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Study Some of HLA Genes Polymorphism Among Patients with *Helicobacter pylori* Infection

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By

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بسم الله الرحمن الرحيم

(وَيَسْأَلُونَكَ عَنِ الرُّوحِ ۖ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُم مِّنَ الْعِلْمِ إِلَّا قَلِيلًا)

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Certification

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Dedication

First and foremost, I thank God (Allah), the Almighty for endowing his immense blessing that helped me in each step of my progress toward the successful completion of my research work

To my father how gave me confidence and was the blessing of the father..

To my mother and who has overwhelmed me with her love and kindness. The main reason behind my success…

To my companion and friend of all days, with their good and bad times. To the one who was always the first to encourage and support me, my dear husband….

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Summary

Helicobacter pylori infection is very common and it is estimated that about half of the world's population is infected with this bacterium causes stomach ulcer ,gastritis and stomach cancer.

in This Case –Control study included 250 subjects divided as 200 patients attending gastroenterology and hepatology center of merjan medical city hospital to diagnosis bacterial infection in stomch aged(15-71) during November2022 to January2023 ,in addition to 50 apparently healthy subject as control group with age and sex similar to case group. from all subject collected 250 blood specimens and 250 stool specimens. 5ml of venous blood specimens that separated into two group . 2.5 ml put in EDTA tube for DNA extraction to detect HLA polymorphism .while 2.5ml put in gel tube for serum separation to detect HLA-DRB and HLA-C levels by ELISA and diagnosis *H.pylori* by Ab rapid test. In addition to detect the bacteria by Ag rapid test through stool specimens according to bacterial culturing and PCR to detection URE A .of the 200 specimens 80(40%) showed positive result for the Ab test and 120(60%) gave negative . 45(22.5%) positive result for the Ag test and according to bacterial culturing while 155(77.5%) gave negative Ag rapid test and bacterial growth.

This study showed an decrease in the serum level of HLA-DRB1 in patient which was (1905) ng/L ,compared with serum levels of healthy control which were (2849) ng/L, $P \leq 0.0003$) ,while HLA-C study showed an increase in the serum level of HLA-C in patient which was (50.90) ng/L compared healthy control which were (28.77) ng/L, $P \leq$ (0.000) .

The genotype distribution of the SNPs in that HLA-C rs2395471 SNPs was 8% GG, 60% GA, and 32% AA, with H. pylori infection. and the genotype distribution in healthy controls was 0% GG, 40% GA, and 60% AA, with T-allele and A-allele frequencies of 8(0.16%) and 42(0.84%) in patients and 19(0.38%) for G-allele frequency and 31(0.62%) for A-allele frequencies, respectively. When comparing the genotype distribution for HLA-C rs2395471 GG result was (P=0.2 OR=5.42, Cl=0.24 to 118). AA indicated that there were no statistically significant differences in the HLA-C rs2395471 SNP between patients and healthy controls (GG,GA). demonstrates that (HLA-C) rs2395471SNPs contributed to infection at a significant p-value (0.04), indicating that there was a significant difference in AA genotypes.

The genotype distribution for the SNPs in HLA-C, rs2249741SNPs, was 20% AA, 52% AC, and 28% CC in patients infected with *H. pylori*. and the genotype distribution in healthy control was 0% AA, 24%AC, and 76% CC, with A-allele and C-allele frequencies 23(0.46)%, 27(0.54)% in patients and healthy control were 6(0.12)% for A-allele frequency and 44(0.88)% for C- allele frequencies, and when comparing the genotype distribution for HLA-C rs2249741SNP, AA result was (P=0.08, OR=13.68,Cl=0.71-262.18) There were no statistically significant differences in HLA-C rs2249741SNP between patients and healthy controls when comparing the (AA) wild genotype to the (AC,CC) homozygous genotype and heterozygous genotype. demonstrates that (HLA-C) rs2249741SNPs was associated with infection by *H. pylori* when p-values of 0.04 and 0.001 were significant .

the genotype distribution for SNP in the HLA-C rs2249742 SNP consisted of 44% CC, 44% CT, and 12% TT, with *H. pylori* infection.

and the genotype distribution in healthy control was 0% CC, 28% CT, and 72% TT with C-allele and T-allele frequency33(0.66)%, 17(0.34)% in patients and healthy control were 7(0.14)% for C-allele frequency and 43(0.86) for T-allele frequencies, and when comparing the genotype distribution for HLA-C rs2249742SNP, CC result was (P=0.01, OR=40.44, Cl=2.21-738.01)This finding (CC,TT) demonstrates that there were significant differences in HLA- C (CT), heterozygous genotype non-significant SNPs. It can be demonstrated that there was a significant difference between the CC and TT genotypes at significant p-value(0.01) and (0.000), indicating that (HLA-C) rs2249742 SNPs was associated with infection by *H.pylori* .

the genotype distribution of rs1794511 genotype SNPs in the HLA-DRB1 rs2249742 genotype SNPs consisted of 64% TT, 24%TG, and 12% GG, with H. pylori infection. and the genotype distribution in Healthy control was 100% TT, 0% TG, and 0% GG, with T-allele and G-allele frequency38(0.62) %, 9(0.38)% in patients and healthy control were 50(1)%. for T-allele frequency and 0 for G-allele frequencies, and when comparing the genotype distribution for DRB1 rs2249742 HLA-DRB1 genotype SNPs, TT result was (P=0.02, OR=0.03, Cl=0.0019 to 0.62) This finding demonstrates that there was significant differences (TG.GG) heterozygous genotype and homozygous genotype non-significant SNPs. p-values (0.06) and (0.1) .Polymorphism in HLA-C and HLA-DRB1 was significantly associated with the disease risk.

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Symbol	List of Abbreviations Description
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme-linked immunosorbent assay
HLA	Human Leukocyte Antigens
IFN	Interferon
IL	Interleukin
MHC	Major histocompatibility complex
NK	Natural Killer
OR	Odds ratio
PPI	Proton- pump inhibitor
SNP	Single nucleotide polymorphism
TBE	Tris-Borate EDTA

Chapter One

Introduction

1.1.Introduction

Helicobacter pylori (H. pylori) is a Gram-negative microaerophilic bacterium that colonizes the human stomach and can caused to chronic gastritis, gastric, peptic ulcer adenocarcinoma and mucosa-associated lymphoid tissue lymphoma. About half of the world's population is estimated to be infected with this pathogen (Zamani *et al.,* 2018).

Human leukocyte antigen (HLA) molecules play a critical role in the adaptive immune response to *H. pylori* infection. HLA molecules are cell surface proteins that present antigenic peptides to T cells, allowing them to recognize and respond to specific pathogens HLA molecules are highly polymorphic, meaning that there are many different variants of HLA genes in the human population. Different HLA variants can present different antigenic peptides, and the ability of an individual's HLA molecules to present *H. pylori* antigens can affect their susceptibility to infection and the outcome of the immune response. (Matsueda *et al***.**2014)

It is important to note that the relationship between HLA gene polymorphisms and *H. pylori* infection is complex and multifactorial. Other factors, including environmental and bacterial virulence factors, also play significant roles in determining an individual's susceptibility to *H. pylori* related diseases HLA genes may affect the host's ability to recognize and present H. pylori antigens to immune cells, potentially influencing the immune response against the infection. These variations can lead to differences in the individual's susceptibility to *H. pylori* infection and the development of associated diseases.(Kocak *et al.*,2020)

In addition to influencing disease susceptibility, HLA molecules can also affect the immune response to *H. pylori* Studies have shown that HLA class II molecules, such as HLA-DR and HLA-DQ, are important for the activation of CD4+ T cells, which play a critical role in the adaptive immune response to *H.*

pylori. The specific HLA alleles expressed by an individual's APCs may affect the types of *H. pylori* antigens that are presented to T cells, influencing the specificity and strength of the immune response. Overall, the polymorphic nature of HLA molecules can have significant effects on the immune response to *H.pylori* infection and the development of *H. pylori*-related diseases. Understanding the role of HLA in the immune response to *H. pylori* may be important for developing more effective diagnostic and therapeutic strategies for these diseases. (Chazy and Taha.,2022)

1.2. Aims of Study

The present study was aimed to study the immunogenic association of HLA level and polymorphism on people infected with *H.pylori* and the achievement of this aim by the following objectives:

1. Blood and stool specimens collection from patients infected with *H.pylori* and healthy as control group.
2. Isolation and identification of bacteria from stool by serological test, culturing and PCR to detect URE A.
3. Detection serum levels of HLA-C and HLA-DRB1 by ELISA.
4. Human DNA extraction to study HLA-C and HLA-DRB1 polymorphism by DNA sequence assay.

Chapter Two
Review of Literature

2 . Literatures Review

2 .1 . Historical review :

The discovery of *H. pylori* by Warren and Marshall was preceded by nearly a hundred year of inconspicuous publications relating to spiral bacteria, achlorhydria, gastritis, gastric urease, and antimicrobial therapy for ulcers (Marshall,1988). Investigation of gastric bacteria properly began in the latter half of the 19th century when microscope resolution had sufficiently advanced (Kidd and Modlin ,1998).

Bottcher and Letulle firstly hypothesized that bacteria caused ulcer disease in 1875, after they discovered bacteria in the floor and margins of gastric ulcers. but they were unable to cultivate them and perhaps because of this they did not present their discovery to the world. In 2018 Marshall drank the bacterial suspension himself to prove himself that bacteria are the cause of stomach ulcers and gastritis. After a few days he struck nausea and a foul smell from his mouth then vomiting appeared. The endoscopy results on the eighth day of the trial were clear - advanced enteritis (Fedorowicz *et al*., 2020).

Similar spiral organisms were found in stomach of humans and other species by several scientists between then and the 20th century. For instance, in 1893 Bizzozero noted spirochetes in the gastric mucosa of dogs, which were named *H.bizzozeroni*. Kasai and Kobayashi in 1920 isolated spirochetes in cats and transmitted them to rabbits to produce ulcers (Kidd and Modlin ,1998)

Warren in 1979 identified *Campylobacter pylori* as the putative causative agent of human gastritis (Kidd and Modlin ,1998 ; Marshall and Warren,1984) Culture of the organism (*H. pylori*) was elusive until 1982

when it was obtained by Barry Marshall (Marshall,1988). Earlier attempts to culture the organism proved abortive because incubation was usually limited to 48 hours. Success at culture was incidental, as one of them spanned a holiday period and hence, lasted for 5 days, thereby yielding a growth. History was then made in April 1982 at the Royal Perth Hospital in Australia where *H. pylori* was cultured. Examination of the plate showed a pure growth of 1mm transparent colonies. Gram stains of the colonies showed slightly curved organisms and not spiral as in the smear of the specimen, which made Marshall to doubt whether it was the organism in question that was grown.

Further studies on the organism and its RNA sequence in ribosomes helped correct the earlier misconception that the organism belonged to the *Campylobacter* family (initially called *Campylobacter pyloridis*). At the 5th International *Campylobacter* workshop in Mexico in February, 1989 the *Campylobacter* taxonomy committee agreed that *H. pylori* should no longer be included in the *Campylobacter* group. There was initial difficulty in nomenclature before Steward Goodwin who was head of the Microbiology Department at Royal Perth Hospital at that time reportedly suggested *Helicobacter*, and this was published in 1989.

The World Health Organization classified *H. pylori* as a class 1 (definite) carcinogen implicated in the etiopathogenesis of gastric malignancies in 1994 (Houghton and Wang ,2005) . (Parsonnet *et al* .,1994) also describe an association between *H.pylori* and gastric lymphoma completed sequencing of the entire 1,667,867 base pairs of the *H. pylori* genome in 1997 (Tomb *et al*.,1997) And in 2005 Warren and Marshall were awarded the Nobel Prize in Physiology or Medicine for their work on *H. pylori* and PUD(The Nobel Prize in Physiology or Medicine 2005).

2 . 2 . Classification of *H. pylori*

Taxonomy aims to provide a meaningful biological framework upon which a wide range of other sciences is built. However, the inaccurate delineation of taxa serves to confound workers in a variety of disciplines (On, 2001).

Cultivation of new stomach bacteria in 1982 marked a new turning point understanding of the environment and diseases of the digestive system. Description Marshall and Warren, spiral or curved bacilli in histological sections of numerous samples of the human gastric mucosa. A number of these samples gave culture positivity to the Gram-negative microaerophilic bacteria. The second international workshop held in Brussels, Belgium, in September 1983 on *Campylobacter* infection gave great importance to its correct classification. The *campylobacter* organism is similar in many aspects, including the curved morphology, growth on rich media failure to fermentation of glucose, sensitivity to metronidazole, and G + C content of 34%(Solnick and Vandamme, 2001)

2 . 3 . General Characteristics and Growth Requirement of *H. pylori*

*H*elicobacter pylori (*H. pylori*) is a gram-negative, microaerophilic bacterium that is adapted to survive in the acidic environment of the stomach. Here are some general characteristics and growth requirements of *H. pylori* Morphology it is a curved or spiral-shaped bacterium that is approximately 3-4 micrometers long and 0.5 micrometers wide.

H. pylori is motile, and it uses several flagella to move through the mucus layer of the stomach. (Qin *et al.*, 2017) .

H. pylori is a fastidious bacterium has specific growth requirements. It grows best at a pH of 7.0-7.4 and at temperatures between 37-42°C. *H. pylori* requires a microaerophilic environment with low oxygen tension (5-

10%) and high carbon dioxide levels (5-10%) for optimal growth. It also requires specific nutrients, such as iron and amino acids, for growth. (Al-Thahab and Al-Awsi, 2018) .

H.pylori can be cultured using specialized media, such as Columbia agar supplemented with 5-10% sheep blood and selective antibiotics, such as vancomycin and trimethoprim. (Scott *et al.,2002).*

H. pylori has a relatively small genome, with approximately 1,500 genes. It has a high degree of genetic variability, with several subtypes or strains that vary in their genetic makeup and virulence factors .Overall *H. pylori* is a fastidious bacterium that is adapted to survive in the acidic environment of the stomach. Its specific growth requirements and genetic variability may have implications for the development of strategies to prevent or treat *H. pylori*-related diseases (Lee *et al.*, 2017).

2 . 4 .Transmission :

The exact mode of transmission is not fully understood, but it is thought to occur through oral-oral or fecal-oral routes.

In areas with high *H. pylori* prevalence, the bacteria is often acquired during childhood, typically before the age of 10. The exact mechanisms of transmission can vary depending on cultural and environmental factors, but it is thought to involve close contact with family members or caretakers who are infected with *H. pylori* (Aziz *et al*., 2015) .

The bacteria can be transmitted through fecal-oral contact, such as consuming contaminated food or water. In addition, *H. pylori* can survive in the environment for several days, and contaminated surfaces or objects may also contribute to transmission(Bürgers *et al.*, 2008) .

Individuals who live in crowded conditions, have poor sanitation, or are exposed to contaminated food or water are at higher risk of *H. pylori* infection. In addition, certain cultural or dietary practices may also increase the risk of transmission(Gebara *et al.*, 2006).

Understanding the mechanisms of transmission and identifying individuals at risk of infection may be important for developing strategies to prevent or reduce *H.pylori* transmission (Stefano *et al.*, 2018).

2 . 5. *H.pylori* virulence factor and pathogensis

i -Lipopolysaccharides (LPS): they are glycolipids found in the cell envelope of gram negative bacteria of which *H.pylori* is one. Lipopolysaccharides are *endotoxins* which stimulate the release of cytokines through their lipid A component. They also interfere with gastric epithelial cell–laminin interaction which may lead to loss of mucosal integrity, inhibit mucin synthesis, and stimulate pepsinogen secretion (Moran,2007).

ii - Leukocyte recruitment and activating factors: these are soluble surface proteins with chemotactic properties produced by the organism. They help to recruit monocytes and neutrophils to the lamina propria and to activate these inflammatory cells. These include *H. pylori* neutrophil-activating protein, expressed by the *napA* gene, and the immunologically active porins (Brisslert *et al.,2005)*

iii - Vacuolating Cytotoxin (VacA): VacA is secreted by *H. pylori* and can target and disrupt various cells in the stomach, including epithelial cells and immune cells. It induces the formation of vacuoles (hence the name "Vacuolating cytotoxin") in these cells leading to cellular damage and alterations in cell function .The effects of VacA on the host cells are complex and involve multiple mechanisms. It can disrupt the integrity of the gastric epithelium, interfere with cellular signaling pathways, impair the function of immune cells, and modulate the host immune response The presence of VacA has been associated with an increased risk of developing gastric diseases, including gastritis, peptic ulcers, and gastric cancer. It is

believed to contribute to the ability of *H. pylori* to colonize and persist in the stomach, as well as promote the progression of *H. pylori*-associated diseases by inducing inflammation and tissue damage (Maeda *et al.,2002)*

iv - Cytotoxin-Associated Antigen (CagA): is a protein produced by certain strains of *H. pylori* bacteria. CagA is considered a virulence factor because it plays a role in the pathogenesis and severity of *H. pylori* associated diseases When *H. pylori* infects the stomach, strains that carry the cagA gene can inject the CagA protein into the epithelial cells lining the stomach. Once inside the host cell, CagA can interact with various cellular signaling pathways, leading to alterations in cell structure, function, and signaling The presence of CagA-positive *H. pylori* strains has been associated with an increased risk of developing more severe gastric diseases, such as peptic ulcers and gastric cancer. It is believed that CagA contributes to the ability of *H. pylori* to persistently colonize the stomach and trigger chronic inflammation, which can lead to the development of these diseases over time The detection of CagA can be performed using specialized tests, such as serological assays (blood tests) that detect antibodies against CagA or molecular methods that identify the presence of the cagA gene or CagA protein *in H. pylori* isolates It's important to note that while the presence of CagA is associated with an increased risk of severe disease outcomes not all *H. pylori* strains carry the cagA gene, and not all individuals infected with CagA-positive strains will develop complications. Other factors, such as host genetic susceptibility and environmental factors, also play a role in determining disease progression (Rohde *et al.,2003).*

V - Outer Membrane Inflammatory Protein (OipA): it is possessed by most strains with CagA. It acts synergistically with CagA to produce a

more intense inflammatory response than would have otherwise occurred in either (Yamaoka *et al.,2000).*

2 . 6 . factors that enhance mucosal colonization and factors that mediate tissue injury.

- **Flagella:** the possession of spiral shaped, unipolar, sheathed flagella (Hofman *et al.,2004)*allows the organism to move rapidly from the lumen of the stomach, where the *pH* is low,through the mucus layer to an area where *pH* is near neutral to permit optimal growth.
- **Urease:** *H. pylori* has a great capacity for urease production, probably more than almost all other bacterial species. Urease hydrolyses urea to produce ammonia (NH_3) and carbon dioxide (CO_2). The presence of NH_3 reduces the acidity of the stomach; which may be necessary for providing a congenial environment for *H. pylori*. Ure1, a *pH*-gated channel helps to regulate the production of urea (Suerbaum and michetti,2002).
- **Adherence factors:** *H. pylori* has tissue tropism for the gastric epithelium. It possesses fibrillar adhesins, located on its surface which binds closely to the carbohydrate receptors on the mucosal cell leading to the formation of an adherence pedestal. The best-characterized of these adhesins is BabA, which is a 78-kD outer-membrane protein that binds to the fucosylated Lewis B blood group antigen. BabA is relevant in *H. pylori* associated disease and may influence disease severity, although the results of several studies are contradictory (Suerbaum and michetti,2002). This property prevents the organism from being shed during cell and mucus turnover.

2.7. *H. pylori* Associated Diseases

2.7.1 Gastritis

H.pylori is a major cause of chronic gastritis, an inflammation of the stomach lining. Gastritis can lead to symptoms such as abdominal pain, bloating, nausea, and vomiting. (Graham and sung,2006). When *H. pylori* infects the stomach, it can lead to chronic inflammation and damage to the stomach lining. This can result in gastritis, which may be acute (short-term) or chronic (long-term). The inflammation can cause symptoms such a .Abdominal pain or discomfort This is a common symptom of gastritis and is often described as a burning or gnawing sensation in the upper abdomen.Loss of appetite Some individuals with gastritis may experience a decreased desire to eat due to the discomfort associated with eating Bloating and indigestion .Gastritis can cause bloating a feeling of fullness and indigestion after meals Black tarry stools (melena). In some cases gastritis can cause bleeding in the stomach leading to the presence of black, tarry stools (Malaty *et al*.,2000).

