



Sudan University of Science and Technology



College of Graduate Studies

**Molecular Detection of *Helicobacter pylori* *GLmM* Gene
among Gastritis and Duodenitis Patients in Albogaa
Specialized Hospital - Omdurman**

الكشف الجزيئي عن البكتريا الحلزونية البوابية في جين *GLmM* في المرضى الذين
يعانون من إتهاب المعدة والإثني عشر في مستشفى البقعة التخصصي - أم درمان

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By

Abdelhamid Gozali Abdelhamid Osman

B.Sc of Medical laboratory, Omdurman Islamic University, 2016.

Supervisor:

Dr. Hisham Nouraldyem Altayb

Assistant Professor of Molecular Biology

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

الآيَة

(وَإِذَا مَرَضْتُ فَبُهِتَ الَّذِينَ يَلْمِئُونَ
بِئْسَ مَا يَشْفِيهِمْ)

صدق الله العظيم

سورة الشعراء الآية ٨٠

DEDICATION

To the candle which burn to light our life (Our Mothers)

To the ones who we alive for making their dream comes true (Our Father)

To specials who inspire and give us meaning of being (Our Brothers, Sisters and Friends)

Special dedication to all who love us.

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ABSTRACT

Helicobacter pylori has a significant pathogenic role in gastritis and peptic ulcer disease, as well as in gastric adenocarcinoma, mucosa-associated lymphoid tissue (MALTS) lymphoma and possibly cardiovascular disease.

This study aimed to detect *Helicobacter pylori* *GlmM* gene by using polymerase chain reaction (PCR), in patients with gastritis and duodenitis attending to the Albogaa specialize Hospital in Omdurman City during the period from April 2018 to January, 2019.

A total of **50** patients (n=**50**) of both gender and ages ranging from 19 to 81 years old were included in this descriptive cross-sectional hospital based study. Endoscopy biopsy samples were collected. Specimens were preserved and transported in phosphate buffer saline. Genomic DNA was extracted using Guanidine hydrochloride protocol and examined for *Helicobacter pylori* *GLmM* gene by PCR technique. Amplified PCR products were separated by agarose gel electrophoresis. The gel was viewed and photographed on Gel-Documentation System.

Out of the 50 samples examined, 9 (18%) were positive for *Helicobacter pylori* *GlmM* gene, out of them: 3 (12%) were from male and 6 (24%) from female. 7 (21.2%) of them have gastritis, 1 (20%) of them have gastric ulcer, 1 (12.5%) of them have other diagnosis. In patients age group less than 30 years, 5 (31.3%) were positive for *Helicobacter pylori* *GLmM* gene, 30-60 years 2 (14.3%) were positive and age group more than 60 years 2 (11.1%) of them were positive gene detection.

The percentage of *H. pylori* detected by GlmM gene is low when compared to other studies.

The finding of this study indicates that there was no significant association between gastritis and duodenitis with *H. pylori* infection.

مستخلص الإطروحة

للبكتريا الحلزونية البوابية دورا هام في مرض المعدة و القرحة الهضمية بالإضافة إلى الغدة الكظرية المعوية والغشاء المخاطي للمفاوي (MALTS) وربما مرض القلب والأوعية الدموية.

الهدف الرئيس من هذه الدراسة هو الكشف عن جين *GLmM* للبكتيريا الحلزونية البوابية باستخدام جهاز تفاعل البلمرة المتسلسل في المرضى الذين يعانون من التهاب المعدة والاثني عشر الذين راجعوا العيادات الخارجية لمستشفى البقعه التخصصي بمدينة أم درمان في الفترة من أبريل ٢٠١٨ الي يناير ٢٠١٩.

شملت هذه الدراسة الوصفية المقطعية ٥٠ مريضاً من الجنسين تتراوح اعمارهم بين ١٩ إلى ٨١. تم جمع عينات خزعات المعده والاثني عشر باستخدام المنظار من المشاركين بالدراسة. تم حفظ العينات ونقلها في محلول ملح الفوسفات. تم إستخراج الحمض النووي الجيني يدويا باستخدام طريقة الغوانيديين هيدروكلوريد. تم اختبار العينات لمعرفة احتوائها علي جين *GLmM* للبكتريا الحلزونية البوابية باستخدام تفاعل البلمرة المتسلسل. ونواتج التفاعل تم عرضها عن طريق نظام الجل.

من مجموع خمسون عينة فحصت, تسعة منهم (١٨%) اعطو نتائج ايجابية لاختبار الحمض النووي جين *GLmM* للبكتريا الحلزونية البوابية. ثلاثة (١٢%) من البكتريا الحلزونية البوابية كانت ايجابية في الذكور و ستة منهم (٢٤%) كانوا من الإناث و سبعة منهم (٢١.٢%) كان لديهم إلتهاب المعدة, واحد منهم (٢٠%) لديه قرحة معدة. ١ (٢٠%) تشخيصات اخرى. تم ايجاد الجين في ٥ (٣١.١%) من المرضى تحت عمر الثلاثين , وفي ٢ (١٤.٣%) في الفئة ٣٠-٦٠ سنة , و ٢ (١١.١%) في الفئة العمرية اكثر ٦٠ سنة.

إن نسبة البكتريا الحلزونية البوابية المكتشفة بواسطة جين *GLmM* منخفضة بالمقارنة مع دراسات أخرى.

خلصت هذه الدراسة الى انه لا يوجد ارتباط حقيقي بين عدوى جرثومة المعدة والتهابي المعدة والاثني عشر.

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LIST of ABBREVIATION

<i>BabA</i>	Blood group antigen binding Adhesion
<i>CagA</i>	Cytotoxic-associated gene A
CLO	Campylobacter – like organism
DNA	Deoxyribonucleic acid
DST	Drug susceptibility testing
ELIZA	Enzyme-linked immunosorbent assay
GC	Gastric Cancer
<i>Hsp-B</i>	Heat shock protein
IgG	Immunoglobulin G
MALT	Mucosa-associated lymphoid tissue
NSAIDs	Non-steroidal anti-inflammatory drugs
<i>OipA</i>	Outer inflammatory protein A
<i>OMP</i>	Outer membrane protein
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PPIs	Proton pump inhibitors
RUT	Rapid urease test
<i>SabA</i>	Sialic acid binding Adhesion
SPSS	Statistical package for social science
UBT	Urea breathe test
<i>UreC</i>	Urease C gene other name (<i>GLmM</i> gene)
<i>VacA</i>	Vacuolating cytotoxin A
WHO	World Health Organization

CHAPTER ONE
INTRODUCTION

CHAPTER ONE

1. INTRODUCTION

1.1 Background

Helicobacter pylori (*H. pylori*) discovered by Marshall and Warren in 1983 (Warren and Marshall, 1983). It is a gram negative bacterium that colonizes the gastric mucosa and the microvillus of the epithelial cells (Siqueira *et al.*, 2007). *H. pylori* is a spirally shaped bacterium, 0.5-0.9 mm wide by 2-4 mm long like *Campylobacter*'s, it requires carbon dioxide for growth, but it has a tuft of sheathed unipolar flagella, unlike the unsheathed flagella of *Campylobacter*'s (Van Vliet and Ketley, 2001.). *H. pylori* have special growth requirements such as 2 to 5% of O₂ levels, 5 to 10% CO₂ and high humidity. Many laboratories utilize standard microaerophilic conditions of 85% N₂, 10% CO₂, and 5% O₂ for *H. pylori* culture. Growth occurs at 34 to 40°C, with an optimum of 37°C (Gerrits *et al.*, 2006). Infection with *H. pylori* worldwide, but prevalence is very high in economically less developed region (Peleteiro *et al.*, 2014). *H. pylori* infection is estimated to be present in 50% of the world's adult population. In developing countries, the prevalence of infection may reach levels of 80-90% by 20 years of age. This prevalence remains constant for the rest of adult life. In contrast, in developed countries the prevalence of *H. pylori* infection is less than 20% in people below the age of 25 years (Wadi and Fedail, 2008).

