* IgG.

3.12. Experimental Work

3.12.1. Specimen collection

Under strict sterile conditions, 5 ml of whole venous blood was collected after disinfected of skin by 70% alcohol then the blood was poured in plain container and centrifuged at 2000 rpm for 3 minutes to obtain the plasma.

3.12.2. Specimen processing

3.12.2.1. ICT intended use

H. pylori one step test device (plasma) is a rapid chromatographic immune assay for qualitative detection of antibody to *H. pylori* in plasma to aid in diagnosis of *H. pylori* infection.

3.12.2.1.1. Principle

The *H. pylori* one step test device (plasma) is qualitative membrane strip based immune assay for the detection of *H. pylori* antibody in plasma.in this test procedure, anti-human IgG is immobilized in the test line region of the device after plasma is placed in the specimen well, it react with *H. pylori* antigen coated particles in the test. This mixture migrates chromatographically along the length of test strip and interacts with the immobilized anti-human IgG. If the specimen contain *H. pylori* antibody, a colored line will appear in the test line region indicating positive. If the specimen doesn't contain *H. pylori* antibody, colored line will not appear in the region indicating a negative result. Best result will be obtained if the assay was performed within one hour.

3.12.2.1.2. Assay Procedure

1. The test device was removed from foil pouch and used as soon as possible.
2. The test device was placed on clean leveled surface. The dropper was held vertically and three drops of plasma were transferred (approximately 100μL) to the specimen well of the test device and started the timer, air bubbles were avoided in the specimen well.
3. The results were read within 10 minutes.
4. The positive results showed double lines one for control and the other for sample. (Image 2).
5. The negative results only control line will appear. (Image 1).

CHAPTER FOUR

RESULTS

4. Results

4.1 Frequency of *H. pylori* antibody in asthmatic patient and non-asthmatic

Total of 43 asthmatic patients examined, 30 were found positive (70%) for *H. Pylori* IgG antibody and the same result were found in non asthmatic 30(70%).Table and Figure4.1

4.2 The effect of age in detection of *H. Pylori*

Twenty six (30.2%) positive case were detected in age group range between 21-40 years, while 23 (27%) *H. pylori* positive was detected in age group range 41-60 years. Five (5.8%) positive cases were seen in age group range 2-20 years and 6 (6.9%) positive were detected in age group range 61-80 years. Table and figure 4.2 Distribution of *H. pylori* in asthmatic and non-asthmatic according to age group shown in table 4.4.

4.3The effect of gender in detection of *H. pylori*

The results in table 4.3 and figure 4.3 revealed that 26males out of 60 positive patients (43.4%). 34 females out of 60 positive patients (56.6%) were shown positive for *H. pylori* IgG antibody. Distribution of *H. pylori* in asthmatic and non-asthmatic according to gender shown in table 4.5

According to chi-square, there was insignificant association between age, gender of asthmatic patients presence of *H. pylori* IgG antibody $p=1.0>0.05$. Table 6.

Table 4.1 Prevalence of *H. pylori* in asthmatic and non-asthmatic

			Asthmatic		
			Yes	No	Total
H. pylori	Positive	Count	30	30	60
		% of Total	34.9%	34.9%	69.8%
	Negative	Count	13	13	26
		% of Total	15.1%	15.1%	30.2%
	Total	Count	43	43	86
		% of Total	50.0%	50.0%	100.0%