2 .7.2 Peptic ulcer disease

H. pylori infection is a primary risk factor for the development of peptic ulcers, which are open sores that form in the lining of the stomach or the upper part of the small intestine. Peptic ulcers can cause abdominal pain, discomfort, and in some cases, bleeding. (Zhang *et al*, 2017)

2.7.3 Gastric Cancer

Long-term infection with H. *pylori* is associated with an increased risk of developing gastric (stomach) cancer. However, it's important to note that the majority of individuals infected with *H. pylori* do not develop cancer. (Tham *et al*.,1998).

2.7.4 Duodenal ulcer

The review evidence supports a strong causal relation between *H. pylori* infection and duodenal ulcer, as patients are more likely to be infected by virulent strains which later cause duodenal ulceration. Thus, eradication of *H. pylori* infection decreases the incidence of duodenal ulcers, and prevents its recurrence by reducing both basal gastrin release and acid secretion without affecting parietal cell sensitivity *H. pylori* can elevate acid secretion in people who develop duodenal ulcers or hyper secretion of gastric acid can by itself evoke duodenal ulcers (Ahmed and Belayneh, 2019) .

2.7.5 MALT lymphoma

MALT Lymphoma: *H.pylori* infection can lead to the development of MALT (mucosa-associated lymphoid tissue) lymphoma, a type of non-Hodgkin's lymphoma. MALT lymphoma typically occurs in the stomach but can also affect other organs.(Houghton and Wang,2005; Matysiak-Budnik and Megraud,2006).

2.7.6 Dyspepsia

H. pylori infection may be a contributing factor to non-specific symptoms of indigestion and upper abdominal discomfort, a condition known as functional dyspepsia. (Talley and Vakil,2005).

2.7.7 *H. pylori* and Insulin Resistance

Since the immune system is triggered by *H. pylori* infection, some inflammatory cytokines such as tumour necrosis factor _ (TNF-_) and leptin and adipokines createan immune response to this inflammation. Relevant studies have revealed that leptin deficiency can induce the insulin resistance (IR) of high TNF-_ and IL-6 levels (Chen *et a*l.,2015; Kern *et al*.,2003).Inflammatory cytokines induce the phosphorylation of

serine residues on the insulin receptor substrate, causing disruption of insulin function and disrupting the substrate'sinteraction with insulin receptors. Thus, diabetes can occur with the deterioration in bloodglucose regulation (*Maeda et al*.,2002 ; Mansori *et al*., 2020).

2.7.8 *H. pylori* and Anaemia

Iron is an important micronutrient for animals and microorganisms as a cofactor for enzymes involved in oxygen and electron transport and DNA synthesis. The response to infection is mediated by an iron-retaining mechanism that indirectly reduces the redistribution from the cell cytosol to the cell surface, and reduces circulating transferrin and the growth of infecting pathogens (Flores *et al*., 2017). demonstrated that the SabA gene in the pathogenesis of *H. pylori* is highlyexpressed in bacterial isolates from patients with iron deficiency anaemia, proving that this virulence factor has a role in the development of anaemia (Kato *et al*.,2017). Moreover, *H. pylori*

causes hypochlorhydria and atrophic gastritis, in addition to peptic ulcer disease and increasin the risk of gastricmalignancies. In this case, weakening of iron absorption canfactor secretion, hypochlorhydria, or achlorhydria and can lead to intestinal iron and B12malabsorption (Rockey *et al*.,2020 ; Sipponen *et al*.,2003).

2.8 . Diagnostic Techniques:

H. pylori resides in the stomach, colonizes gastric epithelium, and causes several digestive system diseases. Several diagnostic methods utilizing invasive or non-invasive techniques with varying levels of sensitivity and specificity are developed to detect *H. pylori* infection.

Selection of one or more diagnostic tests will depend on the clinical conditions, the experience of the clinician, cost, sensitivity, and specificity. Invasive methods require endoscopy with biopsies of gastric tissues for the histology, culture, and rapid urease test. Among non-invasive tests, urea

breath test and fecal antigen tests are a quick diagnostic procedure with comparable accuracy to biopsy-based techniques and are methods of choice in the test and treatment setting. Other techniques such as serological methods to detect immunoglobulin G antibodies to *H. pylori* can show high accuracy as other non-invasive and invasive biopsies, but do not differentiate between current or past *H. pylori* infections. Polymerase chain reaction (PCR) is an emerging option that can be categorized as invasive and non-invasive tests. PCR method is beneficial to detect *H. pylori* from gastric biopsies without the need for the cultures (Sabbagh *et al*., 2019)

2.8.1 Method based on discovery of the enzyme

2.8.1.1 Rapid urease test (RUT)

The fact that *H. pylori* is both abundant in the stomach and contains urease has been widely used to assist in clinical diagnosis., with a short time after the discovery of *H. pylor*i, rapid urease tests had been developed to allow rapid detection of *H. pylori* using gastric specimens (mucus, biopsy, or brushings)., methods to detect changes in pH either directly or using color changes following incubation of gastric specimens proved both simple and reliable and were most widely adapted (Graham and Miftahussurur, 2018).

Biopsy samples, approximately 2–3 mm each were taken from the antralgastric mucosa and placed on the yellow colored well containing urea and a pH indicator. The production of the urease enzyme by *H. pylori* results in the decomposition of urea into bicarbonate and ammonia which causes the pH to rise and the colour of the dot to change from yellow to red or pink. Positive results were read within 5 to 30 min. Samples that were weakly positive took up to 1 h to develop and no colour change at 1 h was regarded negative (Adu-Aryee *et al.*, 2016).

2.8.1.2 Urea breath testing (UBT)

The urea breath test is a non-invasive, simple and safe test which provides excellent accuracy both for the initial diagnosis of *H.pylori* infection and for the confirmation of its eradication after treatment.Some studies have found no differences between urea breath test performed under non-fasting conditions. The simplicity, good tolerance and economy of the citric acid test meal probably make its systematic use advisable. The urea breath test protocol may be performed with relatively low doses (<mg seem to be sufficient. With the mg or even 50 mg) of urea: 75 100 mg of urea), excellent most widely used protocol (with citric acid and 75 min accuracy is obtained when breath samples are collected as early as 10–15 after urea ingestion (Gisbert and Pajares, 2004 ; Lopes *et al.*, 2014) . Several factors including the patient's condition bacterium, and the test itself can affect the results of the UBT .

Nonetheless, the urea breath test is widely available because breath samples are easy to collect for rapid testing . UBT is useful for epidemiological studies and for assessing the effectiveness of eradication therapy . This method has advantages such as non-invasive, safe, accurate, and with a sensitivity of 95.9% and a specificity of 95.7% (Sabbagh *et al.*, 2019).

2.8.2 Method based on discovery of antigen-antibodies to *H. pylori*

2.8.2.1 Stool antigen test (SAT)

In 1997, it was reported that the detection of *H. pylori* antigens in stools using polyclonal anti-*H. pylori* antibodies (HpSA) with a sensitivity and specificity of 88.8% and 94.5%, respectively (Mégraud and Lehours, 2007 ; Miftahussurur and Yamaoka, 2016). In infected individuals, *H. pylori* sticks to the gastric epithelial wall and is excreted in the feces. This test is a direct test of initial infection that results in the superiority of serologic tests . The test is based on the detection of *H. pylori* antigens in

the stool. There are two types of SATs used for *H. pylori* detection: enzyme immunoassay (EIA)– and immunochromatography assay (ICA)–based methods, using either polyclonal antibodies or monoclonal antibodies .Monoclonal antibody–based tests show better results compared to polyclonal-based tests mainly because of the difficulty in obtaining polyclonal antibodies of consistent quality every time. EIA-based tests provide more accurate and reliable results than ICA-based tests although both tests can be performed with monoclonal antibodies (Thaker *et al*., 2016 ; Sabbagh *et al.*, 2019).

2.8.2.2 *H.pylori* specific antibodies

Serological testing is the most widely available test for the detection of *H. pylori* with a relatively high negative predictive value . Furthermore, serology is the only test that is not affected by local changes in the stomach that could lead to false-negative results in the other tests. Furthermore, in patients treated with PPIs, if it not possible to stop them for at least 2 weeks, a validated IgG serology test (ELISA) may be used. This is the case in the setting of ulcer bleeding, as well as the recent use of antimicrobial and antisecretory drugs (Tonkic *et al.*, 2012).

This type of testing is not appropriate for proving current infection since IgG antibodies are present for such a long period during a patient's life. It can't tell the difference between past and current infections. Serology cannot be used to track eradication for the same purpose. Antibodies will last a lifetime after eradication. (Miftahussurur and Yamaoka, 2016).

2.8.3. Method based on the presence of bacterium

2.8.3.1. Culture

Several selective media are suggested for *H. pylori* culture and isolation. The transport medium, time in transit to the pathology laboratory, temperature during transportation, and the medium used all

influence bacterial viability and recovery all of these factors influence bacterial viability and recovery (Graham and Miftahussurur, 2018).

The culture of *H. pylori* is performed on the gastric biopsy samples to confirm the *H. pylori* infection and is performed only in specialized laboratories . Culturing of gastric biopsy samples to detect *H. pylori* is not a routine method for detecting *H. pylori* . Bacterial culture is carried out mainly for scientific research and when the prior treatments have failed to detect an appropriate bacterium. It is recommended that the mentioned test should be performed before the next treatment line to determine the microbial susceptibility (Sabbagh *et al.*, 2019).

Although the culture is an expensive, complicated, and time-consuming test for *H. pylori* detection, an antibiotic susceptibility test of *H. pylori* by culture is a useful clinical practice for accurate detection. Besides, culture allows the isolation of *H. pylori* for phenotypic and genotypic studies (Wang *et al.*,2015)

2.8.3.2.Endoscopy

A typical endoscopy exam is performed to detect *H. pylori*–related diseases. Endoscopy is the method for obtaining biopsies from the gastric mucosa that can be used in further studies on other invasive methods (Lan *et al.*, 2012) . Also, the endoscopy offers the precise and clear image of gastric mucosa, but it may not have better results than other diagnostic tests (Cho *et al.*, 2013).

2.8.3.3. PCR for *H. pylori* Markers

The polymerase chain reaction (PCR) is an effective molecular technique that can be used in a number of clinical settings, including broad-spectrum infection detection, emerging infection assessment, genotypic bacterial identification, antibiotic resistance, and epidemiological studies (PCR) tests are used for diagnosis. PCR detection of *H. pylor*i has been reported using a variety of clinical samples including

gastric biopsy, gastric juice, saliva, dental plaque, and stools as well as environmental samples.

Whenever possibly, noninvasive tests are preferred over invasive tests. *H. pylori* are excreted in the stool. Culture from stool is variable whereas stool antigen testing is widely used. Stool consists of a complicated mixture of communal bacteria and chemicals and often includes inhibitors of PCR. Nevertheless, simple extraction methods are available to efficiently extract DNA from human stools and nested-PCR targeting the 23S rRNA gene have proven to be highly sensitive for the detection of *H. pylori*. Detection of clarithromycin susceptibility/resistance is important clinically and the mutation of the 23S rRNA gene responsible for resistance can also be detected using stool. This described method can be modified for other clinical samples such as gastric juice or biopsy material (Rimbara, *et al*., 2013).

Antibiotic resistance mutations can be detected using molecular techniques such as PCR, which is an appropriate methodology for pathogen detection and can assist us in selecting an appropriate treatment strategy (Ramírez-Lázaro *et al.*, 2011) .

2.8.3.4 DNA-Enzyme immunoassay:

this is a form of PCR where the PCR amplicons are detected by calorimetric method. It is ELISA-based and involves the use of coated microwells. This method is more rapid than the standard PCR and result can be obtained within a few hours (Vaira *et al*.,1999).

2.9.Treatment

Currently, the main challenge in the field of *H. pylori* infection is antibiotic resistance, which influences the efficacy of eradication regimens. The latest systematic review and meta-analysis reported that the primary

and secondary resistance rates to clarithromycin, metronidazole, and levofloxacin exceeded 15% (alarming levels) in all the World Health Organization (WHO) regions (Savoldi *et al*., 2018) .The traditional proton-pump inhibitor (PPI)-based triple therapy (PPI plus two antibiotics) has been used for eradicating *H. pylori* for more than 20 years. However, PPI-based triple therapy provides low treatment success (intention-to-treat [ITT] analysis below 80% in most studies) (Graham and Fischbach, 2010) In 2012, the Maastricht IV/Florence Consensus Report recommended that PPI-clarithromycin-containing triple therapy should be abandoned in areas with clarithromycin resistance rates above 15% to 20%. In addition, bismuth-containing quadruple therapy (BQT) is recommended as a first-line treatment for eradicating *H.pylori* in areas with high or low clarithromycin resistance because of its high efficiency, safety, and tolerance. a number of studies were conducted to evaluate the efficiency of other regimens (eg, sequential, concomitant, hybrid therapy, high-dose PPI-amoxicillin dual therapy, vonoprazan [VPZ]-based triple therapy, probiotics supplemented triple therapy or combined with BQT) in *H. pylori* eradication. the review by (Malfertheiner *et al*., 2012). Summarize the recent progress in *H.pylori* eradication .However, bacterial antibiotic resistance is still challenging the outcome of *H.pylori* eradication treatment. The “key” antibiotics in the treatment of *H. pylori* infection are clarithromycin and levofloxacin, and the prevalence of *H. pylori* strains resistant to these antibiotics has been increasing over the last decades (Megraud *et al*., 2013) .

2.10. Immune Response to *H. pylori*

Helicobacter pylori (H. pylori) is a bacterium that colonizes the stomach of over half the world's population. While some people may remain asymptomatic, *H. pylori* infection can cause a range of

gastrointestinal disorders, from chronic gastritis and peptic ulcer disease to gastric cancer and mucosa-associated lymphoid tissue (MALT) lymphoma. The immune response to *H. pylori* infection involves both innate and adaptive immunity. The innate immune response is the first line of defense against *H. pylori* and involves the activation of various immune cells such as neutrophils, macrophages, and dendritic cells. These cells recognize *H. pylori* as a foreign invader and initiate a cascade of events that lead to the destruction of the bacterium. (Gall *et al*., 2017).

The adaptive immune response is the second line of defense and involves the activation of T and B lymphocytes. T cells recognize *H. pylori* antigens presented by antigen-presenting cells (APCs) and become activated to produce cytokines and chemokines that recruit other immune cells to the site of infection. B cells, on the other hand, produce antibodies that can directly neutralize *H. pylori* or tag it for destruction by other immune cells.

However, *H. pylori* has developed various mechanisms to evade the host immune system, including the secretion of virulence factors that can modulate the host immune response and facilitate bacterial survival. This can lead to chronic inflammation and tissue damage, which can increase the risk of developing more serious gastrointestinal disorders (Robinson *et al*., 2007).

2.10.1. Innate immune response

The innate immune response plays an important role in the early recognition and response *to H. pylori* infection. *H. pylori* is recognized by a variety of pattern recognition receptors (PRRs) expressed on innate immune cells, including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), and others.

When PRRs on innate immune cells recognize *H. pylori* they trigger a signaling cascade that leads to the production of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor

necrosis factor-alpha (TNF-α). These cytokines, in turn, recruit and activate other innate immune cells, such as neutrophils and macrophages, to the site of infection. (Sabbagh *et al*., 2019).

Neutrophils are among the first immune cells to arrive at the site of *H. pylori* infection and play a critical role in clearing the bacteria. They phagocytose and kill *H. pylori* through the release of reactive oxygen species (ROS) and antimicrobial peptides. Macrophages are also recruited to the site of infection and phagocytose *H. pylori* but they can also contribute to chronic inflammation through the production of pro-inflammatory cytokines. (Zhang *et al*., 2020).

Other innate immune cells, such as dendritic cells and natural killer (NK) cells, also play a role in the innate immune response to *H. pylori* Dendritic cells can present *H. pylori* antigens to T cells, initiating the adaptive immune response, while NK cells can directly kill infected cells.

Overall, the innate immune response to *H. pylori* is critical for controlling the initial infection and preventing the spread of the bacteria. However, chronic *H. pylori* infection can lead to persistent inflammation, tissue damage, and the development of more serious gastrointestinal disorders (Ohno and Satoh-Takayama, 2020) .

2.10.2 Cellular immune response

The cellular immune response is an important component of the adaptive immune response to H. pylori infection antigens can be presented to T cells by antigen-presenting cells (APCs), such as dendritic cells and macrophages, leading to the activation and proliferation of antigen-specific T cells.

CD4+ T cells, also known as helper T cells, are a key component of the cellular immune response to *H. pylori*. Helper T cells can differentiate into

different subsets, including Th1, Th2, Th17, and regulatory T (Treg) cells, depending on the cytokine microenvironment. Th1 cells are important for the clearance of intracellular pathogens by producing interferon-gamma (IFN-γ), while Th2 cells produce cytokines such as interleukin-4 (IL-4) and interleukin-13 (IL-13) that are important for antibody production. Th17 cells produce interleukin-17 (IL-17) and other cytokines that promote inflammation and neutrophil recruitment, while Treg cells suppress immune responses and help to maintain immune tolerance. (Yamamoto-Furusho *et al.* 2020).

In *H. pylori* infection, Th1 cells are thought to play a key role in controlling the infection by producing IFN-γ, which activates macrophages and promotes the production of antimicrobial peptides. However, chronic *H. pylori* infection can lead to the accumulation of Th17 cells, which contribute to persistent inflammation and tissue damage. Treg cells may also play a role in the development of immune tolerance and the prevention of autoimmune responses against the host.

CD8+ T cells, also known as cytotoxic T cells, can recognize and kill infected cells that express *H. pylori* antigens on their surface. This is important for controlling the infection and preventing the spread of *H. pylori* within the host.

Overall, the cellular immune response to *H. pylori* is complex and involves the activation of multiple T cell subsets that can have both beneficial and deleterious effects on the host. The balance between different T cell subsets may be important in determining the outcome of *H. pylori* infection.(Zindl *et al*. 2013).

2.11. Human Leukocyte Antigens (HLA)

Human leukocyte antigens (HLA) are genes in major histocompatibility complexes (MHC) that help code for proteins that differentiate between self and non-self. They play a significant role in diseas an immune defense. They are beneficial to the immune system but can also have detrimental effects. Some of the immune system effects are the interaction with complement, the cytotoxic effect of T cells, and cellular immunity Additionally, they play a role in autoimmunity and continue to be the target of researchers for their further effects and interactions Human leukocyte antigens are of three main types. Class I HLA antigens include HLA-A, B, and C molecules; class II, which includes HLA-DR, -DQ, and -DP loci, are on antigen-presenting cells; and class III contains genes for proteins that have immune functionality HLA antigens, particularly the A, B, and C loci, are highly variable, especially in the extracellular domains, as evidenced by the over 300 class I alleles that researchers have identified. The parts of the HLA antigens that are the most variable reside near the peptide-binding groove. The variability alters the interactions with T-cell receptors and the peptide-binding specificity this changes the function of the HLA antigens, which can alter the immune response and disease resistance.

In addition to their variability and function as peptide receptors, they act at different sites with beta-2-microglobulin, an alpha-beta T-cell receptor, and inhibitory molecules. The various alleles within each class of HLA antigens allow for these additional functions (Nordquist *et al*., 2019).