H. pylori have a significant pathogenic role in gastritis and peptic ulcer disease, as well as in gastric adenocarcinoma, mucosa-associated lymphoid tissue (MALTS) lymphoma and possibly cardiovascular disease.

The mode of transmission of *H. pylori* is poorly understood, although it appears that oral-oral and fecal-oral routes are possible. The organism is rarely isolated from feces. Transmission of *H. pylori* in gastric juice as a result of epidemic childhood vomiting has also been proposed (Yakoob and Hussainy, 2010). Several techniques with different sensitivities and specificities are available for identification of *H. pylori* infection. These methods can be classified as invasive including use of endoscopy for the collection of gastric biopsies and non-invasive, such as identification of antigens, antibodies, specific genomic loci and enzyme activities (Kusters *et al.*, 2006). To date, there is no diagnostic method that can meet on its own criteria for acceptable sensitivity and specificity in the detection of *H. pylori* infection. Instead diagnostic methods are recommended in combination of two or more in order to meet diagnostic criteria (Krogfelt, 2005). Histological analysis is one of the most widely used diagnostic methods allowing for detection of the presence of the bacteria as well as assessment of the type and intensity of inflammation in the gastric mucosa (Hirschl and Makristathis, 2007). The primary limitation of histology is the subjectivity of assessment and the associated inter observer variation. Other limitations are related to variations of the bacterial density and the location in the stomach (Fabre *et al.*, 1994; Datta *et al.*, 2005). Furthermore, the presence of other microorganisms that produces urease can lead to false positive results. Culture of *H. pylori* allows the detection of factors and mechanisms related to pathogenicity, molecular epidemiology, and drug susceptibility testing (DST) (Kullavanijaya *et al.*, 2004).

However, growth in culture can be affected by factors such as concentration of bacteria in the specimen (biopsy), transport conditions, use of antimicrobials and PPI (Malfertheiner *et al.*, 2007).

Molecular methods allow rapid detection of *H. pylori*, as well as the determination of their genotype (Ahmad *et al.*, 2016).

1.2 Rationale

Today with high morbidity and prevalence of *H. pylori* we need molecular method to be used as golden standard to find patient truly infected; So the aim of this study to detected *H. pylori UreC (GLmM)* gene using PCR technique which slightly superior from other diagnostic methods for detection of *H. pylori* from biopsy samples. Also this method can enables to recognize infection when other tests are negative due to low bacterial density. To date, there is no diagnostic method that can meet on it's own criteria for acceptable sensitivity and specificity in the detection of *H. pylori* infection. Instead diagnostic methods are recommended in combination of two or more in order to meet diagnostic criteria (Patel *et al.*, 2014)

1.3 Objectives

1.3.1 General objective

To perform molecular detection of *Helicobacter pylori* among gastritis and duodenitis patients in Omdurman City.

1.3.2 Specific objective

1. To detect *H. Pylori GLmM gene* from gastric biopsy by using PCR technique.
2. To compare between detection of *GLmM gene* and gastritis and duodenitis.
3. To evaluate possible factor such as sex to detect the *GLmM gene*.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO

2. LITERATURE REVIEW

2.1 History of *H. pylori*

The bacterium *H. pylori* was initially named *Campylobacter pyloridis*, then *H. pylori* (after a correction to the Latin grammar) and in 1989 after DNA sequencing and other data showed that the bacterium did not belong in the *Campylobacter* genus, it was placed in its own genus (*Helicobacter*). The name pylori means "of the pylorus" or pyloric valve (the circular opening leading from the stomach into the duodenum) and its Greek word means gatekeeper. In 1875, German scientists found helical shaped bacteria in the lining of the human stomach. The bacteria could not be grown in culture and the results were eventually forgotten (Suerbaum and Josenhans, 2007). In 1893, the Italian researcher Giulio Bizzozero described helical shaped bacteria living in the acidic environment of the stomach of dogs (De Groot *et al.*, 2005). Professor Walery Jaworski of the Jagellonian University in Kraków investigated sediments of gastric washings obtained from humans in 1899. Among some rod like bacteria, he also found bacteria with a characteristic helical shape, which he called *Vibrio rugula*. He was the first to suggest a possible role of this organism in the pathogenicity of gastric diseases (Andersen, 2007). The bacterium was rediscovered in 1979 by Australian pathologist Robin Warren, who did further research on it with Barry Marshall beginning in 1981; they isolated the organisms from mucosal specimens from human stomachs and were the first to successfully culture them.

In their original paper, Warren and Marshall contended that most stomach ulcers and gastritis were caused by infection by this bacterium and not by stress or spicy food as had been assumed before (Goodwin *et al.*, 1989).

2.2 Classification of *H. pylori*

The most important stage in the development of the taxonomy gastric microorganism was the proposal in 1989 to establish a new genus called *Helicobacter* to mean a spiral rod and that *C. pylori* should be transferred to that genus as *H. pylori* (Goodwin *et al.*, 1989).

Classification of *Helicobacter Pylori* :

Kingdom: *Bacteria*

Phylum : *Proteobacteria*

Class : *Epsilon Proteobacteria*

Order : *Campylobacterales*

Family : *Helicobacteraceae*

Genus : *Helicobacter*

Species : *H. pylori*

Binomial name : *Helicobacter pylori*

2.3 Cellular morphology of *H. pylori*

Helicobacter pylori is a microaerophilic, gram negative bacterium of approximately 0.51 (2.5–5 µm). The majority of *H. pylori* possess the basic morphology of an S shape with tuft of 5-7 polar sheathed flagella (Lewinska and Wnuk, 2017). The overall composition of the cell envelope of *H. pylori* is similar to that of other gram negative bacteria. It consists of an inner (cytoplasmic) membrane, periplasm with peptidoglycan, and an outer membrane. The outer membrane consists of phospholipid and lipopolysaccharide. The outer membrane phospholipid moiety contains cholesterol glycosides, which is very rare in bacteria (Kusters *et al.*, 2006). Lipopolysaccharides are complex molecules present in the outer membrane of gram negative bacteria. Structurally Lipopolysaccharides consist of three main components: Lipid A, the core oligosaccharide and the O polysaccharide. Lipopolysaccharide in *H. pylori* has low biological activity as compared to lipopolysaccharide from other gram negative bacteria, which may be explained by unusual composition of lipid A (Parija, 2014). The *H. pylori* genome consists of 1.65 million bp and codes for about 1,500 proteins. However, *H. pylori* populations are extremely different as a result of point mutations, substitutions, insertions and or deletions in their genome (De Falco *et al.*, 2015). *Helicobacter pylori* change his morphology in vitro from a helical form to a coccoid form under various conditions such as extended cultivation, aerobic culture or antibiotic treatment. It has been reported that the coccoid form is non culturable but viable and metabolically active (Osaki *et al.*, 2002).

A variety of culture media have been used for isolation of *H. pylori*, the most commonly used are Colombia, brain heart infusion,

trypticase soy or blood agar media, each supplemented with 5%-10% blood (Al-Sulami *et al.*, 2008). *H. pylori* are biochemically closely related to Campylobacter that are oxidase, catalase, and urease positive (Harry *et al.*, 2001).

