Sudan University of Science and Technology
College of Graduate Studies

A Study Of Molecular Detection of *Helicobacter pylori* IceA gene in Gastric biopsy from Endoscopic patients in AL Faisal and AL Ribat Hospitals in Khartoum State

دراسة عن الكشف الجزيئي عن جين IceA في البكتريا الحلزونية البوابية في خزعات من جدار المعدة من مرضى مناظير بمستشفى الفيصل ومستشفى الرباط في ولاية الخرطوم

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

By:
Fatima Youssif Ali Hassan
B.Sc. in Medical Laboratory Science (Microbiology), University of Science And Technology, (2016)

Supervisor:
Prof. Yousif Fadlallah Hamedelnil
2021

الآية

بسم الله الرحمن الرحيم

قال الله تعالى

﴿وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا﴾

سورة الإسراء, الأية 85

DEDICATION

This work is dedicated to my beloved family; my mother, my brothers, my sister and my late father may Alla give him mercy and forgiveness.

ACKNOWLEDGMENT

I thank firstly the Almighty Allah to make it possible for me to finish this work successfully and my gratitude to my supervisor Prof.Yousif Fadlallah Hamedelnil for sharing his knowledge and his endless support, guidance, valuable comments.

Also my thanks and gratitude goes to D.Maram Mustafa Mohammed for her fully support for this work to be done.

The completion of this research could not have been done without the participation and assistance of so many people whose names may not be all enumerated but I wouldn't forget to thank them.

ABSTRACT

Colonization with *Helicobacter pylori* known to be cause of variety of clinical consequences includes gastritis or even gives rise to severe and potentially fatal complications, and different studies suggested that there is a relationship between the clinical outcomes and some virulence gene that *H.pyloi* has.

The aim of this study is to detect 16_s rRNA gene and the virulence gene iceA of *H.pylori* among Sudanese patients in Khartoum State. The samples were collected from 2 hospitals in Khartoum, AL Faisal and AL Ribat Hospitals. The participants were aged from 15 to 73 years old who visited our target hospitals for endoscopy procedure, which revealed 88.3% with gastritis and 11.3% with gastric ulcer, and the majority of patients in the study (68.8%) claimed to have a previous *H. pylori* infection.

Total of eighty (N=80) gastric biopsy specimens were collected from patients, the gender distribution was (50%) males and (50%) females, the males had the most of the positive results for the 16_s rRNA ( )and iceA genes ( ), among the samples eleven (13.8%) were confirmed as *H.pylori* positive by PCR by the presence of 16_s rRNA of *H.pylori*, and from the positive samples seven (8.8%) were positive also by PCR for iceA gene. In the current study it was found there was no statistically significant association between *H.pylori* and its iceA virulence gene with age or gender. Regarding clinical outcome; no significant association (*p* value= .129) has been detected between iceA gene and gastric ulcer, while there was a significant association (*p* value= .005) between 16_s rRNA gene of *H.pylori* and gastric ulcer.

The study confirmed the relationship between *H. pylori* and gastric ulcer. Further studies are required to consolidate these results with larger samples.

ملخص الأطروحة

الاستعمار عن طريق البكتيريا الحلزونية البوابية عرف كسبب لتتابعات مرضية، ودراسات عدة اقترحت وجود علاقة بين الناتج المرضي كإلتهاب جدار المعدة وتقرحات و سرطان المعدة مع بعض الجينات الضارية التي تمتلكها البكتيريا الحلزونية البوابية.

هدف هذه الدراسة هو الكشف عن وجود جين 16_srRNA و جين iceA الموجودان لدى البكتيريا الحلزونية البوابية لدى مرضى سودانيون في ولاية الخرطوم، وهؤلاء المشاركون في هذه الدراسة كانت أعمارهم تتراوح ما بين خمسة عشر عامًا إلى ثلاثة وسبعون عاما، وكان هؤلاء المرضى قد زاروا مستشفى الرباط التعليمي ومستشفى الفيصل بغرض اجراء عملية المناظير المعوية التي اظهرت وجود التهاب في جدار المعدة لدى الغالبية (88.3%) واخرون اظهرت لديهم وجود قرحة في المعدة (11.3%). وقد صرحت الغالبية من المرضى المشاركون عن سبق إصابتهم بهذه الباكتيريا الحلزونية البوابية.

تم جمع عدد ثمانين عينة من الخزعات التي تم أخذها من المعدة من المرضى المتضمنين في الدراسة ، (50%) من الاناث و (50%) من الذكور الذين كان لهم النصيب الاكبر من النتائج الموجبة لجين 16_S rRNA و جين iceA.من هذه العينات تم تاكيد احدى عشر (13.8%) نتيجة ايجابية للباكتيريا الحلزونية البوابية عن طريق فحص جين 16_s rRNA بطريقة PCR ، ومن هذه النتائج الإيجابية تم ايجاد سبع عينات (8.8%) ايجابيات لجين iceA ايضا عن طريق فحص ال PCR .

من هذه الدراسة اظهر توزيع الجينات بين الجنس والفئات العمرية أنه لا توجد علاقة يعتد بها بين هذه الجينات والجنس والفئات العمرية. أما بخصوص الناتج المرضي اظهر أيضا أنه لا توجد علاقة يعتد بها (P value= 129. بين جين 16_s rRNA و قرحة المعدة، بينما توجد علاقة يعتد بها (P value= .005) بين قرحة المعدة وجين iceA .

هذه الدراسة أكدت وجود علاقة بين الباكتيريا الحلزونية البوابية و قرحة المعدة، لمزيدٍ من النتائج التي يمكن تعميمها، دراسات أخرى يتوجب إنشاؤها لدمج هذه النتائج مع عينات أخرى.

TABLE OF CONTENTS

CHAPTER THREE

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µm	Micro meter
Bab A	Blood Group Antigen-Binding Adhesion
CagA	Cytotoxin Associated Gene
D.W	Distill Water
EDTA	Ethyline Diamine Tetra Acetic acid
ELISA	Enzyme Linked Immune Sorbent Assay
H. pylori	Helicobacter pylori
nm	nano meter
PCR	Polymerase chain reactin
PPI	Proton Pump Inhibitors
RUT	Rapid Urease Test
SPSS	Statistical Package of Social Science
TBE	Tris Borate EDTA
UBT	Urea Breath Test
Vac A	Vacuolating Cytotoxin Gene
IceA	Induced by contact with epithelium
GU	Gastric ulcer
SPP	Species
DNA	Deoxyribonucleic acid
rRNA	Ribosomal Ribonucleic acid
cDNA	Complementary Deoxyribonucleic acid

FAT	Fecal antigen test
U.V	Ultra Violet
rpm	Revolutions per minute
bp	Base pair
PAI	Pathogenicity island
IgG	Immunoglobulin G

CHAPTER ONE

Introduction

CHAPTER I
1.1 INTRODUCTION

About 20 years ago, Barry Marshall and Robin Warren described the successful isolation and culture of a spiral bacterial species, later known as *Helicobacter pylori*, from the human stomach. This discovery resulted in the awarding of the 2005 Nobel Prize in Physiology and Medicine to Robin Warren and Barry Marshall for their discovery of the bacterium *Helicobacter pylori* and its role in gastritis and peptic ulcer disease (Ribeiro *et al*., 2003). *Helicobacter pylori* were isolated in 1983 from stomach biopsy specimens of patients with chronic gastritis. The bacterium colonizes the human gastric mucosa and the infection can persist for decades (Covacci *et al*., 1993). *H. pylori* and all organisms belonging to these genera are small, curved, motile, Gram-negative bacilli. With few exceptions, most of these bacteria also have a requirement for a microaerobic atmosphere (5% to 10% O_2) (Tille, 2014).

The genus *Helicobacter* belongs to the subdivision of the the *Proteobacteria*, order *Campylobacterales*, family *Helicobacteraceae.* To date, the genus *Helicobacter* consists of over 20 recognized species, with many species waiting formal recognition. Members of the genus *Helicobacter* are in most cases catalase and oxidase positive, and many but not all species are also urease positive (Kusters *et al*., 2006). *H. pylori* hold an important acid resistance mechanism through the generation of urease enzyme and one of the principal structural parts of *H. pylori* is the flagella, it is unipolar flagella developed to move through the mucus barrier toward the gastric epithelial surface for colonization (Huwiage *et al.,* 2019).

The exact mode of transmission is unknown. An oral-oral, fecal-oral, and a common environmental source have been proposed as possible routes of transmission (Tille, 2014). Risk factors for *H. pylori* infection in both developing and developed countries are closely related to poor living conditions and genetic susceptibility, low socioeconomic status, poor hygiene conditions, overcrowding, bed sharing, interfamilial clustering, and family history of parental gastric disease (Tongtawee *et al.,* 2016).

The acquisition rate of *H. pylori* appears to be more rapid in developing than developed countries (Khetrapal *et al.,* 2019). In various developing countries, more

than 80% of the population is *H. pylori* positive, even at young ages, while 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 (Kusters *et al.*, 2006).

Already in the late 1980s it was noted that *H. pylori* strains isolated from different patients display a high degree of genetic variability, which has led to the speculation that there may be as many unique *H. pylori* strains as there are unique human hosts that carry them (Suerbaum, 2000). There is continuing interest in identifying *H. pylori* virulence factors that might predict the risk for symptomatic clinical outcomes. It has been proposed that iceA and cagA genes are such markers and can identify patients with peptic ulcers (Yamaoka *et al*,. 1999). *Helicobacter pylori* has a high mutation rate, and strains from unrelated persons exhibit a high level of genetic diversity also *H.pylori* strains from various geographic areas can be classified into distinct populations and subpopulations (Bullock *et al*., 2017).

Colonization with *H. pylori* almost invariably leads to a chronic inflammatory response of the gastric mucosa (type B gastritis) that may be asymptomatic during decades of colonization, or give rise to severe and potentially fatal complications. At least 10% of all persons infected with *H. pylori* will develop overt *H. pylori*-associated disease, such as gastric or duodenal ulcerations or malignant tumors of the stomach (carcinoma, lymphoma) (Suerbaum, 2000).

