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IMMUNE RESPONSE FOR PATIENTS INFECTED WITH HELICOBACTER PYLORI IN IRAQ

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IMMUNE RESPONSE FOR PATIENTS INFECTED WITH HELICOBACTER PYLORI IN IRAQ

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January 2023

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

IMMUNE RESPONSE FOR PATIENTS INFECTED WITH HELICOBACTER PYLORI IN IRAQ

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Master of Science in Biology

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January 2023

In this study 40 patients were used in the experiment, 30 patients infected with *H. Pylori*infection and 10 for control, In this study the blood group, IFN-beta, and IL12 were collcted by Enzyme-linked Immuno Sorbent Assay (ELISA) technique, to define the *H. pylori* infection.The serum concentration of IL12 in patients infected with *H.pylori* was high level (162.314 ± 14.782) with a significant increase (p-value 0.0085), compared with healthy patients (control) (39.385 ± 6.819), and there was a significant increase (p-value 788) in serum concentration of IFN-beta in patients infected with *H. pylori*(168.360 ± 28.693) compared with healthy individuals (control) (50.640 ± 3.779). The Increase in IL12, IFN-beta in patients infected with h.pylori than the healthy patients showed us that the immunity system in infected patients resisted the pathogen, the aim of this study demonstrated the effect of the age risk factor on patients infected with helicobacter pylori and the effect of cytokines IFN-beta, IL12 in the immune response in patients infected with *H. pylori*, as a conclusion in this study older age consider as the main risk factor in patients infected with *H. pylori*and cytokines (IL12,IFN-beta) level demonstrated immune response in patients with *H. pylori*.

2023, 39 pages

Keywords: *Helicobacterpylori*, Dentric cells, Peptic ulcer, Cytokines, IFN-γ

ÖZET

IRAK'TA HELİKOBAKTER PİLORİ ENFEKSİYONU OLAN HASTALARDA İMMÜN TEPKİ

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Biyoloji, Yüksek Lisans

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Ocak 2023

Bu çalışmada 40 hasta, bu çalışmada *H. pylori*enfeksiyonu olan 30 hasta kullanıldı. Kontrol için sağlıklı kişilerden 10 adet numune alındı. Bu çalışma *H. Pylori*enfeksiyonunu belirleyecek, ardından Enzyme-linked Immuno Sorbent Assay (ELISA) tekniği ile kan grubunu, IFN-beta ve IL12'yi saptayacaktır.*H. pylori* ile enfekte hastalarda serum IL12 konsantrasyonu, sağlıklı hastalara (kontrol) (39.385 ± 6.819) kıyasla anlamlı bir artışla (p değeri 0.0085) yüksek düzeyde (162.314 ± 14.782) bulundu. Sağlıklı bireylere (kontrol) (50.640 ± 3.779) kıyasla *H. pylori*(168.360 ± 28.693) ile enfekte hastalarda IFN-beta serum konsantrasyonunda anlamlı artış (p-değeri 788) tespit edildi. *H. pylori* ile enfekte hastalarda sağlıklı hastalara göre IL12, IFN-beta artışı, enfekte hastalarda bağışıklık sisteminin patojene direnç gösterdiğini bize göstermiştir.Bu çalışmanın amacı, yaş risk faktörünün enfekte hastalardaki etkisini ortaya koymaktır. *H. pylori* ile enfekte hastalarda immün yanıtta sitokinler IFN-beta, IL12'nin etkisi, ileri yaş *H. pylori*ile enfekte hastalarda ana risk faktörü olarak kabul edilir ve sitokinler (IL12,IFN-beta) düzeyi helicobacter pylori hastalarında bağışıklık tepkisi göstermiştir.

2023, 39 sayfa

Anahtar Kelimeler: *Helicobacter pylori*, Dendritik hücreler, Peptik ülser, Sitokinler, IFN-y.

PREFACE AND ACKNOWLEDGEMENTS

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Kamil Idan Kamil AL-KINANI

Çankırı-2023

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LIST OF SYMBOLS

%	Percent
/	Divide
+	Plus
µL	Microliter
dL	Deciliter
Fe^{2+}	Ferrous
Fe^{3+}	Ferric
mg	Milligram
mL	Milliliter
ng	Nanogram
nm	Nanometer
O_2	Oxygen
ºC	Degrees celsius
α	Alpha
β	Beta
γ	Gamma

LIST OF ABBREVIATIONS

ABO	System use to define the blood group of test ABO
DCs	Dendritic cells
ELISA	Enzyme-linked immunosorbent assay
IDA	Iron-deficiency anemia
IFN-BETA-	Interferon beta
IL12	Interleukin 12
ISGs	Interferon stimulated genes
MALT	Mucosa-associated lymphoid tissue
MS	Multiple sclerosis
PAMPs	Pathogen-associated molecular patterns
pH	Potential of hydrogen
SEM	Standard error of mean
SPSS	Statistical package for the social sciences

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1. INTRODUCTION

Campylobacter pylori, a gram-negative microaerophilic bacteria, is the bacteria known as *Helicobacter pylori (H. pylori),* which has the capacity to reduce the immune response sensors (Roebothanand Chandra1994. Schreiber*et al*. 2004). A bacteria called *H. pylori* has six unipolar flagella that are sheathed, Flagella are organisms that bacteria use for motility (Kusters *et al*. 2006). Since their description of *helicobacter pylori* in 1983, bacteria have received attention from scientists. Scientists MarshallandWarren (1984) were the first to detect *H. pylori* in 1983. Despite the strong persistent cellular immunological and humoral response to *H. pylori* at the local and systemic level, it settles the gastrointestinal mucosa of its host (Marshall and Warren1984).

H. pylori is a negative spiral bacterium, has been associated with a variety of gastrointestinal conditions, including non-lymphoma Hodgkin's disease of the stomach, duodenal and gastric ulcers, gastric adenocarcinoma, mucosa-associated lymphoid tissue (MALT) lymphoma, etc. In a typical lifestyle, humans ingest many microorganisms daily, but due to the human stomach's acidic ph, these microorganisms cannot colonize the stomach.Additionally, when a person is fasting, the gastric lumen's pH is reduced to a level below 2, which prevents *H. pylori* from crossing the epithelial barrier because it encounters a less acidic environment (than that which is present in the stomach lumen) by inhibiting the growth of bacteria in the gastric mucus layer, which is located beneath the gastric epithelial cells (Schreiber *et al*. 2004, Hazell *et al*. 1986).

H. pylori was categorized by scientists as a non-invasive bacterial organism, the majority of *H. pylori* organisms reside freely inside the gastric mucus layer, while some of the bacteria attach to the apical surface of gastric epithelial cells and are infrequently absorbed by these cells (Amieva *et al*. 2002, Kwok *et al*. 2002,Oh*et al*. 2005).