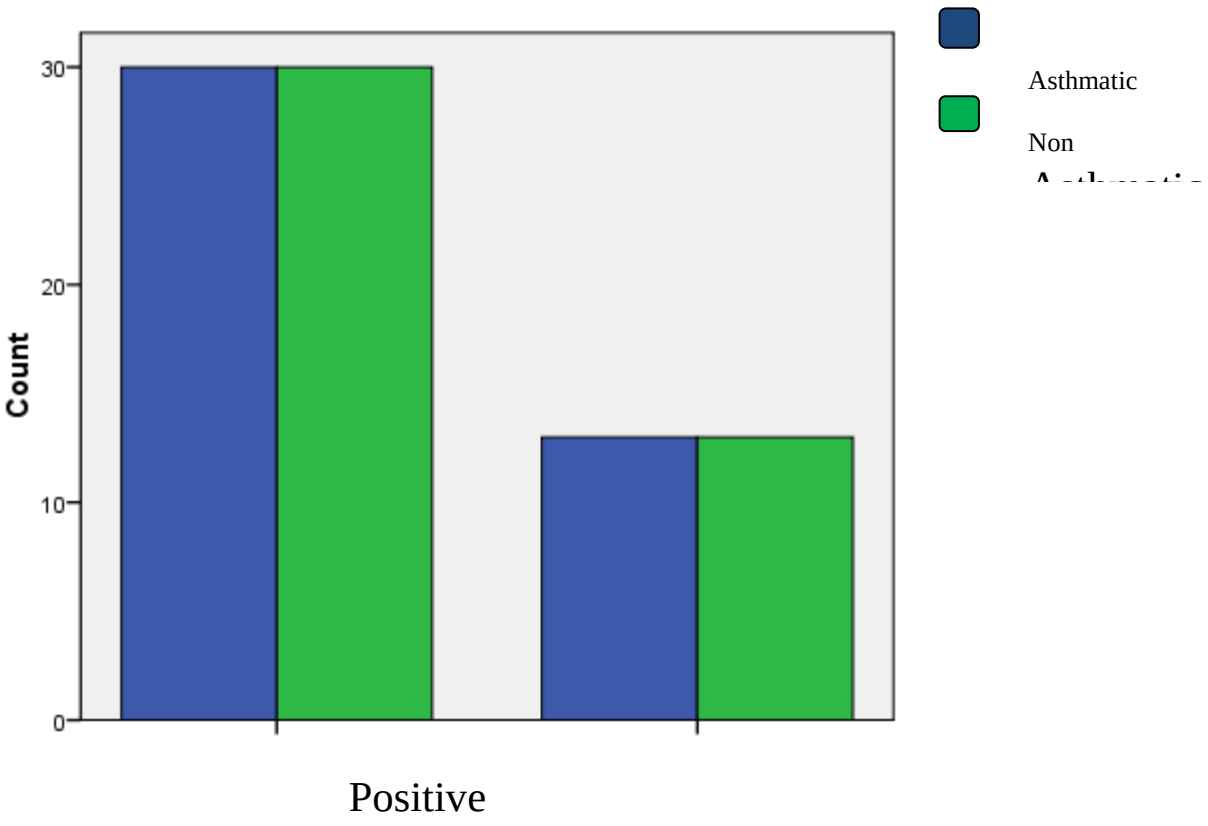

Fig (4.1) Prevalence of *H. pylori* in asthmatic and non asthmatic

Table 4.2 ***H. pylori*** **among different age groups**

Age	*H. pylori* positive	Percentage	*H. pylori* Negative	Percentage	Total
2-20	5	5.8%	4	4.6%	9
21-40	26	30.2%	17	19.7%	43
41-60	23	27%	5	5.8%	28
61-80	6	6.9%	0	0%	6
Total	60	70%	26	30%	86

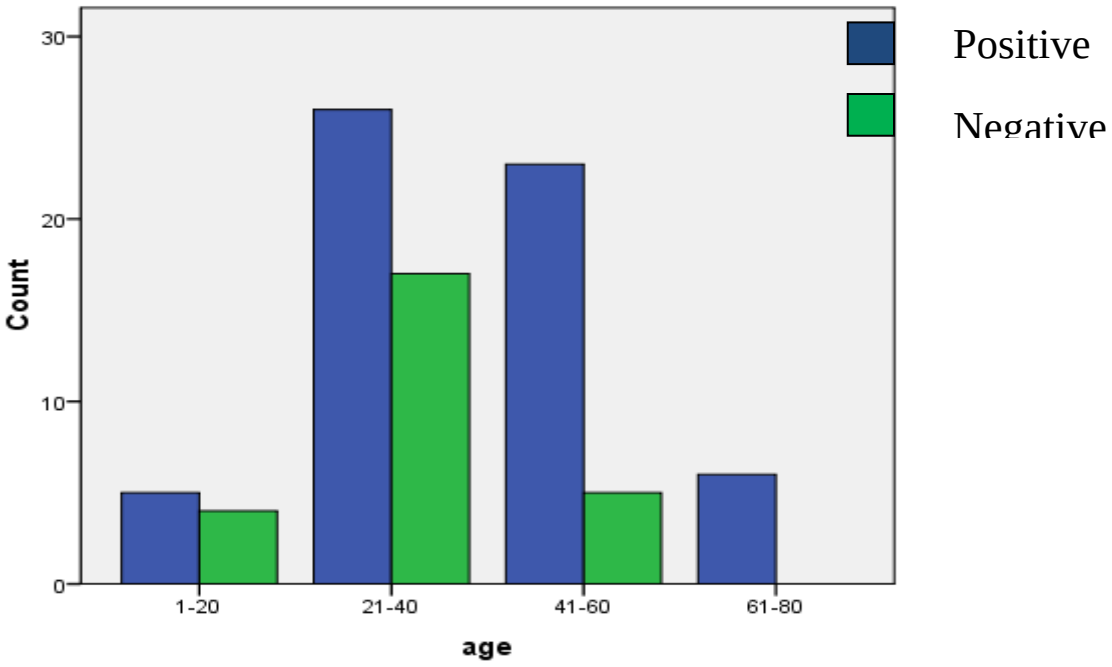

Fig 4.2 Distribution of age according to *H. pylori*.