2.4 Epidemiology of *H. pylori*

Helicobacter pylori are commonest bacterial pathogens in human. Human is only known reservoir of *H. pylori* (Das and Paul, 2007). The prevalence of *H. pylori* infection varies widely according to geographical area, patient age and socioeconomic status. Rates of isolation range between 70-90% in developing countries and 25-50% in developed countries. In various regions of sub-Saharan Africa 61-100% of the population may harbor the pathogen. Although there is geographical and socio-demographic variation in the prevalence of human infection with the organism, prevalence has been reported to be discordant with the incidence of morbidity caused by the infection. In Africa, the prevalence of infection is very high but the incidence of gastric carcinoma and other *H. pylori* associated morbidities are relatively low (Tanih *et al.*, 2010).

However, there have been few published data considering the prevalence of *H. pylori* (Basiri *et al.*, 2014). Natural acquisition of *H. pylori* infection occurs for the most part in childhood via fecal–oral and oral–oral pathway. The vast majority of *H. pylori* infection occurs in the developing countries where up to 80% of the middle-aged adults may be infected.

Among infected individual approximately 10% develop severe gastric lesions such as peptic ulcer disease, 1-3% progresses to gastric cancer (GC) with a low 5 year survival rate and 0.1% develops mucosa-

associated lymphoid tissue (MALT) lymphoma. It is estimated that individuals infected with *H. pylori* have more than two fold increased risk of develop (GC) compared with non-infected ones. Gastric cancer is one of the most common cancers and the third leading cause of cancer related deaths worldwide with more than 70,000 deaths annually (De Falco *et al.*, 2015).

2.5 Virulence factors of *H. pylori*

The clinical outcome of *H. pylori* infection has been associated with virulence factors (Medina *et al.*, 2017). The virulence factors include components (flagellum and adhesin) and substances produced by bacterium (enzymes and cytotoxins), the most important among which are urease and the cytotoxins, *CagA* and *VacA* (Zhang *et al.*, 2011). The presence of these factors is useful as molecular markers in the identification of the high risk for developing severe gastric pathologies (Medina *et al.*, 2017).

2.5.1 Flagella

The bacterial flagellum is a complex motility organ composed of multiple types of protein subunits. Each flagellum consists of three components the basal body, hook and filament. Electron microscopic observation of the *H. pylori* flagellum reveals the presence of a sheath and a terminal bulb. Flagella play an important role in the colonization of the gastrointestinal mucosa (Gu, 2017).

These help the organism to penetrate into gastric mucous layer hence it is protected from acidic environment of the stomach (Parija, 2014).

2.5.2 Urease C gene

The *UreC* gene encodes for a phosphoglucosamine mutase; this gene is unrelated to urease production, so it was renamed *GLmM* gene by (De Reuse *et al.*, 1997). This gene is considered a “housekeeping” gene, and it participates directly in cell wall synthesis (Espinoza *et al.*, 2011). *UreC* gene conserved and applied PCR for a *GlmM* gene segment was almost better than other methods for the detection of *H. pylori* infection, since it has a high degree of sensitivity and specificity (Ho and Windsor, 2000; Brooks *et al.*, 2004; Kalaf *et al.*, 2013).

2.5.3 Cytotoxic-associated gene A

The cytotoxic-associated gene A (*CagA*) 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 *CagA* positive strains of *H. pylori* are more interactive with the host than those that do not (Asl *et al.*, 2018). They responsible for mucosal inflammation and interleukin-8 (IL-8) production and are associated with pathogenesis of gastric cancer (Bibi *et al.*, 2017).

2.5.4 Vacuolating cytotoxin A

One important virulence factor is the Vacuolating cytotoxin (*VacA*) a secreted pore forming toxin that causes epithelial cell vaculation (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 *VacA* toxin bind to host cell and is internalized

(Palframan *et al.*, 2012), that induce vaculation and multiple cellular activities, including membrane channel formation, cytochrome 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).

2.5.5 Adhesins

Adhesins are bacterial cell surface proteins that enable bacterial adherence to cells (Kalali *et al.*, 2014).

Although the infectious process depends on several factors, the adhesion to the gastric mucosa is the first and important step required for both colonization and pathogenesis (Ansari and Yamaoka, 2017). The adherence of *H. pylori* to gastric mucosa is important for protection from mechanisms like acidic pH, mucus, and exfoliation. *H. pylori* adhesive factors belong to largest outer membrane protein (OMP) family of the bacterium namely, the Hop family. The Hop family contains the most well-known adhesins of *H. pylori* like *BabA*, *SabA*, *AlpA/B*, *HopZ* and *OipA*. *BabA* is a 78 KDa protein termed blood group antigen binding adhesion (*BabA*) which can bind Lewis band related terminal fucose residues on blood group antigen O (H antigen), A and B on gastric epithelial cells. *BabA* positive strains colonize more densely and induce stronger IL-8 secretion in the mucosa compared to *BabA* deficient strains. Sialic acid binding adhesin (*SabA*) is a 70 KDa adhesin of *H. pylori* which bind to sialyl-dimeric Lewis^x (Le^x) after initial colonization mediated by *BabA*, *H. pylori* infection lead to up regulation of Le^x expression enabling *SabA* mediated binding. The *SabA* adhesion can further bind the sialylated carbohydrates on granulocytes and induce an oxidative burst in these cells. More over *SabA* bind sialylated structures expressed on

erythrocyte and to hemagglutination. Adherence associated lipoproteins A/B (*AlpA/B*) are coproduced proteins that are involved in adhering to the gastric tissue. *HopZ* is a 74 KDa adhesin that mediates the adherence of *H. pylori* to gastric epithelial cells as bacterial binding is significantly reduced in Hop knockout strains.

The host receptor is as yet unknown. Outer inflammatory protein A (OipA) is a 35 KDa protein. The exact role of OipA is still not clear (Kalali *et al.*, 2014).

2.5.6 Enzymes

H. pylori produce many enzymes, such as urease, mutinies, phospholipases, superoxide dismutase and catalase. The enzyme urease is a very important factor for colonization of *H. pylori* in the gastric mucosa. The enzyme also stimulates monocyte and neutrophil chemotaxis and stimulates production of cytokines. Both mucinase and phospholipase break down gastric mucus, while superoxide dismutase and catalase prevent phagocytic killing of the bacteria (Pajavand *et al.*, 2015).

2.5.7 Heat shock protein (Hsp-B)

The heat shock protein facilitates expression of the enzyme urease (Tao *et al.*, 2014).

2.5.8 Acid inhibitory protein

This protein causes hypochlorhydria by blocking secretions of acid from parietal cell (Stingl *et al.*, 2002).

2.6 Normal habitat of *H. pylori*

Human stomach is considered as a main reservoir of *H. pylori* but according to the previous hypothesis and results of some published investigations, foods with animal origins play an important role in the distribution and transmission of *H. pylori* (Gilani *et al.*, 2017).

2.7 Transmission

It is not known how *H. pylori* is transmitted or why some patients become symptomatic while others do not. The bacteria are most likely spread from person to person through fecal-oral or oral-oral routes possible environmental reservoirs include contaminated water sources. Iatrogenic spread through contaminated endoscopes has been documented but can be prevented by proper cleaning of equipment (Garhart *et al.*, 2002).