One of the main risk factors for *H. pylori* is lower socioeconomic position, which increases the prevalence of infection (Ceylan *et al*. 2007). *H. pylori* is a stomach cancer class I carcinogen according to the WHO (Vahedi *et al*. 2017). *H. pylori*transmission

can take place via sexual, gastric, oral, or fecal pathways. Monographs on the assessment of human carcinogenic hazards numerous autoimmune conditions, such as rheumatoid arthritis and idiopathic thrombocytopenic purpura, may be linked to *H. pylori*; the organism lives in its host for the duration of its life.*H. pylori* is commonly regarded as a free-living organism in the mucus layer of the stomach after entering the stomach, where it penetrates the mucous gastric layer but does not overcome the epithelial barrier (Schreiber *et al*. 2004).

As a result, it is considered a non-invasive bacteria, although*H. pylori* is typically thought of as a free-living organism in the mucus layer, some have reached or attached to the gastric epithelial cells' apical surface and caused a number of gastroduodenal diseases, including peptic ulcers, chronic gastritis, gastric cancers, mucosa-associated lymphoid tissue lymphomas, Iron deficiency anemia, and immunogenic (idiopathic) thrombocytopenic purpura (ITP), two additional gastrointestinal disorders brought on by *H. pylori* infection (IDA), *H. pylori* also causes a number of gastrointestinal diseases, often patients with *H. pylori* bacteria have higher levels of gastrin at rest and after meals, as well as lower levels of gastric mucus and duodenal mucosal bicarbonate secretion, all of which contribute to the development of ulcer infection (Phillipsand Burns1988).

These differences range from 67 to 6 percent in patients with duodenal ulcers. *H. pylori* removal significantly lowers the likelihood of ulcer recurrence, and in patients with stomach ulcers, from 59% to 4% percent (RJ*et al.* 1996).It is still unclear exactly how *H. pylori* infection affects the host to produce pathogenic consequences (Gupta*et al.* 2002).

The stomach and duodenum are the sites of peptic ulcers (Ramakrishnan and Salinas2007), *H. pylori* causes a decrease in somatostatin levels after infecting humans with gastritis. Additionally, the bacterium continuously colonizes the gastric/duodenal mucosa, resulting in innate and particular immunological reactions as well as gastroduodenal disorders as gastritis and peptic ulcers. But if the infection persisted, the chronic active gastritis condition might never go away (Bagheri *et al.* 2017).

The SBA gene in the *H. pylori* bacteria pathogenesis is highly expressed in bacterial isolates from patients with iron deficiency anemia.Proving that this factor has an impact on development and improvement of anemia disease, due to the fact that older patients are more likely to develop gastric ulcer disease than younger ones, gastric ulcer is regarded as an advanced age disease. Gastric ulcers in older patients also tend to be large and occur in more severe locations (Freston and Freston1990).

Most gastric ulcers are benign, but many exhibit clinical signs of stomach cancer. According to a study, the clinical manifestation of giant gastric ulcer illness is an acute or chronic bleed in about half of cases. The death rate associated with bleeding from giant gastric ulcers is also very high, at 37%. The largest incidence of gastric ulcers occurs between the ages of 60 and 70 for men and between the ages of 70 and 80 for women in older people (Strange 1963).

Indicating that early surgical intervention might be warranted and essential. Furthermore, it has been reported that in these patients, the perforation rate is as low as 5% (Rockey *et al.*2020). Additionally, there are numerous causes of *H. pylori* in the world, including atrophic gastritis and hypohydrogenase, which raise the risk of gastric cancer and peptic ulcer disease. On the other side, iron deficiency anemia may result from weakened iron absorption (Shiferaw and Abera2019).

A study conducted in Maysan, Iraq, by Omer Mansib discussed the connection between *H. pylori* infection and family and individual earnings (Al-Hussaini *et al.*2019, Assaad *et al.*2018).

H. pylori infection risk is increased in low-income individuals who share beds and are crowded, have inadequate sanitation, and lack running water. It is also discovered a strong link between eating fast food and illness, with daily fast food consumption outside the home increasing (Gu *et al.*2017, Lindo *et al.*1999).

The incorrect handling of food and transmission from one person to another can be used to explain this relationship, according to a study by Lindo *et al.*(1999)at the university of the West Indies in Kingston, Jamaica.The age risk factor profile for *H. pylori* is shown in Figure 1.1. Approximately 27% (n 5 15) of 5-year-olds were seropositive, compared to 60%-77% (n 5 172) of those in the 5- to 44-year-old age range. Participants aged 45 years and older had the highest seroprevalence (93%, n 5 15). In the study population, *H. pylori* prevalence averaged 68.8% (n 5 202) (Lindo *et al.*1999).

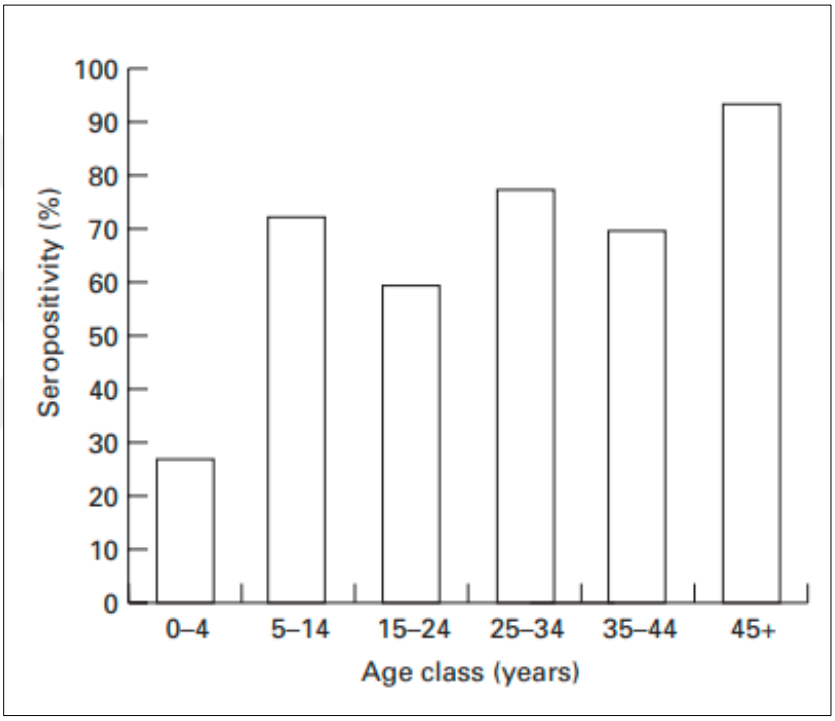

Figure 1.1*H. pylori* with age class (Lindo *et al.* 1999)

The age range factor plays a significant influence in *H. pylori* infection, and older age is considered to be the main risk factor, as illustrated in Figure 1.1 of the study (Lindo *et al.*1999).

China had the greatest percentage of *H. pylori* infections in terms of numbers, many Chinese researchers have become confident about the infections of *H. pylori* in Chinese people and the presence of gastritis, peptic ulcers, or stomach cancer. Up to this point, there are 500 million individuals in China who are still at danger of *H. pylori*

infections.According to numerous studies that have found that 50% of the population is affected, such as peptic ulcers and stomach cancer, corresponding to certain studies from Japan, where intestine metaplasia and atrophy were more common, Many groups believed that germs may be connected to one another in the 1970s (Peterson1991).