2.12.polymorphism and h.pylori infection

The relationship between HLA (Human Leukocyte Antigen) gene polymorphism and *H. pylori* Helicobacter pylori infection has been a

subject of scientific investigation. HLA genes play a crucial role in the immune system by encoding proteins that help the body recognize and present antigens to immune cells Several studies have explored the potential association between HLA gene polymorphisms and susceptibility to *H. pylori* infection, as well as the development of related conditions such as gastritis, peptic ulcer disease, and gastric cancer. Here are some key findings from the research . HLA Class II genes HLA-DRB1 and HLA-DQB1 genes, which belong to the HLA Class II region, have been the focus of many studies. Certain HLA-DRB1 and HLA-DQB1 alleles have been associated with an increased risk of *H. pylori* infection or its related diseases. For example, specific HLA-DRB1 alleles, such as DRB10301 and DRB10405, have been linked to an elevated risk of gastric cancer in *H. pylori*-infected individuals . Cytokine gene polymorphisms *H. pylori* infection triggers an immune response involving the release of various cytokines. Polymorphisms in genes encoding these cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β), have been investigated for their association with *H. pylori*-related diseases. These polymorphisms can influence cytokine production and, in turn, affect the immune response to *H. pylori* infection**.** Host genetic susceptibility Studies have suggested that genetic variations in certain HLA genes may affect the host's ability to recognize and present *H. pylori* antigens to immune cells, potentially influencing the immune response against the infection. These variations can lead to differences in the individual's susceptibility to *H. pylori* infection and the development of associated diseases (Gönen *et al*., 2017)

Chapter Three

Materials and Methods

3.Materials and Methods

3.1Materials

3.1.1.Laboratory Equipment and Instruments

The equipment and instrument used in this study are listed in Table (3-1)

Table (3-1) : Equipment and instruments

No.	Equipment and instruments	Source
1.	Auto vortex	Bioneer , Korea
2.	Autoclave	Haramaya . Japan
3.	Candle jar	Germany
4.	ELISA system	Biotech .USA
5.	Eppendorf centrifuge	Hitich , Germany
6.	Eppendorf tubes	Germany
7.	Gel electrophoresis system	Cleaver , scientific
8.	Hood	Fisher, scientific
9.	Incubator	Memmert . Germany
10.	Light microscope	Olympus . Japan
11.	Micropipette 0.5-20 µL	Japan
12.	Micropipette 100-1000 µL	Japan
13.	Micropipette 20-200 Ml	Japan
14.	Microwave	Germany
15.	NanoDrop spectrophotometer	Hitich , Germany
16.	Petri dish	Sterilin , England
17.	Plain tubes	DMD-DIDPO , Syria
18.	Refrigerator	Japan
19.	Sensitive balance	Sartorius . Germany
20.	Thermocycler (PCR)	Prime . UK
21.	UV- Tran illuminator	USA

3.1.2. Culture media,

Table (3-2) Culture media

No	Culture media	Source
1	Columbia agar	Madrid , Spain
2	Urea agar medium	This medium was used to examine bacteria's capacity to manufacture the urease enzyme (Mahon and Manuselis, 2007)

3.1.3 Kits, biological and chemical materials

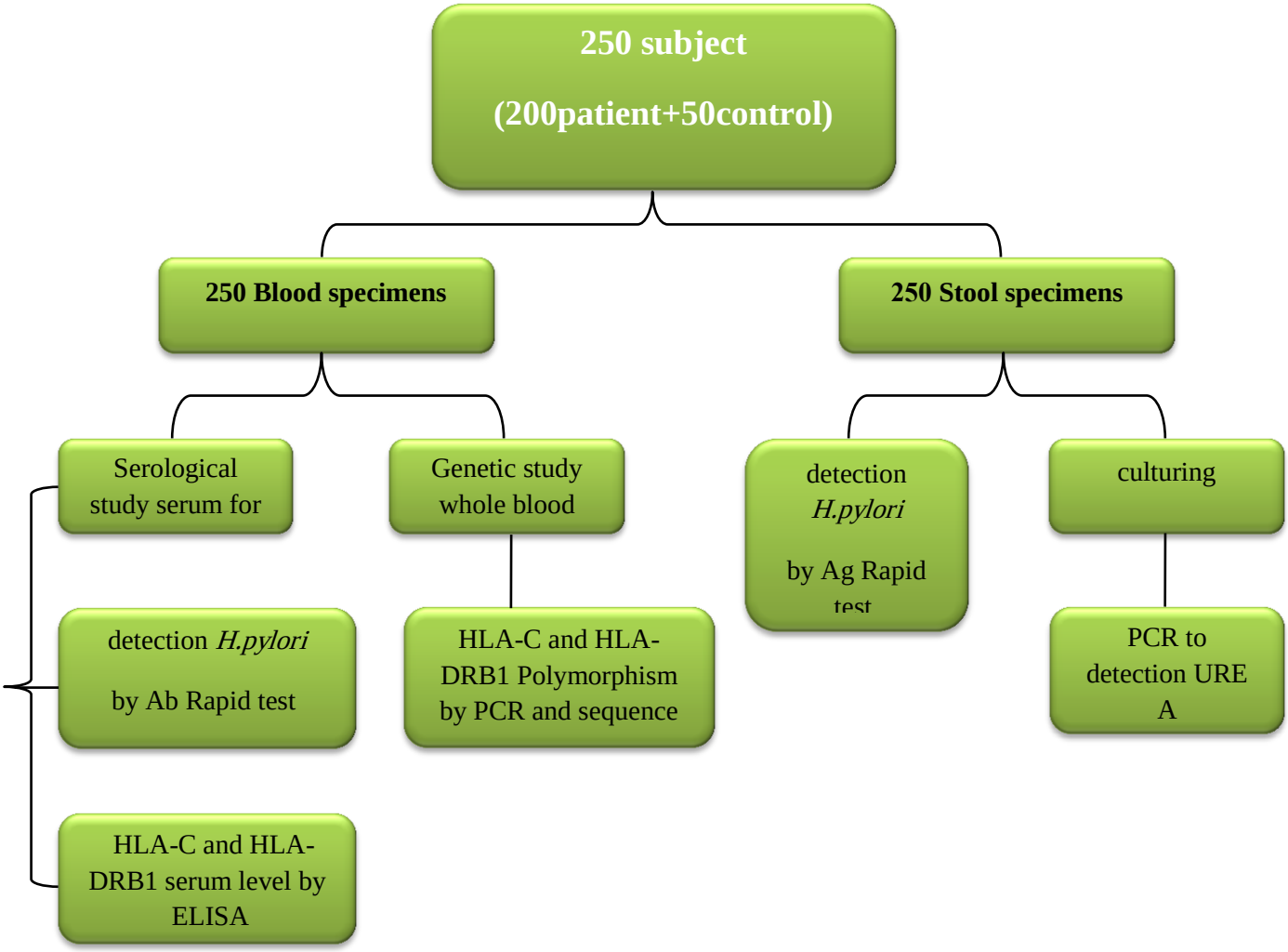
The kits, biological and chemical materials used in this study are listed in the table (3-3)

Table (3-3): Kits, biological and chemical materials

No	Type of Kit	Company (Origin)
10	Agarose, TBE buffer	Condalab (Spain)
1	DNA extraction Kit	Favorgen(Taiwan)
8	DNA ladder	Bioner(Korea)
7	Green master mix	Bioner(Korea)
2	*H. pylori* antibody rapid test kit	ctk, USA
3	*H. pylori* antigen rapid test kit	ctk, USA
4	HLA-C ELISA Kit	Biotech(China)
5	HLA-DRB1 ELISA Kit	Biotech(China)
9	Nuclease Free water	Bioner(Korea
6	primers	Macrogen (Korea)

3.1.4. Antibiotics : The antibiotic used in this study for *h.pylori* selective media were polymyxin(2.5 µg) ,Trimethoprim (5 µg), vancomycin (10 µg)

3.1.5. Study design



3.2 .Methods

3.2.1 Collection of specimens

This Case –Control study included 250 subjects divided as 200 patients attending gastroenterology and hepatology center of merjan medical city hospital to diagnosis bacterial infection in stomch aged (15-71) during November2022 to January2023 ,in addition to 50 apparently healthy subject as control group with age and sex similar to case group. from all subject

collected 5ml of venous blood specimens that separated into two group . 2.5 ml put in EDTA tube for DNA extraction to detect HLA polymorphism .while 2.5ml put in gel tube for serum separation to detect HLA-DRB and HLA-C levels by ELISA and diagnosis *H.pylori* by Ab rapid test. In addition to detect the bacteria by Ag rapid test through stool specimens according to bacterial culturing and PCR to detection URE A .the blood is allowed to coagulate for about 30 minutes at room tempreture and then centrifuged for 5 minutes at 3000 rpm . The serum was then collected in a sterile eppendorf tube and kept frozen at -20C°.

3.2.2Antibody rapid test

This test was done according to manufacturer company the principle of serum antibody rapid test of *H. pylori* was based on qualitative membrane immunoassay for the detection of *H. pylori* antibodies in whole blood, serum, or plasma . In this test, anti-human is immobilized in the test line region of the test. After sample is added to the sample well of the device, it reacts with *H. pylori* antigen coated particles in the test.

This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti-human . If the specimen contains *H. pylori* antibodies, a red colored line will appear in the test line region indicating *H.pylori* infection red color appears only on the letter C (control line)as show in Figure (3.1).

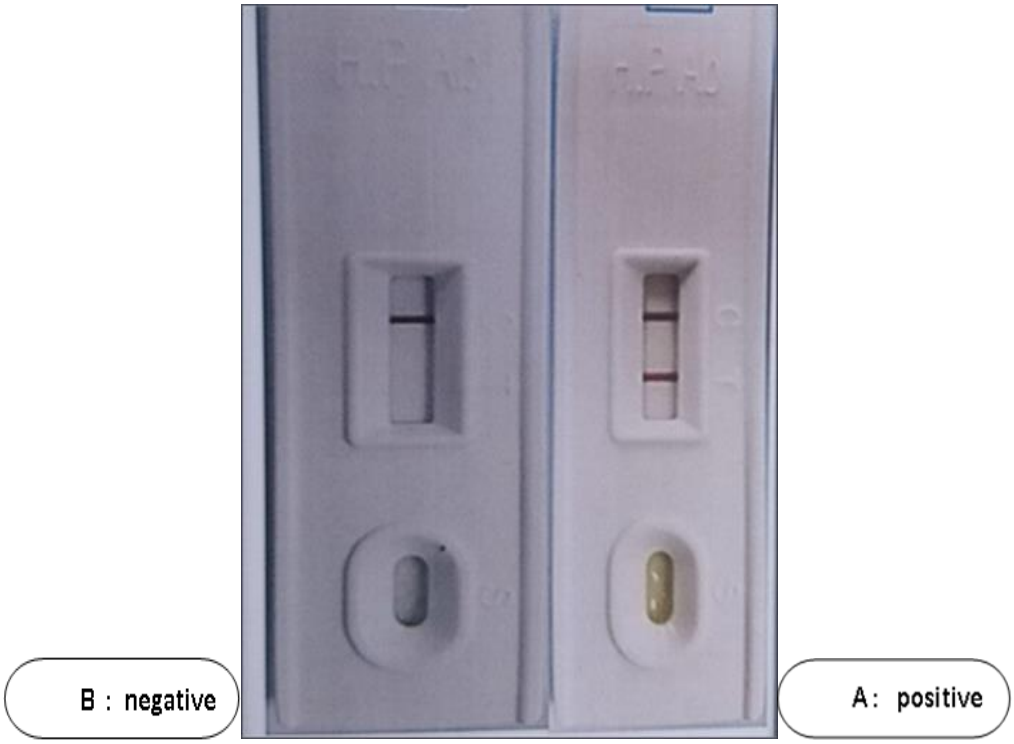


Figure (3.1) : Antibody rapid test for *H.pylori* detection

In the case of infection absence with the bacterium red color appears only on the letter C , while in the case of infection in addition to the package control in red color, a red pack'age will appear on the letter T

3.2.3. Stool antigen rapid test

The test is used to diagnosis the bacteria in stool samples . small samples of stool specimens collected from three different parts of the stool sample wire transferred to s vial with diluents, vigorously agitated and after two minutes of resting the tuba, dropping sound two to three drops into the round window of the test cassette. Riding was made after 10 minutes of incubation at room temperature, and based on the appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicted positive test, only one line in C indicted negative result. A pale colored line in T was also considered positive as it is shown in Figure (3-2).

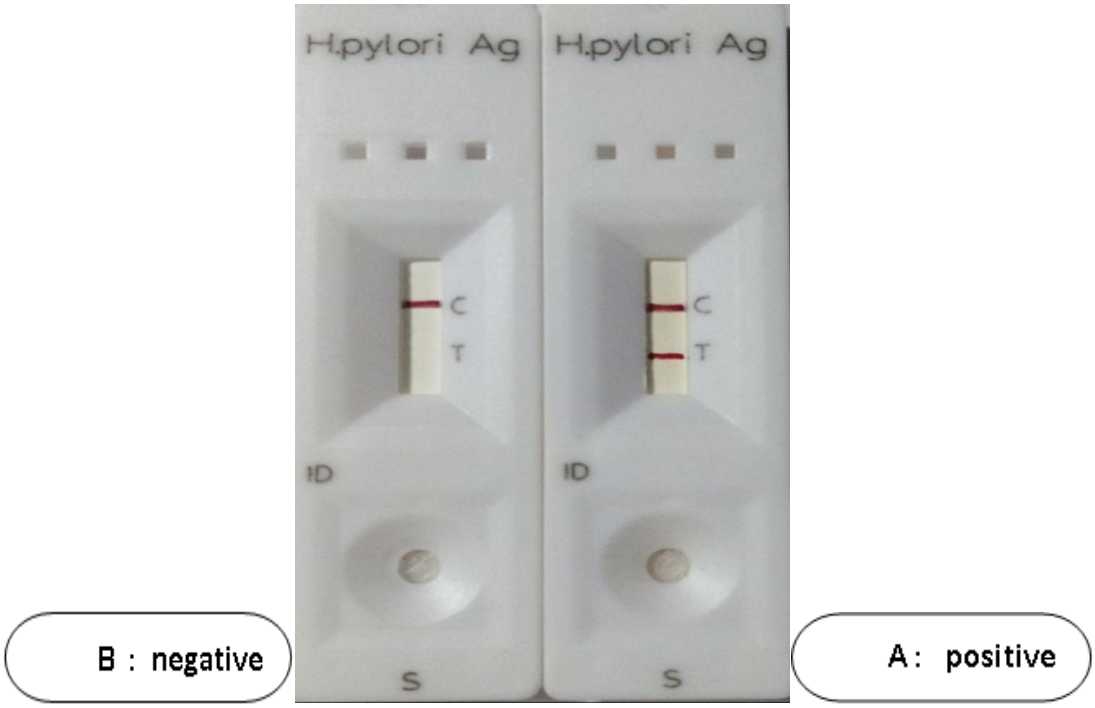


Figure (3-2): Stool antigen rapid tests for *H.pylori* detection
appearance of colored lines across the central window of the cassette, two lines, C (control) and T (test), indicted positive test, only one line in C indicted negative result.

3.2.4.Culturing of *H. pylori*

1-Columbia agar : Forty five (45) gram of blood agar base media were taken and suspended in one litter of D.W and sterilized in autoclave (121C° for 15 min) , and after that left to cool to 45 C° and the following antibiotics were added to it. polymyxin B 2.5 mg/ ml Trimethoprim 5 mg/ml Vancomycin 10 mg/ml And 10% of human blood was added , mixed well then poured in petri dish (25 ml/one petri dish), leaving the media to cool and then saved in refrigerator for its use ((Forbes et al., 2007)

2- Urea Agar Medium :This medium was prepared by adding 15 mL of urea solution sterilized by filtering (0.22µm in diameter) with 100 mL of urea agar base sterilized by autoclaving at 121°C for 15 minutes and cooled at 50°C. The medium was then distributed into sterile test tubes and allowed to solidify in a slant shape after the pH was adjusted to 7.1(Mahon and Manuselis, 2007).

3.3. Identification of *H. pylori*

3.3.1.Morphological identification of *H. pylori*

This is depending on the morphological properties of colonies such as color,size of colony ,shape, condition of growth also microscopic examination as Gram staining ,shape and size of bacterial cell ,and arrangement of these cell.

3.3.2.Biochemical diagnostic test of *H. pylori*

3.3.2.1.Gram stain

Crystal violet (primary stain) Iodine solution/Gram's Iodine (mordant that fixes crystal violet to cell wall) Decolorizer (e.g. ethanol) Safranin (secondary stain) Water (preferably in a squirt bottle).

1-Make a slide of cell sample to be stained. Heat fix the sample to the slide by carefully passing the slide with a drop or small piece of sample on it through a Bunsen burner three times

2-Add the primary stain (crystal violet) to the sample/slide and incubate for 1 minute. Rinse slide with a gentle stream of water for a maximum of 5 seconds to remove unbound crystal violet.

3-Add Gram's iodine for 1 minute- this is a mordant, or an agent that fixes the crystal violet to the bacterial cell wall

4-Rinse sample/slide with acetone or alcohol for ~3 seconds and rinse with a gentle stream of water. The alcohol will decolorize the sample if it is Gram negative, removing the crystal violet. However, if the alcohol remains on the sample for too long, it may also decolorize Gram positive cells

5-Add the secondary stain, safranin, to the slide and incubate for 1 minute. Wash with a gentle stream of water for a maximum of 5 seconds. If the bacteria is Gram positive, it will retain the primary stain (crystal violet) and not take the secondary stain (safranin), causing it to look violet/purple under a microscope. If the bacteria is Gram negative, it will lose the primary stain and

take the secondary stain, causing it to appear red when viewed under a microscope(Coico.,2006)

3.3.2.2.Oxidase test

Oxidase disc was used in this test small portion of bacterial colonies was spread on this disc by wooden stick , positive result appeared as color change to purple , while negative result appeared as no color change . This test depend on the presence of certain bacterial oxidase that would catalyze the transport of electrons between electron donors in bacteria and a redox dye (Tetramethyl-p-phenylene- diamine dihydrochloride) (Forbes *et al*., 2007).

3.3.2.3.Catalase test

The isolates were tested for catalase activity by using slide method in which the sterile wire loop is stacked on the surface of the pure colony and transferred to a microscope slide and one or two drop of 3% H_2O_2 were added onto the organism on the slide and observed for immediate oxygen bubble formation (Forbes *et al*., 2007) .

3.3.2.4.Urease Test

The urease test was applied according to (MacFaddin, 2000). The pure isolates were inoculated heavily on the entire surface of urea agar and stab with loop. The tubes were inoculated at 37°C in the incubator. The formation of purple color was the positive result to examined after 24 hr.

3.4: Immunological study

3.4.1 Estimation of serum Human HLA-C and HLA-DRB1

ELISA kit was applied to the in vitro quantitative determination of Human HLA-C and HLA-DRB1.

1. Test principle

In the ELISA kit, the Sandwich-ELISA method is used to detect HLA-C and HLA-DRB1, respectively. A Human HLA-DRB1 antibody is

pre-coated on a 96-well microtiter plate included in this kit. Samples or standards were mixed with the appropriate antibody in the appropriate wells. A biotinylated detection antibody for HLA-C was then added. To each well, we added an avidin-horseradish peroxidase conjugate (HRP). Washing was performed after the chromatogenic step to remove any remaining free components. Each well was incubated with a different substrate Only the wells containing HLA-C and HLA-DRB1 contain biotinylated detection antibodies and Avidin HRP conjugates. The enzyme-substrate reaction turns yellow after the stop solution is added. The optical density (OD) at an excitation wavelength was measured using spectrophotometric technique (450 nm).

2-Reagent preparation

HLA-C

1- All reagents was brought to room temperature before use.

2- Standard solution preparation: about the 120µl of standard were reconstitute (0.2ng/ml) with 120µl of standard diluent to generate an 80ng/ml standard stock solution, the standard allowed to sit for 15 mins with gentle agitation before making dilutions. Prepared duplicate standard points were by serially diluting the standard stock solution (80ng/ml) 1:2 with standard diluent to produce 40ng/ml, 20ng/ml, 10ng/ml, and 5ng/ml solutions. Standard diluent serves as the zero standards (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. The dilution of standard solutions

Wash Buffer: 20ml of washed buffer concentrate 25x diluted into deionized or distilled water to yield 500 ml of 1x Wash Buffer.