1.2. Rationale

Colonization of the gastric mucosa by *H. pylori* results in an acute inflammatory response and damage to the gastric epithelium.

Inflammation can then progress to several disease states, ranging in severity from superficial gastritis, chronic atrophic gastritis, peptic ulceration, to lymphoma and gastric cancer (Kavermann *et al.*, 2003).

Sensitive detection methods for virulence genes are important because these genes are potential pathological determinants and data collected from such researches needed for epidemiological reasons and for monitoring ordinary laboratory diagnostic methods also the data concerning the geographic distribution of iceA alleles are scarce, and information on the association of the gene with the disease is rare and still controversial.

In Sudan *H. pylori* infection is one of the major health problems and most of the previous studies with routine tests for the detection of *H. pylori* are depending on serological tests which have a cross reaction with other infections.

1.3. Objective

1.3.1 General objective

Molecular detection of *H.*pylori IceA gene in gastric biopsy from endoscopic patients in AL Faisal and AL Ribat Hospitals in Khartoum State.

1.3.2. Specific objectives

1. To identify *H. pylori* from gastric biopsy of patients with gastritis by amplifying 16_s rRNA using PCR .
2. To detect iceA virulence gene using PCR among positive *H.*pylori samples.
3. To correlate between positive *H.*pylori and iceA samples and clinical outcomes.

CHAPTER TWO

Literature Review

CHAPTER II

2. LITERATURE REVIEW

2.1. Helicobacter pylori

2.1.1. Morphology

Helicobacter are considered small, curved, motile, gram-negative bacilli, with few exceptions (Tille, 2014). Normally, this bacterium has a spiral shape, which is crucial for proper colonization of the stomach and cork-screwing penetration of dense mucin covering this organ. However, *H. pylori* may also form curved-straight rods, filamentous forms and coccoid forms. This morphological variability affects nutrient transport and respiration processes, as well as motility, the ability to form aggregates-biofilms, and resistance to adverse environmental factors (Krzyżek and Gościniak, 2018). In gastric biopsy specimens, *H. pylori* organisms are 2.5 to 5.0 µm long and 0.5 to 1.0 µm wide; there are four to six unipolar sheathed flagella, which are essential for bacterial motility. Each flagellum is approximately 30 µm long and approximately 2.5 nm thick Flagella exhibit a characteristic terminal bulb, which is an extension of the flagellar sheath. The flagellar sheath exhibits the typical bilayer structure of a membrane (Hassan, 2019). In prolonged culture the morphology of *H. pylori* alters from the normal helical form to a spherical cell. This morphological transformation may be the result of less than optimal cultural conditions, a reaction to waste products, or it may be a natural phenomenon (Jones and Curry, 1990).

2.1.2. Genetic and virulence determinants

H. pylori and humans have co-evolved for at least 100,000 years, long before human ancestors left Africa (Moodley *et al.,* 2012). During this long history in its hostile gastric niche in humans, *H. pylori* have developed a wide spectrum of strategies to persist in and adapt to changing conditions in and around its host (Suerbaum and Achtman, 1999). *H. pylori* was the first bacterial species for which two complete genomic sequences became publicly available. Only approximately one third of the strain-specific genes are homologous to genes with known functions, the other two thirds are homologous to genes whose functions are unknown or have no known database homologues (Burucoa and Axon, 2017).

More than 50% of the world's population is infected with *H. pylori*, only a minority of carriers develops serious gastroduodenal diseases. There is increasing evidence that the genetic variability of *H. pylori* may have a clinical importance. Several genes have

been identified that may play a role in the pathogenesis of *H. pylori*, such as cagA, vacA s1/m1, iceA1, and babA (Kim *et al*., 2001). Genetic variation among *H.pylori* strains is known to be an important factor influencing the outcome of infection, for example, strains that contain the cag pathogenicity island (PAI), which encodes CagA (a secreted effector protein) and a type IV secretion system, are associated with a higher risk of gastric cancer or peptic ulceration compared to strains that do not contain the cag PAI. Similarly, strains that produce active forms of the VacA toxin and strains that produce specific outer membrane proteins have been linked to an increased risk of gastric cancer or peptic ulceration (Bullock *et al*., 2017).

And in the case of the designated iceA (induced by contact with epithelium) have two main allelic variants of the gene: iceA1 and iceA2. The expression of iceA1 is up-regulated on contact between *H. pylori* and human epithelial cells, and may be associated with peptic ulcer disease (Kim *et al*., 2001). It has been reported that the iceA allelic type was independent of the cagA and vacA status (Yamaoka *et al*,. 1999). Only iceA1 RNA is induced following adherence in vitro (Ribeiro *et al*., 2003).

2.1.3. Modes of transmission

H. pylori have been hypothesized to spread through various routes, though generally believed to spread from person to person (Bui *et al.,* 2016). Transmission routes can be classified into direct, as the, oral– oral, gastro-oral, and fecal–oral, and indirect routes such as via food, animals, and drinking water (Breckan *et al.,* 2016). *H.pylori* is transmitted from person to person within families and that *H. pylori* is clonal over short periods of time after natural transmission (Suerbaum, 2000). Intrafamilial transmission is common especially from mother to child. *H. pylori* have been detected in dental plaque, saliva, tongue, tonsil tissue, root canals, and oral mucosa usually by PCR (Burucoa and Axon, 2017). Yet the transmission routes of *H. pylori* are still poorly understood and have been assumed to take place in early childhood, most likely from mother to child. Drinking water supply has also been discussed as indirect transmission sources as have toilet facilities (Breckan *et al.,* 2016). Among the risk factors for acquisition of *H. pylori* infection, poor socioeconomic status, poor sanitization and hygiene practices, and contaminated food and water, are the most significant ones (Zamani *et al.,* 2017).

2.1.4. Epidemiology

The prevalence of *H. pylori*-related diseases varies geographically and it is partially determined by the virulence of the circulating strains (González *et al.,* 2019).

H. pylori infection is one of the most prevalent infections among humans, with seroprevalence estimates as high as 70% worldwide and up to 80% or more in developing countries (Bui *et al.,* 2016). There is a study included studies published between 1970 and 2016; 183 papers from 62 countries were in the final analysis (11 from Africa, 75 from Asia, 66 from Europe, 13 from Latin America and Caribbean, 13 from North America, and 5 from Oceania) with a total population of 531 880 individuals. In this study the highest prevalence of *H. pylori* was found in Africa (79.1%), Latin America and the Caribbean (63.4%), and Asia (54.7%), and the lowest prevalence in Northern America (37.1%) and Oceania (24.4%). The authors suggested that approximately 4.4 billion individuals were infected globally in 2015 which would account for 60.3% of the global population. There are some factors can be associated with *H. pylori* infection, such as Demographic and socioeconomic status along with diet and lifestyle (Sjomina *et al*., 2018).

H.pylori is more prevalent in the developing countries, and more often among younger people reaching up to 10% of the population in comparison to only 0.5% in more developed world (Salih *et al*., 2017).

2.1.5. Pathogenesis

The intragastric distribution and severity of this chronic inflammatory process depend on a variety of factors, such as characteristics of the colonizing strain, host genetics and immune response, diet, and the level of acid production (Kavermann *et al.,* 2003). *H. pylori* is capable of colonizing the mucous layer of the antrum and fundus of the stomach but fails to invade the epithelium. Motility allows *H. pylori* to escape the acidity of the stomach and burrow through and colonize the gastric mucosa in close association with the epithelium. The change in pH protects the organism from the acidic environment produced by gastric secretions (Tille, 2014). Colonization with *H. pylori* almost invariably leads to a chronic inflammatory response of the gastric mucosa (type B gastritis) that may be asymptomatic during decades of colonization, or give rise to severe and potentially fatal complications. At least 10% of all persons infected with *H. pylori* will develop overt *H. pylori*-associated disease, such as gastric or duodenal ulcerations or malignant tumors of the stomach (carcinoma, lymphoma) (Suerbaum, 2000). *H. pylori* attaches to the mucus-secreting cells of the gastric

mucosa and produce large amounts of ammonia from urea by the organism's urease, coupled with an inflammatory response, leads to damage to the mucosa leading to loss of the productive mucus coating predisposes to gastritis and peptic ulcer. The ammonia also neutralizes stomach acid, allowing the organism to survive. The chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually a B-cell lymphoma (Levinson, 2016).

2.1.6. Infection and complication

Colonization of the gastric mucosa by *H. pylori* results in an acute inflammatory response and damage to the gastric epithelium. Inflammation can then progress to several disease states, ranging in severity from superficial gastritis, chronic atrophic gastritis, peptic ulceration, to lymphoma and gastric cancer (Kavermann *et al.,* 2003). Histological gastritis is essentially universal among *H. pylori*-infected individuals, but only a minority develop a clinically significant outcome, such as peptic ulcer disease or gastric cancer (Yamaoka *et al,*. 1999). The type and severity of diseases depend on many factors, among them: the status of the host's immune system, the pathogenicity of *H. pylori* strains, and the presence of environmental factors (diet, stress, hygiene level, or the presence of co-infections) (Krzyżek and Gościniak, 2018). The infection is strongly associated with non-autoimmune chronic gastritis and peptic ulcer disease (Crabtree *et al.,* 1991). Most of *H.pylori* infections usually are symptomless and without clinical manifestation. However, signs and symptoms associated with the disease are primarily due to gastric or peptic ulcer illness or duodenal inflammation. Furthermore, other symptoms such as nausea, vomiting, and abdominal pain may be attributed to other gastrointestinal diseases (Abbas *et al.,* 2018). Furthermore, possible associations have been reported between *H. pylori* and a number of extragastric manifestations related to cardiovascular, dermatological, neurological, immunological, hematological, hepatobiliary, respiratory, and endocrine and metabolic disorders (Zamani *et al.,* 2017).