The pathogen was not clearly identified because healthy stomachs were relatively sterile and humans with stomach and gastric diseases had spoiled gastric flora and low acidity. However, broad-spectrum antibiotics may be worth a try; Oral neomycin has been used at the Mayo Clinic to treat hepatic encephalopathy patients by decreasing the formation of gastrointestinal ammonia (caused by urease) (Lindo *et al.* 1999).

On the other hand, Dr. John Likodis had utilized an antibiotic "drink" named "Elgaco" in Greece with excellent results and effectiveness in treating numerous patients with duodenal ulcers (Rigas *et al.* 2002).

Finally, around the middle of the 1980s, Spanish researchers conducted a study employing metronidazole for the treatment of duodenal ulcers in a prospective small controlled pilot study that demonstrated an improvement in patients radiation healing (Diaz and Escobar1986).

Interleukin 12 (IL12) converts type 1 helper T (Th1) Oral neomycin has been used at the Mayo Clinic to treat hepatic encephalopathy patients by decreasing the formation of gastrointestinal ammonia (caused by urease). Cells from T naive cells, which assist the immune response by secreting IFN-, IL2, and TNF-. The two high-affinity receptors of IL12 are crucial because Th1 cells that express both receptors and T-lymphocytes that respond to IL12 depend on these receptors to function. As a result, Th1 plays a crucial role in the expression of IL12 receptors (Larosa *et al.* 2019).

Additionally, IL12 promotes production of the enzyme (glucosyltransferase) to regulate P and E-selectin ligands of Th1 cells and has control over a number of genes that maintain the route and homing of Th1 cells (Lu *et al.*2017).

Since it has been demonstrated that IL12 can regulate both innate (NK cells) and adaptive (cytotoxic T lymphocytes) immune responses, its anticancer effects make it a potent new medication in cancer immunotherapy. Additionally, combining IL12 with a variety of other therapeutic modalities, including as antibodies, anti-angiogenic drugs, radiation, adaptive therapy, and anticancer vaccinations, can increase the antitumor effect of IL12 (Gately *et al.* 1998).

First off, IL12 stimulates T cells and NK cells to produce INF-gamma, while concurrently inhibiting IL4's ability to do the same (Sgadari *et al.* 1996). Notably, INF-gamma may activate a defense mechanism in athletes to prevent the growth of malignancies, and produces chemokines that control the development of new blood vessels, like CXCL10 and CXCL9, by causing a variety of cells (fibroblasts, endothelial cells, and monocytes) to be stimulated (Goldstein and Laszlo1988, Lindo *et al.*1999).

In leukemia (including hairy cell and chronic myeloid leukemia), interferon therapy is used to treat various lymphomas, malignancies, including non-muscle invasive bladder cancer, bladder tumors, and other malignancies. Results of the IL12 therapy for cancer patients showed that CXCL10 and CXCL9 are found in the genes of cancer cells (like nodular lymphoma, and cutaneous T-cell lymphoma), *H. pylori* elimination could cut the risk of stomach cancer by 44%, according to recent studies. Additionally, a sizable randomized trial found that after eradication, the prevalence of precancerous lesions is reduced by 39% (Kondo*et al.*2010, Baird2011).

In addition, other trials with longer follow-up periods following eradication revealed that atrophic body gastritis reversed in 50% of patients who were cured of *H. pylori* (Trikudanathan*et al.* 2011, Malatyand Graham1994).

However, there hasn't been any research showing a significantly lower occurrence of stomach cancer with the removal of *H. pylori* (Shin *et al.* 2010). However, some research found that eradication could prevent the growth of preneoplastic lesions (Yin *et al.* 2010). Additionally, autoimmune diseases like rheumatoid arthritis and idiopathic

thrombocytopenic purpura may be brought on by *H. pylori* infection (also called immune thrombocytopenic purpura) (Baird2011, Schreiber *et al*. 2004).

In various studies on the subject, age has been found to be the main risk factor for the bactirum infection (Lim *et al.* 2013). A recent meta-analysis of publications from 73 countries found that 44.3% of people worldwide have *H. pylori* infection (Zamani *et al.* 2018).

According to current thinking, the majority of people carry the *H.pylori* infection for many years after contracting it as children. It's amazing how few people with *H. pylori* infection experience symptoms of gastric disease, such as chronic gastritis, peptic ulcers in the stomach lining and the upper small intestine, mucosa-associated lymphoma, and 1-3% carcinoma (De Marteland Parsonnet2006).

Dendritic cells, normal production of the cytokine interleukin12, which is believed of as messengers between the innate and adaptive immune systems. Interleukin12 plays a significant role in the immune system of all individuals with *H. pylori* infection (Kalińsk*iet al.* 1997).

On the other side, individuals with *H. pylori* infection benefit from interferon-beta, a similar and naturally occurring signal protein. Two factors were chosen to identify the immune response in patients infected with *H. pylori* because there were insufficient studies that covered the immune system response in patients with *H. pylori*(Geis*et al.* 1989).

1.1 Aim of Study

The aim of the current study is to investigate the effect of age on IFN-beta, IL12 of the immunity system in patients infected with *H. pylori.*

2. LITERATURE REVIEW

Helicobacter pylori is a microaerophilic, usually found in human stomachs, Figure 2.1 shows *H. pylori* in stomach, the bacterium is a *gram-negative*, *helical* bacteria (Marshall and Warren1984, Blaser and Atherton2004). Infected individuals with *H. pylori* do not typically exhibit any indications of gastric inflammation (Dooley *et al.*1989). *H. pylori* has been related to peptic ulcer disease and is the most frequent cause of chronic gastritis. When a person is fasting, their gastric luminal pH is below 2, which inhibits the growth of germs (Correa 1992, Chang *et al.* 1991).