2.8 Pathogenicity of *H. pylori*

The mechanism of *H. pylori* pathogenicity is not well understood. The mechanism by which *H. pylori* cause disease in humans can be described as a multi-step process where the bacterium first has to evade the bactericidal activity of the gastric acid barrier and enter the mucous layer (colonization) and then it has to adapt and multiply under the environmental conditions of the gastric mucus (persistence). Several bacterial virulence factors have been associated with the development of gastric diseases. The first step essential in successful infection, is the adhesion of *H. pylori* to the host gastric mucosa and bacterial motility. Adhesion is mediated by *H. pylori* surface bound proteins, termed adhesin that recognize glycan structures (Gly-Rs) expressed on the surface of gastric epithelial cells and are also present on the mucus layer lining the gastric mucosa. The blood group

antigen binding adhesin (*BabA*) recognizes fucosylated blood group antigens including the difucosylated Lewis antigens, such as the Lewis b (Leb) antigen and H type 1 histo-blood group carbohydrate structures expressed in the gastric epithelium and mucus layer (De Falco *et al.*, 2015).

H. pylori is protected from phagocytosis and intracellular killing by enzymes, such as elastase and superoxide dismutase. After adhesion, damage to the epithelial cells is caused by multiple factors such as mucinases, phospholipases, vacuolating aflatxin and by production of urease. Finally Helicobacter infection causes atrophic and event metaplastic changes in the stomach (Pajavand *et al.*, 2015).

2.9 Pathogenesis of *H. pylori*

2.9.1 Gastritis

Gastritis refers to inflammation of the gastric mucosa. Gastritis can be grouped as acute or chronic gastritis. Acute gastritis refers to the transient inflammation of the gastric mucosa. It is most commonly associated with local irritants such as bacterial endotoxins, caffeine, alcohol and aspirin. Depending on the severity of the disorder the mucosal response may vary from moderate edema and hyperemia to hemorrhagic erosion of the gastric mucosa. 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. *H. pylori* gastritis is a chronic inflammatory disease of the antrum and body of the stomach (Tanih *et al.*, 2010).

2.9.2 Peptic ulcer disease

Helicobacter pylori is now 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. Occasionally, an ulcer may penetrate the outer wall of the stomach or duodenum; with spontaneous remissions and exacerbations being common (Chiba, 2004).

2.9.3 Gastric cancer

Infection with *H. pylori* appears to serve as a co-factor in some types of gastric carcinoma. Gastric carcinoma is the major cause of cancer death worldwide. Among factors that increase the risk of gastric cancer is genetic predisposition, carcinogenic factors in diet (e.g., N-nitroso compounds and benzopyrene found in smoked and preserved foods) and autoimmune gastritis. Virtually all tumors are adenocarcinoma arising from mucus secreting cells in the base of the gastric crypts. Stomach cancers are either 'intestinal', arising from areas of intestinal metaplasia or 'diffuse', arising from normal gastric mucosa. Carcinomas are more common and arise against background of chronic mucosal injury (El-Omar *et al.*, 2000).

2.9.4 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* are 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).

2.10 Host immunity of *H. pylori*

Helicobacter pylori colonize the gastrointestinal tract, thus there is need to the immune responses directed toward *H. pylori* in the context of the general defenses are multiple and might be physical, chemical and immune mediated the mucosal epithelium blocks invasion by pathogenic and commensal bacteria by forming multiple layers of physical (tight junction), chemical nitric oxide and immunoprotection (local secretion of defenses, anti- and pro-inflammatory chemokines (cytokines and IgA, IgG and IgM transport). Gastric biopsies from *H. pylori* infected individual showed infiltration of lymphocytes and monocytes, along with significantly increased expression of IL-1, IL-8 and IL-6 in the gastric antrum. The numbers of gastric CD4+ and CD8+ were increased (Beswick *et al.*, 2006). A humeral immune response is elicited in nearly all *H. pylori* infected humans. Serum IgA and IgG antibodies in chronically infected persons are directed toward many different *H. pylori* antigens. A local antibody response directed toward *H. pylori* antigens is also detectable with chronic *H. pylori* infection. These subjects have remarkably higher frequencies of total IgA and IgM secreting cells than the no infected subjects, while the frequencies of IgG secreting cells were virtually the same in the different groups. Notably, *H. pylori* infection induces auto antibodies reactive with gastric epithelial cells through activation of complement, inducing apoptosis or triggering an antibody dependent cellular cytotoxicity reaction leading to the tissue destruction (Moyat and Velin, 2014).

2.11 Diagnosis of *H. pylori* infection

Several diagnostic tests are used to detect *H. pylori* infection. These tests including invasive and non-invasive techniques. The advantages and disadvantages of the various techniques are described below (Benjamin *et al.*, 2000).

2.11.1 Invasive techniques

2.11.1.1 Culture

Although it should be stated that *H. pylori* culture is not a routine procedure in initial diagnosis, in many bacteriology laboratories *H. pylori* isolation via the culture of biopsy samples is a routine second line approach. Because of the demanding character of this bacterium, this method remains challenging. This technique, although highly specific it is not as sensitive as other tests like histology and the rapid Urease test. As well as for purposes of scientific research, cultured live *H. pylori* is used for diagnostic approaches and for the detection of antibiotic resistance if treatment failure is suspected (Taj *et al.*, 2003).

H. pylori require a microaerophilic atmosphere (5% to 10% oxygen, 5% to 12% carbon dioxide and 80%–90% nitrogen with humidity) and a complex culture media. The most commonly used media contains Columbia Wilkins-Chalgren, brain-heart infusion or trypticase agar bases, supplemented with sheep or horse blood (Ndip *et al.*, 2003). Because isolation of this microaerophilic organism from gastric biopsy specimens takes a long time (up to 5–7 days), to overcome the problem of growth of other competitors that exist in the sample, the culture media is supplemented with specific antibiotics. Although *H. pylori* could be cultured from stool specimens. Moreover, it is possible

that the bacterium goes into a viable form that cannot be cultured (coccoid form) which leads to false negative results (Azevedo *et al.*, 2007b; Azevedo *et al.*, 2007a).

2.11.1.2 Histological analysis of biopsy

Routine histological analysis of biopsy samples is common and practical. This technique is helpful because one can visualize the mucosa, permitting detection of histological gastritis and lesions such as MALT-type lymphomas, which are tumors of lymphoid tissues. Also, standard staining techniques (i.e., eosin staining) are usually unreliable for detection of *H. pylori* by microscopy. Adding to the impracticality of this method is that it requires endoscopy and diagnosis cannot be obtained until several days after the procedure (Konturek *et al.*, 2009).

2.11.1.3 Campylobacter like organism (CLO) test

This test is based on the fact that mucosal biopsy specimens can be inoculated into a medium containing urea and phenol red, a dye that turns pink in a pH of 6.0 or greater (Liao *et al.*, 2018). The pH will rise above 6.0 when *H. pylori* (the Campylobacter like organism) metabolize urea to ammonia by way of its urease activity. This test is commercially available and therefore quite inexpensive. Only one-half hour is required for diagnosis of infection and the test has shown 98% sensitivity and 100% specificity (Bojarski *et al.*, 2000).

2.11.1.4 Polymerase chain reaction (PCR) technique

Molecular tests based upon PCR enable the specific detection of nucleic acid and have been used for the diagnosis of *H. pylori* in

clinical specimens. PCR tests for *H. pylori* that used a range of genomic target have been reviewed recently (Aiba *et al.*, 1998).

The advantages of PCR are uncounted, such as a possibility of genotyping of samples to identify different strains of the species, the use of retrospective materials in research, which is of great relevance to avoid the need to repeat the invasive procedure to collect another sample; the detection in samples that contain small DNA quantity, in addition to allowing amplification of resistance genes to antibiotics without the need to perform a conventional antibiotic susceptibility testing (Menoni *et al.*, 2013). The disadvantages are the high cost of the technique and the possibility of sample contamination, but they can be avoided by the use of biosafety standards during all phases of the process. It should also be considered that after antibacterial therapy, the number of bacteria decreases and the microbiological and histological tests can produce false-negative results. However, the PCR is a sensitive method that allows amplification of small amounts of bacterial DNA in various types of biological samples (Simsek *et al.*, 2000).