2.1.6.1. Gastritis

Healthy stomach mucosa contains very few inflammatory cells. However, when *H. pylori* colonizes the stomach, polymorphonuclear cells and neutrophils migrate to the area resulting in an acute inflammatory response (Abdallah, 2018). Persistent colonization of *H pylori* in the human stomach results in release of chemoattractants,

such as IL-8, which stimulate significant infiltration of neutrophils into the gastric mucosa, leading to chronic gastritis (Brandt *et al.,* 2005). One histological pattern induced by *H. pylori* pangastritis, which may subsequently progress to gastric ulceration (GU), atrophy, and intestinal metaplasia and lowers the threshold for distal gastric cancer. H. pylori may alter cellular processes with premalignant potential, such as apoptosis and proliferation (Israel *et al.,* 2001). *H. pylori* causes chronic (type B) gastritis. An increase in mucosal cell proliferation increases the likelihood of a neoplastic clone of epithelial cells emerging where there is chronic epithelial cell injury associated with *H. pylori* gastritis. The intestinal form of gastric cancer, which is the commonest type, develops against a background of chronic gastritis, atrophy, and intestinal metaplasia (Lynch *et al.,* 1995).

2.1.6.2. Gastric Ulcer

H. pylori-positive patients have a 10 to 20% lifetime risk of developing ulcer disease (Khetrapal *et al.,* 2019). And it is responsible for 70% - 85% of all gastric ulcers (Bakhti *et al.,* 2019). Gastric ulcer (GU) is a precancerous pathology with an increased risk of carcinogenesis, the management of peptic ulcer diseases, namely screening and therapy (Ramadan *et al.,* 2020). It is a chronic disease characterized by frequent recurrences. The continuation of antiulcer therapy after ulcer healing results in a reduced rate of ulcer recurrence (Graham *et al.,* 1992).

2.1.6.3. Duodenal Ulcer

The majority of infected individuals do not develop any clinically apparent disease, but there is compelling evidence that 6–20% of these infections result in duodenal ulceration. Duodenal ulcers tend to be associated with a high degree of acid secretion (Chakravorty *et al.,* 2006). All primary duodenal ulcer disease is related to *H. pylori*. Treatment with antibiotics and bismuth salts has resulted in ulcer healing and decreased relapse rates in adults after *H. pylori* infection was cleared from the gastric mucosa (Israel and Hassall, 1993). Duodenal mucosal bicarbonate secretion is the most important protective factor against acid-induced mucosal injury. *H. pylori* infection down regulated the expression and functional activity of duodenal mucosal cystic fibrosis transmembrane conductance regulator and solute linked carrier 26 gene family which are the two key duodenal mucosal epithelial cellular bicarbonate transporters to mediate duodenal bicarbonate secretion (Wen *et al*., 2018).

2.1.6.4. Gastric Cancer

Gastric cancer, one of the five most common causes of cancer death, and it is associated with a 5 year overall survival rate less than 30% (Rugge *et al*., 2017). *H. pylori* was classified as a class I carcinogen in humans by a working group of the World Health Organization International Agency for Research on Cancer (Yamazaki *et al.,* 2005). *H. pylori* is one of the most common among the numerous bacterial species of the stomach (Ilie *et al.,* 2011). *H. pylori* infection developed during early childhood is considered as a significant risk factor for gastric carcinoma in the adult individual. (Abbas *et al.,* 2018) Carcinomas in the stomach are thought to arise as a result of a series of changes occurring within the gastric mucosa, progressing from Helicobacter pylori gastritis, through atrophic gastritis, intestinal metaplasia, and intraepithelial neoplasia, to malignancy (Anagnostopoulos *et al.,* 2007). *H. pylori* is a genetically diverse bacterial, and these genetic differences among these populations might be a factor underlying geographic variation in gastric cancer incidence (Ribeiro *et al*., 2003). Some studies suggested that African strains of *H.pylori* may have reduced virulence. And with the low rate of gastric cancer in Africa that could be due to co-evolution of African strains with African humans over a very long period of time, allowing the microbe and host to form a more harmonious relationship. Several other factors could contribute to a relatively low incidence of gastric cancer in Africa, including a high rate of intestinal parasitic infections that might attenuate *H.pylori* virulence, composition of the gastric or intestinal microbiome, or composition of the diet. A low reported incidence of gastric cancer might also reflect limitations in the availability of diagnostic procedures (such as endoscopy) and incomplete reporting (Bullock *et al*., 2017).

2.1.7. Diagnosis

Include invasive (Culture, Histological examination, Rapid Urease test, and polymerase chain reaction, which requires the use of endoscopy to collect biopsy specimens) and Non Invasive methods (Urea breath test, Stool Antigen test and serology) that they should be reliable, simple and quick to perform (Fan *et al.,* 2020; Milani *et al.,* 2019; Kesli *et al.,* 2010).

2.1.7.1. Invasive Methods

Tissue biopsy material of the stomach for detection of *H. pylori* should be placed directly into transport media such as Stuart's transport medium to prevent drying. Specimens for biopsy may be refrigerated up to 24 hours before processing; tissues should be minced and gently homogenized (Tille, 2014). Conventional endoscopy is limited to detecting lesions on the basis of gross morphological changes. The diagnosis of gastrointestinal pathology therefore depends on biopsy sampling of macroscopically obvious endoscopic features, or blind biopsy sampling of normal appearing mucosa (Anagnostopoulos *et al*., 2007).

2.1.7.1.1. Culture

Culture is difficult to control because of the draconian pre-analytical conditions to be respected and the great difficulty of growing *H pylori* compared with other bacteria (Jehanne *et al.,* 2020). One important characteristic is its fastidious growth requirements. *H. pylori* only grow in microaerophilic or in carbon dioxide-enriched (5–10%) atmospheres, and it exhibits optimal growth at 35–37°C. The bacterium grows very slowly (Stevenson *et al.,* 2000). *H. pylori* usually require 4-7 days of incubation for the small translucent initial circular colonies to develop. It is typically diagnosed according to cellular morphology and positive oxidase reactivity, and by catalase and rapid-urease test results (Al-Thahab, and Al-Awsi, 2018). Isolation and subculture of *H pylori* on various selective and non-selective solid media have been established with the cultivation in large quantities of liquid media (Xia *et al.,* 1993). Culture-guided treatment is standard for infectious diseases where antimicrobial resistance is common as it allows therapy to be tailored to antimicrobial susceptibilities (Bhakta *et al.,* 2018). Plating media that have commonly been used to cultivate *H.pylori* contain either Columbia agar, brain heart infusion agar or charcoal base agar, supplemented with either whole, laked or chocolated blood or serum. There are also numerous broths routinely used to culture the organism, including brain heart infusion broth, Mueller-Hinton broth and Brucella broth (Stevenson *et al.,* 2000).

2.1.7.1.2. Histological Examination

The most reliable method of diagnosis of *H. pylori* infection is either histopathological identification of the bacterium with at least one other positive

biopsy-based test or positive culture (AL Kirdy *et al.,* 2020). Describing histological specimens containing *H. pylori* is variable. The classification grades the stomach biopsy in regard to 4 domains: chronicity (based on presence of lymphocytes) activity (based on presence of neutrophils), glandular atrophy; and metaplasia (Ahmed *et al.,* 2020).

2.1.7.1.3. Rapid Urease test

This test is based on the principle that abundant urease enzyme produced by *H. pylori* hydrolyses urea to ammonia. The consequent rise in the pH of the medium is detected by phenol red indicator (Khetrapal *et al.,* 2019). Detection of urease activity has become an important tool for the diagnosis of *H. pylori* infection (Dahlén *et al*., 2018). Samples should be dried and placed into capped tubes containing 1 mL freshly prepared 10% w/v urea in deionized water at pH 6.8, including two drops of phenol red indicator. The presence of urease activity was observed as a color change from yellow to pink (Nisha *et al.,* 2016). The rapid urease test (RUT) is a popular diagnostic test in that it is a rapid, cheap and simple test that detects the presence of urease in or on the gastric mucosa. Best results are obtained if biopsies are obtained from both the antrum and corpus. False-positive results are rare if the RUT contains an antibacterial agent to prevent growth of urease-containing contaminants (Uotani and Graham, 2015).

2.1.7.1.4. PCR

The diagnosis of *H. pylori* infection can be made by PCR on gastric biopsies (Jehanne *et al.,* 2020). Polymerase chain reaction (PCR) is a laboratory technique that is used to make multiple copies of DNA segment. The technique is very accurate and precise and it can be used to or copy or amplify a specific DNA segment from the mixture of DNA molecules (Al-Thahab, and Al-Awsi, 2018). Limitations of PCR methods include the propensity for false-positive results in part due to the detection of cDNA from non-H.pylori organisms. This is particularly important in environmental samples which may contain previously uncultured organisms or non *H. pylori Helicobacter* spp. False negative results may also occur due to a low number of organisms or to the presence of inhibitors in the sample. This is especially important in stools and environmental samples. A number of target genes have been proposed as candidates

for the PCR detection of *H.pylori*, including the16SrRNAgene, the 26K species-specific antigen gene, the glmM gene (Sugimoto *et al.*, 2009).

2.1.7.2. Non Invasive Methods

2.1.7.2.1. Urea breath test

H. pylori utilizes the strong activity of urease as a protective buffering enzyme to hydrolyze urea into ammonia and carbon dioxide against gastric acid and for survival in low pH environment of human stomach (Fan *et al.,* 2020). UBT is another very sensitive, specific, and diagnostic accurate method. It is expensive and requires trained personnel and complex instrumentation to obtain and read breath samples (Kesli *et al.,* 2010).The test has a sensitivity of 88–95% and specificity of 95–100% (Yari *et al.,* 2016).

2.1.7.2.2. Stool antigen test

Fecal antigen test (FAT) identifies antigens of *H. pylori* through monoclonal and polyclonal antibodies in a fecal sample**.** Currently, most FAT uses monoclonal antibodies. The test can be performed by immuno-enzymatic assay or by immunochromatography assay (Dalla *et al.*, 2016). Today, the stool antigen test is more accessible and has been examined in numerous studies. The sensitivity and specificity of the test have been reported above 90%. But in polyclonal stool antigen, test sensitivity and specificity will drop. However, this test is very useful, as a noninvasive test but have limitations, such as the effect of stool consistency in the test result, and clinical limitations such as bleeding. Monoclonal stool antigen kits are also very expensive (Yari *et al.,* 2016).

