TURKISH REPUBLIC OF NORTH CYPRUS

NEAR EAST UNIVERSITY

HEALTH SCIENCE INSTITUTE

EVALUATION OF THE CORRELATION BETWEEN STOOL ANTIGEN TEST AND HISTOPATHOLOGY REPORT RESULTS OF HELICOBACTER PYLORI PRESENCE IN NEAR EAST UNIVERSITY HOSPITAL APPLICANTS

LUMA HUSNI ALZUBI

MEDICAL MICROBIOLOGY AND CLINICAL MICROBIOLOGY PROGRAM

MASTER OF SCIENCE THESIS

SUPERVISOR

EŞREF ÇELİK MD ASSISTANT PROFESSOR

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APPROVAL

The Directorate of Health Sciences Institute, / INSTITUTE OF GRADUATE STUDIES

This study has been accepted by the Thesis Committee in Medical and Clinical Microbiology Program as a Master of Science Thesis.

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DECLARATION

Hereby declare that the work in this thesis I had no unethical behaviour in all stages from planning of the thesis until writing thereof, I obtained all the information in this thesis in academic and ethical rules, I provided reference to all of the information and comments which could not be obtained by this thesis study and took these references into the reference list and had no behaviour of breeching patent rights and copyright infringement during the study and writing of this thesis.

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I would like to express my special appreciation to MY FATHER AND MY MOTHER.

ÖZET

LUMA ALZUBI. Yakın Doğu Üniversitesi Hastanesi'ne başvuran kişilerde *Helicobacter pylori* varlığının dışkı antijen testi ve histopatoloji raporu sonuçları arasındaki ilişkinin değerlendirilmesi. Yakın Doğu Üniversitesi, Lisansüstü Eğitim Enstitüsü, Tıbbi Mikrobiyoloji ve Klinik Mikrobiyoloji Programı, Yüksek Lisans Tezi, Lefkoşa, 2021

Helicobacter pylori (H. pylori) dünya çapında nüfusun yaklaşık yarısını enfekte etmekte etmektedir ve %40-90 'lık prevalans ile gelişmekte olan ülkelerde en yaygın enfeksiyon nedenleri arasında yer almaktadır. *H. pylori* enfeksiyonunun tanısında çeşitli yöntemler kullanılmaktadır. Bakterilerin teşhisinde altın standart yöntem konusunda fikir birliği yoktur. Çalışmamızda *H. pylori* tanısında kullanılan yöntemlerden biri olan gaita antijen hızlı testi ile mide biyopsisine ilişkin histopatolojik raporlardan elde edilen *H. pylori* pozitif tanısı alan hastalar arasındaki korelasyonu belirlemeyi amaçladık. Bu çalışma, 1 Ocak 2017-31 Aralık 2019 tarihleri arasında Yakın Doğu Üniversitesi Hastanesi'ne dispeptik şikayetlerle başvuran ve mikrobiyoloji laboratuvarında *H. pylori* antijen testi ile incelenen 1227 hastanın dahil edildiği retrospektif bir çalışmadır. Hastaların yaşı, cinsiyeti, şikayetleri (hastaneye yatış nedenleri) ve tanıları insan dışkı örneklerinde ve histopatoloji raporlarında geriye dönük olarak elde edildi. Gaitada *H. pylori* antijen testi incelenen 1227 hastanın 67'sinin (%5,5) *H. pylori* pozitif tanısı aldığı belirlendi. Hastaların 344'üne (%28) biyopsi yapıldığı ve histopatoloji raporlarına göre biyopsi yapılan hastaların 84'ünde (%24,4) *H. pylori* pozitif bulunduğu saptanmıştır. 84 hastanın histopatoloji raporunda *H. pylori* pozitif olmasına karşın, gaita örneklerinde 17'sinin negatif tanı aldığı belirlenmiştir. *H. pylori* antijen testinin gaita örneklerinde duyarlılığı %79,7 ve özgüllüğü %100 idi. Sonuçların geriye dönük olarak değerlendirildiği çalışmamızda, *H. pylori* gaita antijen testinin invaziv, ucuz, kolay uygulanabilir olması ve histopatoloji raporlarına yakın pozitif sonuçlar vermesi nedeniyle *H. pylori* enfeksiyonunun tespitinde kullanılabileceği sonucuna varılmıştır.