2.11.2 Non-invasive technique

2.11.2.1 Breath test

Although *H. pylori* it self can't be detected non-invasively, it's urease activity can be detected by way of a breath test. In this test, urea that is radioactively labeled with carbon 13 and carbon 14 is ingested (Pathak *et al.*, 2012). Bacterial urease splits off labeled carbon dioxide, which can be detected in the breath. The breathe test technique reflects only current infection with *H. pylori* but can demonstrate very rapidly the existence of infection. A disadvantage of this technique is that it may

involve a small amount of exposure to radiation. Although carbon 13 is a stable isotope and does not emit radiation, its detection requires a mass spectrometer, which may not readily available. The breathe test is not yet commercially available (Benjamin *et al.*, 2000).

2.11.2.2 Detection of IgG antibody

When a host infected with *H. pylori* an immune response immediately stimulates IgG and secretory antibody IgA. Therefore, serologic testing for antibodies to *H. pylori* using the enzyme-linked immune. The test is simple, inexpensive and readily available. ELISA detects IgG with a sensitivity of up to 99% and is 100% specific. Since spontaneous clearing of *H. pylori* by IgG or IgA is rare, an elevated antibody titer indicates current infection. This test also detects the decline in antibody titer after removal of the organism; however, the rate of decline of IgG after eradication is still not known. This technique although useful, accurate, low cost and an attractive choice for detecting *H. pylori* infection still has certain limitations (Braden, 2012). In current practice; endoscopy is still required for diagnosis of infection by *H. Pylori*. The full range of non-invasive techniques is expected to be more readily available soon, with the antibody tests ideal for assessing current infection (Burucoa *et al.*, 2013).

2.12 Treatment of *H. pylori*

Eradication of *Helicobacter pylori* is defined as the absence of the organism four or more weeks after eradication therapy. Since the eradication rate for single drug therapy is only 19% and that for double-drug therapy is still only 48%, researchers have found that combining three antibiotics offers a better chance for eliminating the bacterium (Parente *et al.*, 2003).

The highest eradication rate (82%) was achieved by combining bismuth, metronidazole and tetracycline. There are obvious drawbacks to this type of treatment; First of all, it is inconvenient for the patient so; it is difficult for doctors to convince their patients to comply with the therapy. Second, such multidrug therapy is almost always associated with many adverse side effects; namely diarrhea, nausea and vomiting, which occur in approximately 20% of all patients (Logan and Walker, 2001).

2.13 Prevention and control

Since the source of *H. pylori* is not yet known, recommendations for avoiding infection have not been made. In general, based on the results of recent studies, the following measure may help to reduce transmission of *H. pylori* bacteria: practice good hygiene and hand washing especially with food preparation, all patient with chronic gastrointestinal symptoms that may be associated with *H. pylori* infection should be tested and treated to prevent exposure to family members, patient should complete the full course of therapy and maintain proper nutrition to prevent iron deficiency anemia (Cheungk and Wong, 2008).

2.14 Previous studies

A total of 57 samples from other study of molecular detection of *Helicobacter pylori* among gastrodoudenits and peptic ulcer patient done by Bakhit et al. (2014) examined by conventional PCR, 12 (21.1%) were positive for *H. pylori* DNA.

A study of molecular technique for detection and identification of *H. pylori* in clinical specimen conducted by Jessica et al. (2017). A total of 85 samples were analyzed, 56 (65.88%) women and 29 (34.12%) male, age varied from 15 to 89 years.

A total of 44 samples examined by *GLmM* gene conducted by Tamer et al. (2013). Was highly conserved and has been used to identify *H. pylori* in gastric biopsies 43 (98%).

A total of 91 samples examined by *UreC* gene conducted by Mohamed et al. (2013) for routine upper gastrointestinal endoscopy at Firoozgar Hospital (A University hospital). The patients included 39 males and 52 females with a mean age of 45 years (range: 17–87 years).

A study of molecular detection of *Helicobacter pylori* infection in gastric biopsy specimens by PCR conducted by Alnaji et al. (2018). A total of 75 specimens were examined *UreC* gene (19/49) (38.8%).

CHAPTER THREE
MATERIALS and METHODS

CHAPTER THREE

3. MATERIALS and METHODS

3.1 Study design

This was descriptive cross-sectional study and hospital based study.

3.2 Study area and duration

The study was carried out at the Albogaa Specialize Hospital in Omdurman city, during the period from April, 2018 to January, 2019.

3.3 Study population

Male and female patients from all age groups whom were suspect for gastric biopsy verbally consent were enrolled in this study.

3.4 Sampling technique

Biopsy samples were collected by gastroenterologist and preserved in phosphate buffer saline. They preserved in temperature -20°C.

3.5 Sample size

Fifty samples (n=50) of endoscopy biopsies were collected from gastritis, gastric ulcer, duodenal ulcer and gastric maltoma patients.

3.6 Data collection

Data was collected through a structured questionnaire. Questionnaire was designed to record clinical data.

3.7 Sample Collection

3.7.1 Collection and transportation of specimens

Upper endoscopy was performed and multiple gastric biopsy specimens were taken from the stomach antrum, the corpus and the duodenum. Specimens were preserved and transported in phosphate buffer saline.

3.7.2 DNA extraction

Genomic DNA was extracted manually from endoscopic biopsies using Guanidine hydrochloride protocol. According to the following steps: 2ml of phosphate buffer saline (PBS) were used to collect the samples. The samples were centrifuged at high speed (3000rpm) for 10min and the pellet was collected and transferred to a falcon tube. Then 2ml of lyses buffer, 1ml of guanidine hydrochloride, 300 μ l of NH₄ acetate and 10 μ l of proteinase K were added to the pellet. After two hours incubation at 65°C, samples were cooled at room temperature; 2ml of pre-chilled chloroform was added. Then samples were centrifuged for 5mins at (6000rpm). Upper layer was collected to a new falcon tube; 2ml of cold absolute ethanol was added to the samples and then kept at -20°C overnight. After that samples were centrifuged for 10mins at (6000rpm). Then the supernatant was drained. Pellet was washed with 2ml of 70% ethanol then was centrifuged for 10mins at (6000rpm). Supernatant was poured off and pellet was allowed to dry. Pellet was dissolved in 50 μ l of double distilled water H₂O and then was incubated at -20°C until analysis.

3.7.3 PCR amplification with *GLmM* gene primers

Amplification was conducted in a total volume of 25 μ L. The reaction mixture contained 4 μ L master mix, 1.0 μ L of each forward and reverse primers, 2 μ L DNA template, and 17 μ L of double distilled water to a total volume of 25 μ L. The amplification was carried out in a K-690 thermal cycler (Heal force, China) according to the following program: an initial denaturation step at 94°C for 3mins, followed by 40 cycles of denaturation at 94°C for 30sec, annealing, primer specific shown at 53°C for 30sec, and a final extension step at 72°C for 5mins. Amplified PCR products were separated by agarose gel electrophoresis (240 volt, 125 current for 30mins) using 1.5% agarose in Tris Acetate EDTA (TAE) buffer containing 0.5 μ g/mL of ethidium bromide. Molecular size ladder of 100bp (Intron Biotechnology, Korea) was used to determine the size of the bands. The gel was viewed and photographed on a Gel-Doc System.