2.1.7.2.3. Serology

H. pylori infection usually results in the production of specific antibodies that can be measured in serum (Nisha, *et al.,* 2016). Measurements of the *H.pylori* IgG antibody are useful for monitoring treatment because of its high sensitivity and ease of performance (Abbas *et al.,* 2018). These serological tests have often been used to characterize risk factors, prevalence, incidence, and loss of infection in various settings where endoscopy and breath tests have not been readily available (Everhart *et al.,* 2002). Serological test is an example of passive tests detecting the markers of

exposure to *H. pylori* but not indicating the existence of active infection. Because antibodies against *H. pylori* may remain positive long after the eradication therapy, serological testing does not distinguish between current and past infections. This is the main drawback of this test method (Kesli *et al.*, 2010).

2.1.8. Treatment

The recommended treatment regimen for *H. pylori* infections are the first line regimen involving the usage of three drugs: Omeprazole, Clarithromycin, and Amoxicillin for 7-14 days or the use of Omeprazole, Clarithromycin, and Metronidazole for 7-14 days. The second line suggests the use of four drugs: Omeprazole, Bismuth subsalicylate, Tetracycline and Metronidazole for 14 days (Huwiage *et al.*, 2019). First-line therapy for *H. pylori* infection includes clarithromycin triple therapy (clarithromycin, proton pump inhibitor (PPI), and amoxicillin or metronidazole), bismuth quadruple therapy (bismuth salt, PPI, tetracycline, and metronidazole or amoxicillin), or concomitant therapy (clarithromycin, PPI, amoxicillin, and metronidazole) (Nguyen *et al.*, 2019). Patients should be asked about any previous antibiotic exposure(s) and this information should be taken into consideration when choosing a *H. pylori* treatment regimen. The main determinants of successful *H. pylori* eradication are the choice of regimen, the patient's adherence to a multi-drug regimen with frequent side-effects, and the sensitivity of the *H. pylori* strain to the combination of antibiotics administered (Chey *et al.*, 2017).

CHAPTER THREE

Materials and Methods

CHAPTER III

3. MATERIALS AND METHODS

3.1. Study design

This was descriptive; cross sectional, Hospital-based study.

3.2. Study area and duration

Study was carried out in Al-Ribat hospital and Al-Faisal hospital from September 2019 to February 2020 in Khartoum State.

3.3. Study population

The study population was patients with endoscopic findings of gastritis and ulcer. They attended to the Gastroenterology Department of Al-Faisal and Al-Ribat Hospitals for routine upper gastrointestinal endoscopy.

3.4. Sample size

The formula for calculating the gastric biopsy sample size:

$$(\frac{N = Z^2 \times P \times Q}{D^2})$$

N= number of samples. Z= confidence of interval= 1.96

P= prevalence of disease (50%). Q= (1- P)

D= percentage of error which equal .05%

□((N = 〖1.96〗 ^2 × .5 × .5)/ 〖.05〗 ^2)= 384

Limitation of sample size due to high cost, total of 80 gastric biopsy samples were collected in this study.

3.5. Sampling technique

This study was based on non-probability convenience sampling technique.

3.6. Data collection

Data were collected by direct interview (self-administration questionnaire) (Appendix).

3.7. Ethical approval

Approval to conduct this study was obtained from the College of Medical Laboratory Science, Sudan University for Science and Technology and verbal consent from the patients to collect the samples after they have been informed about the value of the study.

3.8. Laboratory Method:

3.8.1. Specimen collection:

The biopsies were collected by the Gastroenterologist and placed in sterile containers with Brain Heart Infusion broth as preservative.

3.8.2. Preservation of specimens:

The samples were frozen under -20ºC until used.

3.8.3. DNA extraction

Guanidine chloride method for extraction was used to extract *H.pylori* DNA from gastric biopsies.

3.8.3.1. Procedure

The biopsies embedded in transport media were washed 3 times with distilled water then centrifuged at 3000 rpm for 15-20 min. To the pellet were collected 400µl of lysis buffer, 5µl of proteinase K, 200 µl of guanidine chloride and 50 µl of ammonium (NH4) acetate were added, vortexed, and incubated at 37C° overnight or at C° for 2 hr. The mixture was cooled to room temperature, and then 400 µl 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 1 ml of cold absolute ethanol were added, shaked, and kept at -20 C° 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 1 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 C° overnight. The extracted DNA integrity was assessed by ethidium bromide stained agarose gel electrophoresis.

3.8.4. Polymerase Chain Reaction (PCR)

3.8.4.1. Procedure

3.8.4.1.1. Detection of 16_s rRNA

Amplification was performed in a final volume of 13 µL of PCR mixture (iNtRO Bio USA) containing forward and reveres primers of 16_s rRNA 0.5 µL for each primer (Rhenium, Jerusalem), 5 µL of Master mix, 5 µL of Distil water and 2 µL of each sample separately.

DNA amplification was carried out as follows:

1- Denaturation at 95°C for 5 minutes in the first cycle, followed by 37 cycles of denaturation at 95°C for 1minute.

2- Annealing for 1 minute at 60°C.

3- Extension for 1 minute at 72°C and for the last cycle the extension was increased to 5 minute to ensure complete extension of the amplified fragment.

3.8.4.1.2. Detection of iceA virulence gene

Positive samples for 16_s rRNA gene were carried for the detection of iceA virulence gene. Amplification was performed in a final volume of 13 µL of PCR mixture (iNtRO Bio USA) containing forward and reveres primers of iceA 0.5 µL of each primer (Rhenium, Jerusalem), 5 µL of Master mix, 5 µL of Distil water and 2 µL of each sample separately.

DNA amplification for the IceA gene was carried out in the same steps as in previous DNA amplification for the 16s rRNA detection.

3.8.4.2. Gel electrophoresis

Preparation of 10X Tris Base EDTA (TBE) buffer; Amount of 108g of Tris base was added to 55g of boric acid and 40ml of 0.5% EDTA and then dissolved into 1L of D.W. And for the preparation of 1X TBE buffer, 10ml of 10X is added to 90 ml D.W and mixed.

Preparation of ethidium bromide, 10mg of ethidium bromide was dissolved into 0.5ml D.W and kept into brown bottle.

For preparation of agarose gel amount of 1.5g of agarose powder were dissolved into 100 ml of 1X TBE and heated for 30 second then the mixture had been cooled, then 2µl of ethidium bromide was added, mixed well and poured in a casting tray that had been taped up appropriately and equipped with spacers and suitable comb to form wells, any bubbles were removed and the gel was allowed to sett at room temperature, after solidification the combs and the spacers were gently removed.

A total of 4µL amount of each PCR product were added to the wells to be separated in the prepared agarose gel. The amplicons were separated at 100 volt for 18 min in a 1.5% (wt/vol) agarose gel; bands were visualized under U.V transilluminator to detect the specific amplified products by comparing with 100 base pairs standard ladders (Fermentans, Germany). A result was considered positive when a band of size 532 bp for 16sRNA gene and 248bp for ICE A *H. pylori* specific gene were visible in the gel. Standard procedures for reducing contamination were strictly followed.

Table 3.1: Primer sequences used for detection of Helicobacter pylori genes and amplicon sizes

16srRNA	16sRNA-F 16sRNA-R	GCTAAGAGATCAGCCTAGTCC TGGCAATCAGCGTCAGGTAAT	532	(Hassan, 2019)
iceA	iceA-F iceA-R	GTGTTTTTAACCAAAGTATC CTATAGCCASTYTCTTTGCA	248	(Van Doorn *et al.,* 1998)

3.8.5. Data analysis

Data were analyzed by chi square test and frequencies using Statistical package for the social sciences (SPSS) version 18.

CHAPTER FOUR

Results

CHAPTER IV

RESULTS

4. Results

In this study, biopsies specimens from the antrum area in the stomach were collected from 80 patients, suffering from variety of gastric discomforts, including nausea, dysphagia and reflux symptoms. The endoscopic findings ranged between mainly gastritis (88.3%) and gastric ulcer (11.3%), the participants were 50% men and 50% women ranging from 15 to 73 years old with the age group 26 to 35 years old being the most. (figure 1 and figure 2)

Molecular detection of *H. pylori* was performed by PCR with primers (iceA, and 16S rRNA) to amplify a 248bp product for iceA gene, and a 532bp product for16S rRNA gene.

Only 11 biopsies showed positive for the 16_s RNA gene (13.8%), among those biopsies 7 (63.6%) were positive for the iceA gene. (Figure 3 and figure 4)

Subjects who had previous *H.pylori* infection were 85.5% and 14.5% of them came positive for 16_s rRNA gene, when our patients asked about the tests used to confirm their infection the majority divided between being not diagnosed before and the ICT blood test. (Table 4.1 and Table 4.2)

Positive 16_s rRNA and iceA gene were more frequent in males than females, and more in the age group between 26 and 35 years old in both genes. (Table 4.3 and Table 4.4)

Only 9(11.3%) patients showed gastric ulcer in the endoscopy which 4(44.4%) of those patients came positive for 16_s rRNA and 2(28.6%) for iceA gene. Table 4.5