Anahtar kelimeler: *Helicobacter pylori*, gaitada antijen testi, histopatolojik raporlar

ABSTRACT

LUMA ALZUBI. Evaluation of the correlation between faeces antigen test and histopathology report results of *Helicobacter pylori* presence in Near East University Hospital applicants. Near East University, Institute of Graduate Studies, Medical Microbiology and Clinical Microbiology Program, M.Sc. Thesis, Nicosia, 2021.

Helicobacter pylori (*H. pylori*) infects approximately half of the population worldwide and is among the most common causes of infection in developing countries with a prevalence of 40-90%. Various methods are used in the diagnosis of *H. pylori* infection. There is no consensus on the gold standard method in the diagnosis of bacteria. In our study, we aimed to investigate the correlation between the faeces antigen rapid test, which is one of the methods used in the diagnosis of *H. pylori*, and those with a positive diagnosis of *H. pylori* obtained from histopathological examination reports on stomach biopsy specimens. This study is a retrospective study that included 1227 patients who applied to the Near East University Hospital with dyspeptic complaints between January 1, 2017 and December 31, 2019 and were examined with a *H. pylori* antigen rapid test in the microbiology laboratory. The patients' age, gender, complaints (reasons for admission to hospital) and diagnosis were obtained by retrospective examination of *H. pylori* antigen test in human faecal specimens results and histopathology reports. It was determined that 67 (5.5%) of 1227 patients whose stool *H. pylori* antigen rapid test were examined were *H. pylori* positive. Biopsy was performed in 344 (28%) of the patients, and according to histopathology reports, 84 (24.4%) of the patients who underwent biopsy were found to be *H. pylori* positive. Although *H. pylori* was positive in the histopathology report of 84 patients, it was determined that 17 of them were negative in faecal specimens. The sensitivity of *H. pylori* antigen rapid test in faecal specimens was 79.7% and the specificity was 100%. In our study, in which the results were evaluated retrospectively, it was concluded that the *H. pylori* stool Ag test may use in the detection of *H. pylori* and infection because it was noninvasive, cheap, easily applicable and gave positive results close to histopathological results.

Keywords: *Helicobacter pylori*, stool antigen test, histopathological reports.

TABLE OF CONTENTS

ABBREVIATIONS

Ag: Antigen

C_{13}: Carbon-13

C_{14}: Carbon-14

CO_2: Carbon dioxide

DNA: Deoxyribonucleic Acid

FDA: Food and Drug Administration

H. Pylori: Helicobacter pylori

H^+: Proton

H_2: Histamine

HCO_3: Bicarbonate

HpSA (-): *Helicobacter pylori* stool antigen negative

HpSA (+): *Helicobacter pylori* stool antigen positive

HpSA: *Helicobacter pylori* stool antigen

IgG: Immunoglobulin G

MALT: mucosa-associated lymphoid tissue

MALToma: mucosa-associated lymphoid tissue lymphoma

NH_3: Ammonia

NH_4^+: ammonium

NIH: National Institutes of Health

NPV: Negative predictive value

OR: Odds ratio

PCR: Polymerase chain reaction.

