Sudan University of Science and Technology
College of Graduate Studies

Detection of *Helicobacter Pylori* By using Culture and PCR Techniques in Khartoum state

الكشف عن بكتريا الملوية البوابية باستخدام تقنيتي الإستزراع و تفاعل البلمرة المتسلسل في ولاية الخرطوم

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

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الآية

بسم الله الرحمن الرحيم

قَالَ تَعَالَىٰ:

﴿ إِنَّمَآ أَمۡرُهُۥٓ إِذَآ أَرَادَ شَيۡـًٔا أَن يَقُولَ لَهُۥ كُن فَيَكُونُ ٨٢ ﴾

صدق الله العظيم

سورة يس الآية: (82)

Dedication

The entire work is dedicated to my parents for their love, prayers, caring and sacrifices for educating and preparing us for our future, my close friend, my husband and family members.

Acknowledgements

First of all, I thank God for all the beneficent and most merciful. I am very thankful to my supervisor Dr. Kawthar Abdalgaleil Mohammed -Salih Ibrahim for her help and guidance. I feel thankful to her for advice and suggestions. Also, thanks extended to Dr. Ahmed Ibrahim Hashim. I would like to express our special thanks to staff of Endoscopy Department in Ibn Sina Hospital. In addition, special thanks to the teaching staff of Microbiology Department and to all of my colleague in Medical Laboratory Science, Sudan University of Science and Technology. I finally, our thanks go to all the people who have supported us to complete my research.

Abstract

Helicobacter pylori (*H. pylori*) is a bacterium found in human epithelial cells of the gastrointestinal tract. Its infection is related to different diseases, such as chronic gastritis, peptic ulcers, gastric lymphoma and adenocarcinoma.

The aim of this study to detect *H. pylori* from stomach biopsy and to compare the diagnostic methods of culture and polymerase chain reaction (PCR).

This is cross sectional study was conducted in Ibn Sina Hospital - Khartoum state Sudan, during the period from June 2018 to -June 2019. A total of 50 samples were collected from patients undergoing endoscopy, Biopsy samples obtained from these patients were subjected to culture on Colombia agar and PCR. The detection of gene (UreC gene) was carried out by PCR. DNA was extracted by the guanidine chloride method. Data analyzed using SPSS software program version 16.

Out of 50 biopsy specimens collected in this study 24(48%) male and 26 (52%) female, 9(18%) was found positive for *H. pylori* gene. All positive samples extracted from the biopsy samples showed target band (294bp) after PCR assay. In the other hand there was no growth of culture.

PCR is a useful method to detect the presence of *H. pylori* in the stomach tissue and more sensitive than culture in diagnosis of *H. pylori.*

المستخلص

جرثومة الملوية البوابية هي بكتريا موجودة في الخلايا الظهارية البشرية في الجهاز الهضمي، وترتبط العدوي بامراض مختلفة مثل التهاب المعدة المزمن ،القرحة الهضمية،سرطان الغدد اللمفاوية في المعدة، وسرطان الغدية.

الهدف من هذه الدراسه الكشف عن جرثومة الملوية البوابية من خزعة المعدة ومقارنة طرق التشخيص للتزريع وتفاعل البلمرة المتسلسل.

أجريت هذه الدراسة العرضية في مستشفي ابن سينا- ولاية الخرطوم –السودان في الفترة من يونيو 2018 الي يونيو 2019 . تم جمع50عينة من المرضى الذين يخضعون للتنظير،واستخدمت عينات الخزعة التي تم الحصول عليها من هؤلاء المرضى للتزريع وتفاعل البلمرة المتسلسل. تم إجراء الكشف عن الجين(UreC gene) بواسطة تفاعل البلمرة المتسلسل. تم إستخراج الحمض النووي عن طريق طريقة كلوريد القواندين. اما تحليل البيانات فقد تم باستخدام برنامج التحليل الإحصائيSPSS

من بين50عينة تم جمعها في هذه الدراسة وجد 24 (48 ٪) من الذكورو 26 (52 ٪) من الإناث و9 (18 ٪) إيجابية لجرثومة الملوية البوابية. وأظهرت جميع العينات الموجبة المستخرجة من عينات الخزعة الفرقة المستهدفة (284نقطةأساس) بعد إختبار البلمرة. من ناحية اخري لم يكن هنالك نمو للإستزراع.

تفاعل البلمرة المتسلسل هو طريقة مفيدة للكشف عن وجود جرثومة الملوية البوابية في انسجة المعدة واكثر حساسية من الإستزراع في تشخيص جرثومة المعدة.

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List of abbreviations

NH4	Ammonium
CagA	Cytotoxin‐associated gene
dATP	Deoxy nucleotide adenosine triphosphate
dCTP	Deoxy nucleotide cytosine triphosphate
dGTP	Deoxy nucleotide guanine triphosphate
DNA	Deoxy nucleic acid
dTTP	Deoxy nucleotide Thymine triphosphate
EIzA	Enzyme linked immunosorbant assay
GIT	Gastro intestinal tract
glmM	Phosphoglucosamine mutase
H.P	Helicobacter pylori
IgG	Immunoglobulin G
IL‐8	Interleukin‐8
LPS	Lipopolysaccharide
MALT	Mucosa-associated lymphoid tissue
MCHUA	Modified Columbia hemoglobin urea agar media
NSAIDs	Non-steroid anti-inflammatory drugs
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PPI	Proton pump inhibitor
P value	Probability value
SPSS	Statistical Package for Social Sciences
TSB	Tryptic soya broth
UBT	Urea breath test

UGIB	Upper gastrointestinal bleeding
ureC	Urease C gene
VacA	Vacuolating Cytotoxin A
WHO	World Health Organization