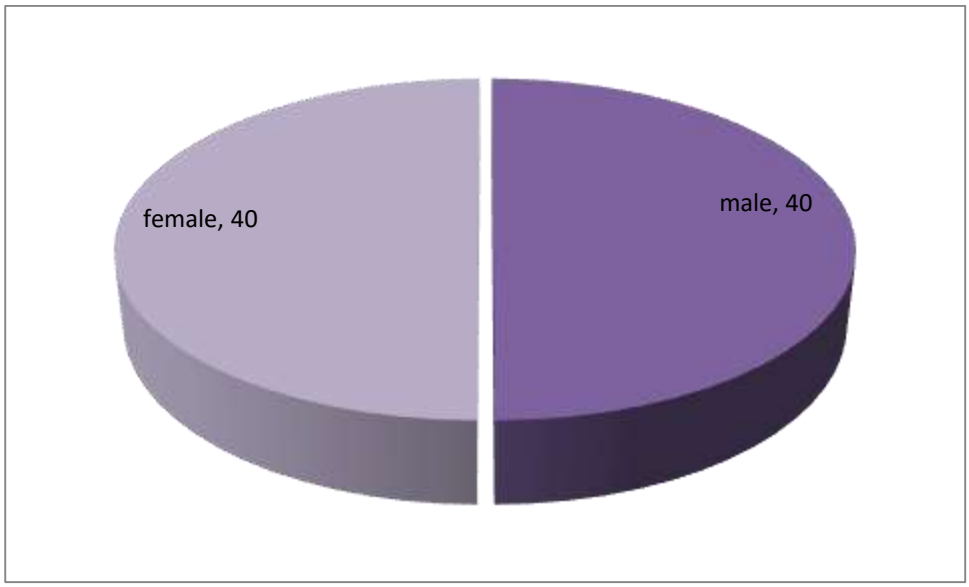

Fig 4-1: Gender distribution in the study subjects

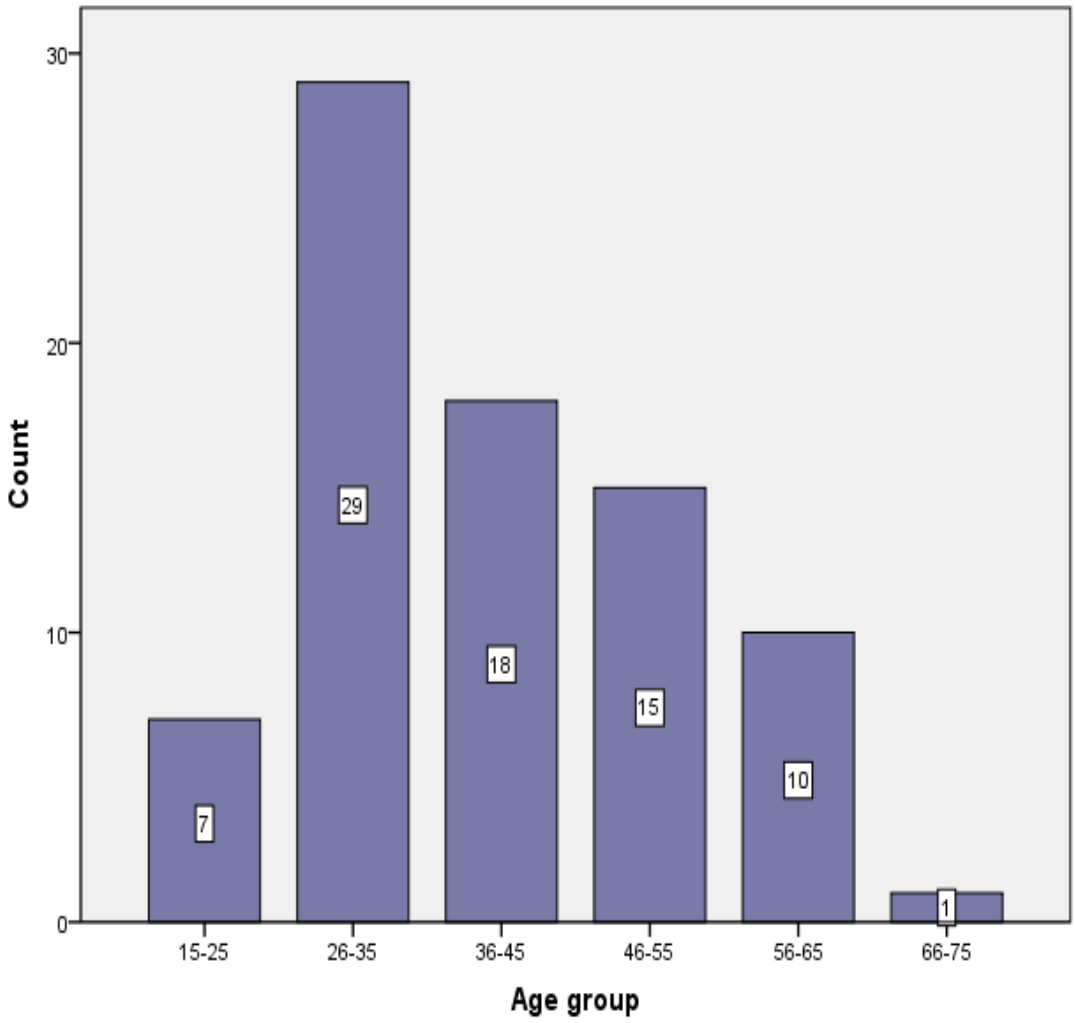

Fig 4-2: Age group wise distribution of participants in the study

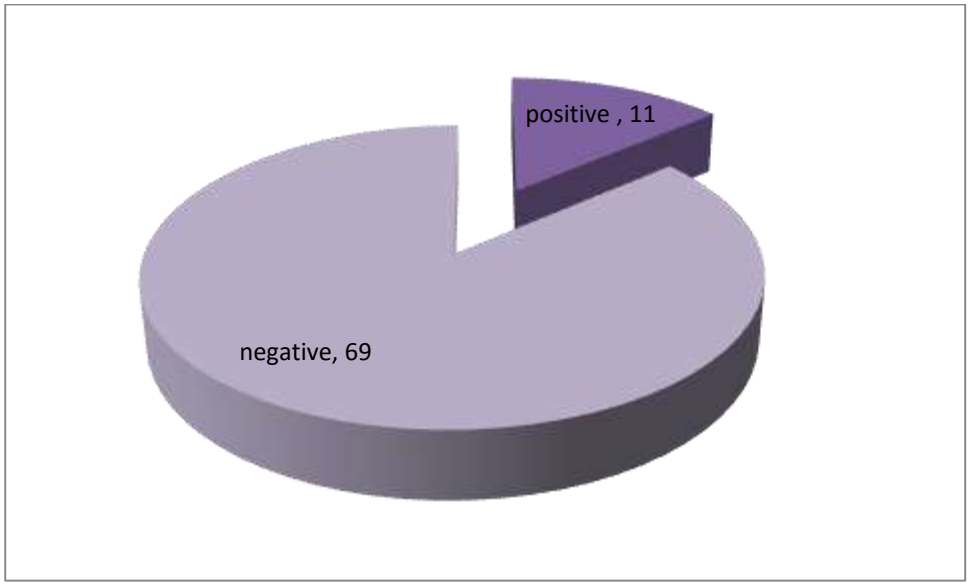

Fig 4-3: PCR results for the detection of *H.pylori* by 16_s rRNA gene

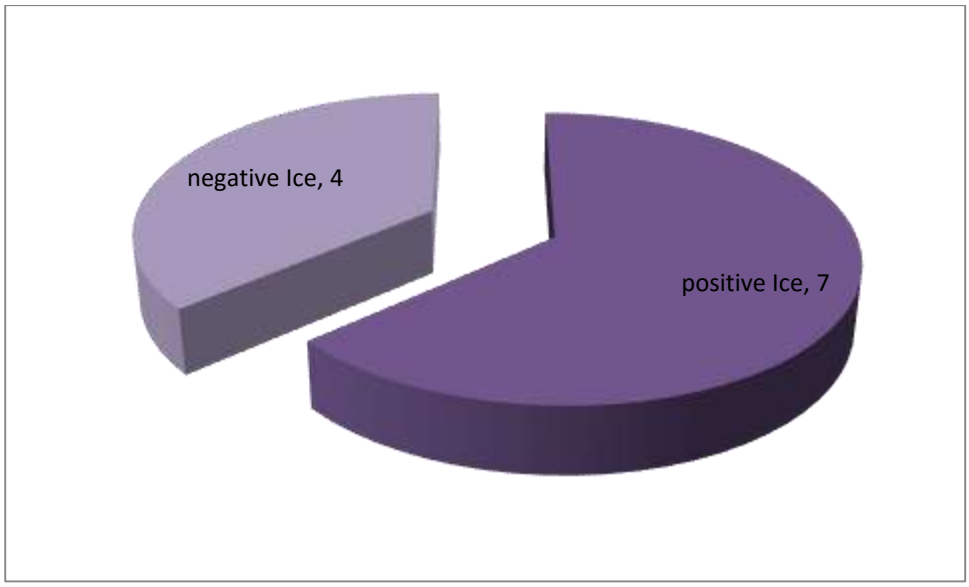

Fig 4-4: PCR results of iceA virulence gene among 16_s rRNA positive biopsies.

Table 4.1: Disruption of 16_s rRNA gene results among the patients with and without past history of *H.pylori* infection.

	Past infection	
	Yes	**No**
Positive *H.pylori*	8(14.5%)	3(12%)
Negative *H.pylori*	47(85.5 %)	22(88%)

Table 4.2: The diagnostic tests applied to the subjects in the past which have been used to confirm the past infection and their distribution in PCR results.

Positive *H.pylori*	**Negative *H.pylori***	**Tests used to diagnose previous *H.pylori infection***	**Positive iceA**	**Negative iceA**
2(18.2%)	23(33.3%)	**ICT blood test**	1(14.3%)	1(25%)
5(45.5%)	6(8.7%)	**Stool Ag**	4(57.1%)	1(25%)
3(27.3%)	22(31.9%)	**Not diagnosed**	2(28.6%)	1(25%)
0(0%)	10(14.5%)	**Not knowing**	0	0
1(9.1%)	8(11.6%)	**ICT blood test and stool Ag**	0	1(25%)

Table 4.3: PCR results of *H.pylori* genes between age groups.

Positive *H.pylori*	**Negative *H.pylori***	**Age group**	**Positive iceA**	**Negative iceA**
0(0%)	7(8.8 %)	**15-25**	0(0%)	0(0 %)
5(6.3 %)	24(30%)	**26-35**	4(36.4 %)	1(9.1%)
2(2.5 %)	16(20 %)	**36-45**	0(0%)	2(18.2%)
2(2.5%)	13(16.3 %)	**46-55**	2(18 %)	0(0%)
2(2.5%)	8(10%)	**56-65**	1(9.1 %)	1(9.1 %)
0(0%)	1(1.3 %)	**66-75**	0(0%)	0(0 %)

Table 4.4: Genes PCR results between both male and female subjects.

	Gender	
	Male	**Female**
Positive *H.pylori*	6(54.5 %)	5(45.5 %)
Negative *H.pylori*	34(49.3 %)	35(50.7 %)
Positive iceA	5(71.4 %)	2(28.6%)
Negative iceA	1(25 %)	3(75%)

Table 4.5: Gastric ulcer findings between positive and negative results for both 16_s rRNA (*p* value= .005) and iceA gene (*p* value=.129).