pH: Power of Hydrogen

PPI: Proton pump inhibitor

PPV: Positive predictive value

Spp: Species

WGO: World Gastroenterology Organisation

WHO: World Health Organization

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CHAPTER ONE

1. INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram (-) bacterium that specifically colonizes the stomach. *H. pylori* infects approximately half of the population worldwide and is among the most common causes of infection in developing countries with a prevalence of 40-90% (WGO, 2011). *H. pylori* infection can lead to peptic ulcer diseases, intestinal metaplasia, dysplasia, or chronic gastritis that can turn into adenocarcinoma (Kotilea et al., 2019). *H. pylori* accounts for approximately 95% and 70% of duodenal ulcers and stomach ulcers, respectively (Rothenbacher et al., 2003). Stomach cancer and mucosa-associated lymphoid tissue (MALT), which are among the most common cancer types of *H. pylori* worldwide, are considered among the important risk factors in the development of lymphoma (Lehours and Yılmaz, 2007; Rothenbacher et al., 2003). The insufficient immune response caused by *H. pylori* infection to destroy the bacteria causes the infection to become chronic and to be effective throughout the life of the individual unless antibiotic treatment is administered.

Various methods (non- and invasive) are used in the diagnosis of *H. pylori* infection. There is no consensus on the gold standard method in the diagnosis of bacteria. Therefore, to use more than one diagnostic method to increase the accuracy of the diagnosis. Accurate diagnosis is very important in terms of both preventing unnecessary antibiotic use and starting treatment immediately (Owen, 2005). When the bacteria are eradicated with appropriate treatment, the ulcer heals and the risk of recurrence of the infection significantly decreases (5, 6). Although there is a temporary symptomatic improvement after treatment with H^+ pump inhibitor or H_2-receptor-antagonists, since *H. pylori* is not eradicated, the risk of recurrence of symptoms and ulcer is quite high.

1.1. Aim of the Research

In our study, we aimed to investigate the correlation between the stool/faeces antigen one-step test, which is one of the methods used in the diagnosis of helicobacter pylori, and those with a positive diagnosis of helicobacter pylori obtained from histopathological examination reports on stomach biopsy specimens.

CHAPTER TWO

2. GENERAL INFORMATION

2.1. History of *H. pylori*

In 1979, pathologist Robin Warren, in his studies of stomach biopsy specimens, noticed bacteria usually located in the mucus lining of the stomach. After the gastroenterologist Barry Marshal was also interested in Warren's observations, the two researchers began studies to isolate the organism from stomach biopsy samples. Because these organisms are curved and gram (-), the researchers tried the isolation methods used for Campylobacter species in collaboration with researchers working in the bacteriology field. Many Campylobacter species were able to grow in 48 h under these environmental conditions, and when no growth was observed, plaques were discarded within 3 days. In this way, cultures from about 30 patients ended up negative. But luckily, a culture incubated for 5 days due to the Easter holiday, and colonies were found at the end of the holiday. Thus, for the first time, the organism we now call *Helicobacter pylori* was isolated on 14.05.1982 (Marshall and Warren, 1984). After the publication of this invention, many researchers around the world quickly confirmed the presence of this bacterium in gastric mucus, thus laying the foundation for studies on *H. pylori.*

Marshall (1984), after isolating *H. pylori*, decided to try the bacteria on himself and, after observing that the gastric mucosa was healthy by endoscopy, he drank the suspension prepared from the bacterial culture produced from the lesion of the patient with peptic ulcer. Acute gastric symptoms began to appear in Marshall a week later. Two weeks later, folded bacteria settled under the gastric mucus layer and inflammation were detected at the end of the endoscopic examination in the biopsy sample taken from the stomach. As a result of Marshall's experiments, the presence and effect of *H. pylori* on the etiology of stomach diseases has been proven (Hopkins and Morris 1994). This bacterium was first named Campylobacter pyloridis by Marshall in 1984 and was later included in a new genus named 'Helicobacter' and named Helicobacter pylori in 1989 (Goodwin et al., 1989). Finally, in 2005 they won the NOBEL prize in medicine for their discovery.