CHAPTER ONE

INTRODUCTION

Chapter one

1. Introduction

1.1Background

In 1984, Marshall and Warren reported the discovery of a bacterium, which was subsequently named *Helicobacter pylori* (Aktepe *et al*., 2011*). Helicobacter pylori* are a small, spiral, Gram negative bacillus that plays a role in the pathogenesis of a number of diseases (kouitheu Mabeku *et al*., 2017), it is a non-spore forming, microaerophilic in nature which requires 5-10% oxygen, and 5 to 10% carbon dioxide for optimum growth in culture medium, it's grow well at a temperature of 30-37°C (Wahab *et al*., 2015). *Helicobacter pylori* is a common pathogen that colonizes the gastric epithelium with a high infection prevalence, it is detected in more than 70% of cases of gastric ulcer and 90% of cases of duodenal ulcer (Kültür and Histopatolojik, 2018). It is highly links to duodenal ulcer, which was classified as a group 1carcinogen in1994 by WHO (Wang, 2014). Acute infection can yield an upper gastrointestinal illness with nausea, pain, vomiting, and fever may be present. The acute symptoms may last for less than 1 week or as long as 2 weeks. After colonization, the *H. pylori* infection persists for years and perhaps decades or evens a lifetime (Jawetz, 2016). There is also a growing awareness that chronic *H. pylori* infection may be associated with an increased risk of extra gastric disease that include host iron deficiency (hematological) (Flores *et al*., 2017), cardiovascular, neurological, metabolic, autoimmune and dermatological diseases (Lewinka and Wnuk, 2017). Multiple routes of transmission of *H. pylori* infection have been postulated, including fecal-oral, gastro-oral, and oral–oral; however, the exact route of transmission of this bacterium is still unknown (El-khier *et al*., 2015). The accurate detection of *H. pylori* is essential for the management of patients and for the eradication of the bacterium following

treatment (Aktepe *et al*., 2011), since the discovery of *H. pylori* several diagnostic methods have become available for determining the presence of *H. pylori* infection, these tests can be assessed by invasive and noninvasive method (Aktepe *et al*., 2011). Invasive (culture, histopathological examination, rapid urease test and molecular tests) (Cagdas*etal*.,2012), which requires endoscopy and are also known as biopsy-based test (Khalifehgholi *et al*., 2013) and non –invasive (urea breath test, serological tests, stool culture and stool antigen/nucleic acid tests methods) may be use (Cagdas *et al*., 2012). Culture stills play a major role in the diagnostic spectrum. It's continues to be the only test allowing for a comprehensive analysis of pathogen characteristics (Gonzalez-vazquez *et al*., 2012). The culture method has been the standard method for detecting this bacterium because it allows selection of appropriate antimicrobial therapy, but it is time consuming, expensive and difficult to perform since *H. pylori* is a fastidious growth-requiring microorganism. (Ottiwet *et al.*, 2010). The advantages of PCR are genotyping of *H. Pylori* and detecting antibiotic resistance genes and gene mutations (Rana *et al.*, 2017). PCR provides excellent sensitivity and specificity, greater than 95%, as compared with other conventional tests and has more accurate results of detecting of *H. pylori* (Wang *et al.*, 2015).

1.2 Rationale

H. pylori infect more than half of the adult population worldwide**.** It's associated with gastrointestinal disorders, ranging from gastritis to peptic ulcer diseases, gastric ulcers, mucosa-associated lymphoid tissue lymphoma and gastric cancer. *H. pylori*, being a type one carcinogen, it's the major risk factor for gastric cancer development (Lee, 2017). The most specific method to detect *H. pylori* is culture of the bacterium from clinical specimen, and it helps to study the morphology and to investigate the antimicrobial susceptibility testing. PCR provides excellent sensitivity and specificity, greater than 95% as compared with other conventional tests and has more accurate results of detecting of *H. pylori* (Wang *et al.*, 2015). In Sudan the prevalence of the *H. pylori* was estimated to be 65.8% , this study was done to detect prevalence and factors associated with *H. pylori* in Eastern Sudan (Abdallah *et al*., 2014).

1.3 Objectives

1.3.1 General objective

To detect *H. pylori* in gastric biopsy by using culture and PCR techniques in Khartoum state.

1.3.2 Specific objectives

1- To detect of *H. pylori* characteristics in gastric biopsy by culture.

2- To detect the presence of *H. pylori* gene in gastric biopsy by PCR.

3- To estimate prevalence of *H. pylori* among patients undergoing upper GIT endoscopy with upper gastrointestinal symptoms.

4- To compare between sensitivity of culture and PCR techniques

5- To detect association between age, sex, family history, past history, *H. pylori* symptoms (abdominal pain, acidity, weight loss, gastritis and ulcer) and *H. pylori* infection).

CHAPTER TWO

LITERATURE REVIEW

Chapter two

2. Literature review

2.1. History of *H. pylori*

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 (Blase, 2006). By the late 19th and early 20th centuries, several investigators had reported the presence of spiral microorganisms in the stomachs of animals. Soon afterward, similar spiral bacteria were observed in humans, some of whom had peptic ulcer disease or gastric cancer. The organism was initially named "*Campylobacter*- like organism," "gastric *Campylobacter*-like organism," "*Campylobacter pyloridis*," and "*Campylobacter pylori*" but is now named *Helicobacter pylori* in recognition of the fact that this organism is distinct from members of the genus *Campylobacte* (Kusters *et al*., 2006).

2.2. Taxonomy and classification

The genus *Helicobacter* belongs to the ε subdivision of the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae*. This family also includes the genera *Wolinella*, *Flexispira*, *Sulfurimonas*, *Thiomicrospira* and *Thiovulum* (Blase,2006). The genus *Helicobacter* contains 36 described species that have been validated at this point in time and is divided according to their major colonization sites as gastric or lower intestinal tract associated bacterial species (Rosa, 2017).

2.3 Structure

H. pylori are non-spore-forming gram-negative bacteria. The cellular morphology may be curved, spiral, or fusiform. The spiral wavelength may vary with the age, the growth conditions and the species identity of the cells. In old cultures or those exposed to air, cells may become coccoid (Alkhidir *et al.*, 2017), microaerophilic bacterium with multiple sheathed flagella at each pole of the bacterial cell. The cells are 2-4 μm long and 0.2-1.0 μm wide. It was cultivated and described for the first time in 1982 (Rosa, 2017).

2.4. Transmission

Living in poor sanitary conditions, in a crowded family and the lack of hygienic drinking water increases the risk of contamination. The main routes of infections are oral-oral, gastro-oral (through vomiting) and fecal-oral transmission (Aksit Bicak *et al.,* 2017). The fecal-oral route is much more controversial. It can be vertical (parents to children) (Mentis *et al*., 2015). Several epidemiological studies have shown that the bacterium is transmitted by persons close to the children, especially mothers (Mentis *et al*., 2015), or horizontal modes of transmission (environmental contamination) (Aksit Bicak *et al.,* 2017). Contaminated water may play a key role in the transmission of *H. pylori* (Bai *et al*., 2016).

2.5. Epidemiological characteristics

H. pylori infection is one of the commonest infections worldwide, occurring in all regions and infecting at least half of the world's population. The prevalence of *H. pylori* infection worldwide is approximately 50%, as high as 80%–90% in developing countries and ≈35%–40% in the United States. Whereas within countries, the prevalence is higher among group with lower socioeconomic status (Ahmed, *et al*., 2018). Most individuals acquire infection during childhood and infection is more common in developing countries (Afzali, 2016). In Europe, the

prevalence of infection was 84.2%, with 61.7% of strains also positive for CagA (Eusebi *et al*., 2014). While in Sudan the prevalence of infection was estimated to be 80%among patients with gastritis. However, there have been few published data considering the prevalence of *H. pylori* (Abdallah *et al*., 2014).