	Gastric ulcer	
	Yes	**No**
Positive *H.pylori*	4(44.4%)	7(10%)
Negative *H.pylori*	5(55.5 %)	64(90 %)
Positive iceA	2(28.6 %)	5(71.4%)
Negative iceA	2(50%)	2(%50)

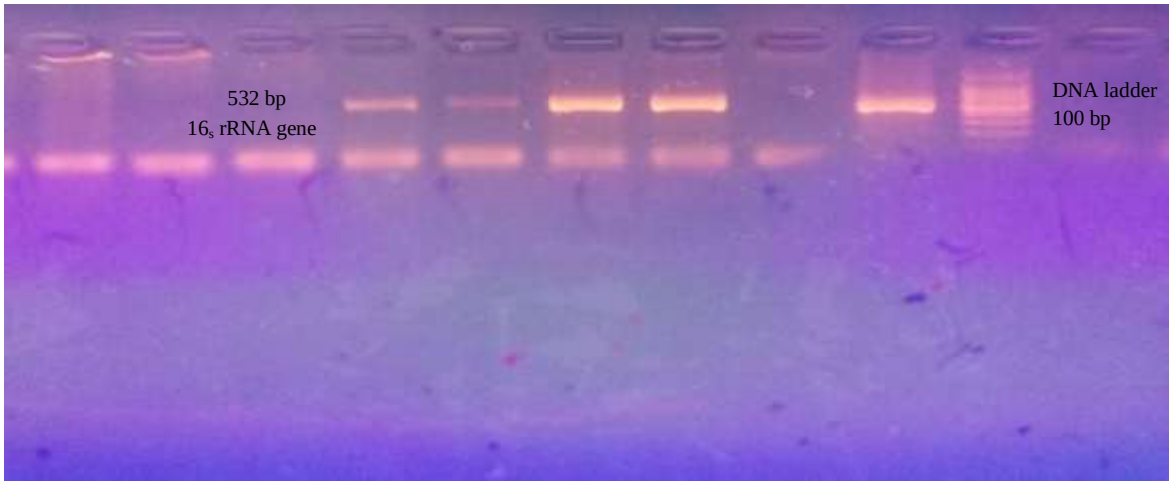

Fig 4-5: Detection of 16_s rRNA gene by gel electrophoresis

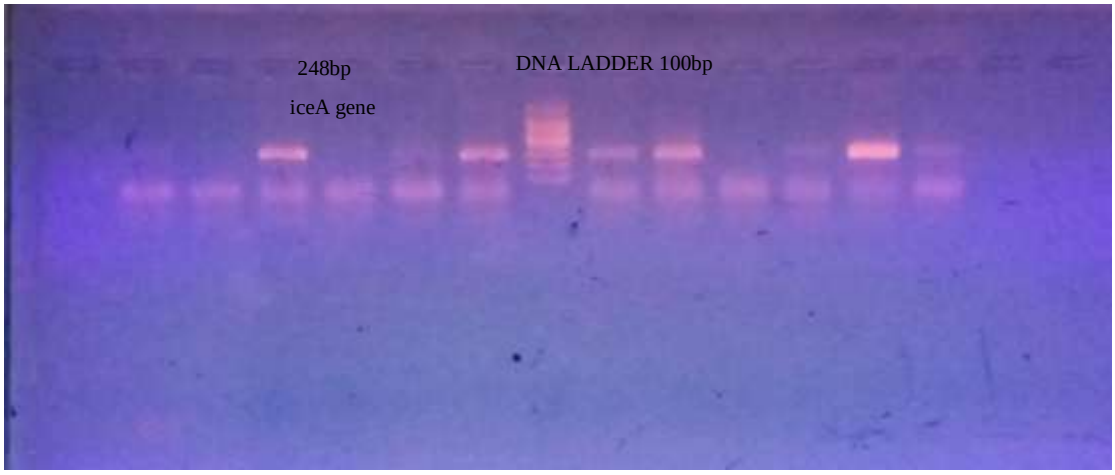

Fig 4-6: Detection of iceA gene by gel electrophoresis

CHAPTER FIVE

Discussion, Conclusion and Recommendations

CHAPTER V

Discussion, Conclusion and Recommendation

5.1. Discussion

H. pylori cause chronic gastritis and has been associated with several serious diseases of the gastrointestinal tract, including duodenal ulcer and gastric cancer. Since its "discovery" in 1982 by Warren and Marshall, *H. pylori* has been the topic of extensive research (Brown, 2000). The present study has focused on detecting the virulence gene iceA of *H. pylori* from gastric biopsy specimens from patients with upper gastrointestinal diseases and their relationship with clinical status. *H. pylori* was detected by the presence of the genes 16_s rRNA and then analyzed for the presence of iceA virulence gene.

In this study polymerase chain reaction was used to detect presence of *H. pylori* DNA in eighty biopsy specimens from patients with endoscopic findings mainly of gastritis, and the results showed that 63.6% was found to be positive for iceA gene, and 13.8% for the 16_s rRNA gene.

Ahmad and Mahdi (2020) in Iraq found that most of the positive cases (60%) were males, the aged distribution for positive cases were mostly between 25-35, the prevalence of genes were 10 (100%) for the region of 16_S rRNA gene and 7 (70%) for the region of iceA1 gene of *H. pylori* which were amplified by PCR, as in this study there is resemblance in the aspects of the percentage of positive results of iceA gene and the majority of positive results were males and were in age between 26 and 35 years old.

Another study was conducted in Turkey in 87 patients with functional dysplasia and duodenal ulcer reported in their results about 25.3% were positive for iceA gene and their results showed no relation between functional dysplasia and duodenal ulcer with iceA gene (Baglan *et al.,* 2006). Comparing our results with this study we have not found any duodenal of functional dysplasia in our subjects.

While in the direction of the gastric ulcer frequency in the positive iceA gene results, a study was performed in England in 2005 in gastric ulcer patients found iceA gene was predominant as in 57% of the total samples among other genes they investigated (Kauser *et al.,* 2005), though our results presented only 28.6% of the positive iceA gene in gastric ulcer patients.

Whereas in Ashour *et al.* (2001) in Brazil, they investigate by PCR the frequency of iceA alleles in Helicobacter pylori strains isolated from 142 patients with gastritis, duodenal ulcer, or gastric adenocarcinoma. iceA was identified in bacterium samples obtained from all patients. Eleven (7.7%) of them were infected with multiple strains. Among the patients with nonmixed infection, iceA2 allele was detected in 118 (90.1%). iceA2 allele was associated with ulcer (*P* value= 0.02). The gene was also more frequent in strains obtained from males (*P* value= 0.02).

In Saudi Arabia one hundred twenty-eight gastric biopsy specimens were positive in genotyping PCRs. The iceA1 and iceA2 genes were detected in rates of 42.2% (54/128), 32.8% (42/128), respectively. The iceA genes were significantly associated with gastritis and gastric ulcer (Akeel *et al.,* 2019), while in this study we only found a significant association between 16_s rRNA and gastric ulcer and no significant association with the iceA virulence gene and gastric ulcer.

A study conducted in Egypt in 2018 present results of 46.29% from their studied patients were iceA gene positive also among the patients with gastritis 16.7% were iceA1/A2 positive, while the percentages of iceA1/A2 positive in peptic ulcer patients were 18.8% (Abu-Taleb *et al.,* 2018).

A recent study in 2020 in Pakistan revealed in their results that iceA gene was 24.5% and it was the least common among the other genes that was investigated. Peptic ulcer disease and gastritis were higher among patients having iceA positive strains as compared to other genes patients. Males were more affected 118 (60.2%) as compare to females 78(39.8%). ice-A positive strains were the significant cause of gastritis, (*p* value= 0.001), Peptic ulcer disease was significantly higher among patients having iceA positive strains, (*p* value= 0.001) (Bajwa *et al.,* 2020).

5.2. Conclusion

H.pylori and its iceA virulence gene were found to be present in small percent in Sudanese patients with gastric discomfort in Khartoum State. The presence of *H.*pylori can be related to gastric ulcer while iceA virulence gene was not related to gastric ulcer by the results.

5.3. Recommendations

1- Conduct an extended research with lager sample size to evaluate and correlate the *H.pylori* results with the clinical outcomes.

2- Implementation of PCR as confirmatory test to be applied for positive *H.pylori* results done by other tests.

3- Set an educational program about *H.pylori*, its risks and the importance of early and complete treatment in the internal medicine clinic for the patients.

REFERENCES

Abbas, M., Sharif, F.A., Osman, S.M., Osman, A.M., ElSanousi, S.M. and Magzoub, M. (2018). Prevalence and associated symptoms of *Helicobacter pylori* infection among schoolchildren in Kassala State, East of Sudan. *Interdisciplinary Perspectives on Infectious Diseases***.**

Abd Al Rahem, S.A. and Elhag, W.I. (2018). Molecular detection of *Helicobacter pylori* in drinking water in Khartoum State (Sudan*). African Journal of Medical Sciences.* **3** (5)

Abdallah,T.M.M. (2018). Phenotypic and molecular detection of *Helicobacter pylori* in Fresh Milk Produced from Various Farms in Khartoum State. master's thesis, Sudan University of Science and Technology, Khartoum

Abu-Taleb, A.M.F., Abdelattef, R.S., Abdel-Hady, A.A., Omran, F.H., El-korashi, L.A., El-hady, H.A. and El-Gebaly, A.M. (2018). Prevalence of *Helicobacter pylori* cagA and iceA Genes and Their Association with Gastrointestinal Diseases. *International Journal of Microbiology.*

Ahmad, F.K., Aladily, T.N., Altamimi, M., Ajour, M., Alsaber, N. and Rawashdeh, M. (2020). Helicobacter pylori Prevalence and Impact: A Histology-Based Report About Children from an Endemic Country. *International Journal of General Medicine***. 13:** 207–214

Ahmad, I.S and Mahdi, N.B. (2020). Determination of some virulence genes in *Helicobacter pylori* isolated from dyspeptic patients by PCR in Kirkuk City, Iraq. *Eurasia J Biosci.* **14:**1727-1732