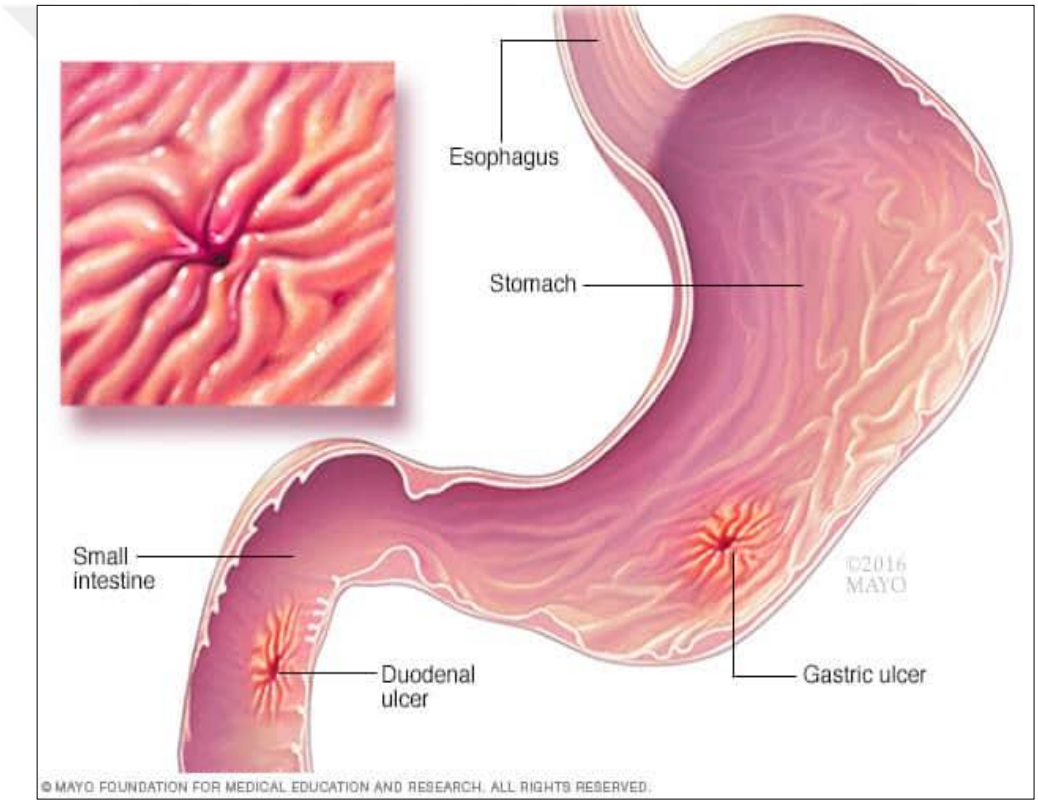

Figure 2.1*H. pylori* in stomach (Yue*et al*. 2016)

H. pylori is a bacterium with a long history that was initially discovered last decades of the 20th century in the stomach mucus layer that overlying the gastric epithelial cells (Marshall and Warren 1984).

H. pylori had been observed at the beginning of the development of microbiology when the microscopic observation was crucial, but it was then forgotten likely because there was more than just enough to study with bacteria easily can grow in culture. The bacteria had been misled into thinking that the stomach was a sterile organ, in low ph, no bacteria could grow in such a condition, and as a result, many believe ideas that the bacteria observed were simply not there (Perez-Perez *et al.* 1990).

A study in Iraq and Najaf shown the significance of the cellular and humoral immune responses in the treatment of patients with *H. pylori*. Smokers can use *H. pylori* infection to make an early diagnosis of stomach cancer, and various studies have indicated that eliminating the infection can improve the management of peptic ulcers (Arkkila *et al.*2003,Seo*et al.*2015).

Without a doubt, there is a connection between, gastritis, *H. pylori* and peptic ulcer infection. According to (Jones *et al.*1986) more than 50% of people over the age of 60 in the world exhibit an immune response to *H. pylori* infection by producing antibodies. A study conducted in India revealed that in patients over 60, the prevalence was found to be close to 90% (Malatyand Graham1994).

Typically, *H. pylori* causes gastritis in NSAID consumption, whether overt or covert, in older patients according to (O'Riordan *et al.* 1991). There are numerous ways to check for helicobacter pylori infection, infection is present (CLO test) (Ig) A and (IgG) antibodies from antral biopsy tissues were tested for urease (the presence of immunoglobulin serological markers).Insufficient bacterial distribution in the stomach, which can occur in elderly people, has no effect on the detection of IgG antibodies.In CD4+ T cells, STAT4 activities by IL12, STAT4 is in charge of T-bet transcription and Th1 cell differentiation, and particular cytokines, Th1 chemokines, and T-bet (master regulator) influence the expression of certain receptors (Thieu *et al.*2008, Schreiber *et al*. 2004).

When IFN-gamma and STAT4 combine together to increase CXCR3 transcription, T-bet alone, CCL3-4, and IL-12R1 also favorably regulate IFN-expression (Jenner *et al.* 2009).

Perforin, IFN-gamma, and granzyme are secreted by cytotoxic NK cells and CD8+ T cells, which inhibit tumor development and cause cancer cells to apoptosis (Zwirner and Ziblat 2017). Lung cancer treatment with IL12 and IFN-gamma is proven to be successful (Mirlekar and Pylayeva-Gupta2021). IL12 stimulates NK cells, which in the tumor niche increase the expression of CD69 and CD25 (Yue *et al.* 2016).

IL12 also upregulates increases the multiple histocompatibility complex-1 (MHCI), stimulates M1 macrophage polarization and the production of the chemokines CXCL9, CXCL10, and CXCL11 for the effector immune cells on tumor cells (Yu *et al.* 2016, Roebothanand Chandra1994).

In order to demonstrate that the IL12 cytokines are crucial in patients with *H. pylori* infection, IL12 were chosen as a parameter to detect the infection.When a person has a genetic predisposition, they develop the chronic neuroinflammatory disease known as multiple sclerosis (MS) in the central nervous system (CNS). It's possible that a mix of infectious and non-infectious environmental variables sets off the pathogenic process (Smyk*et al.* 2014).

The Hp Sydney Strain-1 antigen injection research recently published (Hp-SS1 antigen) in an experimental MS model revealed that it has immunomodulating properties, indicating a very likely involvement for Hp infection in the pathophysiology of the illness (Boziki *et al.* 2012).

Myeloid dendritic cell concentrations in peripheral blood are reduced by interferon-beta, which also differentiates and modifies the function of dendritic cells and other APCs to inhibit antigen presentation and T-cell response stimulation (Jiang *et al.* 1995, Schreiber *et al*. 2004).

However, beta interferons contribute to the maintenance of plasmacytoid dendritic cells that secrete type I interferon (Nuyts*et al.* 2013). However, they also cause TLR9 to be downregulated, which reduces TLR9-mediated interferon-alpha release from plasmacytoid dendritic cells that promotes Th1 (Balashov *et al.* 2010).

However, interferon-beta increases the expression of TLR3, TLR7, and MyD88 on plasmacytoid dendritic cells, the TLR adaptor protein thought to improve immunological control and reduce the risk of virus-mediated MS relapses (Derkow *et al.* 2013).

Interferon beta cytokine has been selected as a parameter, and it has been demonstrated that it plays an important role in patients with *H. pylori* infection's immune response.One of studies that demonstrate that age range is the primary risk factor in *H. pylori* infection, age was linked to an increased risk of infection, age range was determined to be the main risk factor in this analysis for patients with *H. pylori* infection (Lim *et al.* 2013).

Over the past 20 years, the first diagnosis of peptic ulcer illness and hospital admission for complications of peptic ulcer disease occur nearly exclusively in people over 65 (Sonnenberg 1987, Til Vis*et al.* 1987).

The high infection incidence at the orphanage shows that person-to-person transmission of *H. pylori* occurs, which is consistent with other studies such as one in Thailand that revealed the infection rate is higher than in industrialized nations (Kondo*et al.*2010).

Among *H. pylori*infected patients and healthy people, to illustrate the function of (IL12, IFN-beta) cytokines in the immunological response of *H. pylori* patients in Iraq with consideration of the age range factor is the main risk factor in patients infected with H. pylori, the sample collection proved challenging (Roebothanand Chandra1994).

With a small database, there weren't many research in Iraq that discussed the immune response in *H. pylori* infected patients (Kato *et al.*2017).