HLA-DRB1

1- All reagents was broughted to room temperature before use.

2- Standard solution preparation: the 120µl of the standard (16.28ng/L) reconstituted with 120µl of standard diluent to generated a 4800ng/L standard stock solution. Was allowed the standard to sit for 15 mins with gentle agitation before making dilutions. Prepared duplicate standard points by serially diluting the standard stock solution (4800ng/L) 1:2 with standard diluent to produce 2400ng/L, 1200ng/L, 600ng/L, and 300ng/L solutions. Standard diluent serves as the zero standards (0 ng/L).

Wash Buffer: Diluted 20ml of washed buffer concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved

3.4.2:Assay procedure

ELISA Assay Procedure

1. Prepared all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.

2. Determined the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.

3. Added 50µl standard to the standard well. Note: Don't added a biotinylated antibody to a standard well because the standard solution contains a biotinylated antibody.

4.40µl sample to sample wells and then add 10µl anti-HLA antibody to sample wells, then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate for 60 minutes at 37°C.

5.Removed the sealer and wash the plate 5 times with wash buffer. Soak wells with 300ul wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6.Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

7.Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8.Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution**.**

3.5Calculating of Results of ELISA Test

The standard curve was created by plotting the mean OD value for each standard on the y-axis against the concentration on the x-axis and drawing a best-fit curve through the points on the graph, when specimens have been diluted, the concentration calculated from the standard curve was multiplied by the dilution factor. the OD of the specimen that surpassed the upper limit of the standard curve was tested after appropriate dilution as shown in the figure (3-4) and (3-5).

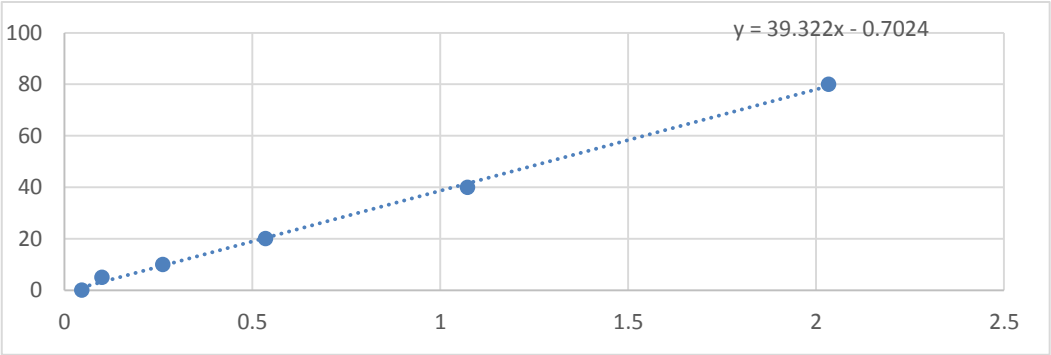


Fig (3-4) Standard Curve of HLA-C

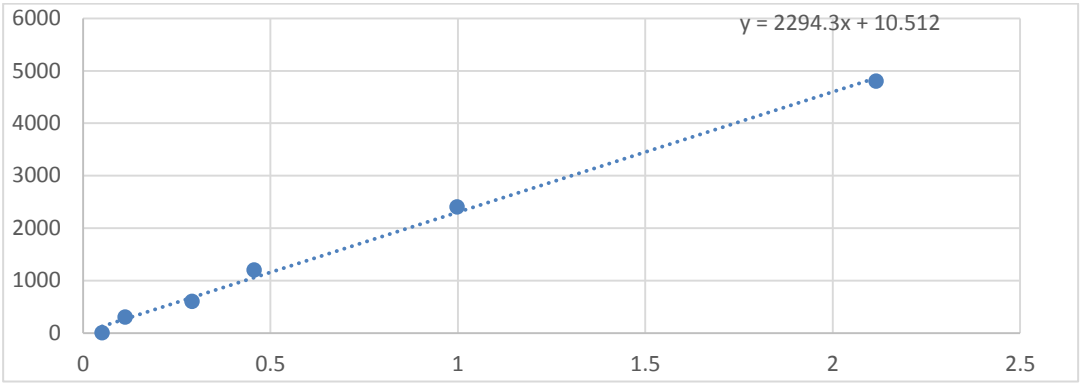


Fig (3-5) Standard Curve of HLA-DRB1

3.6: Genetic Study

3.6.1: Genomic DNA extraction for bacteria:

Favor PrepTM Genomic DNA Mini Kit was used to extract genomic DNA fromisolates following the manufacturer's protocol. Inoculum of each isolates wasprepared at density up to 10^9. Bacterial pellets were harvested via centrifugation at14000 rpm for 1 min. The harvested cells were resuspended thoroughly in 200 µl of FATG buffer (for gram negative isolates) FABG (200µl) was added respectively to the bacterial cells. The resulting homogeneous cells suspension was incubated for 10 minutes at 70ºC and vortexed for 10 sec every 3 min. until the sample lysate is clear. DNA was extracted from thehomogeneous suspension by the addition of 96~100% of ethanol and then transferredto the FABG column assembled inside a 2 ml

The flowthrough was discarded .The FABG column was dried via further centrifugation at 14000 rpm for 3 min.over a new 2 ml column collection tube to remove any residual ethanol solution. The dry FABG column was then transferred to a new 1.5 ml microcentrifuge tube, and100µl of preheated elution buffer or TE was added directly to the FABG column membrane for 3~5 min, followed By centrifugation for 1 min at 14000 rpm to elute DNA. The purity of eluted genomic DNA was measured by nanodrop ,then stored at-20ºC until use.

3.6.2:Genomic DNA extraction frozen human blood:

Favor the PrepTM Genomic Mini Kit obtained genomic DNA from frozen human blood following the manufacturer's protocol.

1- A 1.5 mL microcentrifuge tube was used to transfer approximately 200 µL of thawed frozen blood from an EDTA tube. Following this step, the sample was incubated in the incubator for 15 minutes at 60 ° C with 30 µL of proteinase K (10mg/ml, not provided).

2- As soon as the blood turned greenish-black from the addition of 200 µL of FABG Buffer for cell lysis, the sample was incubated for 15 minutes at 70 ° C. and vortexed or shaken to ensure that the cells were fully lysed before being placed back into the assay. Every three minutes, the sample was inverted to create a new one. For DNA elution, the Elution Buffer was placed in an incubator set to 70°C.

3- In order to ensure that no precipitation formed during DNA binding was added 200 µL of ethanol (96-100%) to the sample and vortexed for 10 seconds. This was followed by one minute of 1400-rpm centrifugation on the FABG column after it was carefully transferred to the FABG column. Discarded the Flow-Through Two milliliters of new collection tubes were used to hold the FABG column in place.

4-After adding 400 µL of W1 Buffer to the FABG tube, the tube was centrifuged at 1400 rpm for 30 seconds. After discarding the flow-through, the FABG column was reinserted into the collection tube. well and toward the positive (red) electrodes. Add power by turning on the switch.

5-To dry the column, 600 µL of wash Buffer was added to the FABG tube and centrifuged at 1400 rpm for 30 seconds. The flow-through was then discarded and the FABG tube was placed in the collection tube and centrifuged for an additional 3 minutes .

6- After that, the dry FABG column was transferred to a new 1.5 ml microcentrifuge tube, and 100 µL of preheated elution buffer or TE was added directly to the FABG column membrane. After 10 minutes of incubation at 37°C, the DNA was extracted by centrifugation at 1400 rpm for 1 minute. DNA was kept at 4°C until it was needed.

3.6.3: Primer preparation

The nuclease-free water was added in accordance with the manufacturer's instructions (macrogen/Korea) to obtain a (300 picomole/microliter) concentration of primer stock solution. By diluting the stock solution with nuclease-free water, the working solution was obtained at a concentration of 10 picomole/microliter (Green and Sambrook,2012).

3.6.4: Reaction mixture

Amplification of DNA was carried out in a final volume of 50 µL reaction mixture as mentioned in the Table (3-4).

Table 3-4: contents of the Reaction Mixture

No.	Contents of the reaction mixture	Volume	Volume
1	Green master mix	12.5 µL	25 µL
2	Upstream primer (10pmol/ µL)	1.5 µL	3 µL
3	Downstream primer (10pmol/ µL)	1.5 µL	3 µL
4	Nuclease free water	6.5 µL	14 µL
5	DNA template	3 µL	5 µL
Total volume		25 µL	50 µL

Table 3-5. Sequence and PCR conditions of UreA , HLA-C and HLA-DRB1

Primer	Sequence 5 to 3	product	Conditions Tm ºC / Time s/min		NO. CY-CLE	Reference
UreA-F	**5'GAGAATGAGATGAAACTCACCC3'**	632bp	Step 1	95ºC/10min	1	(Samareh. ,*et al2016*)
			Step 2	94ºC/30 s	35	
			Step 3	54 ºC/60 s		
UreA-R	**5'-TTGTCTGCTTGTCTATCAACC-3'**		Step 4	72 ºC/60 s		
			Step 5	72 ºC/10min	1	
			Step 6	4		
HLA-C-F	**5' GGGGAACTTGATGCCAGACA-3'**	407bp	Step 1	95ºC/300 s	1	This study
			Step 2	95ºC/30 s	35	
			Step 3	62.5 ºC/30 s		
HLA-C-R	**5'- GTGATGACTACAGGCTCCCG-3'**		Step 4	72 ºC/30 s		
			Step 5	72 ºC /300 s	1	
			Step 6	4		

HLA-DRB1-F	5' TCTCCCTCCTAATGGTAACTGAG-3'	409bp	Step 1	95ºC/300 s	1	This study
			Step 2	95ºC/30 s	35	
			Step 3	63 ºC/30 s		
HLA-DRB1-R	5'-GAGTTTCCCCTGAGAGTGCT -3'		Step 4	72 ºC/30 s		
			Step 5	72 ºC /300 s	1	
			Step 6	4		

3.6.5: Polymerase chain reaction (PCR)

The target DNA was amplified using specific primer pairs in a conventional PCR as previously described (Table 3-5) PCR product (amplicon) is obtained by repeating three consecutive steps for a specific number of cycles, which can then be visualized after agarose gel electrophoresis,

3.6.6:TBE Buffer (Tris-Borate-EDTA)

DNA gel electrophoresis was commonly performed using a TBE running buffer. The stock solution of TBE was prepared and stored at a concentration of 10x working solution. the 1x concentration was used when preparing gel for electrophoresis , the final concentration of the TBE solution was prepared by adding 100ml of 10x TBE buffer to 900 ml of sterile D.W. (Sambrook and Russel, 2001)..

3.6.7: Agarose Gel Electro

Agarose gel electrophoresis is the most effective way of separating DNA fragments. The concentration of agarose in a gel depends on the sizes of the DNA fragments need to be separated, ranging between 0.5%-2% (Lee *et al.,* 2012). A 0.7% gel was used to obtain good separation of genomic DNA (5-10

kb) after extraction while 1.5%-2% was used to gain good resolution for small fragments of PCR product (0.2-1 kb). However, the specific weight of agarose was added to 100ml of 1×TBE buffer and then melted in microwave until the solution becomes clear. Once the agarose was cooled to 50-55°C, 5µl of simplysafe dye (10 mg/ml) was added to 100 ml of melting agarose gel to get final concentration 0.5µg/ml (Sambrook and Russel, 2006).

The agarose was pouredin the gel tray with sealed ends, comb placed properly, and then left to dry. The samples were loaded in a separate well of the gel, with marker in one well. Electrodes were connected correctly and the run was applied according to the gel electrophoresis percentage and size of gel, (The time of agarose gel electrophoresis is 45 minute for genomic DNA and 1 hour and 30 minute for PCR product).

1. The gel-casting tray in plastic tray, check that the teeth of the comb are approximately 0.5mm above the gel bottom.

2. Prepare 500ml of TBE (1X) by adding 50ml of TBE (10X)stock solution to afinal volume of 500ml of deionized water.

3. 100ml of the buffer placed into a 500ml flask and add 0.8g of agarose. Melt the agarose by heating (microwave) the solution on hot plate for approximately 10min. Carefully swirl the agarose solution to ensure that the agarose is dissolved,that is no agarose particles are visible.

4. The agarose solution cooled to approximately 50°C and added 2-3 µl of Simple safe stock solution. Slowly pour the agarose into the gelcasting tray. Remove anyair bubbles by yellow tip.

5. The comb Positioned approximately 1.5cm from the edge of the gel. when the agarose leted for approximately 20–30 minutes. After the agarose has solidified remove the comb with a gentle back and forth motion, taking care not to tear the gel.

6. The gel-casting tray removed and the tray placed on the central supporting platform of the gel box.

7. Electrophoresis buffer were added to the buffer chamber until it reaches a level of

0.5–1cm above the surface of the gel.

8. Load the samples into the wells using a yellow tip. Place the tip under the surface of the electrophoresis buffer just above the well. Expel the sample slowly, allowing it to sink to the bottom of the well. Take care not to spill the sample into a neighboring well. Note: samples must be loaded in sequential sample wells. When loading fewer samples than the number of wells it is preferable to leave the wells nearest the edge of the gel empty.

9. First load 5µl of ladder molecular weight marker to each side of the gel(flanking the sample line) and 20 µl of DNA specimen in the other well.

10. Place the lid on the gel box and connect the electrodes. DNA will travel towards the positive (red) electrode positioned away from the well. Turn on the power supply.

11. Continue electrophoresis until the tracking dye moves at least 10 cm of the gel length.

3.6.8: Sequencing of PCR product

Forty microliters of HLA-C and Hla-DRB1 products were sent to Macrogene/ Korea for Sanger sequencing. After trimming each sequence, the result of the trimmed sequence was blasted in NCBI to check the similarities and differences with the database. Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http://www.geospiza.com) was used to check the polymorphism of the genes above.

3.7: Biosafety and Hazard Material Disposing

Biosafety aspects were followed during the work including wearing all personal safety equipment such as masks ,and gloves, when taking samples from patients. Then all contaminated syringes and supplies were disposed of

supplies by autoclaving and then incineration. All benches were cleaned with alcohol (70%) before and after work.

3.8: Ethical Approval

1-The study was done and the cases were collected after getting the agreement of the patients (verbal acceptance).

2- Approval of Babylon Science Collage Ethical Committee

3-Before starting the study, permission was taken from Babylon's health presidency.

3.9 Statistical analysis

Number and percentage were used to express categorical variables. Parametric variables were given as mean ± standard deviation (SD) and significant differences were assessed using the least significant difference (LSD) test. Nonparametric variables were expressed as the median and interquartile range (IQR), and the Mann-Whitney U test (to compare two groups) was used to assess significant differences between medians multinomial logistic.

Chapter Four

Results and Discussion

4- *Result and Discussion*

This Case –Control study included 250 subjects divided as 200 patients attending gastroenterology and hepatology center of merjan medical city hospital to diagnosis bacterial infection in stomch aged(15-71) during November2022 to January2023 ,in addition to 50 apparently healthy subject as control group with age and sex similar to case group. from all subject collected 250 blood specimens and 250 stool specimens. 5ml of venous blood specimens that separated into two group . 2.5 ml put in EDTA tube for DNA extraction to detect HLA polymorphism .while 2.5ml put in gel tube for serum separation to detect HLA-DRB and HLA-C levels by ELISA and diagnosis *H.pylori* by Ab rapid test. In addition to detect the bacteria by Ag rapid test through stool specimens according to bacterial culturing and PCR to detection URE A .115(57.5%) of them were male, and 85 (42.5%) of female, as shown in Table(4.1).

Table (4.1): Demographic of study population based on sex

sex	Total samples N	Patients n N(%)	Control (%)
Male	140(56%)	115(57.5%)	25(50%)
Female	110(44%)	85(42.5%)	25(50%)
Total	250	200	50

Fifty(25%) subjects gave a negative result for bacterial infection through laboratory diagnosis, and they do not have any symptoms or diseases of the digestive system used as a control group. This results were similar to (Williams and Pounder., 1999)who was reported that *H. pylori* infections have been demonstrated worldwide and affect all age groups. It is estimated that 50% of the world's population are infected, similar results were observed by(Amer *et al*., 2014)who was studding *H. pylori* infections in immune compromised children and he was proven that no significant difference between the two groups regarding sex. Also, the infection incidence of men more than women, as a study showed by(Replogle *et al*., 1995) The diseases associated with *H. pylori* infection, such as peptic ulcer disease and gastric cancer, afflict men more frequently than women.

4-2. Distribution according to age groups

The total patients and control are divided to three groups. Distribution of patients and control, according to the age groups were reported higher rate of infection groups (24-47years) while lower rate of infection were recorded in first age group< 24 years as show in Table (4.2).

Table (4.2): Distribution of patients and control according to age groups

Age groups	Distribution		Total	Cumulative Percent
	Patients	Control		
<24 years	10	10	20	8%
24-47 years	100	20	120	48%
48-71years	90	20	110	44%
Total	200	50	250	%100

There are several factors that contribute to higher rates of *H. pylori* infection among young people. Here are some possible reasons Behavioral Factors Behavioral factors among young individuals can contribute to an increased likelihood *of H. pylori* infection. For example, consuming contaminated food or sharing food with others, drinking untreated water, and poor personal hygiene practices can increase the risk of infection Household Transmission Young individuals are often part of households where *H. pylori* infection is present. Close contact with infected family members, sharing utensils or food, and living in crowded conditions can facilitate the transmission of the bacterium Social Factors Young people especially in certain cultural or socioeconomic contexts may engage in behaviors that increase the risk of *H. pylori* transmission. This could include communal eating, sharing of food or drinks, or participation in activities where close contact is common.limited immunity young (Al Sulami *et al*., 2008) .

individuals may have less developed immune systems compared to adults, which could potentially make them more susceptible to *H. pylori i*nfection. As the immune system matures with age, the risk of infection may decrease Environmental Factors Certain environments or living conditions can contribute to the spread of *H. pylori* Factors such as poor sanitation inadequate access to clean water, and overcrowding can facilitate the transmission of the bacterium, particularly among young people living in resource-limited settings It's important to note that while young people may have higher rates of *H. pylori* infection, many individuals remain asymptomatic and do not develop complications. However, those who do experience symptoms or complications may require treatment with antibiotics, along with other appropriate management strategies(mabe *et al*.,2022).

4-3: Diagnosis of *H.pylori*

4-3-1.Rapid diagnostic test of *H.pylori*

4-3-1-1.Antibody diagnostic test

In this study, 200 blood specimens were taken for the purpose of laboratory examination to test for *H. pylori* assay 80 (40%) specimens were given a positive result and120 (60%) specimens were given a negative result as shown in the table (4-3) The main advantage of this method was widely available and inexpensive but disadvantage include that positive results may be reflect previous rather than current infection and useful in antibiotic monitoring(Laheij *et al.*, 1998).

4-3-1-2.Stool Antigen Diagnostic Test

Forty-five 45(22.5%)positive specimens were given from a total of 200 and 155 negative samples as shown in the table (4-3). Through work, some flaws in this method have been observed, which is the difficulty of collecting specimens (stool) from patients, as they refrain from giving the specimens , and false negative results may be due when antibiotics are used recently. The results were homologous to (Al-Thahab and Lateef.,2018), who show that the sensitivity and specificity of stool Ag test were100% for both of them.

A study conducted in Turkey reported that HpSA had a sensitivity of 98% and specificity of 100% .Stool antigen tests (SATs) are noninvasive diagnostic modules for *H. pylori* infection. Both European and Japanese guidelines have shown that EIA-based SATs using monoclonal antibodies are useful for primary diagnosis as well as for the assessment of eradication therapy. ICA-based tests do not require particular equipment and are therefore useful in developing countries (Shimoyama, 2013).

4.3.2.Culturing of stool for detection *H.pylori*

4.3.2.1 Stool culture : Forty five specimens were diagnosed that gave a positive result cultured on special media for culture of *H. pylori*, such as (Colombia agar). The results showed Table (4-3).