The primers used for the amplifications of *H. pylori* gene were (*GLmM*-F 5- AAGCTTTTAGGGGTGTTAGGGGTTT -3) and (*GLmM*-R 5- AAGCTTACTTTCTAACACTAACGC -3). The primers obtained from (Macrogen, Korea) (Bickley *et al.*, 1993).

3.7.4 PCR products detection

Amounts of 4 μ L from each PCR mixture separated in a 1.5% agarose gel, then stained with 3 μ L ethidium bromide and viewed under Gel-Doc system. A result was considered positive when a band of the size 294bp was visible in the gel.

3.8 Ethical consideration

Permission to conduct this study was obtained from college of graduate studies and college of Medical Laboratory Science, Sudan University of Science and Technology.

3.9 Data analysis

Collected data was analyzed using the statistical package of social science (SPSS) program version 16. Chi-square statistical analysis was used to Mean \pm STD to determine *P.value* for significance range (*P. value* < 0.05 consider significant).

CHAPTER FOURE

RESULTS

CHAPTER FOUR

4. Result

Total of 50 samples were collected, among these patients the frequency of male were 25 (50%) while 25 (50%) were female, there was no significant association between gender and *H. pylori* infection ($P\text{-value}>0.05$) (Figure 2). Out of 25 male 3 (12%) of them were positive for gene detection (*GLmM* gene), While 6 (24%) of 25 female were positive for *GLmM* gene ($P\text{-value}>0.05$). (Table 1). The age group were classified into three age groups; less than 30 years 16 (32%), 30-60 years 16 (32%) and more than 60 years 18 (36%). The ages of patients range from 19 to 81 years old and showed insignificant relation between age and *H. pylori* infection ($P\text{-value}>0.05$) (Figure 3). Out of 16 patients age group (less than 30) years 5 of them (31.3%) were positive, 16 patients age from (30-60) years 2 of them (14.3%) were positive and age group (more than 60) years 2 of them (11.1%) were positive for *GLmM* gene detection of *H. pylori* infection ($P\text{-value}>0.05$). (Table 2).

Total of 33 (66%) of volunteer had gastritis, 5 (10%) have gastric ulcer, 1 (2%) has duodenal ulcer, 3 (6%) have gastric maltoma and 8 (16%) have other diagnosis with no significant relation between *H. pylori* infection and diagnosis ($P\text{-value}>0.05$). (Figure 4). Out of 33 patients with gastritis 7 of them (21.2%) of them were positive for *GLmM* gene detection, out 5 patients with gastric ulcer 1 of them (20%) was positive for *GLmM* gene detection, while gene not detected in duodenal ulcer and gastric maltoma and out of 8 patients with other diagnosis 1 of them (12.5%) was positive for *GLmM* gene detection.

Which show there was insignificant association between diagnosis and *H. pylori* infection ($P\text{-value}>0.05$). (Table 3).

Found 9 (18%) out of 50 samples were positive for *H. pylori* infection *GLmM* gene by using PCR. (Figure 1).

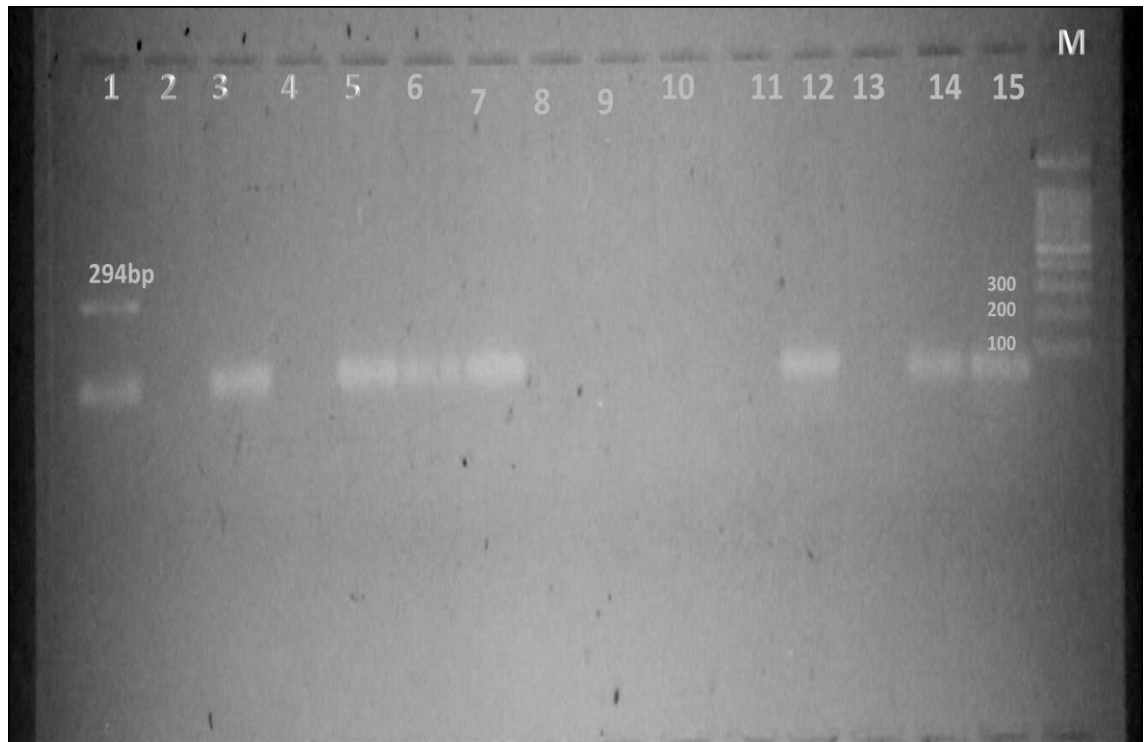


Figure (1): Gel electrophoresis of *H. pylori* *GLmM* gene (294 bp). 1.5% agarose gel. Lane 1: Positive sample. Lanes from 2 to 15: Negative samples. M: 100bp DNA marker (ladder).

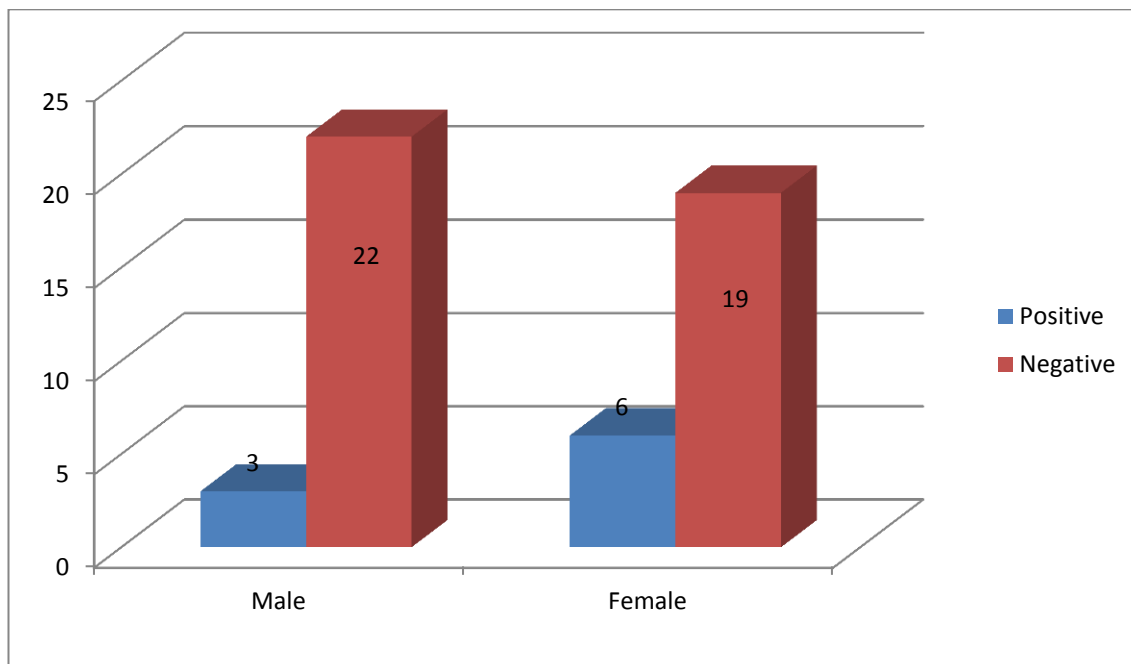


Figure (2): Association between gender and PCR results.