3. MATERIALS AND METHODS

In this study 40 outpatients ere collected from both genders with different age range factors and separated them into three different group ages range, with two cytokines (IFN-beta, Interlukin-12) as parameters by Enzyme-linked immunosorbent assay (ELISA).

3.1 Materials

To arrive to main aim of current medical investigation, it has been many material for this perpose, patients and others requirements are summarrized in some below sections.

3.1.1 Patients

In current study, thirty infected patients were used with both genders, ten patients were used as a control, so 40 patients were used in this study.Patients were taken care in (Yarmouk teaching hospital, Al-Kindi Teaching Hospital, other laboratories) during the period of 5 march /2022 – 25 june/ study include (30) patients with *H. pylori* infection and healthy people (10) as a control,different group ages range between 15-20 (10 samples), 21-26 (10 samples), 27>(10 samples).

3.1.2 Healthy individuals (control)

Ten healthy patients were used in this study.Patients participating in this medical research were classified and selected on the basis of Wagner's classification and approval. The ethical commitment was taken from the participants. Individuals were selected and divided randomly into groups

3.1.3 Collection of blood

By using a sterile disposable syringe; 5 mL of blood was collected from all patients and healthy individuals (control), then for 5min centrifuged at 3000 rpm. Serum was aspirated from the tube, then stored at -25^{o}C until using it for Enzyme immunoassay.

3.1.4 Serum

Utilizing a serum separator tube, samples should be allowed to clot for two hours at room temperature or overnight at 4 ^{o}C before centrifugation at roughly 1,000 xg for 20 minutes. Assay freshly prepared serum right away, or save samples in an aliquot and store them for later use at -20°C or -80°C. Steer clear of frequent freeze-thaw cycles.

3.1.5 Kits

1. Enzyme-linked Immunosorbent Kit For Interleukin 12(IL12). ORIGIN USA
2. Enzyme-linked Immunosorbent AssayKitFor InterferonBeta(IFNb). ORIGIN USA

3.2 Methods

3.2.1 Reagentpreparation

To generate a double dilution, 7 tubes ere prepared with 0.25 mL of Standard Diluent, 7 Tubes containing 0.25mL standard diluent and produce a double dilution series are shown in Figure 3.1. Before the subsequent transfer, properly combine each tube. Set up seven points of diluted standards, such as 200 pg/mL, 100 pg/mL, 50 pg/mL, 25 pg/mL, 12.5 pg/mL, 6.25 pg/mL, and 3.12 pg/mL. The last set of EDTA tubes with standard diluent is the blank, which is set at 0 pg/mL. Using Assay Diluents A and B, respectively, dilute them 100 times to the working concentration before using washing solution. To make 600mL of Wash Solution (1%), combine 20mL of Wash Solution concentrate (30%) with 580mL of deionized or distilled water.

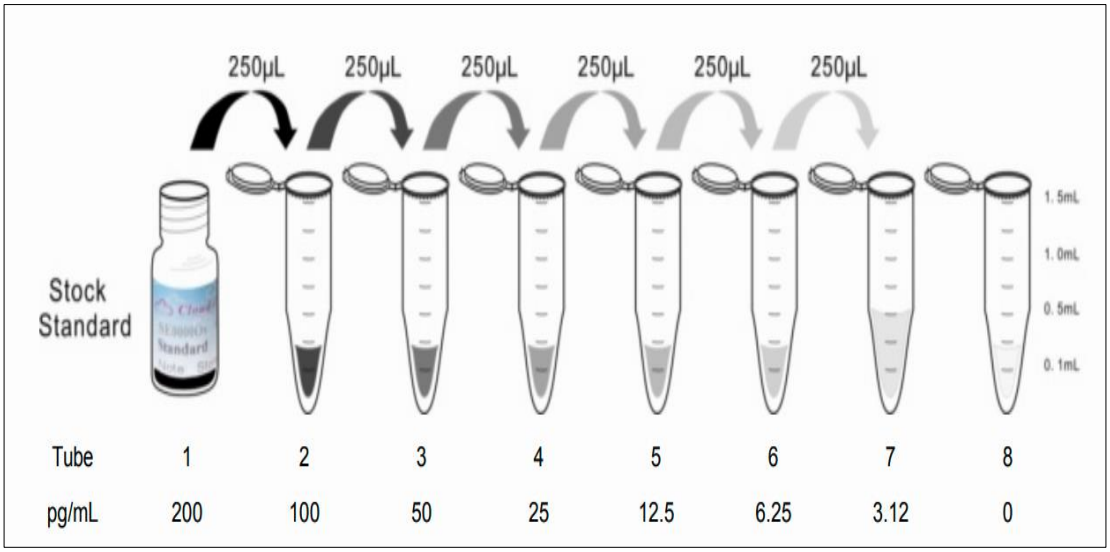

Figure 3.1 7 Tubes containing 0.25mL standard diluent and produce a double dilution series

3.2.2 The enzyme-linked immunosorbent assay (ELISA)

These tests were achieved according to the manufacturing company (cloud-clone corp.) For infb, IL12 follows:

1. Set up seven standard wells and one blank well. Fill the relevant wells with 100µL of dilutions of a standard (see Reagent Preparation), blank, and samples, respectively. Use the Plate sealer to cover. At 37 ºC, incubate for one hour.
2. Drain the contents of each well without washing.
3. Include 100 mL of the detection agent. Fill each well with a working solution, seal the wells with the plate sealer, and then incubate for an hour at 37 °C.
4. Using a multi-channel spray bottle, aspirate the solution and wash with 350 l of 1 Wash Solution into each well.Give it a half-minute to sit in a pipette, manifold dispenser, or autowasher. By setting the plate down on absorbent paper, all liquid that is still present in the wells is fully removed. Three complete washings. After the last wash, aspirate or decant any leftover Wash Buffer. The plate should be turned over and blotted with paper towels.
5. 100 mL of the Detection Reagent B working solution should be added to each well. The wells should then be covered with a plate sealer and left to sit at 37 ℃ for 30 minutes

6. Five times in total, repeat step four's aspiration/wash procedure.
7. To each well, add 90µL of the substrate solution. Add a fresh Plate sealer on top. For 10 to 20 minutes incubate at37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.
8. 50µL of Stop Solution should be added to each well. By adding Stop solution, the liquid will turn yellow. Tap the plate's edge to stir the liquid. Gently tapping the plate will guarantee full mixing if the color change does not appear to be uniform.
9. Check that there are no bubbles on the liquid's surface and remove any water or fingerprints from the plate's bottom. Run the microplate reader after that, and start taking measurements at 450 nm immediately.

3.3 Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software, version 16, was used to conduct the analysis. The mean and standard error of the mean (SEM) were used to present the data, and one-way ANOVA (Analysis of Variance) was used to statistically evaluate them before the Dunnett test. Dunnett test comparisons were carried out to determine the importance of group differences. P 0.05 was chosen as the criterion for statistical significance.

4. RESULTS AND DISCUSSION

4.1 Results

Helicobacter pylori (H. pylori) is a Gram-negative microaerophilic spiral bacterium, it cause gastric disease in the form of chronic gastritis, peptic ulcer in the lining of human stomach or the upper part of small intestine, mucosa associated lymphoma, and develop carcinoma, and cause multiple sclerosis.