Table (4-3) Demographic and Diagnosis of *H.pylori*

Sample	Test					
	(AB)Rapidtest N%		(Ag)Rapidtest %N		Culture %N	
	+ve	-ve	+ve	-ve	+ve	-ve
Control(50)	0(0%)	50(50%	0(0%)	50(50%	0(0%)	50(50%
Patient(200)	80(40%)	120(60%)	45(22.5%)	155(77.5%)	45(22.5%)	155(77.5%)

This study was similar to the study of (Parsonnet *et al*., 1999 , Dore *et al*., 2000)as their study showed percentages of 21% and 25% respectively. The growth of *H. pylori* bacteria appeared on the culture media used in the form of small, circular colonies and has a transparent cream color, these characteristics were similar to the characteristics mentioned by(Xia *et al*., 1994).

4.4.Morphological and biochemical charactristic

H. pylori is Gram-negative, elongated, and comes in various of shapes, such as rode, spiral, filamentous, and coccoid, Most bacteria possess a cell wall that, due to its covalently closed, net-like structure, maintains a specific shape and thus also imposes it on cells .Through the microscopic examination, different shapes were observed where our bacteria appeared in two forms with respect to the rods, which are (curved rods and straight

rods). It also indicated in the detection of the mechanism for forming the cell shape, a (CreS) protein (Caulobacter crescents) which is a protein responsible for the formation of the curved rod shape of bacteria (Ausmees *et al*., 2003)A thin peptidoglycan (PG) or mureinsacculus that surrounds the cytoplasmic membrane keeps the cell in shape(Typas *et al*., 2012)

Table (4-4). Main Biochemical Tests of *H .pylori*

Biochemical test	Result
Gram stain	Negative
Catalase test	Positive
Oxidase test	Positive
Urease test	Positive

4.5.Gentic detection of *H.pylori* by PCR

Genetic test based on PCR enable the specific detection of nucleic acid and have been used for diagnosis of *H. pylori* in clinical specimens . Because its high sensitivity. PCR is suitable for diagnosis when an organism present in small number , slow growing or difficult to identify .

The ureA gene encodes for the urease enzyme, which is a key virulence factor produced by *H. pylori*. Urease plays a crucial role in *H. pylori* ability to colonize and survive in the acidic environment of the stomach. PCR (polymerase chain reaction) is the best a molecular technique used to amplify and detection specific DNA sequences PCR can

be employed to detect the presence of the ureA gene in a clinical specimens (Szymczak.,*et al* 2020).

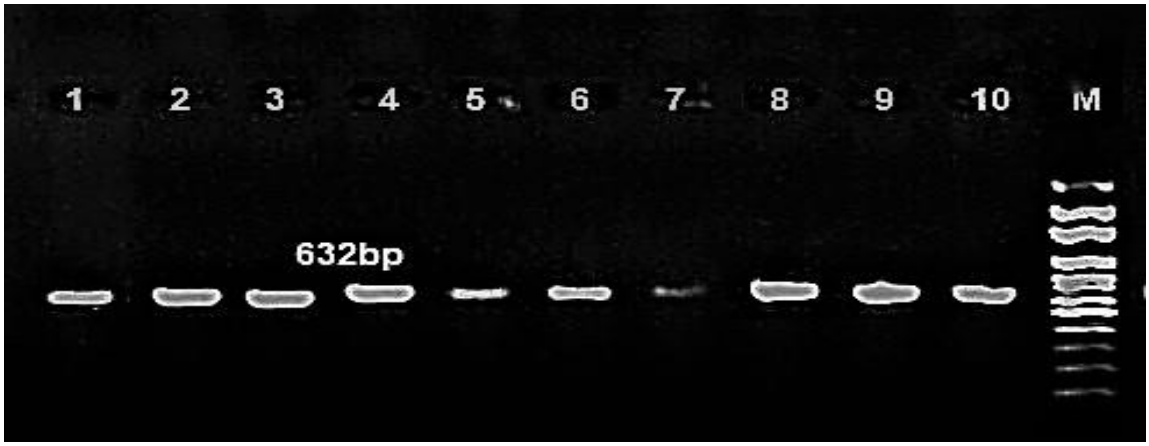


Figure 4-1 : (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to Ure A amplicon (632bp) , 1-10 represented , specimens M (DNA marker size (100bp)

4-6 Immunological study

4-6-1 Estimation of HLA-C serum level among *H.pylori* infection.

The results revealed a significant difference (P ≤0.05) in the serum levels of HLA-C in patient when compared with healthy control this study showed an increase in the serum level of HLA-C in patient which was (50.90) ng/L ,compared with serum levels of healthy control which were (28.77) ng/L, P ≤ 0.000) as shown in Table(4.5)

Table 4-5 Estimation of HLA-C serum level(ng/ml)in blood of patients compared with control

Type of patients	No.	HLA-C ng/ml Mean ±SD	P ≤ value
Patients	80	50.90±12.89	0.000
Healthy Controls	50	28.77± 6.09	

Significant (P≤0.05)

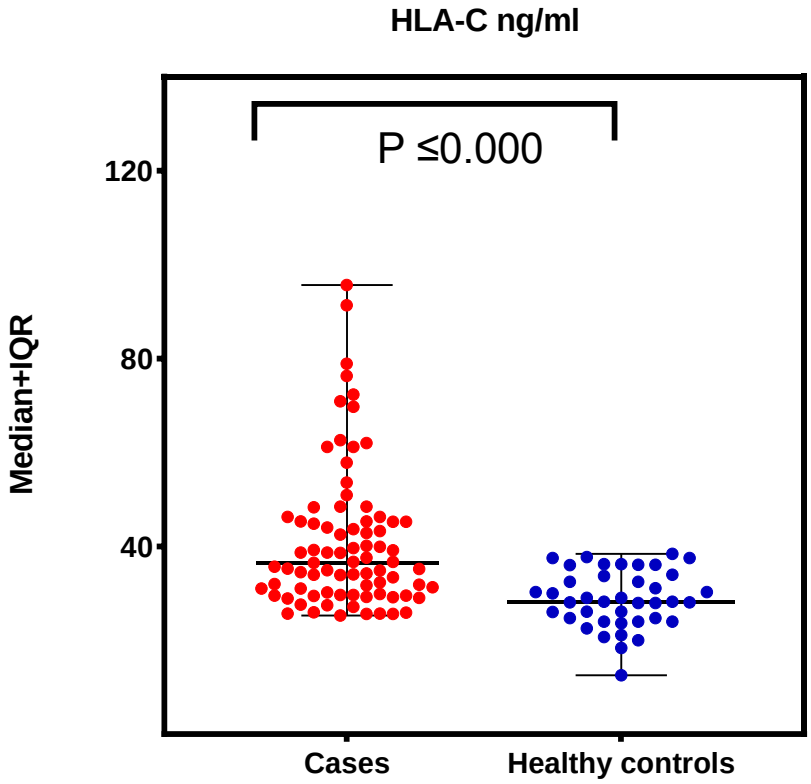
This study found an increase in serum HLA-C levels among Helicobacter pylori patients Itis one of the factors that contribute to the development infections in stomach ulcer patients. it may be a risk factor. The main function of HLA-C is to inhibit innate and adaptive immune responses by interacting with its ligands on target cells to transmit inhibitory signals. Natural killer cells, CD4+ and CD8+ T cells, B cells, macrophages, dendritic cells, and neutrophils are among the target cells functionally influenced by HLA-C. These findings were consistent with previous observations that HLA-C serum levels were up-regulated in the serum of patients with severe disease (Al-Bayatee, and Ad'hiah,2021)

The results were confirmed by(Gönen., *et al* 2017). The current study revealed that patients with duodenal and active gastritis had increased frequencies of HLA class I antigens. For the remaining tissue group antigens, there was no discernible difference between the patient and control groups.

The results of the (Najafimehr,*et al*2020) This study meta-analysis of prospective cohort studies demonstrated that HLA class I overexpression may be helpful marker for the clinical decision-making process regarding gastrointestinal cancer treatment and outcomes. Further studies using additional putative prognostic markers in combination with HLA class I may be required to evaluate their potential in predicting patient outcomes

Human leukocyte antigens class I (HLA- I) which are expressed on the tumor cell's surfaces, are applicable in the surveillance of the T cell immune responses. HLA- I molecules are critical for the presentation of antigen peptides derived from tumor cells to cytotoxic T lymphocytes (CTLs). HLA- I expression have a key role in the tumor cell recognition by

CTLs and determining expression of this antigen helps to predict risk of progression and recurrence of cancers (Speetjens., *et al*2008)



Figure(4.2): Scatter dot plots of concentration HLA-C in patient with *H.pylori* infection and healthy controls. Horizontal lines indicate medians, while vertical lines indicate interquartile range (IQR). Significant differences were assessed with the Mann-Whitney U test (to compare two groups),

4.6.2 Estimation of HLA-DRB1 serum level among *H.pylori* infection.

The results revealed a significant difference (P ≤0.05) in the serum levels of HLA-DRB1 in patient when compared with healthy control this study showed an decrease in the serum level of HLA-DRB1 in patient which was (1905) ng/L ,compared with serum levels of healthy control which were (2849) ng/L, P ≤ 0.0003) as shown in Table (4-6).

Table 4-6 Estimation of HLA-DRB1 serum level(ng/ml)in bloodof patients compared with control

Type of patients	No.	HLA-DRB1 ng/L Mean ±SD	P ≤ value
Patients	80	216.1±1905	0.0003
Healthy Controls	50	177 ± 2849	

Significant (P≤0.05).

It was determined that HLA-DRB1 play a critical role in *H. pylori* infection. For this reason, it is obvious that performing HLA studies in different populations for the same disease is important to determine the risks of and preventive factors for diseases and to plane the treatment.

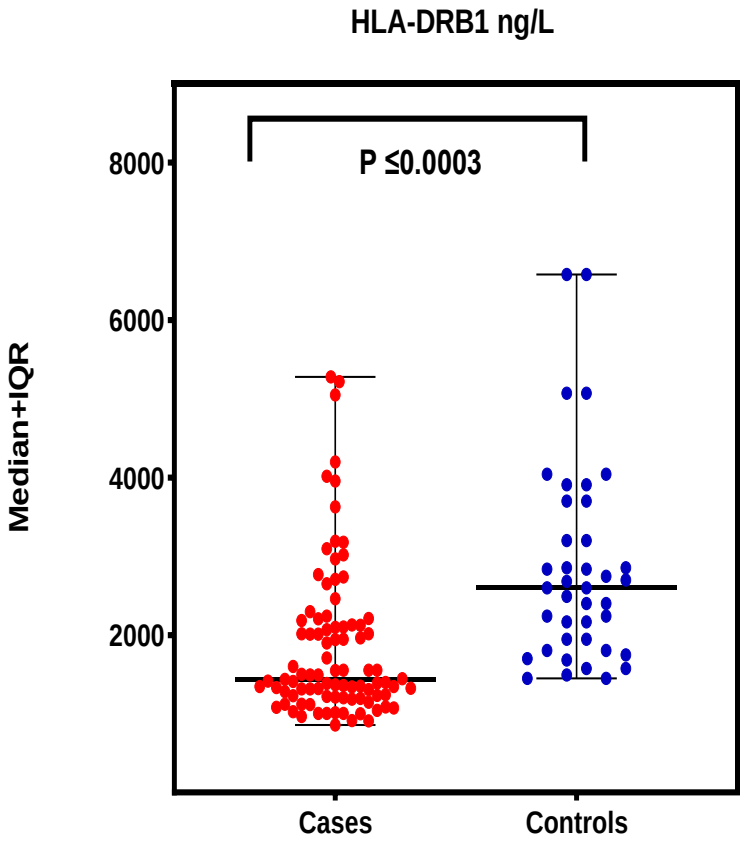
The results were confirmed by (Gönen.,*et al*2017) first to simultaneously investigate the HLA class I and II antigens in *H. pylori*-positive pediatric patients with active gastritis and duodenal ulcer and the relationship with the HLA-B antigen was demonstrated. However, the present study has a small specimens size; thus, further studies with larger sample sizes are required in the long term.

Several studies have examined the association between HLA-DRB1 alleles and *H. pylori* infection. While the findings are not entirely consistent, some evidence suggests that certain HLA-DRB1 alleles may confer protection against *H. pylori* infection or be associated with a lower risk of developing *H. pylori*-related diseases(Zhang,s *et al*.,2014)

The mechanisms underlying the protective effects of specific HLA-DRB1 alleles are not yet fully understood. It is believed that these alleles may influence the immune response against *H. pylori* by presenting

antigens more effectively to T cells, leading to a more robust and efficient immune response against the bacterium

However, it is important to note that the association between HLA-DRB1 alleles and *H. pylori* infection can vary among different populations and ethnic groups. Other factors such as environmental influences, host genetic variations in other immune-related genes, and *H. pylori* strain-specific factors also play significant roles in determining susceptibility to the infection.(Khan.,*et al*2022)



Figure(4.3): Scatter dot plots of concentration HLA-DRB1 in patient with *H.pylori* infection and healthy controls. Horizontal lines indicate medians, while vertical lines indicate interquartile range (IQR). Significant differences were assessed with the Mann-Whitney U test (to compare two groups)

4.7 Genetic study

Humane DNA extraction for detecting and identifying specific PCR amplified fragments (HLA-C and HLA-DRB) is shown in Figures(4.5,6)

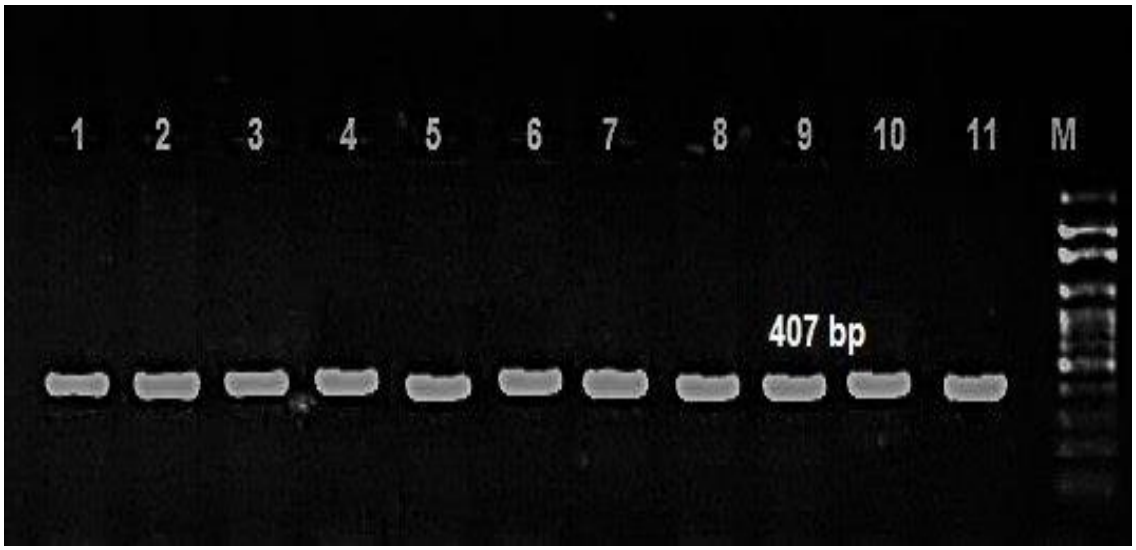


Figure 4-4 : (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to HLA-C amplicon (407bp) , 1-11 represented , specimens M (DNA marker size (100bp)

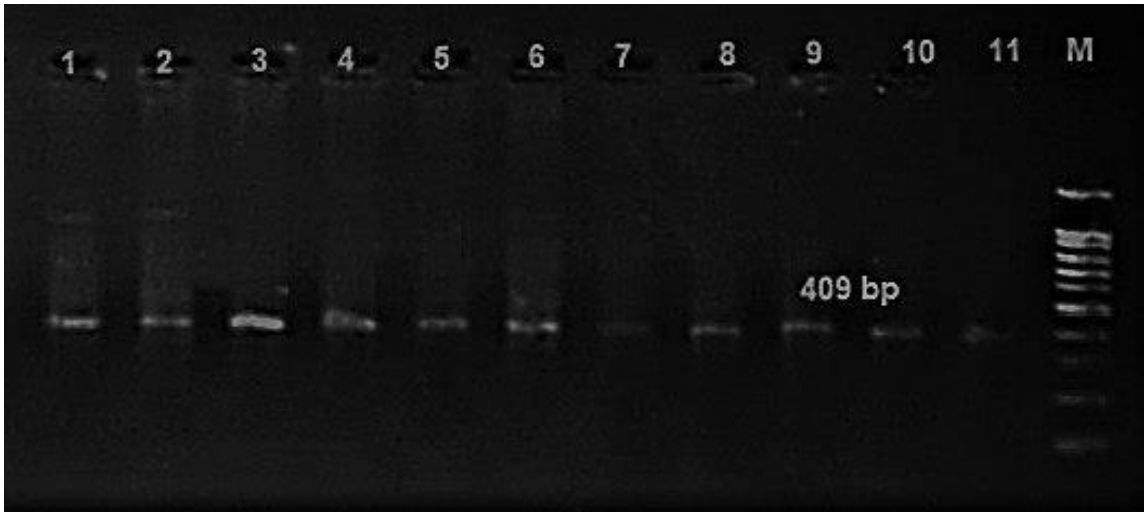


Figure 4-5 : (1.5 %) Agarose gel electrophoresis at 72 volt for 80 minutes of PCR to HLA-DRB1 amplicon (409bp) , 1-11 represented specimens , M (DNA marker size (100bp)

4.7.1 . Association of HLA-C SNPs among patients with *H. pylori* infection.

Trimming of Sanger sequencing results for HLA-C product. Multiple alignments for each were prepared. using Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http://www.geospiza.com) to know the genotype differences between among patients with *H. pylori* infection. and compared with Healthy control . Fig.(4-6) (4-7) (4-8)and (4-9) shows SNPs distributions on the HLA –C gene.