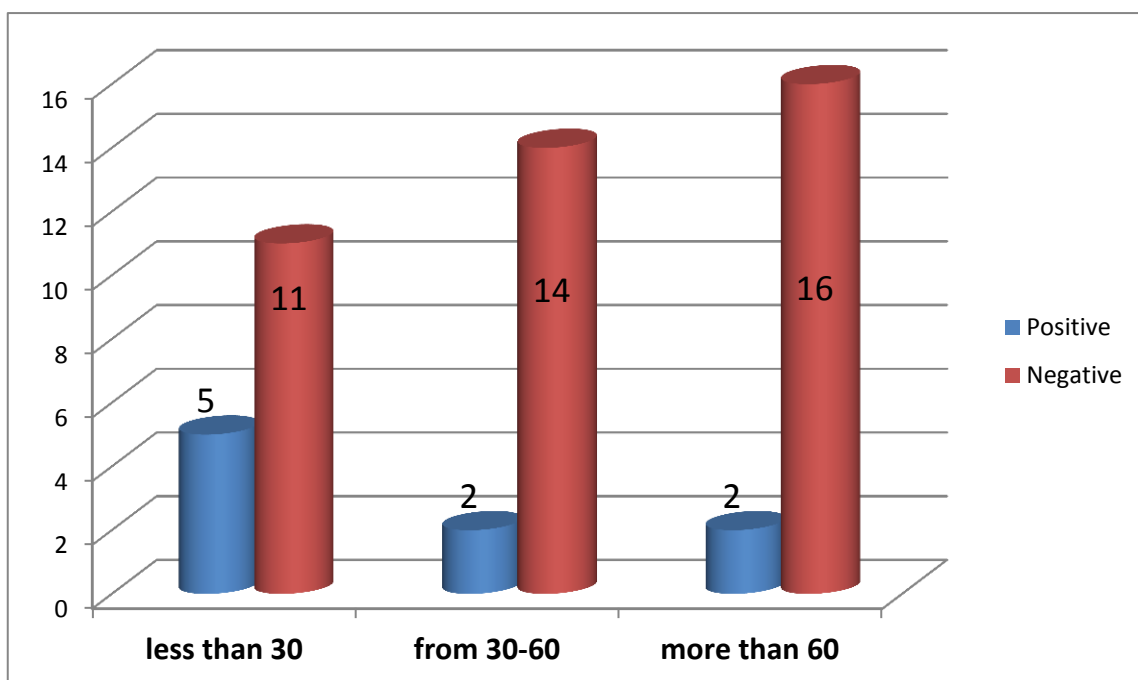


Figure (3): Association between age groups and PCR results.

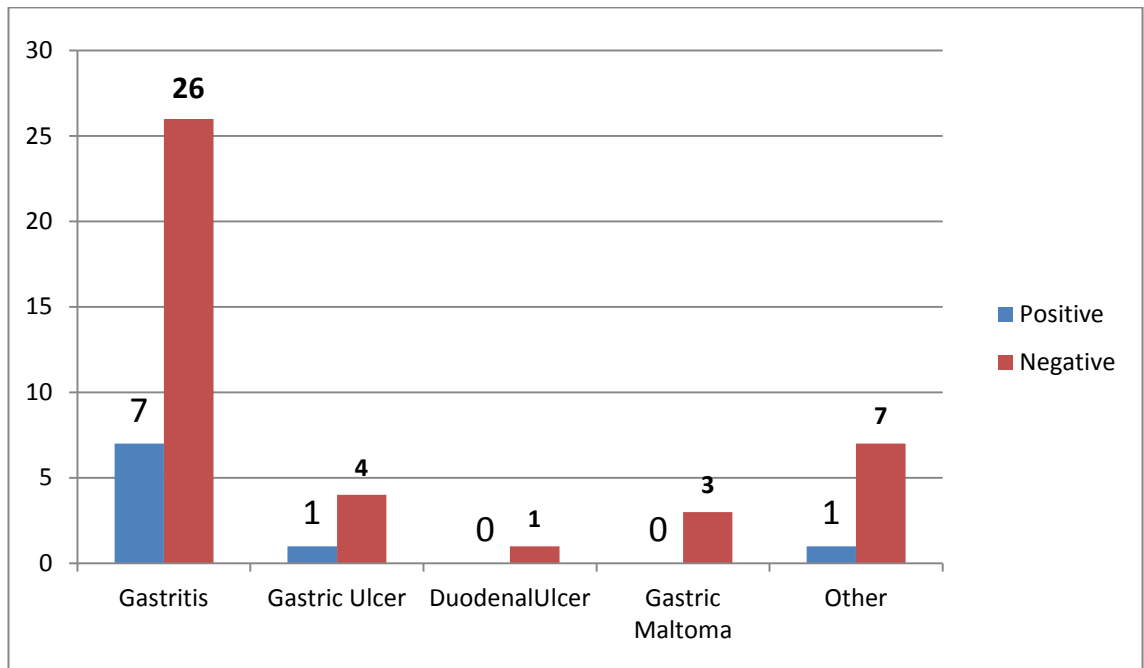


Figure (4): Association between diagnosis and PCR results.

Table (1): PCR results for *H. pylori* in gender of patients.

Gender	PCR results		Total	P value
	+ve	-ve		
Male	3	22	25	0.463
Female	6	19	25	
Total	9	41	50	

Table (2): PCR results for *H. pylori* in age groups of patients.

Age Group	PCR Results		Total	P-value
	+ve	-ve		
< 30	5	11	16	0.757
30-60	2	14	16	
> 60	2	16	18	
Total	9	41	50	

Table (3): PCR results for *H. pylori* with diagnosis of patients.

Diagnosis	PCR results		Total	P value
	+ve	-ve		
Gastritis	07	26	33	0.864
Gastric ulcer	01	04	05	
Duodenal ulcer	00	01	01	
Gastric maltoma	00	03	03	
Other	01	07	08	
Total	09	41	50	

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

5. Discussion

5.1 Discussion

The present study show that out of 50 samples, 25 (50%) were male and 25 (50%) were female, there is no significant association between gender and *H. pylori* infection. A study in Turkey 2007 done by Seyda et al. (2007) showed that there is no relation between gender and *H. pylori* infection which agree with this study.

The present study show that out of (5/16) (31.3%) patients age group less than 30 years, (2/16) (14.3%) patients age group (30-60) years and (2/18) (11.1%) patients age group more than 60 years, there is no significant association between age group and *H. pylori* infection. A study from Lebanon 2017 done by Antoine et al. (2017) showed no relation between age and *H. pylori* infection which agrees with the results of this study.

The present study show that age group less than 30 years showed increased rate of *GLmM* gene detection than other age groups. Other study in India done by Sarma et al. (2017) showed that the age group between 30-60 years showed increased rate of *GLmM* gene detection than other age groups but overall the study showed that there was insignificant relation between age groups and gene detection which agree with the results of this study, more than. However, there was insignificant association between the age and gender of patients and the infection with *H. pylori* ($P\text{-value}>0.05$), which agree with this study.