2.6. 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).

2.6.1. Gastritis

There are mainly two major forms of gastritis. Type A gastritis, which involves the fundus is associated with pernicious anemia and type B gastritis, for which mainly the *H. pylori* is the etiological agent (Sipponen and Maaroos, 2015)**.**

2.6.2. Acute gastritis

Acute phase of colonization with *H. pylori* may be associated with transient non-specific dyspeptic symptom, such as fullness, nausea, and vomiting, and with considerable inflammation of both the proximal and distal stomach mucosa or pangastritis (Kusters *et al*., 2006). Acute gastritis is usually a self-limiting disorder; complete regeneration and healing usually occur within several days (Tanih *et al*., 2010).

2.6.3. Chronic gastritis

Chronic gastritis is characterized by the absence of grossly visible erosions and the presence of chronic inflammatory changes leading eventually to atrophy of the glandular epithelium of the stomach. The changes may become dysplastic and

possibly transform into carcinoma. *H. pylori* and a number of factors such as chronic alcohol abuse, cigarette smoking, and chronic use of non-steroid anti-inflammatory drugs (NSAIDs) may contribute to the development of the disease (Tanih *et al*., 2010)**.** Worldwide the most common cause of chronic gastritis is infection with *H. pylori*, which causes progressive damage to the gastric mucosa and is now accepted as playing a causative role in a number of important diseases, including duodenal ulcer disease, gastric ulcer disease, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue (MALT) lymphoma. *H. pylori*– induced gastritis is considered as the most important risk factor for peptic ulcer and its complications as well as for gastric cancer (Sugano *et al.*, 2015).

2.6.4. Peptic Ulcer Disease

H. pylori is now accepted to be the infective pathogen that causes most of the gastric and duodenal ulcers. It has been documented that virtually all persons with duodenal ulcer and 70% of those with gastric ulcer have *H. pylori* infection. Duodenal ulcers occur five times more commonly than gastric ulcers. Peptic ulcer can affect one or all layers of the stomach or duodenum. It may penetrate only the mucosal surface, or it may extend into the smooth muscle layer. Occasionally, an ulcer may penetrate the outer wall of the stomach or duodenum; with spontaneous remissions and exacerbations being common (Tanih *et al*., 2010).

2.6.5. Gastric cancer

H. pylori infection is necessary but not sufficient for development of *H. pylori*-associated gastric cancer, similar in concept to hepatitis B and C viruses and human papilloma virus. The infection is required for gastric cancer to develop, but *H. pylori* infection alone is not sufficient for gastric carcinogenesis (Graham and Debakey, 2015). Colonization of the human stomach by *Helicobacter pylori* and its role in causing gastric cancer is one of the richest examples of a complex

relationship among human cells, microbes, and their environment. The observation that cell-associated *H. pylori* can colonize the gastric glands and directly affect precursor and stem cells supports these observations. (Amieva and Peek, 2016). There are several mechanisms by which *H. pylori* contributes to development of gastric cancer. Gastric adenocarcinoma is one of many cancers associated with inflammation, which is induced by *H. pylori* infection, yet the bacteria also cause genetic and epigenetic changes that lead to genetic instability in gastric epithelial cells(Graham and Debakey, 2015).

2.6.6 Gastric Malt Lymphoma

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

2.7 Pathogeneses and virulence factors of *H. pylori*

2.7.1 Pathogenesis of *H. pylori*

Pathogenesis of *H. pylori* infection is based on the long-term host to bacterial interaction and affected by the virulence factors of the bacterium, environmental and host factors (age, sex, blood type). Mucosal inflammation is the basic principle mechanism underlying the disease development in which tissue destruction may be initiated and maintained by both the bacterial toxins (CagA, VacA, LPS) and immune responses by the host (Lee, 2017). Immune evasion with bacterial modulation of host response affects the long-term host colonization, which is also

affected by urease and/or motility of the bacterium, presence of lipopolysaccharide and various bacterial enzymes (Lee, 2017).

2.7.2. virulence factors of *H. pylori*

2.7.2.1. Cytotoxin‑associated gene A (CagA)

The cytotoxic-associated gene A (cag A) is present in almost 50% of *H. pylori* strains and is constituent of genomic pathogenicity island (cag-PAI) responsible for type IV secretion system (Bibi *et al*., 2017). The cag A-positive strains of *H. pylori* are more interactive with the host (Alkhidir *et al*., 2017). They responsible for mucosal inflammation and interleukin-8 (IL-8) production and are associated with pathogenesis of gastric cancer (Bibi *et al*., 2017).

2.7.2.2. Vacuolating Cytotoxin A (VacA)

One important virulence factor is the vacuolating cytotoxin, VacA, a secreted pore-forming toxin that causes epithelial cell vacuolation (Aviles-Jimenez *et al*., 2004). All identified *H. pylori* strains possess the vacA gene which codifies for the VacA toxin; this toxin has a vast array of functions that span induction of apoptosis to modulation of the immune system (Medina *et al*., 2017). After secretion from the bacteria via a type V auto transport secretion system, the 88 KDa Vac A toxin bind to host cell and is internalized (Palframan *et al*., 2012), that induce vaculation and multiple cellular activities, including membrane channel formation, cytochrome c release from mitochondria which lead to apoptosis, and binding to cell membrane receptors followed by the initiation of a pro-inflammatory response (Shiota *et al*., 2013).

2.7.2.3 UreC gene

Urease is one of the key enzymes in *H. pylori* pathogenesis. It has a molecular weight of 550 kDa and consists of three subunits of 26.5 kDa (Ure A), 61 kDa (Ure

B), and 13 kDa (Ure C). Urease is necessary for *H. pylori* to maintain a pH-neutral microenvironment around the bacteria, necessary for survival in the acidic stomach. Urease Induces self-destruction of *H. pylori* in vitro in non-acidic media. Urease is strongly immunogenic and chemotaxic for phagocytes. Superoxide dismutase has been isolated from *H. pylori*, which breaks down superoxide produced in polymorphonuclear leukocytes and macrophages and thereby prevents the killing of these organisms. Urease may be excreted from *H. pylori* to the surrounding environment and may protect this pathogen from the humoral immune response (Alkhidir *et al*., 2017)**.**

2.8. Laboratory diagnosis

2.8.1. Collection and Transport of Specimens

Upper endoscopy is performed and multiple gastric biopsy specimens are taken from the antrum, the corpus and duodenum. Transport of specimens is in normal saline (Boulos *et al*., 2002).