Table 4.3 *H. pylori* in males and females

Gender	*H. pylori* positive	Percentage	*H. pylori* Negative	Percentage	Total
Male	26	43.3%	12	46.1%	38
Female	34	56.6%	14	53.8%	48
Total	60	69.7%	26	30.2%	86

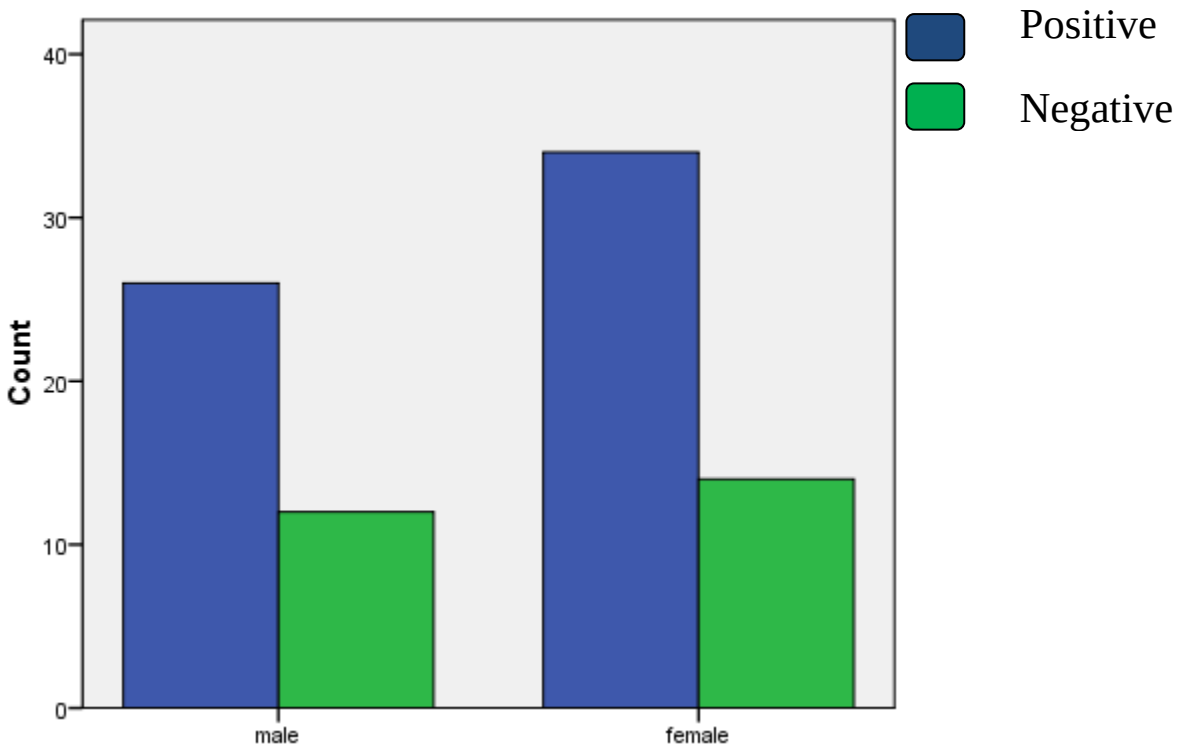

Fig 4.3 Distribution of *H. pylori* according to gender

Table 4.4 *H. pylori* in asthmatic and non- asthmatic age group

Age	asthmatic	Percent	Non asthmatic	Percent	Total
1-20	5	5.81%	4	4.65%	9
21-40	23	26.74%	20	23.26%	43
41-60	41	16.28%	14	16.28%	28
61-80	1	1.16%	5	5.81%	6
Total	43		43		86