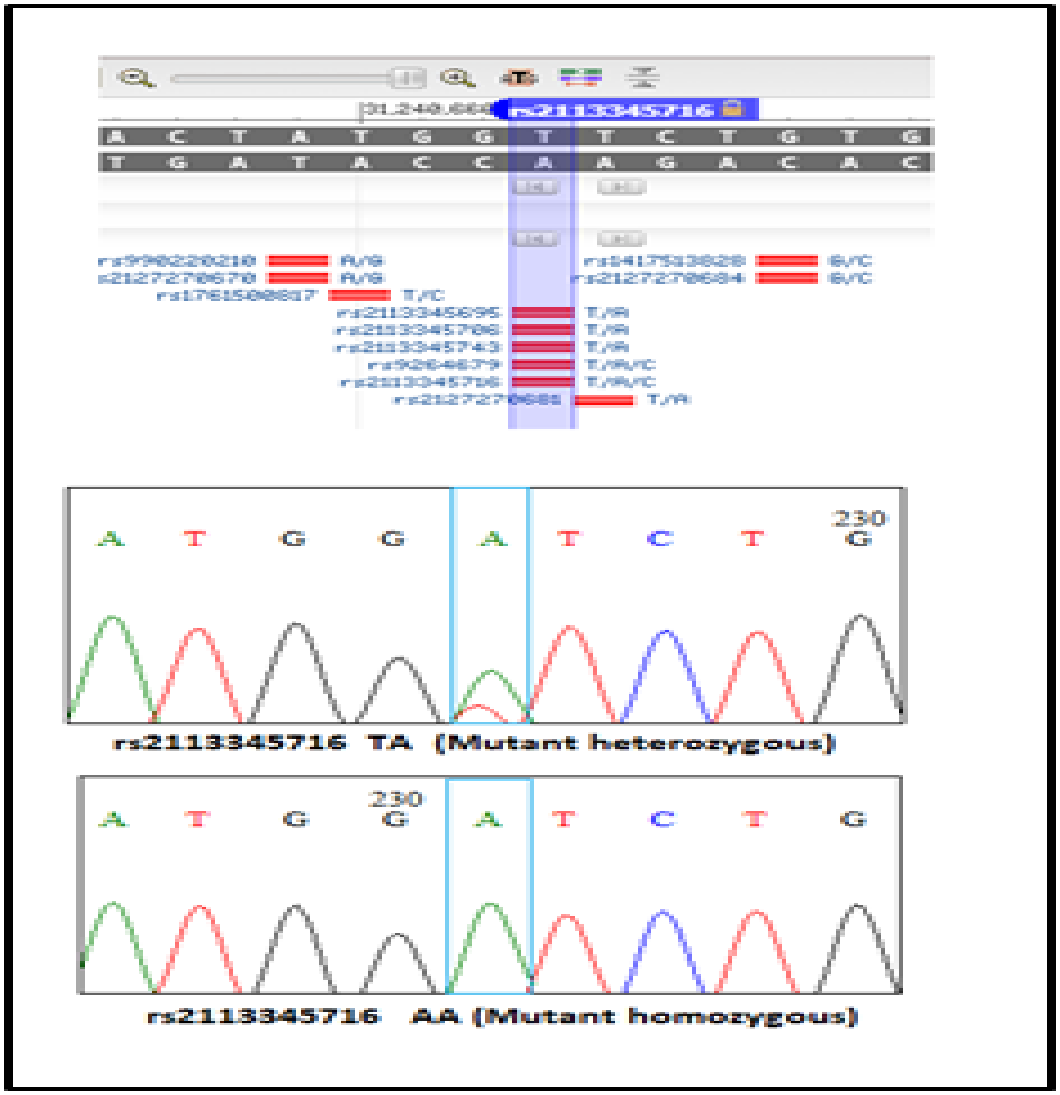


Fig.4-6. Distributions of rs2113345716 genotype SNPs on HLA-C gene in study population

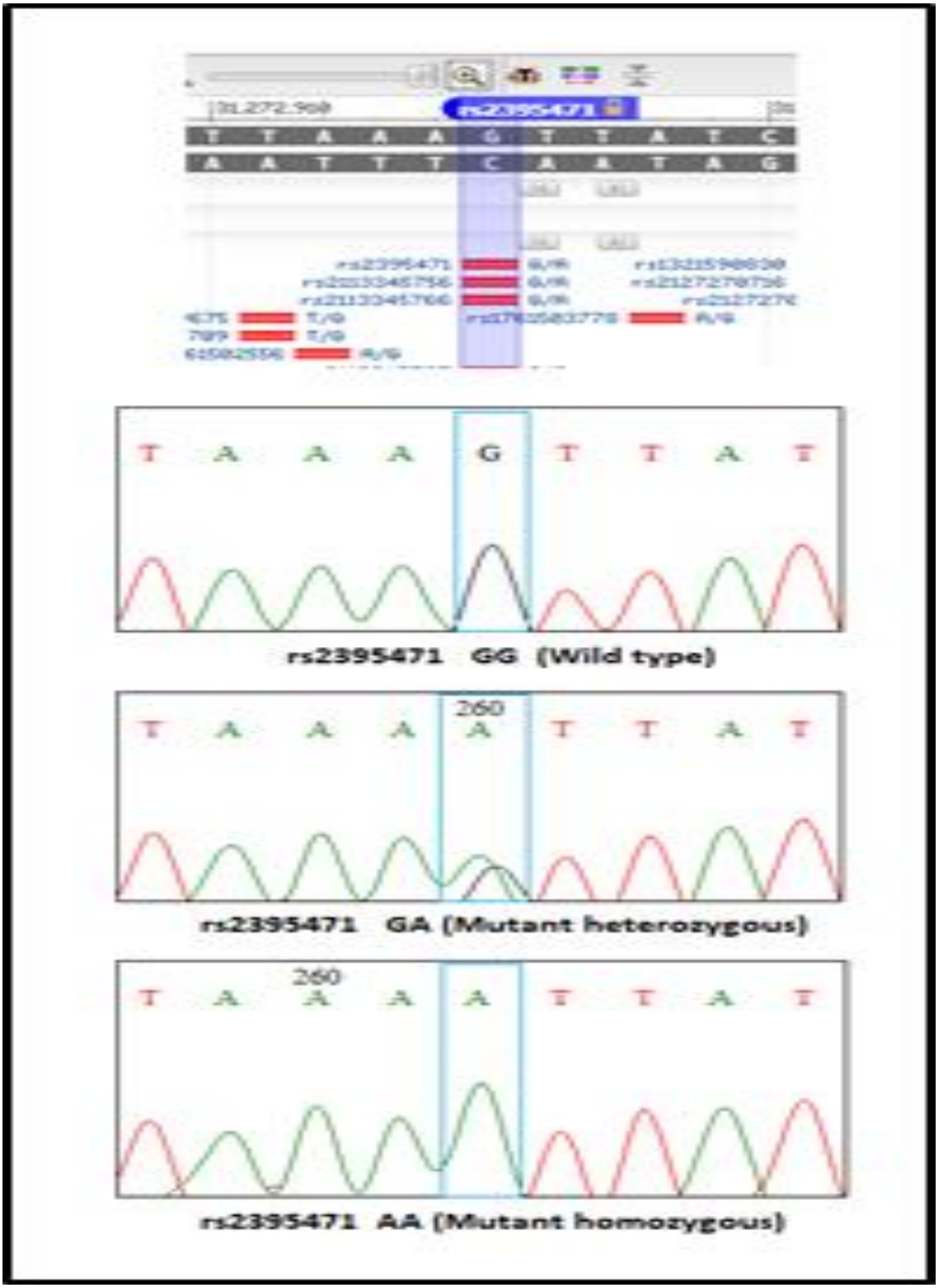


Fig.4-7.Distributions of rs2395471 genotype SNPs on HLA-C gene in study population

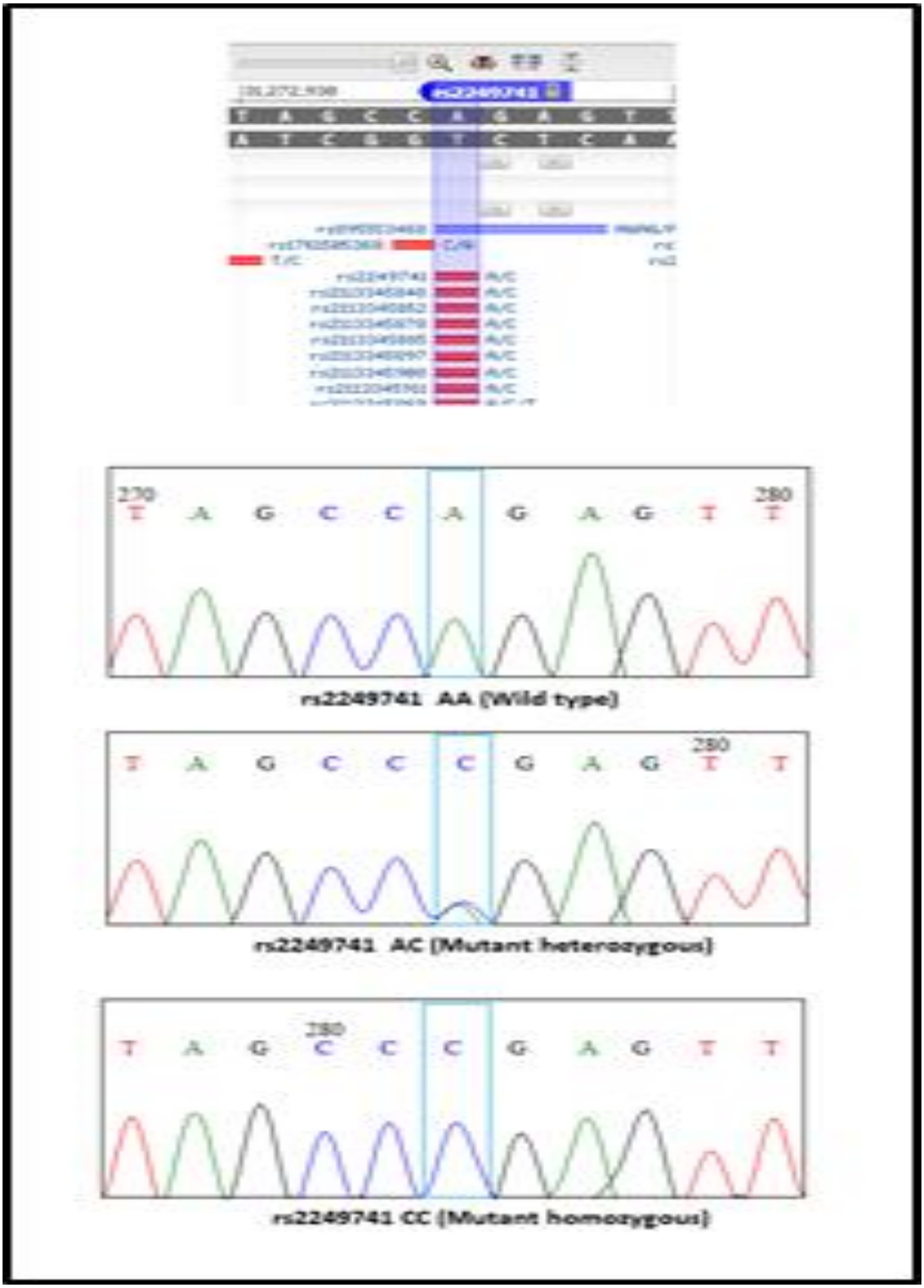


Fig.4-8.Distributions of rs2249741 genotype SNPs on HLA-C gene in study population

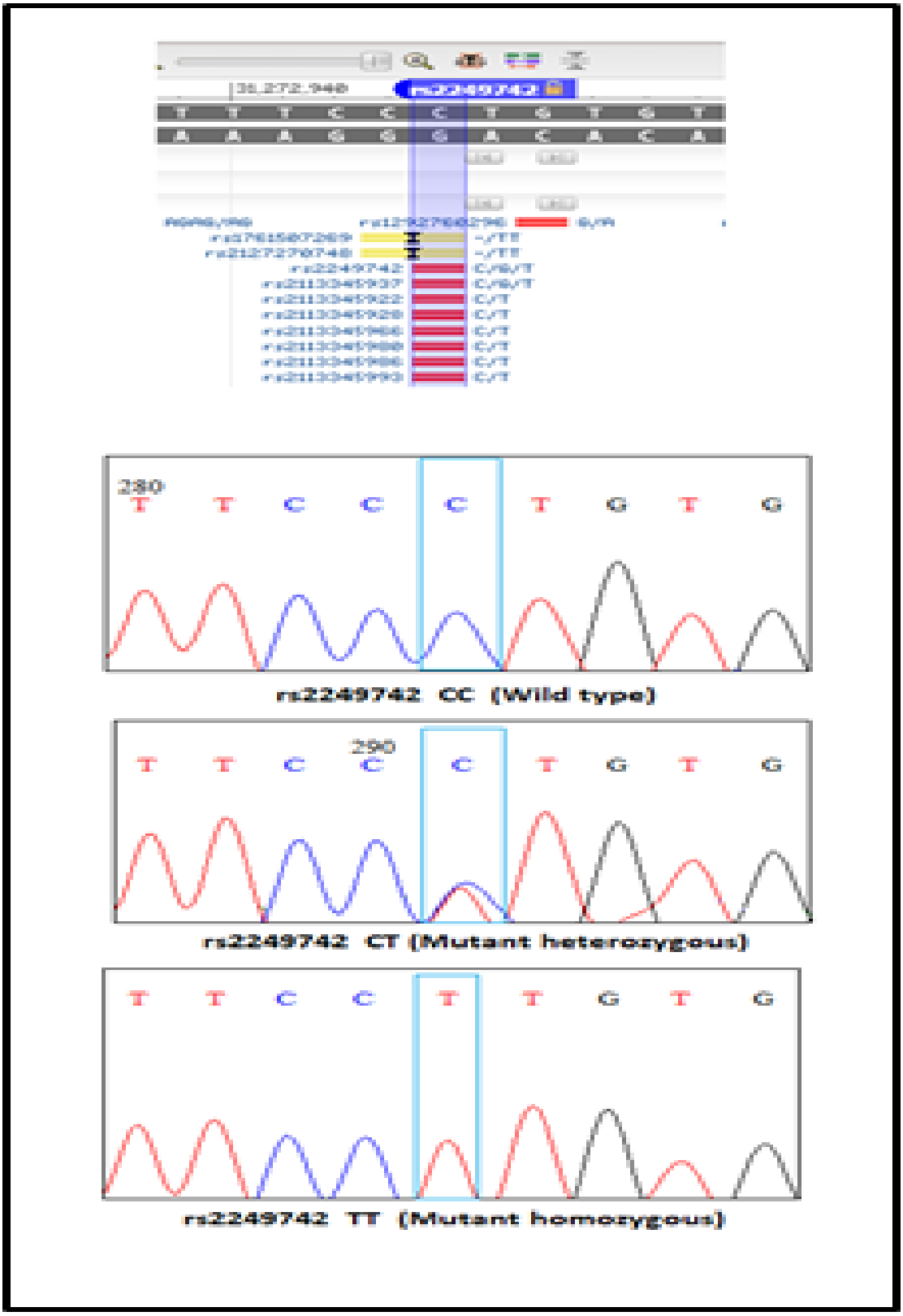


Fig.4-9.Distributions of rs2249742 genotype SNPs on HLA-C gene in study population

A-**HLA-C rs2113345716 genotype SNPs**

The genotype distribution of the HLA-C rs2113345716 genotype SNPs with *H. pylori* infection was 8% TT, 16% TA, and 76% AA, as shown by the first SNP in this study. and the genotype distribution in healthy controls was 0% TT, 32% TA, and 68.5% AA, with T-allele and A-allele frequencies of 8(0.16)%, 42(0.84)% in patients and 8(0.19)% and 34(0.81)% in healthy controls, respectively. Furthermore, when comparing the genotype distribution for HLA-C rs2113345716, the TT result was (P=0.28, OR=5.42, Cl=0.2474 that differences in HLA-C rs2113345716 SNP (TT,AA,TA) were not significant. According to these results, there was no association between (HLA-C) rs2113345716 genotype SNPs and Helicobacter *H.pylori* infection. as shown in table (4-7)

Table(4-7):Distributions of genotype and allele frequencies in HLA-C rs2113345716, SNPs among patients with *H. pylori* infection and healthy control

rs2113345716 genotypes		patients n=25 (%)	Healthy Control n=25 (%)	P-Value	OR	CI=95%
TT (wild type)		**2(8.00)**	**0**	**0.28**	**5.42**	0.2474 to 118.96
TA (Mutant heterozygous)		**4(16.00)**	**8(32.00)**	**0.19**	**40.0**	0.1039 to 1.57
AA (Mutant homozygous)		**19(76.00)**	**17(68.5)**	**0.52**	**1.49**	0.4293 to 5.17
Alleles (%)	**T**	**8(0.16)**	**8(0.19)**	**0.7**	**0.82**	0.2751 to 2.38
	A	**42(0.84)**	**42 (0.81)**			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤ 0.05 calculated for estimation significant difference of patients and control genotypes and alleles

B-HLA-C rs2395471 genotype SNPs

The genotype distribution of the second SNP in that HLA-C rs2395471 genotype SNPs was 8% GG, 60% GA, and 32% AA, with H. pylori infection. and the genotype distribution in healthy controls was 0% GG, 40% GA, and 60% AA, with T-allele and A-allele frequencies of 8(0.16%) and 42(0.84%) in patients and 19(0.38%) for G-allele frequency and 31(0.62%) for A-allele frequencies, respectively. When comparing the genotype distribution for HLA-C rs2395471 GG result was (P=0.2 OR=5.42, Cl=0.24 to 118. AA indicated that there were no statistically significant differences in the HLA-C rs2395471 SNP between patients and healthy controls (GG,GA).Table (4-8) demonstrates that (HLA-C) rs2395471 genotype SNPs contributed to infection at a significant p-value (0.04), indicating that there was a significant difference in AA homozygous genotypes.

Table(4-8):Distributions of genotype and allele frequencies in HLA-C rs2395471SNPs among patients with H. pylori infection and healthy control

rs s2395471 genotypes		patients n=25 (%)	Healthy Control n=25 (%)	P-Value	OR	CI=95%
GG (wild type)		2(8.00)	0	0.2	5.42	0.24 to 118.96
GA (Mutant heterozygous)		15(60.00)	10(40.00)	0.1	2.25	0.72 to 6.97
AA (Mutant homozygous)		8(32.00)	15(60.00)	0.04	0.31	0.0983 to 1.00
Alleles (%)	G	19(0.38)	10(0.2)	0.03	2.45	0.9988 to 6.01
	A	31(0.62)	40(0.8)			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤ 0.05 calculated for estimation significant difference of patients and control genotypes and alleles

C- HLA-C rs2249741genotyp SNPs

The genotype distribution for the third SNP in HLA-C, rs2249741 genotype SNPs, was 20% AA, 52% AC, and 28% CC in patients infected with H. pylori. and the genotype distribution in Healthy control was 0% AA, 24%AC, and 76% CC, with A-allele and C-allele frequencies 23(0.46)%, 27(0.54)% in patients and healthy control were 6(0.12)% for A-allele frequency and 44(0.88)% for C- allele frequencies, and when comparing the genotype distribution for HLA-C rs2249741SNP, AA result was (P=0.08, OR=13.68, There were no statistically significant differences in HLA-C rs2249741SNP between patients and healthy controls when comparing the (AA) wild genotype to the (AC,CC) homozygous genotype and heterozygous genotype. Table (4-9) demonstrates that (HLA-C) rs2249741 genotype SNPs was associated with infection by *H. pylori* when p-values of 0.04 and 0.001 were significant.

Table(4-9). Distributions of genotype and allele frequencies in HLA-C rs2249741 SNPs among patients with *H. pylori* infection and healthy control

rs2249741 genotypes		patients n=25 (%)	Healthy Control n=25 (%)	P-Value	OR	CI=95%
AA (wild type)		**5(20.00)**	**0**	**0.08**	**13.68**	0.71 to 262.18
AC (Mutant heterozygous)		**13(52.00)**	**6(24.00)**	**0.04**	**3.43**	1.02 to 11.47
CC (Mutant homozygous)		**7(28.00)**	**19(76.00)**	**0.001**	**0.12**	0.03 to 0.43
Alleles (%)	**A**	**23(0.46)**	**6(0.12)**	**0.000**	**6.24**	2.25 to 17.29
	C	**27(0.54)**	**44(0.88)**			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤ 0.05 calculated for estimation significant difference of patients and control genotypes and alleles

D-HLA-C rs2249742 genotype SNPs

the genotype distribution for the fourth and final SNPs in the HLA-C rs2249742 genotype SNPs consisted of 44% CC, 44% CT, and 12% TT, with H. pylori infection. and the genotype distribution in Healthy control was 0% CC, 28% CT, and 72% TT, with C-allele and T-allele frequency33(0.66)%, 17(0.34)% in patients and healthy control were 7(0.14)% for C-allele frequency and 43(0.86) for T-allele frequencies, and when comparing the genotype distribution for HLA-C rs2249742SNPs, CC result was (P=0.01, OR=40.44, Cl 2.21 to 738.01)This finding (CC,TT) demonstrates that there were significant differences in HLA- C (CT), heterozygous genotype non-significant SNPs. It can be demonstrated that there was a significant difference between the CC and TT genotypes at significant p-values (0.01) and (0.000), indicating that (HLA-C) rs2249742 genotype SNPs was associated with infection by *H.pylori*, as shown in Table(4-10).