2.8.2 Methods of Diagnosis of *H. pylori* Infection

Patients with *H. Pylori* infection may clinically present with dyspepsia, heartburn, abdominal pain, diarrhea, or halitosis. A specific diagnosis can be made by available invasive and non-invasive methods. Both methods have good sensitivity and specificity (Rana *et al.*, 2017).

2.8.2.1 Invasive methods

Gastroduodenoscopy is very essential in symptomatic individuals who are not responding to therapy and those aged older than 45 years according to European guidelines. It is not only diagnostic but also therapeutic in upper gastrointestinal bleeding (UGIB) cases. These invasive tests have good sensitivity and specificity. (Rana *et al.*, 2017).

2.8.2.1.1 Rapid urease test (RUT)

To perform this test, a biopsy sample from the stomach is placed into a media containing urea, phenol red (pH indicator), buffers, and bacteriostatic agents. If *H. pylori* is present in the sample, it will produce urease enzyme, which will hydrolyze urea to ammonia. As a result, the pH of the medium will increase and the color will change from yellow to red. Sensitivity and specificity were found to be acceptable (98% and 97% respectively), provided the biopsy sample was collected from an ulcerated antrum site of the stomach. False-negative results may

occur when very low numbers of *H. pylori* are present or if the bacteria have a patchy distribution (Somily and Morshed, 2015).

2.8.2.1.2 Histology

Histology remains the gold standard for diagnosis of *H. Pylori* gastritis and detection of *H. pylori* organisms, with a sensitivity and specificity >95%. Giemsa staining is a widely used technique, and immunostaining would increase the sensitivity and specificity to 100% and 98-99%, respectively. A biopsy sample from the body of the stomach is preferred compared to one from the antrum in patients taking acid suppressing drugs, or it can be performed after 2 weeks after drug discontinuation. The availability of immunohistochemistry is limited; however, it has advantages in diagnosing *H. Pylori* infection in RUT negative cases and *H. Pylori* CagA genotypes. (Rana *et al*., 2017).

2.8.2.1.3 Culture

H. pylori is one of the most fastidious and slow-growing bacteria. Although culture is considered the gold standard, very few laboratories routinely culture *H. pylori* due to its complex nature, slow growth, and special growth requirements. Routine culture as a diagnostic tool is not recommended. However, in patients in whom standard second-line antimicrobial therapy has failed, culture is essential to determine which antibiotics the organism is sensitive to. *H. pylori* requires 85% N_2, 10% CO_2, and 5% O_2 for it is optimal growth. Growth occurs at 34°C to 40°C, with an optimum temperature of 37°C, and requires three to five days. It can also be grown in a candle jar. Although its natural habitat is the acidic gastric mucosa, *H. pylori* grows best at a neutral PH (7.0), although it will survive brief exposure to pH of less than four (Somily and Morshed, 2015). The commonly used media include *H. Pylori* agar, Skirrow agar, Columbia blood agar, Brucella agar, Brain heart

infusion or Trypticase soy agar, supplemented with sheep or horse blood (Wang *et al.,* 2015).

2.8.2.1.4 Polymerase chain reactions

The advantages of PCR are genotyping of *H. Pylori* and detecting antibiotic resistance genes and gene mutations. The sensitivity and specificity of this method is 97% and91.8%, respectively, even in peptic ulcer disease compared to RUT (47.7%) and histology (71.6%). Therefore, it can be diagnostic as well as investigative regarding the choice for appropriate therapeutic drugs (Rana *et al*., 2017). PCR allows researchers and clinicians to identify *H. pylori* in small samples that have few bacteria present and it can be performed on samples obtained by both invasive and noninvasive methods. Moreover, PCR can be performed faster than many other diagnostic methods, used to identify diverse bacterial genotypes, and employed in epidemiological studies. A considerable drawback of PCR is that it can detect DNA segments of dead bacterium in the gastric mucosa of patients after treatment (Garza-González *et al.*, 2014).

2.8.2.2 Non-invasive methods

2.8.2.2.1 Urea breath test

In this test 13carbon or 14Ccarbon labeled urea is fed to patient where in stomach is broken down by urease enzyme produces by *H. pylori* if present in the stomach. The released radioactive $13CO_2$ or $14CO_2$ diffusing in the blood released in the lungs. The expired air is collected to measure the activity of labeled Carbon. Currently, improved infrared spectrometers have shown which has the extra advantage of low cost also as compared to mass spectrophotomete. UBT is used to evaluate eradication therapy after giving anti *H. pylori* regimens. The sensitivity is quite good in post therapy and it may be explained by the fact that the UBT gives positive results when other biopsy based tests fail (Patel *et al.,* 2014).

2.8.2.2.2 *Stool antigen test*

The *H. pylori* stool antigen test is a new rapid, non-invasive test that is based on monoclonal immunochromatographic assay. A new, rapid, office based, one step monoclonal (Korkmaz *et al.*, 2015), immunoassay for detection of *H. pylori* antigen in stool has shown promising results in pretreatment and post-treatment settings and provides results within 10 minutes. Further data and cost effectiveness analysis are needed before the one step test can be recommended for use in primary care (Granstrom *et al.*, 2008).

2.8.2.2.3 Immunoassay Tests to Detect *H. Pylori* Antibodies

The ELIZA test has been the most prevalently used. Most commercial ELIZA tests are based on detecting IgG, with sensitivity and specificity values ranging from 60% to 100%. In general, tests containing complex antigen mixtures of various strains show the highest sensitivity. Serology tests are relatively cheap and readily available (Garza-González *et al.*, 2014).

2.9 Treatment

The most commonly prescribed initial treatment regimen for *H. pylori* infection has been a three-drug regimen that includes clarithromycin, a proton pump inhibitor (PPI), and amoxicillin or metronidazole for 14 days. This regimen is now only recommended in regions where *H. pylori* clarithromycin resistance is known to be 15% and in patients with no history of prior macrolide exposure (Alba *et al*., 2017. Patients with an allergy to penicillin or who have previous macrolide exposure may be prescribed a nitro imidazole, tetracycline, bismuth, and a PPI for 10 to 14 days (Alba *et al*., 2017). Other treatment regimens include sequential therapy (5 to 7 days of amoxicillin and a PPI followed by 5 to 7 days of clarithromycin, a nitro imidazole, and a PPI), concomitant therapy (clarithromycin, a nitro imidazole, amoxicillin, and a PPI for 10 to 14 days), hybrid therapy (7 days

of amoxicillin and a PPI followed by 7 days of clarithromycin, a nitro imidazole, amoxicillin, and a PPI), levofloxacin triple therapy (amoxicillin, levofloxacin, and a PPI for 10 to 14 days), and fluoroquinolone sequential therapy (5 to 7 days of amoxicillin and a PPI followed by 5 to 7 days of a fluoroquinolone, a nitro imidazole, and a PPI (Alba *et al*., 2017).