Akeel, M., Shehata, A., Elhafey, A., Elmakki, E., Aboshouk, T., Ageely, H., and Mahfouz, M. (2019). *Helicobacter pylori* vacA, cagA, and iceA genotypes from southwestern region, Saudi Arabia: distribution and association with clinical outcomes and histopathological changes. *BMC Gastroenterology.* **19**(1):1-11

AL Kirdy, F., Rajab, M., and El-Rifai, N. (2020). *Helicobacter pylori* infection: clinical, endoscopic, and histological findings in Lebanese pediatric patients. *International Journal of Pediatrics.*

Al-Thahab, A. and Al-Awsi, G. (2018). Detection Of *Helicobacter Pylori* In Pregnant Women By Stool Culture Method. *Biochem. Cell. Arch***. 18**(1): 49-54

Anagnostopoulos, G.K., Yao, K., Kaye, P., Fogden, E., Fortun, P., Shonde, A., Foley, S., Sunil, S., Atherton, J.J., Hawkey, C. and Ragunath, R. (2007). High−resolution magnification endoscopy can reliably identify normal gastric mucosa, *Helicobacter pylori*−associated gastritis, and gastric atrophy. *Endoscopy.* **39**(3): 202-207

Ashour, A.A.R., Collares, G.B., Mendes, E.N., de Gusmăo, V.R., Queiroz, D.M.M., Magalhães, P.P., de Carvalho, A.S.T., de Oliveira, C.A., Nogueira, A.MM.F., Rocha, G.A., and Rocha A.M.C. (2001). iceA Genotypes of *Helicobacter pylori* strains isolated from Brazilian children and adults. *Journal of clinical microbiology.* **39**(5): 1749-1750

Atherton, J.C. and Spiller, R.C. (1994). The urea breath test for *Helicobacter pylori*. *Gut.* **35**(6): 723-725

Baglan, P.H., Bozdayi, G., Ozkan, M., Ahmed, k., Bozdayi, A.M. and Ozden, A. (2006). Clarithromycin resistance prevalence and iceA gene status in *Helicobacter pylori* clinical isolates in Turkish patients with duodenal ulcer and functional dyspepsia. *J microbial.* **44**(4): 409-416

Bajwa, M.A., Idrees, M., and Maheshwari, P.M. (2020). Association of dupA, iceA, homB genes of *Helicobacter pylori* with Gastritis, Peptic Ulcer Disease and Gastric Cancer. *Journal of Pharmaceutical Research.* **32**(25):1-6

Bakhti, S.Z., Raei, N., Latifi-Navid, S., Zahri, S. and Yazdanbod, A. (2019). Inverse relationship between *Helicobacter pylori* cagG pathogenicity island and risk of gastric ulcer. *British Journal of Biomedical Science.* **76**(2): 95-97

Bhakta, D., Graham, D.Y., Chan, J. and El-Serag, H., (2018). Lessons from using culture guided treatment after referral for multiple treatment failures for *Helicobacter pylori* infection. *Clin Gastroenterol Hepatol*. **16**(9): 1531-1532

Brandt, S., Kwok, T., Hartig, R., Kőnig, W. and Backert, S. (2005). NF-κB activation and potentiation of proinflammatory responses by the *Helicobacter pylori* CagA protein. *PNAS.* **102**(26): 9300-9305

Breckan, R.K., Paulssen, E.J., Asfeldt, A.M., Kvamme, J.M., Straume, B. and Florholmen, J. (2016). The all-age prevalence of *Helicobacter pylori* infection and potential transmission routes. a population-based study. *Helicobacter. Wiley Online Library.* **21**(6)

Brown, L.M. (2000). *Helicobacter pylori*: Epidemiology and Transmission. *Epidemiol Rev.* **22** (2)

Bui, D., Brown, H.E., Harris, R.B. and Oren, E. (2016). Serologic Evidence for Fecal–Oral Transmission of *Helicobacter pylori***.** *Am. J. Trop. Med. Hyg***. 94**(1): 82–88

Bullock, K.K., Shaffer, C.L., Brooks, A.W., Secka, O., Forsyth, M.H., McClain, M.S. and Cover, T.L. (2017). Genetic signatures for *Helicobacter pylori* strains of West African origin. *PLoS ONE.* **12**(11)

Burucoa, C., and Axon, A. (2017). Epidemiology of *Helicobacter pylori* infection. *Helicobacter.* **22**(1)

Chakravorty, M., Ghosh, A., Choudhury, A., Santra, A., Hembrum, J. and Roychoudhury, S. (2006). Interaction between IL1B gene promoter polymorphisms in determining susceptibility to *Helicobacter pylori* associated duodenal ulcer. *HUMAN MUTATION.* **27**(5): 411-419

Chey, W.D., Leontiadis, G.I., Howden, C.W. and Moss, S.F. (2017). ACG clinical guideline: treatment of *Helicobacter pylori* infection. *Am J Gastroenterol.* **112**: 212-238

Covacci, A., Censini, S., Bugnoli, M., Petracca, R., Burroni, D., Macchia, G., Massone, A., Papinit, E., Xiang, Z., Figurat, N. and Rappuoli, R. (1993). Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc. Natl. Acad. Sci*. **90**(12): 5791-5795

Crabtree, J.E., Shallcross, T.M., Heatley, R.V. and Wyatt, J.I. (1991). Mucosal tumour necrosis factor and interleukin-6 in patients with *Helicobacter pylori* associated gastritis. *Gut.* **32**(12):1473-1477

Dahlén, G., Hassan, H., Blomqvist,S. and Carlén, A. (2018). Rapid urease test (RUT) for evaluation of urease activity in oral bacteria in vitro and in supragingival dental plaque ex vivo. *BMC Oral Health.* **18**(1): 1-7

Dalla Nora M., Hörner R., De Carli, D.M., Rocha, M.P., Araujo, A.F and Fagundes, R.B. (2016). Is the Immunocromatographical antigen test effective for primary diagnosis of *Helicobacter pylori* infection in dyspeptic patients. *Arq Gastroenterol***. 53**(4).

Everhart, J.E., Kruszon-Moran, D. and Perez-Perez, G. (2002). Reliability of *Helicobacter pylori* and CagA serological assays. *Clinical and diagnostic laboratory immunology.* **9**(2)

Fan, CC., Chen, CH., Chou, C., Kao, TY., Cheng, A.N., Lee, A.YL. and Kuo, CL. (2020). A time-saving–modified Giemsa stain is a better diagnostic method of *Helicobacter pylori* infection compared with the rapid urease test. *J Clin Lab Anal.* **34**(4)

González, I., González, L., Rojas, A., Rodríguez, B.L., Romero, J., Reyes, O., Morales, E., Alonso, J., Perez-Castro, R., Sabatier, C.A., Valdés, E., Torres, L.E. and Cofre, C. (2019). Pathogenic potential of *Helicobacter pylori* strains can explain differences in H. pylori associated diseases rates from Chile and Cuba. *Bangladesh Journal of Medical Science*. **18(**3): 577-585

Graham, D.Y., Lew, G.M., Klein, P.D., Evans, D.G., Evans, Jr., D.J., Saeed, Z.A. and Malaty, H.M. (1992). Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. *Annals of Internal Medicine.* **116**(9): 705-708.

Hassan, F.A. (2019). Molecular Detection of *Helicobacter pylori* UreC and 16sRNA genes in Saliva from Stool Antigen Positive Patients in Saad Rashwan Medical Center in Khartoum State. master's thesis, Sudan University of Science and Technology College, Khartoum.

Huwiage, G.M., Nami, A.A. and Akadh, A.H. (2019). Stool antigen (HpSA) test in detection of Helicobacter pylori in Tripoli Libya. *International Journal of Medicine and Medical Research.* **5**(2): 5-14

Ilie, M., Popa, M., Chifiriuc, M.C., Baltac, A., Constantinescu, G. and Tãnãsescu, C. (2011). *Helicobacter pylori* cultivation from gastric biopsies and susceptibility to antibiotics used in empirical therapy. *Roum Arch Microbiol Immunol.* **70**(2): 60-4

Israel, D.A., Salama, N., Arnold, C.N., Moss, S.F., Ando,T., Wirth, H-P., Tham, K.T., Camorlinga, M., Blaser, M.J., Falkow, S. and Peek, Jr. R.M.(2001). *Helicobacter pylori* strain-specific difference in genetic content identified by microarray, influence host inflammatory responses. *The Journal of Clinical Investigation*: *J. Clin. Invest*. **107**(5): 611–620

Israel, D.M. and Hassall, E. (1993). Treatment and long-term follow-up of *Helicobacter pylori*-associated duodenal ulcer disease in children. *The Journal of Pediatrics*. **123**(1): 53-58

Gisbert, J.P. and Pajares, J.M. (2004). 13C-urea breath test in the diagnosis of Helicobacter pylori infection – a critical review. *Aliment Pharmacol Ther.* **20**: 1001–1017

Jehanne, Q., Bénéjat, L., Mégraud, F., Emilie Bessède, E. and Lehours, P. (2020). Evaluation of the Allplex™ *H pylori* and ClariR PCR Assay for *Helicobacter pylori* detection on gastric biopsies. *Helicobacter.* **25**(4)

Jones D.M. and Curry A. (1990). The genesis of coccal forms of *Helicohacter pylori*. *Gastritis and Peptic Ulcers.* 29-37

Katelaris, P. H., Forbes, G. M., Talley, N. J. & Crotty, B. (2002). Randomized comparison of quadruple and triple therapies for *Helicobacter pylori* eradication: The QUADRATE study. *Gastroenterology*. **123**(6): 1763–1769

Kauser, F., Hussain, M.A., Ahmed, I., Srinivas, S., Devi, S.M., Majeed, A.A., Rao, K.R., Khan, A.A., Sechi, L.A. and Ahmed, N. (2005). Comparative genomics of *Helicobacter pylori* isolates recovered from ulcer disease patients in England. *BMC Microbiology.* **5**(32)