Table(4-10): Distributions of genotype and allele frequencies in HLA-C rs2249742 SNPs among patients with H. pylori infection and healthy control

rs2249742 genotypes		patients n=25 (%)	Healthy Control n=25 (%)	P-Value	OR	CI=95%
CC (wild type)		**11(44.00)**	**0**	**0.01**	**40.44**	2.21 to 738.01
CT (Mutant heterozygous)		**11(44.00)**	**7(28.00)**	**0. 2**	**2.02**	0.6226 to 6.55
TT (Mutant homozygous)		**3(12.00)**	**18(72.00)**	**0.000**	**0.05**	0.0120 to 0.23
Alleles (%)	**C**	**33(0.66)**	**7(0.14)**	**0.000**	**11.92**	4.4299 to 32.09
	T	**17(0.34)**	**43(0.86)**			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤ 0.05 calculated for estimation significant difference of patients and control genotypes and alleles

If OR=Odd ratio =1 there is no relationship between the SNPs and infection OR=Odd ratio < 1 the SNPs considered a protective factor

OR=Odd ratio >1 the SNPs considered a risk factor

Understanding HLA-C polymorphism is essential in various fields of immunology, genetics, and medicine. It provides insights into immune responses, transplantation compatibility, and disease associations. Ongoing research continues to uncover the specific functional implications of HLA-C polymorphism and its contributions to human health and disease.

Other studies have suggested that HLA polymorphisms may play an important role in the development of gastric disorders, particularly in the regulation of inflammatory and immune responses. The mechanism of HLA and the effects of immunogenic variables in gastrointestinal disorders, as well as the interaction between immunogenetic and environmental factors in the development of gastrointestinal disease, necessitate both population-based and experimental methods. (Gönen, *et al*., 2017).

H. pylori gastritis has been linked to HLA class II antigens in multiple investigations conducted over the past decade. According to findings from multiple populations, each population should characterize its own HLA category associated with an increased risk (Nizhevich *et al*.,2010, Wang *et al*.,2015) .

We identified only a handful of studies that identified relevant HLA class I alleles and found that HLA-CW*03 significantly increased the risk of GC in individuals infected with *H. pylori*. In contrast to bacterial peptides, it has been demonstrated that Cytotoxic T lymphocytes (CTLs) can recognize tumor-specific antigens on HLA class I molecules on tumor cells (Kaneko *et al*.,2011) .

4.7.2 . Association of HLA-DRB1 SNPs among patients with *H. pylori* infection.

Trimming of Sanger sequencing results for HLA-DRB1 product. Multiple alignments for each were prepared

using Finch TV version 1.4.0 (Geospiza, Inc.; Seattle, WA, USA; (http://www.geospiza.com) to know the genotype differences between among patients with H. pylori infection. and compared with Healthy control . Fig.(4-10) shows SNPs distributions on the HLA –DRB1 gene

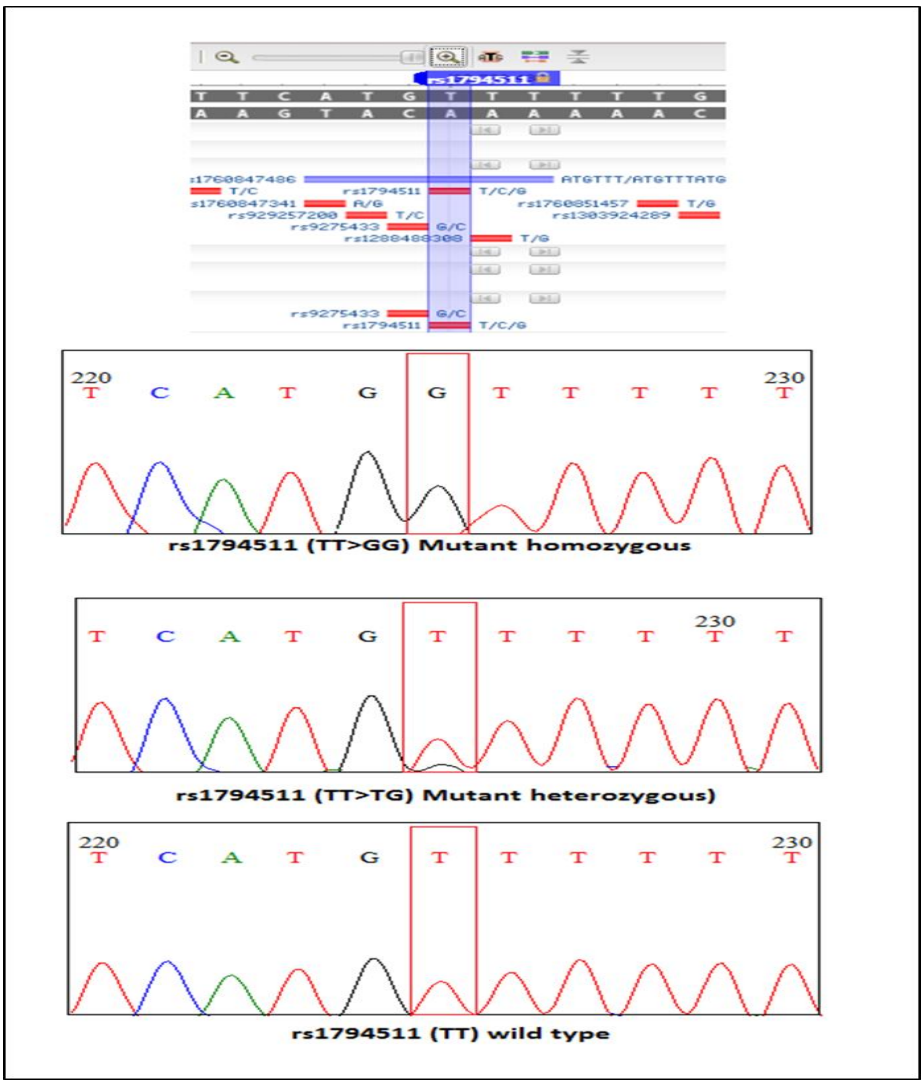


Fig.4-10.Distributions of rs2395471 genotype SNPs on HLA-DRB1 gene in study population

-HLA-DRB1 rs1794511SNPs

the genotype distribution of rs1794511 genotype SNPs in the HLA-DRB1 rs2249742 genotype SNPs consisted of 64% TT, 24%TG, and 12% GG, with *H. pylori* infection. and the genotype distribution in Healthy control was 100% TT, 0% TG, and 0% GG, with T-allele and G-allele frequency**38(0.62)** %, 9(0.38)% in patients and healthy control were 50(1)% for T-allele frequency and 0 for G-allele frequencies, and when comparing the genotype distribution for DRB1 rs2249742 HLA-DRB1 genotype SNPs, TT result was (P=0.02, OR=0.03, Cl=0.0019 to 0.62) This finding demonstrates that there was significant differences . (TG.GG) heterozygous genotype and homozygous genotype non-significant SNPs. p-values (0.06) and (0.1), as shown in Table(4-11).

Table(4-11): Distributions of genotype and allele frequencies in HLA-DRB1 rs1794511SNPs among patients with *H. pylori* infection and healthy control

rs1794511 genotypes		patients n=25 (%)	Healthy Control n=25 (%)	P-Value	OR	CI=95%
TT (wild type)		16(64)	25(100)	0.02	0.03	0.0019 to 0.62
TG (Mutant heterozygous)		6(24)	0	0.06	17.00	0.90 to 320.38
GG (Mutant homozygous)		3(12)	0	0.1	7.93	0.38 to 162.07
Alleles (%)	T	38(0.62)	50(1)	0.02	0.04	0.002 to 0.71
	G	9(0.38)	0			

OR=Odd ratio, Cl (95%)=confidence interval, p-value ≤ 0.05 calculated for estimation significant difference of patients and control genotypes and alleles

HLA-DRB1 polymorphism influences the repertoire of antigens that can be presented to CD4+ T cells. The variations in HLA-DRB1 alleles enable individuals to recognize and mount immune responses against a diverse range of pathogens. Disease Associations HLA-DRB1 polymorphism has been extensively studied in relation to various autoimmune diseases. Certain HLA-DRB1 alleles are associated with an increased risk or protection against autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and others. The specific HLA-DRB1 alleles linked to these diseases can vary among different populations. (Serafim.,*et al* 2020).

Conclusions and Recommendations

Conclusions

1-This study found an increase in serum level of HLA-C among patients with *H.pylori* infection.

2-The study It was found decrease in serum level of HLA-DRB1. among patients with *H. pylori* infectio .It may be a protective factor and rs1794511 genotypes SNPs on HLA-DRB1gene was significant differences ssociated with the disease.

3-rs2395471,rs2249741,rs2249742genotypesSNPs on HLA-C gene was contributed with infection by *H .pylori* and development infections in stomach ulcer patients. it may be a risk factor .

4-Polymorphism in HLA-C and HLA-DRB1 was significantly associated with the disease risk.

Recommendations

1-Study associated of HLA-DRB1 with gastric cancer.

2-Study relationship between other HLA typing such as HLA-A,B and DQ.

References

Adu-Aryee, N. A., Aabakken, L., Dedey, F., Nsaful, J., and Kudzi, W. (2016). Comparison of endoscopic based diagnosis with helicobacter urease test for Helicobacter pylori infection. BMC Research Notes, 9 (1), 421

Ahmed, S., and Belayneh, Y. Muche. (2019). Helicobacter pylori and Duodenal Ulcer: Systematic Review of Controversies in Causation. Clinical and Experimental Gastroenterology, 12, 441–447.

Al Sulami, A., Al Kiat, H. S., Bakker, L. K., and Hunoon, H. (2008). 'Primary Isolation and Detection of Helicobacter Pylori from Dyspeptic Patients: A Simple, Rapid Method.' EMHJ-eastern Mediterranean health journal, 14(2), 268–276.

Al-Bayatee, N. T., and Ad'hiah, A. H. (2021). Soluble HLA-G is upregulated in serum of patients with severe COVID-19. Human Immunology, 82(10), 726–732

Al-Thahab, A. O., and Lateef Al-Awsi, G. R. (2018). Detection of Helocobacter pylori in pregnant women by stool culture method. Biochemical and Cellular Achieve., 18 (1), 49–54.

Amer, Amer, N., Hassan, Janan G., Ihsan et al. Saimary. (2014). Is Helicobacter pylori infections in immunocompromised children a risk factor for cancer Medical Journal of Islamic World Academy of Sciences 22(1): 36–41.

Ausmees, N., Kuhn, J. R., and Jacobs-Wagner, C. (2003). The bacterial cytoskeleton: An intermediate filament-like function in cell shape. Cell, 115(6), 705–713.

Aziz, R. K., Khalifa, M. M., and Sharaf, R. R. (2015). Contaminated water as a source of Helicobacter pylori infection: A review. Journal of Advanced Research, 6 (4), 539–547.

Brisslert, M., Enarsson, K., Lundin, S., Karlsson, A., Kusters, J. G., Svennerholm, A. M., . . . Quiding-Järbrink, M. (2005). Helicobacter pylori induce neutrophil transendothelial migration: Role of the bacterial HP-NAP. FEMS Microbiology Letters, 249 (1), 95–103

Bürgers, R., Schneider-Brachert, W., Reischl, U., Behr, A., Hiller, K. A., Lehn, N., . . . Ruhl, S. (2008). Helicobacter pylori in Human oral cavity and Stomach. European Journal of Oral Sciences, 116(4), 297–304.

Chen, L. W., Chien, C. Y., Yang, K. J., Kuo, S. F., Chen, C. H., and Chien, R. N. (2015). Helicobacter pylori ınfection ıncreases ınsulin resistance and metabolic syndrome in residents younger than 50 years old: A community-based study. PLOS ONE, 10(5), e0128671

Cho, J.-H., Chang, Y. W., Jang, J. Y., Shim, J. J., Lee, C. K., Dong, S. H., . . . Cho, J. Y. (2013). Close observation of gastric mucosal pattern by standard endoscopy can predict H Elicobacter pylori infection status. Journal of Gastroenterology and Hepatology, 28 (2), 279–284

Dore, M. P., Osato, M. S., Malaty, Hoda M., and Graham, D. Y. (2000). Characterization of a culture method to recover Helicobacter pylori from the feces of infected patients. Helicobacter, 5(3), 165–168.

Fedorowicz, S. A. et al. (2020). Helicobacter pylori: Historical Therapy and Current Treatment Helicobacter pylori–Dotychczasowa Terapia I Leczenie Współczesne.

Flores, S. E., Aitchison, A., Day, A. S., and Keenan, J. I. (2017). Helicobacter pylori infection perturbs iron homeostasis in gastric epithelial cells. PloS One, 12(9), e0184026.

Forbes, B. A., Sahm, D. F., and Weissfeld, A. S. (2007). Diagnostic microbiology. St. Louis, MI: Mosby.

Gall, A., Gaudet, R. G., Gray-Owen, S. D., and Salama, N. R. (2017). TIFA signaling in gastric epithelial cells initiates the Cag Type 4 secretion system-dependent innate immune response to Helicobacter pylori infection. mBio, 8(4).

Gebara, E. C. E., Faria, C. M., Pannuti, C., Chehter, L., Mayer, M. P., and Lima, L. A. (2006). Persistence of Helicobacter pylori in the oral cavity after systemic eradication therapy. Journal of Clinical Periodontology, 33(5), 329–333.

Ghazy, A. A., and Taha, A. E. (2022). Association of human leukocyte antigen DQ-rs3920AG genotype with Helicobacter pylori recurrence in Kafrelsheikh, Egypt. Journal of Applied Microbiology, 133(3), 1688–1696.

Gisbert, J. P., and Pajares, J. M. (2004). 13C-urea breath test in the diagnosis of Helicobacter pylori infection–a critical review. Alimentary Pharmacology and Therapeutics, 20(10), 1001–1017.

Gönen, S., Sari, S., Kandur, Y., Dalgiç, B., and Söylemezoğlu, O. (2017). Evaluation of human leukocyte antigen class I and II antigens in helicobacter pylori-positive pediatric patients with active gastritis and duodenal ulcer. Arquivos de Gastroenterologia, 54(4), 297–299.

Graham, D. Y., and Fischbach, L. (2010). Helicobacter pylori treatment in the era of increasing antibiotic resistance. Gut, 59(8), 1143–1153.

Graham, D. Y., and Miftahussurur, M. (2018). Helicobacter pylori urease for diagnosis of Helicobacter pylori infection: A mini review. Journal of Advanced Research, 13, 51–57.

Graham, D. Y.,and Sung, J. Y. (2006). Helicobacter pylori. In M. Feldman, L. S. Friedman and L. J. Brandt (Eds.), Sleisenger and Fordtran's gastrointestinal and liver disease; pathophysiology, diagnosis, management (8th ed) (pp. 1049–1066). Philadelphia: Saunders.

Green, M. R., and Sambrook, J. (2012). Molecular cloning. A Laboratory Manual 4th.

Hofman, B., and Kaiser, K. (2004). The making of the "Big Bang"and its aftermath: A political economy perspective. Reforming intergovernmental fiscal relations and the rebuilding of Indonesia: The "Big Bang"program and its economic consequences (pp. 15–46).

Houghton, J., and Wang, T. C. (2005). Helicobacter pylori and gastric cancer: A new paradigm for inflammation-associated epithelial cancers. Gastroenterology, 128(6), 1567–1578.

Kaneko, K., Ishigami, S., Kijima, Y., Funasako, Y., Hirata, M., Okumura, H., . . . Natsugoe, S. (2011). Clinical implication ofHLA class I expression in breast cancer. BMC Cancer, 11, 454.

Kato, S., Osaki, T., Kamiya, S., Zhang, X. S., and Blaser, M. J. (2017). Helicobacter pylori sabA gene is associated with iron deficiency anemia in childhood and adolescence. PLOS ONE, 12(8), e0184046.

Kern, P. A., Di Gregorio, G. B., Lu, T., Rassouli, N., and Ranganathan, G. (2003). Adiponectin expression from human adipose tissue: Relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes, 52(7), 1779–1785.

Khan, T., Rahman, M., Ahmed, I., Al Ali, F., Jithesh, P. V., and Marr, N. (2022). Human leukocyte antigen class II gene diversity tunes antibody

repertoires to common pathogens. Frontiers in Immunology, 13, 856497.

Kidd, M., and Modlin, I. M. (1998). A century of Helicobacter pylori: Paradigms lost-paradigms regained. Digestion, 59(1), 1–15.

Kocak, B. T., Saribas, S., Demiryas, S., Yilmaz, E., Uysal, O., Kepil, N., . . . and Kocazeybek, B. (2020). Association between polymorphisms in HLA-A, HLA-B, HLA-DR, and DQ genes from gastric cancer and duodenal ulcer patients and cagL among cagA-positive Helicobacter pylori strains: The first study in a Turkish population. Infection, Genetics and Evolution, 82, 104288.

Laheij, R. J. F., Straatman, H. M. P. M., Jansen, J. B. M. J., and Verbeek, A. L. M. (1998). Evaluation of commercially AvailableHelicobacter pylori serology kits: A review. Journal of Clinical Microbiology, 36(10), 2803–2809.

Lan, H.-C., Chen, T. S., Li, A. F., Chang, F. Y., and Lin, H. C. (2012). Additional corpus biopsy enhances the detection of Helicobacter pylori infection in a background of gastritis with atrophy. BMC Gastroenterology, 12(1), 182.

Leboffe, M. J., and Pierce, B. E. (2015). Microbiology: Laboratory theory and application. Morton Publishing.

Lee, J. H., Park, J., Park, M. R., Na, Y. H., and Cho, S. (2017). A comparative study of Helicobacter pylori growth on different agar-based media. Korean Journal of Helicobacter and Upper Gastrointestinal Research, 17(4), 208–212.

Lee, S. H., Yang, J., Goddard, M. E., Visscher, P. M., and Wray, N. R. (2012). Estimation of pleiotropy between complex diseases using single-nucleotide polymorphism-derived genomic relationships and restricted maximum likelihood. Bioinformatics, 28(19), 2540–2542

Lopes, A. I., Vale, F. F., and Oleastro, M. (2014). Helicobacter pylori infection-recent developments in diagnosis. World Journal of Gastroenterology, 20(28), 9299–9313.

Lundgren, A., Suri-Payer, E., Enarsson, K., Svennerholm, A. M., and Lundin, B. S. (2003). Helicobacter pylori-specific CD4+ CD25high regulatory T cells suppress memory T-cell responses to H. pylori in infected individuals. Infection and Immunity, 71(4), 1755–1762.

MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria, Williams and Wilkins p. PA113. Philadelphia.

Maeda, S., Yoshida, H., Mitsuno, Y., Hirata, Y., Ogura, K., Shiratori, Y., and Omata, M. (2002). Analysis of apoptotic and antiapoptotic signalling pathways induced by Helicobacter pylori. Gut, 50(6), 771–778.

Mahon, C. R., and Manuselis, G. (2007). Urea agar procedure. Textbook of diagnostic microbiology (3rd ed), Saunders, W. B., Japanese Government, and Ministry of Health, Labour and Welfare. The Japanese pharmacopeia (15th ed) p. 20 Supplement 2. Ministerial Notification No. 425.

Malaty, H. M., Graham, D. Y., Isaksson, I., Engstrand, L., and Pedersen, N. L. (2000). Are genetic influences on peptic ulcer dependent or

independent of genetic influences for Helicobacter pylori infection? Archives of Internal Medicine, 160(1), 105–109.

Malfertheiner, P., Megraud, F., O'Morain, C. A., Atherton, J., Axon, A. T., Bazzoli, F., … European Helicobacter Study Group. (2012). Management of Helicobacter pylori infection—The Maastricht IV/Florence consensus report. Gut, 61(5), 646–664.

Mansori, K., Moradi, Y., Naderpour, S., Rashti, R., Moghaddam, A. B., Saed, L., and Mohammadi, H. (2020). Helicobacter pylori infection as a risk factor for diabetes: A meta-analysis of case-control studies. BMC Gastroenterology, 20(1), 77.

Marshall, B. J. (1988). The Campylobacter pylori story. Scandinavian Journal of Gastroenterology. Supplement, 146, 58–66.

Marshall, B. J., and Warren, J. R. (1984). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet, 1(8390), 1311–1315.

Matsueda, S., and Graham, D. Y. (2014). Immunotherapy in gastric cancer. World Journal of Gastroenterology, 20(7), 1657–1666.

Matysiak-Budnik, T., and Mégraud, F. (2006). Helicobacter pylori infection and gastric cancer. European Journal of Cancer, 42(6), 708–716.