2.10. Prevention and control

Training the parent, especially mothers and grandmothers, about sanitation rule, washing hands thoroughly, eating food that is properly prepared and drinking water from a safe, clean source are important steps for preventing *H. pylori* infection. Probiotics improve gastrointestinal flora and prevent the infection and it using as a prophylactic functional food for preventing infections in children and their mothers in daily life could be effective. In future the decreased gastric *H. pylori* colonization by vaccination with *H. pylori* antigen and adjuvant was possible (Culver *et al*., 2017).

2.11. Previous studies

High rate of infection was reported by Al-Sulami and his colleagues (2013) in Iraq 66.5%iIn which they collected and cultivated 200 gastric biopsy samples from symptomatic patients.142 patients out of 200 patients were found positive for culture, of these 142 isolates, 133 isolates showed positive results for 16SrRNA gene by PCR While 9 (6.33%) were negative.

Study done by (Mapstone and Quirke, 1993) in Northern England to Identify of *Helicobacter pylori* DNA in the mouths and stomach of patients with gastritis using PCR in found that: Fifteen of 23 patients had a *Helicobacter* associate gastritis on histological examination. Of these, two had contamination during extraction (shown by positive results in their extraction control specimens). Of the

remaining 13 patients, 12 had a PCR positive gastric aspirate specimen and 5 of them had at least one positive oral specimen.

Study done by Zwet and his colleagues (1993) in Netherland to detect Sensitivity of Culture compared with that of Polymerase Chain Reaction for detection of *Helicobacter pylori* from antral biopsy samples. Seventeen of the 20 ulcer patients had culture-positive biopsy specimens. For all of these specimens, PCR was positive.

Lage and his colleagues in (1995) in America Diagnosed of *Helicobacter pylori* Infection by PCR, comparison with other invasive techniques and detection of *cagA* gene in gastric biopsy specimens found that Thirty-eight (36.5%) of the biopsy specimens yielded cultures positive for *H. pylori*. The specific ureC gene fragment was amplified in biopsy specimens from 40 (38.5%) patients. The biopsy specimens of all culture-positive patients were positive ureC by PCR.

Study done by Weiss and his colleagues (2008) in Evanston, chicago, USA to detection of *H. pylori* in gastritis patient found that In gastric biopsy specimens, *H pylori* was detected in 29 (52%) of 56 cases by PCR.

Study done by wahab and his colleagues (2018) in Pakistan to Detection of *H. pylori* by PCR method using ureA and ureC gene in gastric biopsy sample found that biopsy specimen. ureA gene-based PCR positive in 25/50(%) Out of the 50, 10 samples showed positive results for ureC gene. The positive predictive value was determined to be 20% and negative predictive value was found to be 80%

Study done by Linpisarn and his collegues (2003) in Thailand to detect of *Helicobacter pylori* in gastric biopsy samples by Polymerase Chain Reaction with a simple DNA extraction method found that H. *pylori* from seventy-two antral biopsy specimens was detectable by PCR assay in 54.2% of the cases.

Study done by Khalifehgholi and his colleagues (2011) in Iran to Comparison of five diagnostic methods for *Helicobacter pylori* found that a total of 46 (50.5%)

patients tested were positive by at least 2 of the 3 biopsy-based methods. The best sensitivity (95.6%) belonged to histology and RUT. The sensitivities of other tests including PCR, serology and stool antigen test were 93.5%, 91.3% and 73.9%, respectively.

Study done by Neova and his colleagues (2017) in Brazil to make Molecular technique for detection and identification of *Helicobacter pylori* in clinical specimens, a comparison with the classical diagnostic method found that total of 85 samples were collected from patients undergoing endoscopy. From the total samples subjected to RUT, 15 (17.64%) were positive and 70 (82.35%), negative. In PCR for detection of gene 16S ribosomal ribonucleic acid (rRNA) of *H. pylori*, 66 (77.64%) presented positive results and 19 (22.35%), negative results. For the analysis of the presence of UreA gene in all samples, positive results were found in 70 (82.35%), and negative in 15 (17.64%).

Another study in Suudan biopsies of each of the 69 patients were cultured. Thirty-nine (57%) were positive for *H.pylori*. Similarliy, biopsies of the control (31) were cultured, five (16%) were *H.pylori* positive Mirghani and his collegues(1994).

CHAPTER THREE

MATERIAL AND METHODS

Chapter three

3. Material and Methods

3.1. Study design

This was descriptive-cross sectional (Hospital-based) study.

3.2. Study area and duration

Study was done at Ibn Sina Hospital, Khartoum state, during the period June 2018 to June 2019.

3.3. Study population

Patients undergoing upper GIT endoscopy with upper gastrointestinal symptoms.

3.4 Inclusion criteria:

All adult with upper gastrointestinal symptoms undergoing upper GIT endoscopy (Immunocompromise where included).

3.5. Exclusion criteria:

Patients without upper gastrointestinal symptoms and children under 18 years old.

3.6. samle size

Due to difficult of collection of sample and time were take 50 sample.

3.7. Sample technique:

Non-probability convenience.

3.8. Method of Data collection

Personal data were obtained by reviewing medical questionnaire and by using direct interviewing.

3.9. Ethical consideration:

The ethical clearance was approved by the Ethical and Scientific Committee of Medical Laboratory Science College, Sudan University of Science and Technology; permission to carry out the study was taken from Ibn Sina Hospital and verbal consent were taken from patients undergoing upper GIT endoscopy.

3.10 Samples collection

Gastric biopsy collected by endoscopy and transferred immediately in brain heart Infusion broth medium with glycerol for culture and preserved at -20^{o}C deep freezing until processing for extraction and PCR.

3.11. Culture on Colombia Blood Agar Base

The 50 stomach biopsy specimens were centrifuged in normal saline or phosphate buffer saline at 3000rpm for 2minutes, then supernatant was discarded and two to three drops of sediment was cultured on colombia blood agar using sterile wire loop by ordinary method (primary, secondary, tertiary and zigzag) under aseptic condition.