In this study 40 patients were used in the experiment, 30 patients infected with *H. pylori* infection were used and 10 for control, in this study we collect the blood group, IFN-beta, and IL12 by Enzyme-linked Immuno Sorbent Assay (ELISA) technique.

4.1.1 Total patients

For the 40 outpaients, patients with *H. pylori* infection had serum concentrations of IL12 that were high (162.314 ±14.782) and significantly higher (p-value 0.0085) than healthy (controls) (39.385 ± 6.819) as shown in Figure 4.1, and there were significant increase (p-value 0.788) Figure 4.2 in serum concentration of IFN-beta in patients infected with *helicobacter pylori* (168.360 ± 28.693) compared with healthy individuals (control) (50.640 ± 3.779).

Increase in IL12,IFN-beta in patients infected with *H.pylori* than the healthy patients shown us that the immunity system in infected patients was resist the pathogen. The aim of this study demonstrated the effect of age on IFN-beta, IL12 of the immunity system in patients infected with *H. pylori* as demonstrated in Figure4.1 and Figure 4.2.

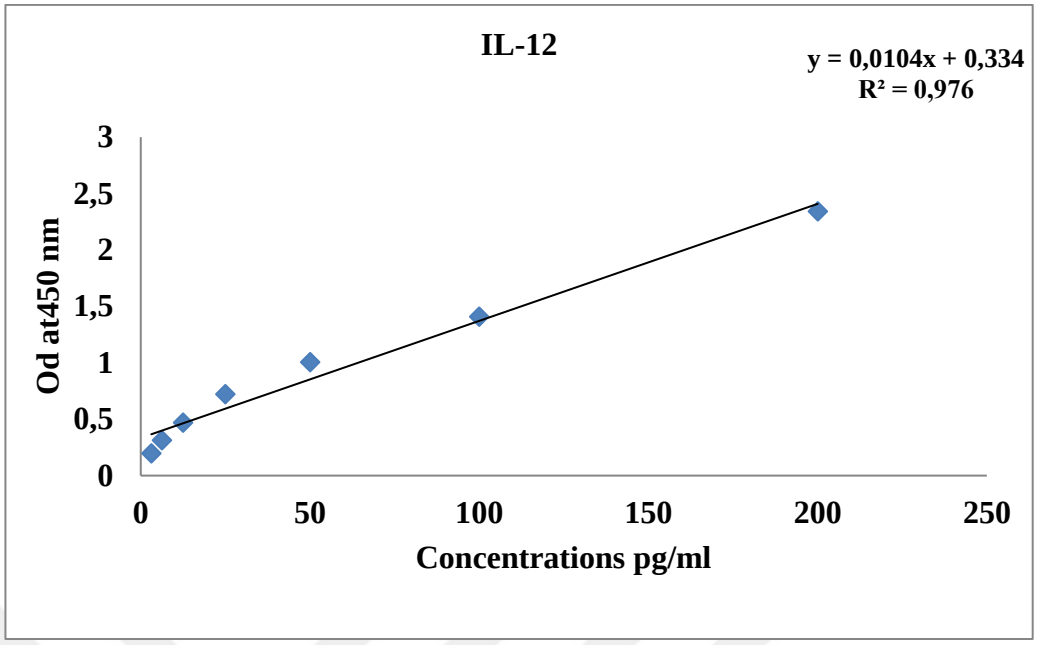

Figure 4.1 IL12 Serum concentration of patients

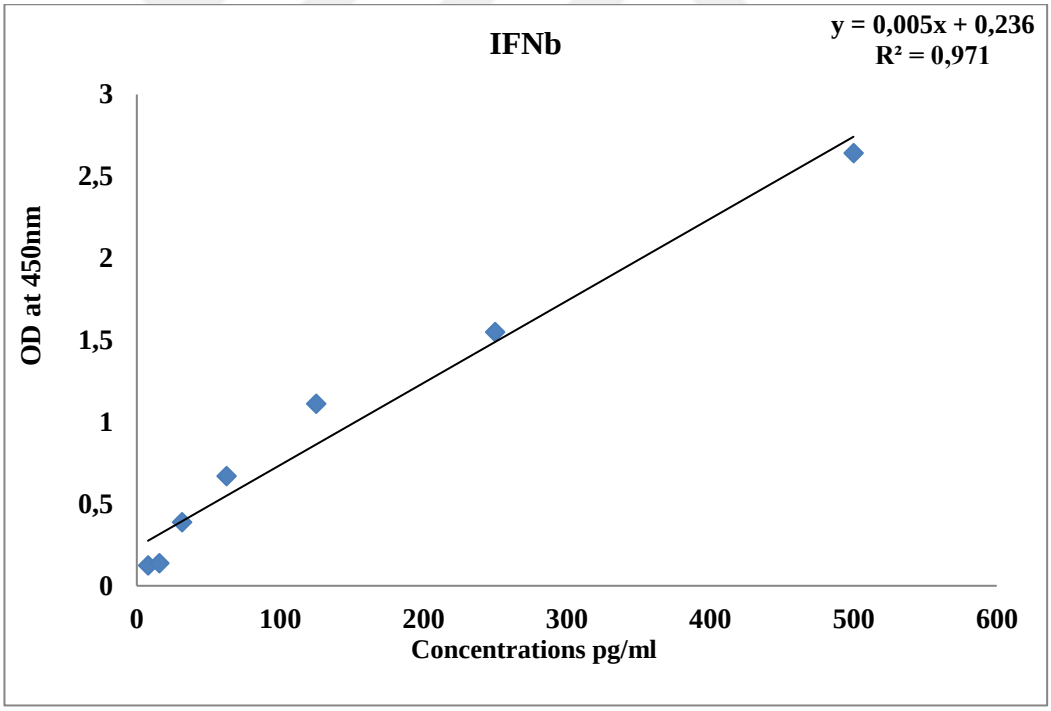

Figure 4.2 IFN-beta serum concentration of patients

4.1.2 Interleukin12

According to the age range there was increase in(mean +SD)in patients infected with *H.pylori* between 15-20 (10 samples) (155.13+11.19) with significant increase (P-value 0.00012) compared with the control 15-20(3 samples) (33.33+2.84).

Also, an increase in increase in(mean +SD)in patients infected with *H.pylori* between 21-26 (10samples)(169.67+15.29)with significant increase (P-value 0.0002) compared with control 21-26 (3 samples) (42.31+5.74), in older age from 27 > there were was increase in(mean +SD) patients infected with *H.pylori* (27)>(10samples) (162.14+15.08) with significant increase (P-value 0.00005)as compared with control27>(4samples) (41.54+8.53) (Table 4.1 and Table 4.2).