Mégraud, F., and Lehours, P. (2007). Helicobacter pylori detection and antimicrobial susceptibility testing. Clinical Microbiology Reviews, 20(2), 280–322.

Megraud, F., Coenen, S., Versporten, A., Kist, M., Lopez-Brea, M., Hirschl, A. M., … Study Group participants. (2013). Helicobacter pylori

resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut, 62(1), 34–42.

Miftahussurur, M., and Yamaoka, Y. (2016). Diagnostic methods of Helicobacter pylori infection for epidemiological studies: Critical importance of indirect test validation. BioMed Research International, 2016, 4819423.

Moran, A. P. (2007). Lipopolysaccharide in bacterial chronic infection: Insights from Helicobacter pylori lipopolysaccharide and lipid A. International Journal of Medical Microbiology, 297(5), 307–319.

Najafimehr, H., Mohamed Ali, K., Safari, S., Yousefifard, M., and Hosseini, M. (2020). Estimation of basic reproduction number for COVID-19 and the reasons for its differences. International Journal of Clinical Practice, 74(8), e13518.

Nizhevich, A. A., Shcherbakov, P. L., Akhmadeeva, E. N., Sataev, V. U., Elicheva, Z. M., Usmanova, I. Z., and Tsyglintseva, N. P. (2010). Immune polymorphism analysis of HLA class II antigens in ulcer diseases associated with Helicobacter pylori in children. Eksp. Klin. Gastroenterol., 1(1), 58–63.

Nordquist, H., and Jamil, R. T. (2019). Biochemistry, HLA antigens.

Ohno, H., and Satoh-Takayama, N. (2020). Stomach microbiota, Helicobacter pylori, and Group 2 innate lymphoid cells. Experimental and Molecular Medicine, 52(9), 1377–1382.

On, S. L. W. (2001). Taxonomy of Campylobacter, Arcobacter, Helicobacter and related bacteria: Current status, future prospects and immediate concerns. Symposium Series, 90(30)(Suppl. 6), 1S–15S.

Parsonnet, J., Hansen, S., Rodriguez, L., Gelb, A. B., Warnke, R. A., Jellum, E., . . . Friedman, G. D. (1994). Helicobacter pylori infection and gastric lymphoma. New England Journal of Medicine, 330(18), 1267–1271.

Parsonnet, J., Shmuely, H., and Haggerty, T. (1999). Fecal and oral shedding of Helicobacter pylori from healthy infected adults. JAMA, 282(23), 2240–2245.

Qin, Z., Lin, W. T., Zhu, S., Franco, A. T., and Liu, J. (2017). Imaging the motility and chemotaxis machineries in Helicobacter pylori by cryo-electron tomography. Journal of Bacteriology, 199(3), e00695–e00616.

Ramírez-Lázaro, M. J., Lario, S., Casalots, A., Sanfeliu, E., Boix, L., García-Iglesias, P., . . . Calvet, X. (2011). Real-time PCR improves Helicobacter pylori detection in patients with peptic ulcer bleeding. Plos one, 6(5), e20009.

Replogle, M. L., Glaser, S. L., Hiatt, R. A., and Parsonnet, J. (1995). Biologic sex as a risk factor for Helicobacter pylori infection in healthy young adults. American Journal of Epidemiology, 142(8), 856–863.

Rimbara, E., Sasatsu, M., and Graham, D. Y. (2013). PCR detection of Helicobacter pylori in clinical samples. In. Methods in Molecular Biology (Clifton, N.J.) (pp. 279–287). Berlin: Springer.

Robinson, K., Argent, R. H., and Atherton, J. C. (2007). The inflammatory and immune response to Helicobacter pylori infection. Best Practice and Research. Clinical Gastroenterology, 21(2), 237–259.

Rockey, D. C., Altayar, O., Falck-Ytter, Y., and Kalmaz, D. (2020). AGA technical review on gastrointestinal evaluation of ıron deficiency anemia. Gastroenterology, 159(3), 1097–1119.

Rohde, M., Püls, J., Buhrdorf, R., Fischer, W., and Haas, R. (2003). A novel sheathed surface organelle of the Helicobacter pylori cag type IV secretion system. Molecular Microbiology, 49(1), 219–234.

Sabbagh, P., Mohammadnia-Afrouzi, M., Javanian, M., Babazadeh, A., Koppolu, V., Vasigala, V. R., . . . Ebrahimpour, S. (2019). Diagnostic methods for Helicobacter pylori infection: Ideals, options, and limitations. European Journal of Clinical Microbiology and Infectious Diseases, 38(1), 55–66.

Sadirovna, S. M. (2020). Immunogenetic aspects Helicobacter pylori associated gastroduodenal pathology in children. American Journal of Medical Sciences and Pharmaceutical Research, 2(8), 58–63.

Samareh-Fekri, M., Hashemi Bajgani, S. M., Shafahi, A., Asadi-Zarandi, M., Mollaie, H., and Jamali Paghalhe, A. (2016). Detection of Helicobacter pylori in the bronchoalveolar lavage of patients with lung cancer using real-time PCR. Jundishapur Journal of Microbiology, 9(11)

Sambrook, J., and Russell, D. W. (2001). Molecular cloning—Sambrook and Russel. Antibiotics, 1, 2, 3 Schmitz, F. J., and Jones, M. E. (1997).

Sambrook, J., and Russell, D. W. (2006). Agarose gel electrophoresis. CSH Protocols, 2006(1)

Savoldi, A., Carrara, E., Graham, D. Y., Conti, M., and Tacconelli, E. (2018). Prevalence of antibiotic resistance in Helicobacter pylori: A systematic review and meta-analysis in World Health Organization regions. Gastroenterology, 155(5), 1372–1382.e17.

Scott, D. R., Marcus, E. A., Weeks, D. L., and Sachs, G. (2002). Mechanisms of acid resistance due to the urease system of Helicobacter pylori. Gastroenterology, 123(1), 187–195.

Serafim, C., Araruna, M. E., Júnior, E. A., Diniz, M., Hiruma-Lima, C., and Batista, L. (2020). A review of the role of flavonoids in peptic ulcer (2010–2020). Molecules, 25(22), 5431.

Shimoyama, T. (2013). Stool antigen tests for the management of Helicobacter pylori infection. World Journal of Gastroenterology, 19(45), 8188–8191.

Sipponen, P., Laxén, F., Huotari, K., and Härkönen, M. (2003). Prevalence of low vitamin B12 and high homocysteine in serum in an elderly male population: Association with atrophic gastritis and Helicobacter pylori infection. Scandinavian Journal of Gastroenterology, 38(12), 1209–1216.

Solnick, J. V., and Vandamme, P. (2001). 'Taxonomy of the Helicobacter Genus.' Helicobacter pylori: Physiology and genetics (pp. 39–51).

Speetjens, F. M., Kuppen, P. J., Sandel, M. H., Menon, A. G., Burg, D., van de Velde, C. J., . . . and Nagelkerke, J. F. (2008). Disrupted expression of CXCL5 in colorectal cancer is associated with rapid tumor formation in rats and poor prognosis in patients. Clinical Cancer Research, 14(8), 2276–2284.

Stefano, K. et al. (2018). Helicobacter pylori, Transmission Routes and Recurrence of Infection: State of the Art. Acta Bio Medica, 89(Suppl. 8), 72–76.

Suerbaum, S., and Michetti, P. (2002). Helicobacter pylori infection. New England Journal of Medicine, 347 (15), 1175–1186.

Szymczak, A., Ferenc, S., Majewska, J., Miernikiewicz, P., Gnus, J., Witkiewicz, W., and Dąbrowska, K. (2020). Application of 16S rRNA gene sequencing in Helicobacter pylori detection. PeerJ, 8, e9099.

Talley, N. J and Vakil, N.,(2005) Practice Parameters Committee of the American College of Gastroenterology. Guidelines for the management of dyspepsia. American Journal of Gastroenterology, 100(10), 2324–2337.

Thaker, Y., Moon, A., and Afzali, A. (2016). Helicobacter pylori: A review of Epidemiology, Treatment, and Management. Journal of Clinical Gastroenterology and Treatment, 2 (2), 1–5.

Tham, T. C., Chen, L., Dennison, N., Johnston, C. F., Collins, J. S., Ardill, J. E., and Buchanan, K. D. (1998). Effect of Helicobacter pylori eradication on antral somatostatin cell density in humans. European Journal of Gastroenterology and Hepatology, 10 (4), 289–291.

Tomb, J. F., White, O., Kerlavage, A. R., Clayton, R. A., Sutton, G. G., Fleischmann, R. D., . . . Venter, J. C. (1997). The complete genome sequence of the gastric pathogen Helicobacter pylori. Nature, 388(6642), 539–547.

Tonkic, A., Tonkic, M., Lehours, P., and Mégraud, F. (2012). Epidemiology and diagnosis of H Elicobacter pylori infection. Helicobacter, 17 Suppl. 1, 1–8.

Typas, A., Banzhaf, M., Gross, C. A., and Vollmer, W. (2012). From the regulation of peptidoglycan synthesis to bacterial growth and morphology. Nature Reviews in Microbiology, 10(2), 123–136.

Vaira, D., Holton, J., Menegatti, M., Ricci, C., Landi, F., Ali', A., . . . Miglioli, M. (1999). New immunological assays for the diagnosis of Helicobacter pylori infection. Gut, 45(Suppl. 1), I23–I27.

Wang, J., Zhang, Q., Liu, Y., Han, J., Ma, X., Luo, Y., . . . Hu, Y. (2015). Association between HLA-II gene polymorphism and Helicobacter pylori infection in Asian and European population: A meta-analysis. Microbial Pathogenesis, 82, 15–26.

Wang, Y.-K., Kuo, F. C., Liu, C. J., Wu, M. C., Shih, H. Y., Wang, S. S., . . . Wu, D. C. (2015). Diagnosis of Helicobacter pylori infection: Current options and developments. World Journal of Gastroenterology, 21(40), 11221–11235.

Williams, M. P., and Pounder, R. E. (1999). Helicobacter pylori: From the Benign to the Malignant. American Journal of Gastroenterology, 94(11) Suppl., S11–S16.

Wu, M. S., Hsieh, R. P., Huang, S. P., Chang, Y. T., Lin, M. T., Chang, M. C., . . . Lin, J. T. (2002). Association of HLA-DQB1*0301 and HLA-DQB1*0602 with different subtypes of gastric cancer in Taiwan. Japanese Journal of Cancer Research: Gann, 93(4), 404–410.

Xia, H. X., Keane, C. T., and O'Morain, C. A. (1994). Culture of Helicobacter pylori under aerobic conditions on solid media. European Journal of Clinical Microbiology and Infectious Diseases, 13(5), 406–409.

Yamamoto-Furusho, J. K., Fonseca-Camarillo, G., Barrera-Ochoa, C. A., and Furuzawa-Carballeda, J. (2020). Synthesis of interleukin-10 in patients with ulcerative colitis and Helicobacter pylori infection. Gastroenterology Research and Practice, 2020, 4171083.

Zamani, M., Ebrahimtabar, F., Zamani, V., Miller, W. H., Alizadeh-Navaei, R., Shokri-Shirvani, J., and Derakhshan, M. H. (2018). Systematic review with meta-analysis: The worldwide prevalence of Helicobacter pylori infection. Alimentary Pharmacology and Therapeutics, 47(7), 868–876.

Zhang, F., Gan, R., Zhen, Z., Hu, X., Li, X., Zhou, F., . . . Zhang, B. (2020). Adaptive immune responses to SARS-CoV2 infection in severe versus mild individuals. Signal Transduction and Targeted Therapy, 5, 1–11.

Zhuang, Y., Cheng, P., Liu, X. F., Peng, L. S., Li, B. S., Wang, T. T., . . . Zou, Q. M. (2015). A pro-inflammatory role for Th22 cells in Helicobacter pylori-associated gastritis. Gut, 64(9), 1368–1378.

Zindl, C. L., Lai, J. F., Lee, Y. K., Maynard, C. L., Harbour, S. N., Ouyang, W., . . . Weaver, C. T. (2013). IL-22–producing neutrophils contribute to antimicrobial defense and restitution of colonic epithelial integrity during colitis. Proceedings of the National Academy of Sciences of the United States of America, 110(31),

وزارة التعليم العالـي و البحث العلمـي

جامعـــة بـابـــل

كـلـيـــة العلـوم

قسم علـوم الحيـاة

دراسة تعدد الاشكال الوراثية لبعض جينات HLA بين اصابات الملوية البوابية

رسالة مقدمة الى

مجلس كلية العلوم / جامعة بابل كجزء من متطلبات نيل درجة الماجستير في العلوم / علوم الحياة

من قبل

نـور فـراس قـيس عـيسى

بكالوريوس علوم حياة / 2020

بإشراف

ا.م.د. نور سلمان كاظم

2023 م 1445 هـ

الخلاصة :-

الملوية البوابية: تعد عدوى الملوية البوابية شائعة جدًا وتشير التقديرات إلى أن حوالي نصف سكان العالم مصابون بهذه البكتيريا التي تسبب قرحة المعدة والتهاب المعدة وسرطان المعدة.

تضمنت هذه الدراسة جمع العينات من الاشخاص المرضى والاشخاص الذين يبدو عليهم انهم اصحاء بلغ عدد الاشخاص 250 شخصًا مقسمين إلى 200 مريضًا يراجعون مركز أمراض الجهاز الهضمي والكبد بمستشفى مدينة مرجان الطبية لتشخيص العدوى البكتيرية في المعدة الذين تتراوح أعمارهم بين (15-71) خلال نوفمبر 2022 إلى يناير 2023، بالإضافة إلى 50 شخصًا يتمتعون بصحة جيدة كمجموعة ضابطة. تم جمع 250 عينة دم و 250 عينة براز من جميع أفراد العينة. 5 مل من عينات الدم الوريدي مقسمة إلى مجموعتين. يوضع 2.5 مل في أنبوب EDTA لاستخلاص الحمض النووي للكشف عن تعدد أشكال HLA. بينما يوضع 2.5 مل في أنبوب هلامي لفصل المصل للكشف عن مستويات HLA-DRB وHLA-C بواسطة ELISA وتشخيص بكتيريا H.pylori بواسطة اختبار Ab السريع. بالإضافة إلى الكشف عن البكتيريا عن طريق اختبار Ag السريع من خلال عينات البراز وفقا للزراعة البكتيرية و PCR للكشف عن URE A. من ال200 عينة 80 (40%) أظهرت نتيجة إيجابية لاختبار Ab و 120 (60%) أعطت نتيجة سلبية. 45 (22.5%) نتيجة إيجابية لاختبار Ag وبحسب الزراعة البكتيرية بينما 155 (77.5%) أعطت نتيجة سلبية لاختبار Ag السريع والنمو البكتيري .

أظهرت هذه الدراسة انخفاضًا في مستوى مصل HLA-DRB1 في المرضى حيث بلغت (1905) نانوغرام / لتر مقارنة بمستويات مصل الدم في الاشخاص الاصحاء والتي كانت (2849) نانوغرام / لتر اي ان هنالك فرق معنوي P= 0.0003 ، بينما أظهرت دراسة HLA-C زيادة في مستوى مصل المرضى حيث بلغت (50.90) نانوغرام / لتر ، مقارنة بمستويات مصل الدم في الاشخاص الاصحاء والتي كانت (28.77) نانوغرام / لتر اي ان هناك فرق معنوي P= 0.000

كانت نتيجة تعدد الشكل الجيني HLA-C كما يلي توزيع النمط الجيني HLA-C rs2395471 SNPs 8 ٪ GG ، 60٪ GA ، 32٪AA للمرضى المصابين بالبكتيريا الحلزونية البوابية. وكان توزيع التركيب الوراثي في الضوابط الصحية 0٪ GG و 40٪ GA و 60٪ AA ، مع ترددات G-allele و A-allele المرضى 19 (0.38٪) لتردد G- أليل و 31

(0.62٪) لترددات أليل A على التوالي.عند مقارنة توزيع التركيب الوراثي لـ HLA-C rs2395471 كانت نتيجة GG OR = 5.42 ،P = 0.2 ،Cl = 0.24-118.9. أشارت AA إلى عدم وجود فروق معنوية ذات دلالة إحصائية في HLA-C rs 2395471 SNPs بين المرضى و الاشخاص الاصحاء (GG، GA). يوضح أن HLA-C rs2395471SNPs ساهم في الإصابة بقيمة احتمالية معنوية (p=(0.04 ، مما يشير إلى وجود اختلاف كبير في الأنماط الجينية AA.

وكان توزيع النمط الجيني rs2249741SNPs 20٪ AA ، 52٪AC ، و 28٪ CC في المرضى المصابين بالبكتيريا الحلزونية. وكان توزيع التركيب الوراثي في التحكم الصحي 0٪ AA، 24٪ AC ، 76٪CC ، مع ترددات A-allele و (0.46) 23 C-allele٪ (0.54)27٪ في المرضى و الاشخاص الاصحاء كانت 6 (0.12) ٪ لتردد أليل A و 44 (0.88) ٪ لترددات C- أليل ، وعند مقارنة توزيع التركيب الوراثي لـ HLA-C rs2249741SNPs ، كانت نتيجة AA ,P = 0.08، OR = 13.68 ، لا توجد فروق ذات دلالة إحصائية في HLA-C rs2249741SNP بين المرضى والاشخاص الاصحاء عند مقارنة النمط الجيني البري (AA) بالنمط الجيني المتماثل (AC، CC) والنمط الجيني متغاير الزيجوت . يوضح أن HLA-C rs2249741SNPs كان مرتبطًا بالعدوى بالبكتيريا الحلزونية عند القيم p= 0.04 و p=0.001

كان توزيع النمط الجيني HLA-C rs2249742 SNPs من 44٪ CC و 44٪ CT و 12٪ TT ، مع الإصابة بالبكتيريا الحلزونية البوابية. وكان توزيع التركيب الوراثي في الاشخاص الاصحاء 0٪ CC، 28٪CT ، و 72٪ TT ، مع تردد C-allele (0.66) 33 T-allele٪ ، 17 (0.34)٪ في المرضى والتحكم الصحي كان 7 (0.14) ٪ لتردد C-allele و 43 (0.86) لترددات T-allele ، وعند مقارنة توزيع النمط الجيني لـ HLA-C rs2249742SNP ، كانت نتيجة CC P = 0.01، OR = 40.44، Cl=2.21 يوضح هذا الاكتشاف (CC، TT) أن هناك فروقًا ذات دلالة إحصائية في ، والنمط الجيني متغاير الزيجوت CT لايود فيه فرق معنوي .يمكن إثبات وجود فرق كبير بين الأنماط الجينية CC و TT عند قيم p معنوية (0.01) و (0.000) ، مما يشير إلى أن HLA-C rs2249742SNPs تعدد الأشكال كان مرتبطًا بالعدوى بالبكتيريا الحلزونية.

يتألف توزيع النمط الجيني لـ SNPs من النمط الجيني 1794511rs في تعدد الأشكال الوراثي 1HLA-DRB 2249742rs من 64٪ TT و 24٪ TG و 12٪ GG ، مع عدوى H. pylori. وكان توزيع التركيب الوراثي في الاشخاص الاصحاء 100٪ TT، 0٪ TG ، و 0٪ GG ، مع تردد T-allele و 38G-allele)0.62(٪ ، 9)0.38(٪ في المرضى و الاشخاص الاصحاء كانت 50 (1) ٪ لتردد T-allele و 0 لترددات G-allele ، وعند مقارنة توزيع النمط الجيني لـ 1DRB 2249742rs 1HLA-DRB SNPs ، كانت نتيجة 0.02TT =0.03p OR= 0.0019Cl -0.62 هذه النتيجة توضح أن هناك اختلافات كبيرة. (TG.GG) التركيب الوراثي متغاير الزيجوت والنمط الجيني متماثل الزيجوت.(0.06) و (0.1) .ارتبط تعدد الأشكال في HLA-C و HLA-DRB1 بشكل كبير مع مخاطر المرض .