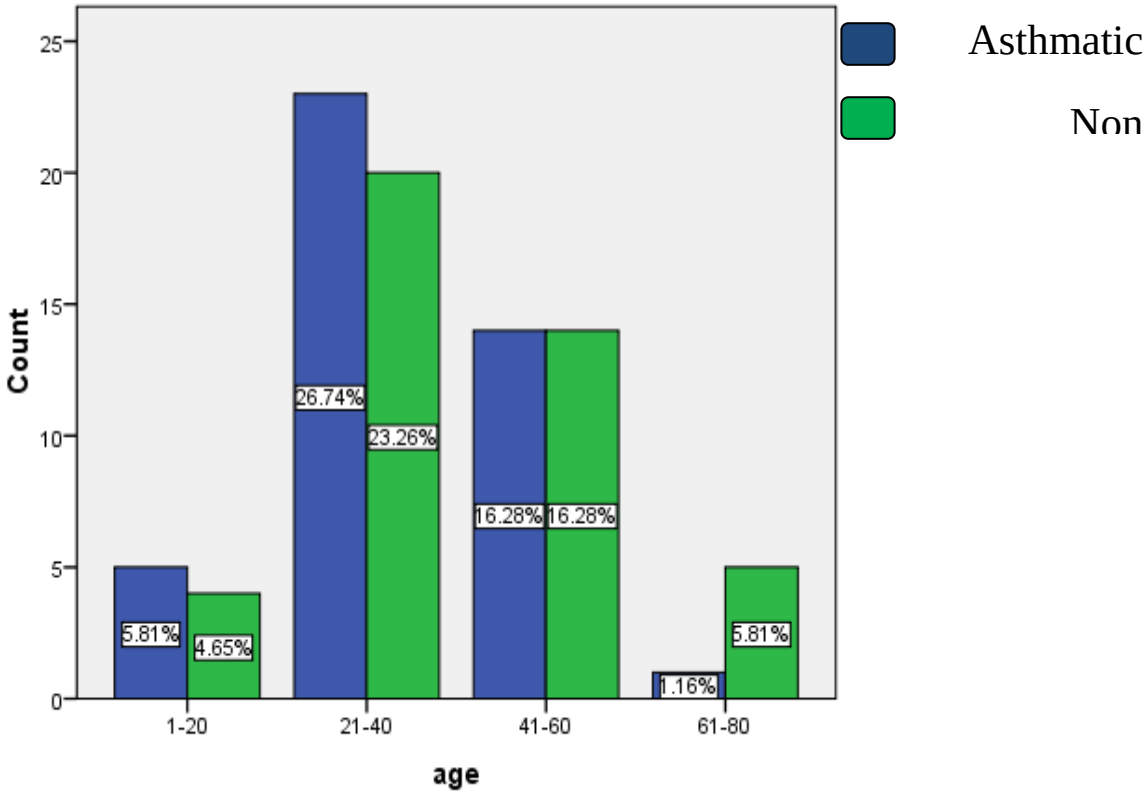

Fig 4.4 Prevalence of *H. pylori* in relation to age in asthmatic and non-asthmatic.

Table 4.5: Frequency of patient's gender.

Gender	Asthmatic	Percentage	Non Asthmatic	Percentage	Total
Male	20	23.26 %	18	20.93%	38
Female	23	26.47%	25	29.07%	48
Total	43	50%	43	50%	86