All plates incubated at 37^{0}C under microaerophilic conditions using microerophilic kits up to 5 days and, the urease activity of *H. pylori* observed within 24 hours in this media. While, growth was obtained after 3 days following the incubation.

Colonies were sub cultured on colombia agar. The purified colonies were identified based on gram stain reaction, colony morphology and biochemical tests (catalase, oxidase, and urease tests).

3.12. DNA extraction

Helicobacter pylori DNA was extracted from the biopsy specimens using guanidine chloride extraction method. Each 2 ml tryptic soya broth (TSB) was centrifuged at 3000 rpm for 15-20 min. The pellet was collected and washed twice by phosphate buffer saline (PBS) to remove excess media. 2 ml of lysis buffer, 10µl of proteinase K, 1 ml of guanidine chloride and 300 µl of ammonium (NH_4) acetate were added to the pellet, vortexed, and incubated at room temprature overnight or at 65^0C for 2 hr. The mixture was cooled to room temperature, and then 2 ml of pre-chilled chloroform were added, vortexed, and centrifuged at 3000 rpm for 5 min. The upper layer of the mixture was transferred to new tube and 10 ml of cold absolute ethanol were added, shaked, and kept at -20^0C for 2hr or overnight. Then the tube was centrifuged at 3000 rpm for 15-20 min., the supernatant was drained carefully, and the tube was inverted on a tissue paper for 5 min. The pellet was washed with 4 ml of 70% ethanol, centrifuged at 3000 rpm for 5 min. The supernatant was poured off and the pellet was allowed to dry for 10 min. Then it was re-suspended in 50 µl of distilled water, briefly vortexed, and kept at -20^0C overnight. The extracted DNA integrity was assessed by ethidium bromide stained agarose gel electrophoresis and the DNA purity was assessed by Nanodrop technique (Abd alrahem *et al.*, 2018).

3.13. PCR technique

This was performed using the following *Helicobacter pylori* urease gene (*ureC*) primers:

5′– AAG CTT TTA GGG GTG TTA GGG GTT T–3

3′– AAG CTT ACT TTC TAA CAC TAA CGC –5′

PCR product size 294 pb. Amplification was performed in a final volume of 20 µL of PCR mixture containing 0.8 µm of each primer, 10 mM of each deoxy nucleotide triphosphate (dATP, dGTP, dTTP and dCTP), 10 mMtrisHCl, 50 mMKCl, 0.1% triton X– 100, 1.5 mM MgCl2, one unit of DNA polymerase and 4 µL of template DNA.

DNA amplification was carried out as follows:

Denaturation at 94°C for 5 min in the first cycle, followed by annealing for 30 sec at 65°C then extension for 30 sec at 72°C. The extension for the last cycle was increased to 5 min to ensure complete extension of the amplified fragment, denaturation for 30 sec at 94°C for a total of 35 PCR cycles. Then the PCR products were resolved by 2% agarose gel electrophoresis6. shows the PCR assay for some extracted DNA samples (Abd alrahem *et al.*, 2018).

3.14 Data analysis

Collected data were analyzed using the statistical package of social science (SPSS) program version (16). Chi-square statistical analysis were used to determine *P. value* significance range. Data represented by mean ± SD frequency, *P*-Value less than 0.05 were regarded as significant.

CHAPTER FOUR

RESULTS

Chapter four

4 Results

Out of 50 patients undergoing upper gastrointestinal endoscopy in Ibn Sina Hospital were randomly selected to participate in this study. Gastric biopsy specimens from the patients were subjected to Culture and Polymerase Chain Reaction (PCR) for the detection of *H. pylori*. The result showed that: 9(18.0%) were positive by PCR while 41(82%) were negative. In the other hand there was no growth of *H. pylori*. (Figure 4.1)

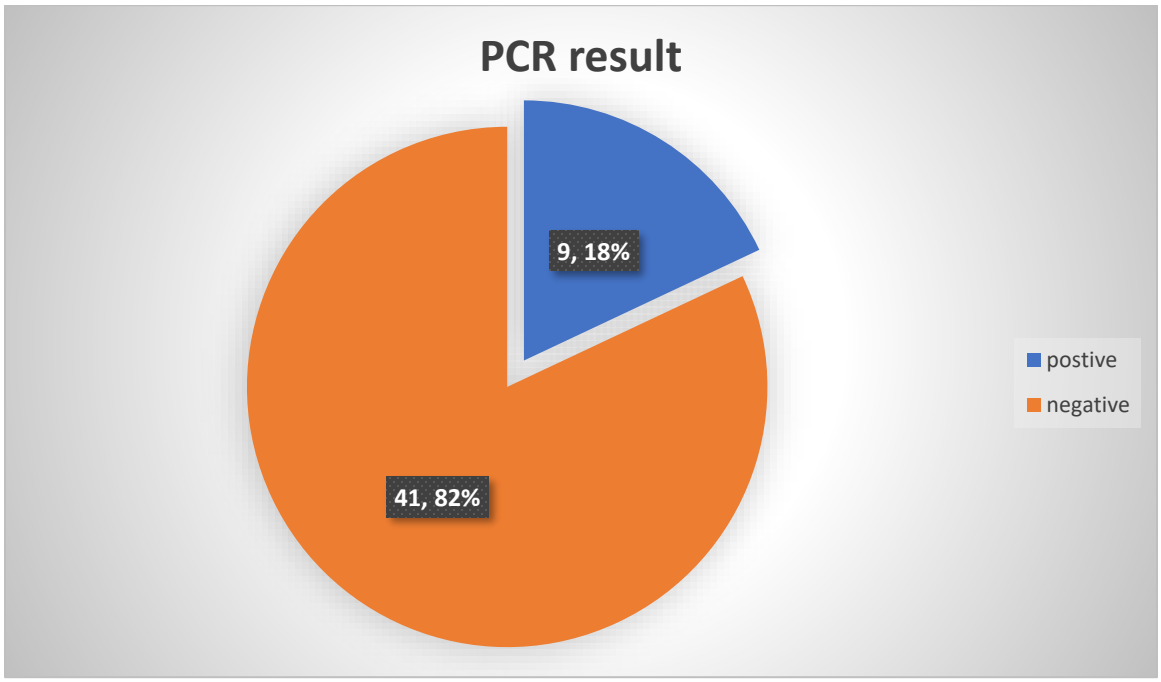

Figure (4.1) Detection of *Helicobacter pylori* gene from gastric biopsies by using PCR:

4.1 Association of gender, age groups, family history and past history of patients on detection of *H. pylori:*

H. pylori were detected in 4/50 (8%) male compare with 5/50(10%) female. The mean age of study subject was 50± 20 and classified to three age groups: 20-40 ,41-6 and 61-80. PCR of *H. pylori* was found in 3/50 (6%) among the age group

20-40 years, 4/50 (8%) among age group 41-60 and 2/50 (4%) among the age group 61-80, and was statistically insignificant (P> 0.05). (Table 4.1).