Kavermann, H., Burns, B.P., Angermüller, K., Odenbreit,S., Fischer,W., Melchers, K. and Haas, R. (2003). Identification and characterization of *Helicobacter pylori* genes essential for gastric colonization. *J.Exp.Med*. **197**(7): 813-822

Kesli, R., Gokturk, H.S., Erbayrak, M., Karabagli, P. and Terzi, T. (2010). Comparison of the diagnostic values of the 3 different stool antigen tests for the noninvasive diagnosis of *Helicobacter pylori* Infection**.** *Journal of Investigative Medicine.* **58**(8)

Khetrapal, S., Shukla, S., Rana, S., Jairajpuri, Z.S. and Acharya, S. (2019). Utility of Rapid urease test in diagnosis of *Helicobacter pylori* infection. *International Journal of Medical Science and Clinical Invention***. 6**(4): 4417-4422

Kim, S.-Y., Woo, C.W., Lee, Y.-M., Son, B.R. , Kim, J.W., Chae, H.B., Youn, S.J. and Park, S.M. **(2001)** Genotyping CagA, VacA Subtybe, IceA, and BabA of *Helicobacter pylori* isolates from Korean patients, and their association with gastroduodenal diseases. *J Korean Med Sci.* **16**(5): 579-584

Krzyżek, P. and Gościniak, G. (2018). Morphology of *Helicobacter pylori* as a result of peptidoglycan and cytoskeleton rearrangements. *Gastroenterology Rev* . **13***(3):182-195*

Kusters, J. G., Van Vliet, A.H.M., Ernst J. and Kuipers, E. J. (2006). Pathogenesis of *Helicobacter pylori* Infection. *Clin Microbiol Rev.* **19**(3): 449-490

Levinson, W. (2016). Review of Medical Microbiology and Immunology. 14th edition. New York P: 162

Lynch, D.A.F., Mapstone, N.P., Clarke, A.T.M., Sobala, G.M., Jackson, P., Morrison, L., Dixon, M.F., Quirke, P. and Axon, A.T.R. (1995). Cell proliferation in *Helicobacterpylori* associated gastritis and the effect of eradication therapy. *Gut.* **36**(3): 346-350

Milani, M., Moaddab, Y. and Sharifi, Y. (2019). One piece biopsy for both rapid urease test and cultivation of *Helicobacter pylori*. *Journal of Microbiological Methods.* **164**(105674)

Moodley, Y., Linz, B., Bond, R.P., Nieuwoudt, M., Soodyall, H., Schlebusch, C.M., Bernhőft, S., Hale, J., Suebaum, S., Mugisha, L., Van der Merwe, S.W. and Achtman, M. (2012). Age of the association between *Helicobacter pylori* and men. *PLoS pathog.* **8**(5)

Nguyen, C.T., Davis, K.A., Nisly, S.A. and Li, J. (2019). Treatment of *Helicobacter pylori* in special patient populations. *Pharmacotherapy*. **39**(10).

Nisha, K.J., Nandakumar,K., Shenoy, K.T. and Janam, P. (2016). Periodontal disease and *Helicobacter pylori* infection: a community-based study using serology and rapid urease test. *Journal of Investigative and Clinical Dentistry*.**7**: 37–45

Ramadan, R.A., Zaki, M.A., Ooda, S.A., Abo Khalifa, H.M. and Ragab, W.S. (2020). Comparison of Serum Trefoil Factor-3 to endoscopy in diagnosing *Helicobacter Pylori* associated gastric ulcer. *Asian Pacific Journal of Cancer Prevention.***21**(7)

Ribeiro, M.L., Godoy, A.P.O., Benvengo, Y.H.B., Mendonça, S. and Pedrazzoli Jr.S. (2003). Clinical relevance of the cagA, vacA and iceA genotypes of *Helicobacter pylori* in Brazilian clinical isolates. *FEMS Immunology δ Medical Microbiology.* **36**(3): 181-185

Rugge, M., Genta, R.M., Di Mario, F., El-Omar, E.M., El-Serag, H.B., Fassan, M., Hunt, R.H., Kuipers, E.J., Malfertheiner, P., Sugano, K. and Graham, D.Y. (2017). Gastric Cancer as Preventable Disease. *Clinical Gastroenterology and Hepatology*. **5**(23)

Salih, K.M.A, Elfaki, O.A, Hamid, Y.H. M., Eldouch, W.M.A., Diab, M. and Abdelgadir, S.O. (2017). Prevalence of *Helicobacter pylori* among Sudanese children admitted to specialized children hospital. *Sudan J Paediatr.* **17**(1): 14–18

Sjomina, O. Pavlova, J. Niv, Y. and Leja, M. (2018). Epidemiology of *Helicobacter pylori* infection**.** *Helicobacter.* **23**(51)

Stevenson, T.H., Castillo, A., Lucia, L.M. and Acuff, G.R. (2000). Growth of *Helicobacter pylori* in various liquid and plating media. *The Society for Applied Microbiology, Letters in Applied Microbiology.* **30**: 192-196

Suerbaum, S. (2000). Genetic variability within *Helicobacter pylori.* *Int.J.Med.Microbial*. **290**(2): 175-181

Suerbaum, S. and Achtman, M. (1999). Evolution of *Helicobacter pylori:* the role of recombination. *Trends in microbiology.* **7**(5): 182-184.

Sugimoto, M., Wu, JY., Abudayyeh, S., Hoffman, J., Brahem, H., Al-Khatib, K., Yamaoka, Y. and Graham, D.Y. (2009). Unreliability of results of PCR detection of *Helicobacter pylori* in clinical or environmental samples. *Journal of clinical microbiology*. **47**(3): 738–742

Tille, P. M. (2014). Bailey and Scott's Diagnostic Microbiology. 13th edition. Missouri P: 419-420

Tongtawee, T., Kaewpitoon, S., Kaewpitoon, N., Dechsukhum, C., Leeanansaksiri, W., Loyd, R.A., Matrakool, L. and Panpimanmas, S. (2016). . Characteristics and risk factors of *Helicobacter pylori* associated gastritis: A prospective cross-sectional study in Northeast Thailand. *Gastroenterology Research and Practice.*

Uotani, T. and Graham, D.Y. (2015). Diagnosis of *Helicobacter pylori* using the rapid urease test. *Ann Transl Med.* **3**(1):9.

Van Doorn, LJ., Figueiredo, C., Sanna, R., Plaisier, A., Schneeberger, P., D Boer, W., and Quint, W. (1998). Clinical relevance of the cagA, vacA and iceA status of *Helicobacter pylori. Gastroenterterology.* **115**(1): 58-66

Wen, G., Deng, S., Song, W., Jin, H., Xu, J., Liu, X., Xie, R., Song, P. and Tuo, B. (2018). *Helicobacter pylori* infection downregulates duodenal CFTR and SLC26A6 expressions through TGFβ signaling pathway. *BMC Microbiology.* **18**(87)

Westblom, T.U., Madan, E. and Bentley, R. (1991). Egg Yolk Emulsion Agar, a new medium for the cultivation of *Helicobacter pylori***.** *Journal of microbiology.* **29**(4): 819-821

Xia, H., English, L., Keane, C., and O'Morain, C. (1993). Enhanced cultivation of *Helicobacterpylori* in liquid media**.** *Clin Pathol*. **46**: 750-753

Xia, H.X., English, L., Keane, C.T. and O'Morain, C.A. (1993). Enhanced cultivation of *Helicobacter pylori* in liquid media. *Clin Pathol.* **46**(8): 750-753

Yamaoka, Y., Kodama, T., Gutierrez, O., Kim, J.G., Kashima, K. and David, Y. (1999). Relationship between *Helicobacter pylori* iceA, cagA, and vacA status and

clinical outcome: studies in four different countries**.** *Journal of clinical microbiology.* **37**(7): 2274-2279

Yamazaki, S., Yamakawa, A., Okuda, T., Ohtani, M., Suto, H., Ito, Y., Yamazaki, Y., Keida, Y., Higashi, H., Hatakeyama, M. and Azuma, T. (2005) Distinct diversity of vacA, cagA, and cagE genes of *Helicobacter pylori* associated with peptic ulcer in Japan. J*ournal of clinical microbiology*. **43**(8): 3906-3916

Yari, F., Abiri, R., Aryan, E., Jouybari, T.A., Navabi, J. and Alvandi, A. (2016). Loop-Mediated Isothermal Amplification as fast method for the detection of *H.pylori J. Clin. Lab. Anal*. **30**: 464–470

Zamani, M., Amin Vahedi, A., Zahra Maghdouri, Z. and Shokri-Shirvani, J. (2017). Role of food in environmental transmission of *Helicobacter pylori*. *Caspian J Intern Med*. **8**(3): 146-152

APPENDIXES

Questionnaire

Demographic data: No. " "

- Name: _____Tel number:_____
- Age: _____
- Sex: □ Male
  □ Female
- Residence: _____
- Date.....ID number.....
- Symptoms:
  - Abdominal pain □Yes □No
  - Nausea □Yes □No
  - Recurrent Vomiting □Yes □No
  - Dysphagia
  - Difficulty in swallowing
  - Reflux symptoms: □Yes □No
  - Acidity
  - Heart pain
  - Regurgitation □Yes □No
  - Weght loss □Yes □No
  - Dyspepsia □Yes □No
  -
  - Family history of gastric cancer
    □Yes □No
- Result of RUT (if done) .....
- Past history of H.pylori □Yes □No

If yes diagnosed by:

□ ICT blood test □ Stool Ag □ UBT □ I dont know

- Type of treatment:

□ Triple □ Quadruple

□ Others: _____

- Type of antibiotic.....
- Frequency of treatment: _____
- Endoscopic findings:
- Gastritis (. . . .) Gastric ulcer (. . . .) Duodenal ulcer
- (. . . . . .) Gastric mass or abnormal tissue(. . . .) Gastric irrosion(. . .)
  Unexplained anamia(. . .) Idiopathic thrombocytopenic purpura
- Others
- Location of biopsy:
- Antrum (. .) . . . . Body. (. . .) . . . Fundus. (. . .) Incisura(. . .)

Fig 7: PCR machine

Fig 8: Hot air oven

Fig 9: Gel electrophoresis system

Fig 10: centrifuge

Fig 11: Sensitive balance