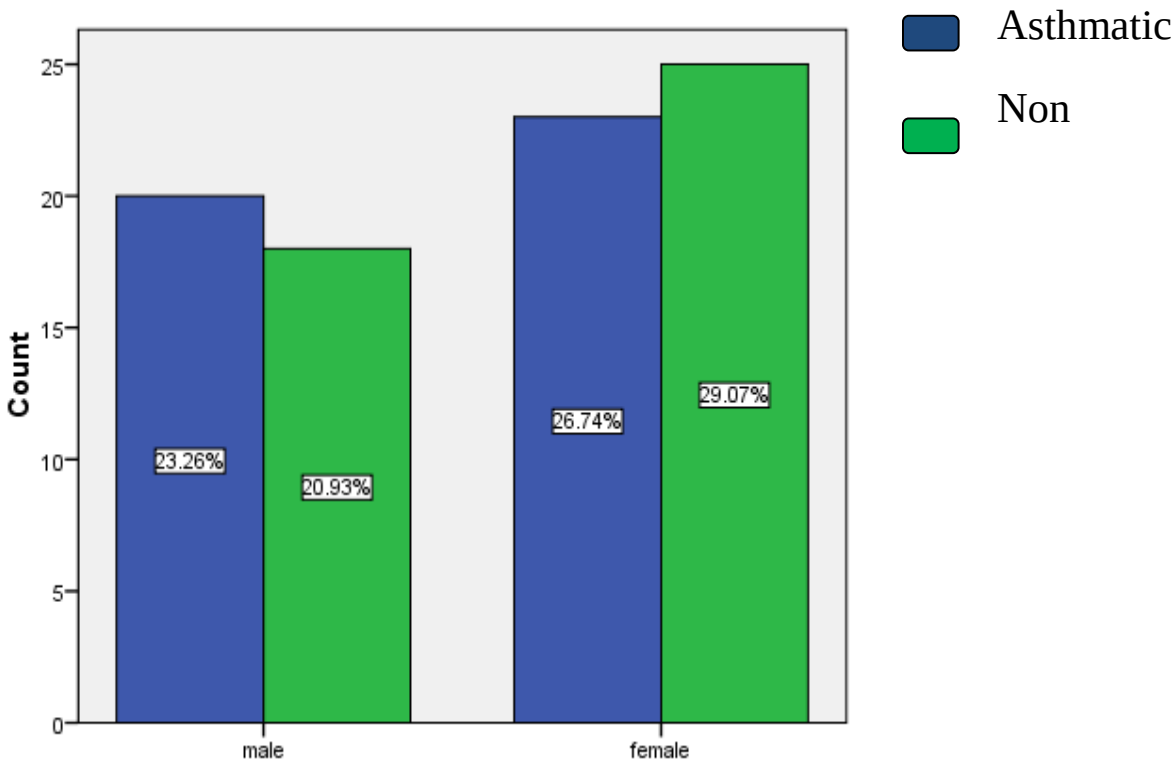

Fig 4.5 Distribution of asthmatic and non asthmatic patient according to gender.

Table (6) Chi-Square Tests

	Value	df	Asymp.Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.000[a]	1	1.000		
Continuity Correction[b]	.000	1	1.000		
Liklihood Ratio	.000	1	1.000		
Fisher's Exact Test				1.000	.593
Linear –by- Linear Association	.000	1	1.000		
N of Valid Cases[b]	86				

CHAPTER FIVE

DISCUSSION

Discussion

The prevalence of *H. pylori* shows large geographical variations in various developing countries, more than 80% population is *H. pylori* positive , even at young ages, whereas in industrialized countries remains under 40% (Perez-Perez *et al,* 2004).

Forty three patients with asthma (n=43) were randomly tested in the present study, 20 of them were males (23.2%), and 23 were females (26.4%) with mean age of 34 years. Insignificant association between *H. pylori* and asthma (*p*= 1.06).No similar studies in Sudan related to our study were found.

However, results obtained in this study were similar to those obtained by Tsang *et al,* (2000) in China, Jun *et al*, (2005) and with Alvai *et al* (2010) in Iran. The result disagree with Yan *et al*,(2012) in Japan, Zhou *et al*, (2013) in British and Eman *et al,(*2012) in Egypt . This variation of results could be attributed to ethnic differences and the small sample size used in our study and also may be due to geographic and climatic differences.

On the other hand the study observed higher percentage of infection among age groups 21-40, 41-60 years in agreement with Kabir (2007) in Sweden, this high percent may be due to the vast majority of individuals acquire this infection during childhood .the low percentage showed among age groups 2-20 , 61-80 may be due to undeveloped or weak immune system respectively.

The study showed females were more affected than males which was in agreement with that done in Egypt by Manal *et al, (*2007*)* and other study by Huang *et al*,(2004) in Malaysia . In contrast, Leandro *et al*, (2005) found that the prevalence was significantly higher in boys.

Conclusion

Statistical analysis showed insignificant association between *H. pylori* infection and asthma disease risk factors males, females and all ages were subjected to infection by this bacterial disease.(p=1.00). Females were more affected than males.

Recommendations

1- Carry out same study using advanced techniques like ELISA and PCR.
2- Initiate and establish National protocol for diagnosis of *H. pylori*.
3- Larger sample size is needed to accurately determine the prevalence.

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APPENDIX

Sudan University for science and Technology Collage of Graduate Studies

Department of Microbiology

Questionnaire

Title: Detection of *Helicobacter Pylori* Immune Globulin G antibody Using Immune-chromatography Test in Asthmatic Patient in Khartoum state

By: Hanady Ali Babeker

Super Visor: yousif Fadlalla

Date:…… Age:…….

Name:…… Index number:……..

Social Status:

single ☐ Married ☐

Residence:……..` Occupation:……..

-**Did you have asthma**:

Yes ☐ No ☐

If yes: how long time you suffering? ……..

What the causes triggering your asthma?.....

-**Have any Git problem**?

Yes ☐ No ☐

-If yes specify:

Gastritis ☐ Peptic ulcer ☐

Heartburn ☐ Pain in the upper abdominal

-**Previous diagnosis of *H. pylori* infection**:

Yes ☐ No ☐

-If yes the result was:

Positive ☐ Negative ☐