Table 4.1 The mean, SD, and P-value of IL12 cytokine in patients infected with *H. pylori*

Groups / **Age**	**Patients (mean+SD)**
15-20 (10 samples)	168.3 + 20.94
21-26 (10 samples)	163.8 + 34.1
27 > (10 samples)	173 + 31.7
P-value	0.788

Table 4.2 The mean, SD, and P-value of IL12 cytokine for control (healthy individuals - 10 samples)

Groups / Age	Control (mean+SD)
15 – 20 (3samples)	48.73 + 3.93
21-26 (3 samples)	50.3 + 4.48
27 > (4 samples)	53 + 2.16
P-value	0.442

4.1.3 Interferon-beta

According to the age range there was increase in (mean +SD) in patients infected with *H. pylori* between 15-20 (10 samples) (168.3+20.94) with significant increase (P-value 0.00011) compared with the control 15-20(3 samples) (48.73+3.93).

Also, an increase in (mean+SD) in patients infected with *H.pylori* between 21-26 (10samples)(163.8+34.1) with significant increase (P-value 0.00007) compared with control 21-26 (3samples) (50.3+4.48), in older age 27 > there were an increase in(mean +SD)in patients infected with *H.pylori* (27>)(10samples) (173+31.7) with significant increase (P-value 0.00016) as compared with control27> (4samples) (53+2.16) (Table 4.3 and Table 4.4).

Table 4.3 The mean, SD, and P-value of IFN-beta cytokine in patients infected with *H. pylori*

Groups / Age	Patients(mean+SD)
15-20	155.13+11.19
21-26	169.67+15.29
27>	162.14+15.08
P-value	0.0085

Table 4.4 The mean,SD, and P-value of IFN-beta in control (healthy individuals-10 samples)

Groups / Age	Control(mean+SD)
15-20 (3samples)	33.33+2.84
21-26 (3samples)	A (42.31+5.74)
27> (3 samples)	41.54+8.53
P-value	0.19

For total patients p-value of infected and control patients IL12 was (0.00011) for the age group between (15-20), in group between (21-26) p-value was (00007) of patients infected with*H. pylori* and control,and group 27> of patients with helicobacter pylori and control with p-value (0.00016) as shown in Table 4.5.

Table 4.5 The mean,SD, and P-value of samples and the parameters of IL-12 cytokine in patients infected with*H. pylori* and control (healthy individuals)

Groups / Age	Patients (mean+SD)	Control (mean+SD)	P-value
15-20	168.3+20.94	48.73+3.93	0.00011
21-26	163.8+34.1	50.3+4.48	0.00007
27>	173+31.7	53+2.16	0.00016
P-value	0.788	0.442	

For total patients p-value of infected and control patients IFN-beta was (0.00012) for the age group between (15-20), in group between (21-26) p-value was (0.0002) of patients infected with *H. pylori* and control, and group 27> of patients infected with *H. pylori* and control with p-value (0.00005) as shown in Table 4.6.

Table 4.6 The mean,SD, and P-value of IFN-beta cytokine in patients infected with *H. pylori* and control (healthy individuals)

Groups / Age	Patients(mean+SD)	Control(mean+SD)	P-value
15-20	155.13+11.19	33.33+2.84	0.00012
21-26	169.67+15.29	A (42.31+5.74)	0.0002
27<	162.14+15.08	41.54+8.53	0.00005
P-value	0.0085	0.19	0.788

4.2 Discussion

It is believed that *H. pylori*, a *gram-negative* bacteria that it is the reason behind one of the most prevalent chronic bacterial illnesses in humans. Although it is known that around half of the world's population is infected with *H. pylori*, infection rates vary from country to country, low socio-ecological condition of the community has always been linked to a high incidence of *H. pylori* (Pacifico *et al.*2010).

H. pylori is much more or less common in certain nations, the incidence varies significantly across various socioeconomic categories. In the Czech Republic, there were 2509 patients between the ages of 5 and 100 who had *H. pylori* 41.7% of the time. *H. pylori* prevalence was not sex-related, although it was correlated with older age and worse socioeconomic status (Burešet *al.* 2006).

In this study, the results of serum concentration were compared between thirty patients with *H. pylori* infection and ten healthy people as a control, with two parameters, IL12 and IFN-beta, with age range factor because it is the highest risk factor.Over the previous 20 years, several Western writers have demonstrated that patients over the age of 65 nearly exclusively receive the initial diagnosis of peptic ulcer disease and are admitted to the hospital due to its consequences, there have been several studies referring to risk factors of *H. pylori* infection (Sonnenberg 1987, Til Vis*et al.*1987).

As a result, unlike in the 1940s and 1950s, when it was more common among young people, peptic ulcer disease now affects men and women who are of old age indicated

that the baseline and stimulated stomach hydrogen ion discharges did not diminish when individuals with atrophic gastritis and *H. pylori* infection were excluded (Katelaris *et al.*1993,Goldschmiedt*et al.*1991).

After correcting for the effects of atrophic gastritis and smoking, a more thorough perspective study in 22 adults above the age of 65 indicated that there was obviously no significant age effect on gastric acid output in any age group (Feldman *et al.*1996). Although serum gastrin levels were similar, in patients with atrophic gastritis, they were higher. However, pepsin production was dramatically reduced in the older patients.

In the table1 of the current study there were 30 patients with 3 age groups, between (15-20) and between (21-26) and (27>) were different in serum concentration of interleukin-12 as appears in Table4.1, there was significant increase (p-value 0.0085) serum concentration were high level as compared with the control(healthy individuals) (p-value 0.19), The level of interleukin 12 factor was significantly elevated in *H. pylori* positive group than in control group of patients. This rise in results indicates the role of IL12 in the immunity system because IL12 help to differentiate naive cells (which is one of the most important wights blood cell) into T helper cells in patients infected *H. Pylori*.

In general, T helper cells are thought to be crucial for the B cell's ability to alter antibody classes, for breaking cross-tolerance in dendritic cells, for activating and growing cytotoxic T cells, and for increasing the bactericidal activity of phagocytes like neutrophils and macrophages.

In IL12 age group between (15-20) (155.13+11.19) (p-value 0.00012) shown that there was a decrease rate of interleukin 12 as compared the age group between (21-26) (169.67+15.29), that given an evidence that interlukin 12 of group (21-26) patients is high level,and in group (21-26) as compeared with group (27>) shown that it was the highest which give us a prove that dendritic cells, macropheges are more activated which mean interleukin12 is high level.

The current Najaf investigation for samples from both sexes reveals that the cellular and humoral immune responses jointly play a crucial role in patients with *H. pylori* (Hamzahand Aljanaby2020), this concurs with our work, which shown that the immune response plays a significant role in patients with *H. pylori* infection, taking into account additional characteristics like the patients' age or smoking habits.

A variety of factors can influence how a bacterium becomes infected, according to Oppmann et al. (2000) discovery that cytokines do not function as IL12's role, rather than the presence of memory effectors in naive T cells, ıt was discovered that the stimulation of naive T cells' TCR was sufficient to create IL-12R2 but not IL-23R in IL23, which serves to both stimulate and activate T lymphocytes (Oppmann *et al.*2000).