The present study found that the most commonly endoscopic finding was gastritis with 21.2%. Other study done by Antoine et al. (2017) found that the most commonly endoscopic finding was gastritis with 78.3% he also found that there is significant relation between duodenitis and *H. pylori* infection, which disagree with this study. This variation may be due to differ in sample size between the two studies. There was insignificant association between duodenitis and *H. pylori* infection in this study.

Study done by Roshana et al. (2014) showed that there was significant relation between duodenitis and *H. pylori* infection. Which disagree with this study.

A study carried out by Alnaji et al. (2018) in Babylon university showed a detection of *GLmM* gene was 19 out of 49 (38.8%) which is higher than the figure which was obtained in this study (18%).

5.2 Conclusion

The findings of this study indicate that there was no significant association between gastritis and duodenitis with *H. pylori* infection and there was no significant association between antibiotic user with *H. pylori* infection, the PCR is a sensitive method that allows amplification of small amounts of bacterial DNA from gastric biopsy .

5.3 Recommendations

1- More studies are needed for the detection of other virulent genes of *H. pylori*.

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

3- Sequencing and phylogenetic analysis of *H. pylori* DNA should be done among gastritis and duodenitis patients in all Sudan states.

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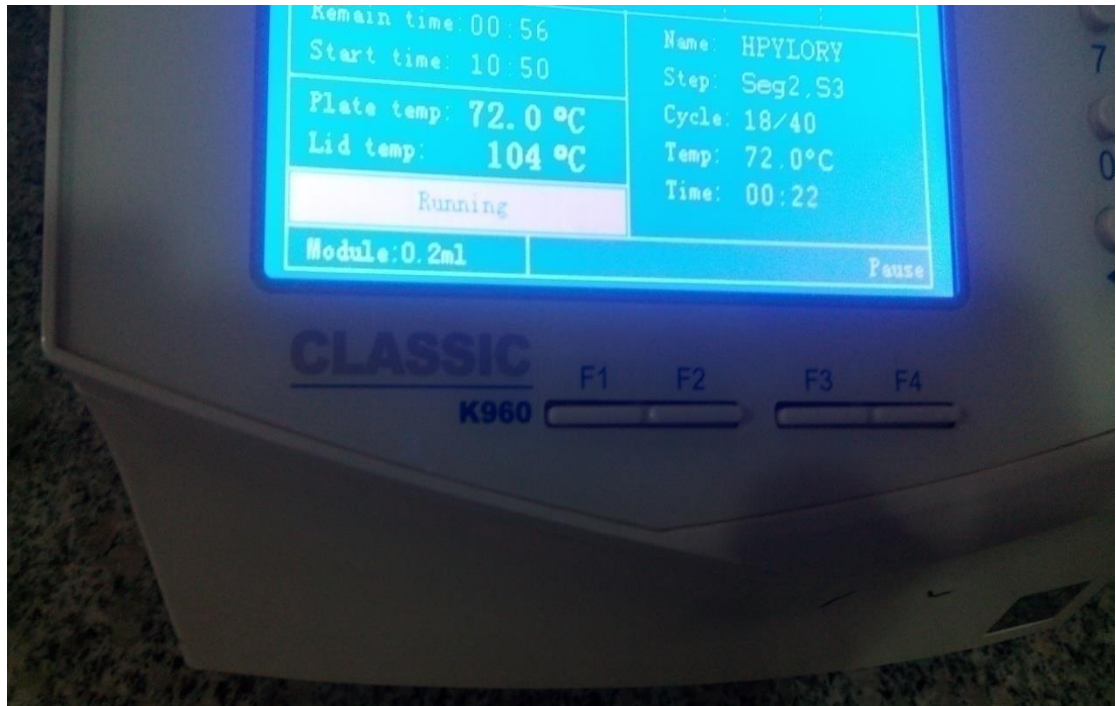
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APPENDICES

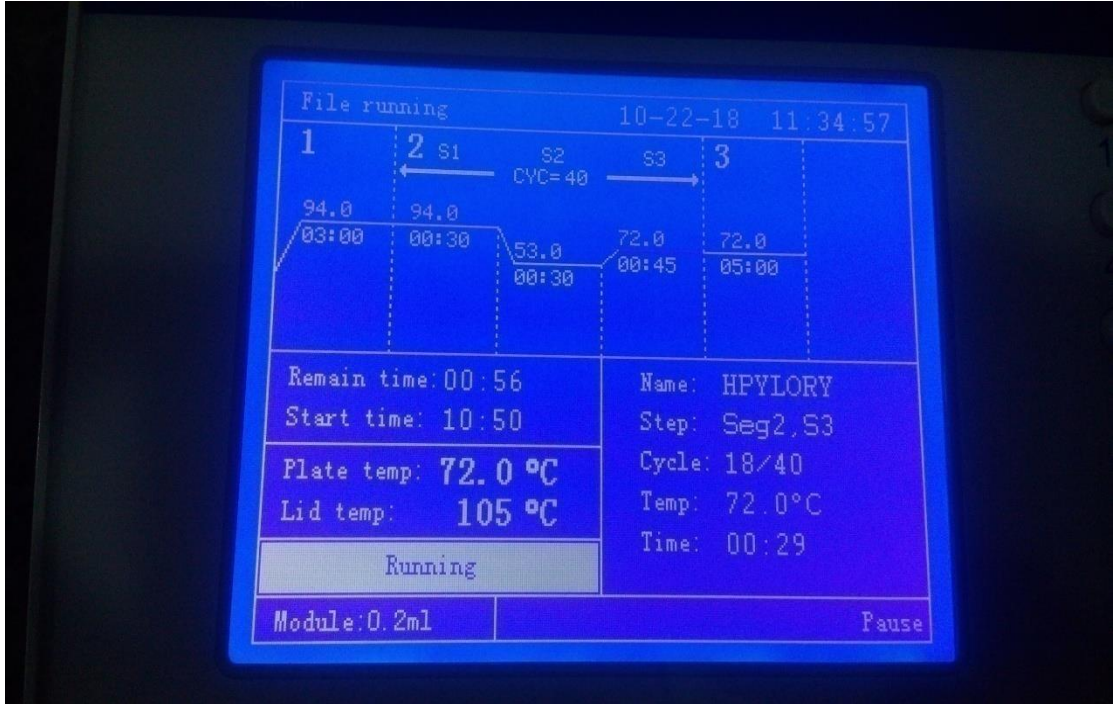
Appendices

Appendix (1)



PCR Machine (Heal force, China)

Appendix (2)



PCR Program

Appendix (3)

Questionnaire

- Date: **- Hospital:**

- Participant name:

- ID number

- Age:years

- Sex:

Male ()

Female ()

- Residence:

Khartoum () Khartoum North () Omdurman () Other ()

- Anatomic location:

Antrum ()

Corpus ()

Incisura angularis ()

- Signs and symptoms:

Difficulty swallowing () Nausea ()
Unexplained weight loss () Recurrent vomiting ()
Dyspepsia () Others ()

- Treatment:

Yes () No ()

- Clinical diagnosis (endoscopy):

a. Gastritis () **b.** Gastric Ulcers () **c.** Duodenal Ulcer ()
d. Gastric maltoma {MALT lymphoma} () **e.** Other ()

- Family history of gastric cancer Yes () No ()

- Lab investigation and results:

- ❖ Idiopathic thrombocytopenic purpura ()

- ❖ Unexplained iron deficiency anemia ()

- ❖ Other.....