UreC gene was detected in 1 (2%) of patient with family history and 7 (14%) with past history, statistically showed in significant (P> 0.05). (Table 4.1).

Table (4.1) The association of gender, age groups, family history and past history of patients on detection of *H. pylori:*

Variable		PCR result		Culture result		P. value
		Positive	Negative	Growth	No growth	
Sex	Male	4 (8%)	20 (40%)	0	50 (100%)	0.813
	Female	5 (10%)	21 (42%)	0	50 (100%)	
Age groups	20-40	3 (6%)	16 (32%)	0	50 (100%)	0.94
	41-60	4 (8%)	16 (32%)	0	50 (100%)	
	61-80	2 (4%)	9 (18%)	0	50 (100%)	
Family history	Yes	1 (2%)	3 (6%)	0	50 (100%)	0.715
	No	8 (16%)	38 (76%)	0	50 (100%)	
Past history	Yes	7 (14%)	20 (40%)	0	50 (100%)	2.498
	No	2 (4%)	21 (42%)	0	50 (100%)	

4.2 Comparison between symptoms of *H. pylori* and PCR result

UreC gene was detected in 8(16%) of patient suffering from abdominal pain, 5 (10%) acidity and weight loss and 9 (18%) with gastritis and statistically showed in significant (P> 0.05) (Table 4.2).

Table (4.2) Comparison between symptoms of *H. pylori* and PCR

Variable		PCR result		Culture result		P value
		Positive	Negative	Growth	No growth	
Abdominal pain	Yes	8(16.0%)	35(70.0%)	0	50 (100%)	0.777
	No	1(2.0%)	6(12.0%)	0	50 (100%)	
Acidity	Yes	5 (10%)	24 (48%)	0	50 (100%)	0.870
	No	4 (8%)	17(34%)	0	50 (100%)	
Weight loss	Yes	5 (10%)	14 (28%)	0	50 (100%)	0.236
	No	4 (8%)	27 (54%)	0	50 (100%)	
Endoscopic finding	Gastritis	9 (18%)	40 (80%)	0	50 (100%)	0.526
	Ulcer	0	1 (2%)	0	50 (100%)	

4.3 Calculate of sensitivity and specificity

Calculate by using rule of sensitivity and specificity

Sensitivity rule: true positive/true positive +false negative

Specificity rule: true negative /true negative + false positive

Sensitivity of PCR : TP/TP+FN= 9/9+0= 1%

Specificity of PCR: TN/TN+FP= 41/41+0=1%

Sensitivity of culture was 0

Specificity of culture was 0

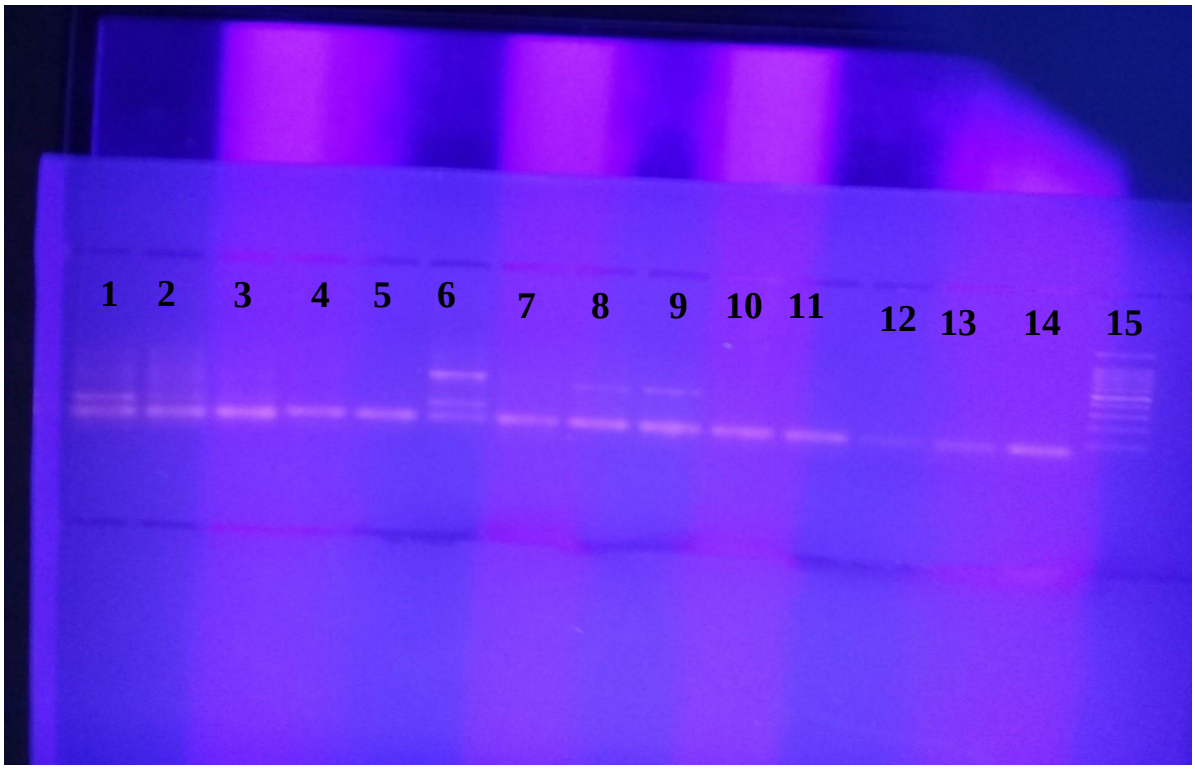

Figure 4.2 Multiplex PCR for *H. pylori*

Lane no.2, 3, 6, 7, 8, 9, 10,11,14, positive sample showed 294 band for *Ure C* gene. Lane no. 15 DNA ladder showed 100 pb for *Ure C* gene, lane no.1 control positive, lane no. 12 control negative, other lanes are negative samples.