A study also demonstrates that which states that reduction of the CD4+ T cell population in the stomach mucosa will limit *H. pylori* persistence (Bagheri *et al.* 2019). This idea suggests that maintaining *H. pylori* infection requires an intact mucosal cell immunity. Therefore, even if there is no proof that poor T cell immunity may lead to a loss of *H. pylori* infection, the concurrent decline in *H. pylori* prevalence with CD4+ T cells is quite similar to the premise of this theory. As antigen-presentation cells of the mammalian immune system, dendritic cells (DCs) digest antigen material and present it to T cells on the cell surface, acting as messengers between the innate and adaptive immune systems.

Group between (21-26) was the highest group of serum concentration level. Therefore, the highest IL12 counts among the patients infected with *H. pylori* suggest the increase in the immune system mediated by T cells (Abaturovand Gerasymenko2014).

Endothelial, epithelial, and immune cells recognize pathogen-associated molecular patterns using their pattern-recognition receptors (PRRs), which include TLR, when the microbial infection occurs (PAMPs). IFN-beta, a cytokine critical for stimulating and regulating innate and adaptive immune responses in the immune system, is promoted by this interaction (Raymond*et al.*2017, Ivashkivand Donlin2014).

The interferon α/β receptor (IFNAR) triggers nearly 300 interferon-stimulated genes (ISGs). These ISGs specifically block essential stages of the viral life cycle (Mahieu and Libert2007), trigger innate immune cells to become active, encourage host cell death, and advance the maturation of the adaptive immunological response (Yan and Chen2012). There was a difference in the rate of IFN-beta in patients with *H. pylori* infection compared to healthy individuals (controls), and this rise indicated that IFN-beta plays a role in the immune system in these patients.

In the 3 groups of patients first between (15-20), between (21-26), and between (27>) shown there was a deference in the IFN-beta of all the groups,these belong to many risk factor, group (15-20) (168.3+20.94) was high level as compared with group (21-26) (163.8+34.1) that's show us a proved that the IFN-beta more activated in ages between (15-20) than the (21-26) group which mean IFN-beta is high level in (15-20) age group.

Group (27>) (173+31.7) was the highest level rate of serum concentration as compared with the other groups, this difference in rate in interferon-beta compared with interleukin-12 belong to many risk factors. It's important to keep in mind that some arguments against the link between smoking and *H. pylori* infection contend that smoking's enhanced acid and pepsin output can shield the stomach mucosa from *H. pylori* infection, this depends on the patient's character, such as whether they were smokers or anemic, and several problems may occur in the immune system. When compared to 2001, Norway's daily smoker population fell to half in 2011. Consequently, in just the past ten years, this risk factor has significantly diminished. Smoking habits are declining across the board from 16 to 64 years old, but they are most prominent in the 16 to 44 age range (Al-Hussaini *et al.*2019).

Patients who are elderly or young will exhibit different clinical signs of the peptic ulcer illness. Elderly people may not experience the normal, nonspecific, and atypical symptoms of peptic illness. Older people frequently experience complications from other illnesses like diabetes and disabilities like the neurological cerebrovascular disease accidents' aftereffects, which could change or diminish the symptoms of peptic illnesses. In older patients, there are changes in the clinical picture. The signs of

chemical peritonitis brought on by the action of gastric contents may be low or less noticeable in older patients due to such coexisting disease if the patient had surgery or was taking current medications, such as anti-inflammatory drugs or corticosteroids. An example of these differences is shown with acute gastroduodenal perforation (Til Vis*et al.* 1987).

According to some research, males are more likely to have *H. pylori* infections than females, which may be because they are exposed to more environmental sources of infection found that there was a male majority of *H. pylori* infection in elderly individuals but not in children. Gender differences were identified in a French study, where men had higher infection rates than women, despite the EUROGAST study group being unable to identify a connection between sex and *H. pylori* infection (Forman*et al.*1993, De Marteland Parsonnet2006).

However, other studies have not discovered a connection between sex and *H. pylori* infection (Feldman *et al.*1996), Although Al-Hashemi *et al.*reported that patients with peptic ulcer disease were more likely to have *H. pylori* infection than those with non-ulcer dyspepsia a study by (Lindo *et al.*1999) demonstrated the importance of age range in patients with helicobacter pylori infection, This is depicted in Figure 4.1 (Lindo *et al.*1999).

This is agree with our current study which explains the role of age range factor as the main risk factor of *H.pylori* infection.The age range of the *H. pylori*infected patients in this study might be regarded as one of the most significant risk factors. A study at (the Dnipropetrovsk Medical Academy of the Ministry of Healthcare of Ukraine) included 224 (79.1%) patients with *H. pylori*positive status, with 59 (20.9 %) patients in the control group who were not *H. pylori* positive during a breath test and study for the presence of specific immunoglobulin (*H.pylori* negativestatus), the study's findings demonstrated that there were no discernible gender differences between the study and control groups, indicating that helicobacter infection does not depend on gender as a risk factor in patients.This is agree with our study, at the same time, according to the study, there is a higher chance of contracting CagA(+) strains of *H. pylori* and

developing an infection when a person is 12 to 14 years old (RR = 3.25). This concurs with our study's definition of the age range factor as the primary risk factor in *H. pylori* infection patients. Across the Eurogast study, which included more than 3000 asymptomatic participants from 17 people in Europe, the seroprevalence of *H. pylori* infection was 62.4% in the older age group and 34.9% in the young age group. There was no association between gender and infection (Forman*et al.* 1993).

This is matching our study that explained the age range factor has been demonstrated to be the main risk factor in patients infected with *H. pylori*, and this is consistent with our study, which found that gender was not a significant risk factor for *H. pylori* infection in patients. A study of the prevalence of *H. pylori* in children and adults suggests a different cause, reported prevalence rates in children (C) and adults (A): Jamaica (27%C/70%A), Barbados (22%C/72%A) and Bahamas (54%C/58%A)(Edwards *et al.* 1997).

In 309 asymptomatic patients aged 1-82 years, there was a study conducted in Turkey that revealed a 70% prevalence of *H. pylori* infection and a link between the infection and poor socioeconomic position. Gender was unrelated to positivity. There were infection rates of 42% in those under 10, 55% in those between 10 and 19, 66% in those between 20 and 29, 78% in those between 30 and 39, 79% in those between 40 and 49, 91% in those between 50 and 59, 100% in those between 60 and 69, and 80% in those over 70 (Abasiyanik *et al.* 2004).

They stated that 89% of those infected with 89% of those in low socioeconomic class were found to have the virus, compared to 70% of those in middle status and 41% of those in high status*H. pylori* seroprevalence in Burkina Faso participants varied from 86 to 100% for individuals aged 0.5 to 15 years, and from 40 to 58% for adults aged 16 to 65 years (Cataldo *et al.* 2004).

There is disagreement over whether gender affects the likelihood of *H. pylori* infection, although a study did suggest that the physiological differences between males and

females may have an impact on the immune system's mechanism of defense against the bacterium's pathogenesis (Ibrahim *et al.* 2017).

5. CONCLUSIONS AND RECOMMENDATION

Helicobacter pylori is a disease of advanced age because older individuals tend to have it more often than younger ones do. Advanced age is considered to be the main risk factor, and age range plays an important role in patients with *H. pylori*, and the cytokines IL12 and IFN-beta play a significant role in the immune response of those patients.

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