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Chapter Five

5. Discussion, Conclusion and Recommendations

5.1 Discussion

The present study found that, the prevalence of *H. pylori* by PCR is high compare with culture. our findings agreed with previous study in Pakistan stated that, biopsy samples from different age groups were investigated for *H. pylori* infection by PCR, used the ureC as the target, low rate of *H. pylori* showed positive results for ureC gene (Wahab *et al.*, 2015). Also agreed with previous study in Iran stated that, low rate of *H. pylori* infection was detected in patients with upper gastrointestinal disorders by PCR (Esfahani *et al*., 2008). On the other hand our findings disagreed with previous study in Sudan stated that, high rate of *H. pylori* infection by PCR were reported by (El-khier *et al.*, 2016) .Also, high rate of *H. pylori* infection was reported by PCR in USA by Ousman *et al* (2011). *H. pylori* DNA from the gastric tissue by the polymerase chain reaction (PCR) is quite satisfactory, can yield high level of sensitivity and specificity, can detect as few as H. *pylori* colony forming units. This study used ureC genes as the PCR target. Found ureC gene for PCR as the most sensitive and specific for the detection of *H. pylori* in gastric biopsy specimens. The reason for variation in prevalence could be due to: different in environment and geographical area, the use of different drugs prior to the endoscopy which are the known suppressors of *H. pylori*, reduce the number of organisms, may be due to delay in processing of the biopsy specimens. On the other hand there was no growth of *H. pylori* by culture, our findings disagreed with previous study stated that, high rate of infection was reported by culture in Iraq (Al-Sulami *et al*., 2013), which disagreed with our study, this may be due to the special conditions required for specimen transport and growth, and

the long interval between specimen culture and test results. *H. pylori* were detected in female as same as male and was statistically insignificant (P> 0.05). According to age groups *H. pylori* was found in 41-60 more than 20-40 more than 61-80 and was statistically insignificant (P> 0.05). In our study there is no association between age, gender, and *H. pylori* infection, our findings agreed with previous study stated that: there was no significant difference between *H. pylori* infected and *H. pylori* subjects with respect to the age and gender distribution in Iran (Salimzadeh *et al.*, 2015) This result also agreed with study done in Serbia (Leszczynska *et al*., 2010,(Petrovic *et al*., 2011) , (Akbar and Eltahawy) , 2005). And study done in Saudi Arabia, aimed to estimate the prevalence of *H. pylori* among patients suffering from upper gastrointestinal symptoms, *H. pylori* status in patients was determined by histology, rapid unease test, and ELISA. The study showed that there was a significance correlation between age, gender and *H. pylori* infection (Abo-Shadi *et al*., 2013) which disagree with our study, this may be due to variation in sample size and geographical area.

According to clinical presentation frequency of abdominal pain as same as acidity and weight loss (frequency of *H. pylori* was equal) was statistically insignificant (P> 0.05), revealed that there was no significance association between symptoms of gastric pain, acidity and weight loss, and *H. pylori* infection. In study done in Australia by (Rosenstock *et al*., 1997, Werdmuller *et al*., 2000),Our result disagreed with this study stated that to assess the relation between *H. pylori* infection and gastro-intestinal symptoms and syndromes, random sample size was high reported that there was association between abdominal pain, and *H .pylori* infection, this may be due to variation in sample size and geographical area and uses more than test. Acidity and weight loss may be due to other diseases.

Frequency of past history as same as family history and was statistically insignificant (P> 0.05) and there was no association between past infection, family

history and *H*. pylori infection. Patients with past infection with *H*. *pylori* and showed positive result by PCR may be due to patient didn’t recover from the past infection or may be this is a new infection. If patient positive past infection and negative PCR result may be due to patient had been recovered from past infection or use antibiotic treatment. On the other hand patient with family history and showed positive *H*. *pylori* may be due to transmission of infection from family by contamination. All positive patients were gastritis patient because *H*. *pylori* infection results in chronic gastritis and, eventually, diseases, such as peptic ulcer, gastric cancer, and MALT lymphoma.

5.2Conclusion

This study concluded that, frequency of H. *pylori* in Khartoum state is low, also we have confirmed that PCR technique better than culture in diagnosis, and there was no association between upper gastrointestinal symptoms (abdominal pain, acidity and weight loss) and *H. pylori i*nfection, on the other hand there is no association between past history, family history and *H. pylori* infection.

5.3: Recommendations

1. Gastritis patients should be screened for *H. pylori* by PCR technique.

2. *H. pylori* DNA detection should be carried out in gastritis patients who tested negative for *H. pylori* antibodies to confirm the result.

3.Larger sample size is needed to accurately determine the rate of infection.

4. Should be used control of culture media for H. *pylori* to accurate the result of culture growth.

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Appendix (1)

Sudan University of Science and Technology

College of Graduates Studies

Questionnaire No ( )

Detection of *Helicobacter pylori* by using culture and PCR techniques in Khartoum state

الكشف عن بكتريا الملوية البوابية بإستخدام تقنيتي الإستزراع وتفاعل البلمرةالمتسلسل في ولاية الخرطوم

Name:…… NO (……..)

Age:…… Sex: ……

date:……

Medical history:

Abdominal pain…….yes……..No

Acidity……...yes……..No

Weight loss……yes……..No

Family history of *H. pylori*…yes…….No

Past history of *H. pylori*…….Yes……No

Endoscopy finding:

Gastritis…….yes……No

Gastric ulcer……Yes……No

Culture result: …..Growth……..Not growth……

PCR result:……Positive……Negative……

Appendix (2)

Chemicals and reagent:

1/ Lysis buffer: cell lysis.

2/ Proteinase K: breakdown of protein.

3/ Chloroform: phase separation in DNA extraction.

4/Absolute ethanol: DNA precipitation

5/70% ethanol: washing the precipitated DNA.

6/ Distilled water: reagent preparation and re-suspension of DNA.

7/ Agarose gel: gel electrophoresis.

Appendix (3)

Preparation of Culture media: preparation of Columbia agar by two method:

1\ Classic Columbia agar :

2\ Modified Columbia hemoglobin urea agar (MCHUA) media:

Add component 41g of Columbia agar base ,20 g of urea and 0.0012 of phenol red as indicator and complete volume with 740ml of distilled water and sterilize the media by autoclave at121 ºC for 15 minutes , without adding antibiotic solution and hemoglobin solution ,cool to 50- 55 ºC, aseptically add 250 ml of hemoglobin solution and 10 ml of antibiotic solution , mix thoroughly and pour into sterile disposable petridish.

Preparation of agarose gel: 0.75g of agarose powder were dissolved into 100ml of 10X TBE [TBE was prepared by taken amount of 108g of Tris base and added to 55g of boric acid and 40ml of 0.5% EDTA and then dissolved into 1L of D.W].

Preparation of ethidiumbromide: Ethedium bromide was prepared by taken 10mg of ethedium bromide and dissolved into 0.5ml D.W then kept into brown bottle.