2.2. Microbiological Characteristics of *H. pylori*

The *H. pylori* organism is a microaerophilic, gram (-) bacterium. When cultured in liquid medium, bacterial morphology is in the form of a rod. Spiral-shaped bacteria are sparse or absent. *H. pylori* in gastric biopsy specimens is 2.5-5 µm long, 0.5-1 µm wide, and has 6 unipolar sheathed flagella. Each flagellum is 30 µm in length and an average of 2.5 nm in thickness. Flagella has characteristic bulb-shaped swellings ('terminal bulb') at the tip, indicating the elongation of the flagella sheath (Figure 2.1.). Flagella sheath is like the typical double layer membrane structure. This sheath is an important structure that distinguishes Helicobacter genus from Campylobacter (Dunn et al. 1997).

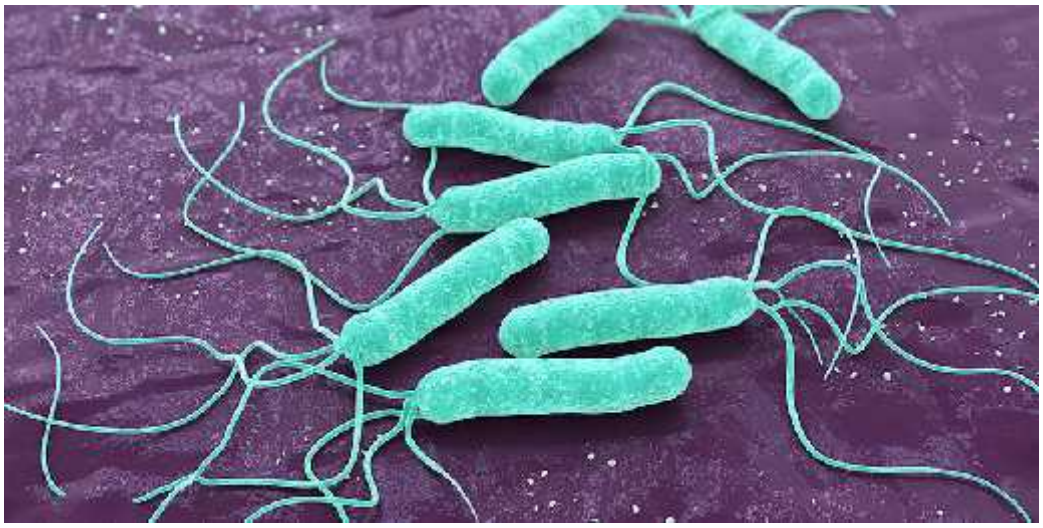

Figure 2.1. Image of *H. pylori* (https://badgut.org/information-centre/a-z-digestive-topics/nobel-prize-for-h-pylori-discovery/, Accession date: 2 March 2021).

Bacteria, solid or liquid seen in spiral form in stomach biopsy specimens. It appears as slightly curved, 'S' or 'U' shaped rods when produced in media. There are two morphological forms of *H. pylori*, spiral and coccoid (Figure 2.2 a and b)). Bacteria in spiral form turn into coccoid form due to reasons such as nutritional deficiency, increased oxygen amount, alkaline pH, high temperature, exposure to different antibiotics and long time in culture. When viewed with an electron microscope, the coccoid forms are U-shaped and the two arms are united with the

membrane structure. Coccoid forms are metabolically active but cannot be cultured in vitro. Some literature has indicated that 5-20 days coccoid form of the bacterium cannot be re-cultured in vitro (Brenciaglia et al., 2000). However, the coccoid form of the bacterium is considered to be alive as it contains membrane and polyphosphate energy sources. It is also thought that this form of the bacterium is responsible for the transmission of infection and recurrence of antimicrobial therapy (Brenciaglia et al., 2000). *H. pylori*, which is similar to the Campylobacter genus in terms of spiral morphology in stomach biopsy samples; It differs from Campylobacter bacteria in that it has more than one flagellum surrounded by a sheath on a single pole, its fatty acid profile is different and the cell wall surface is flat (Owen, 2005).

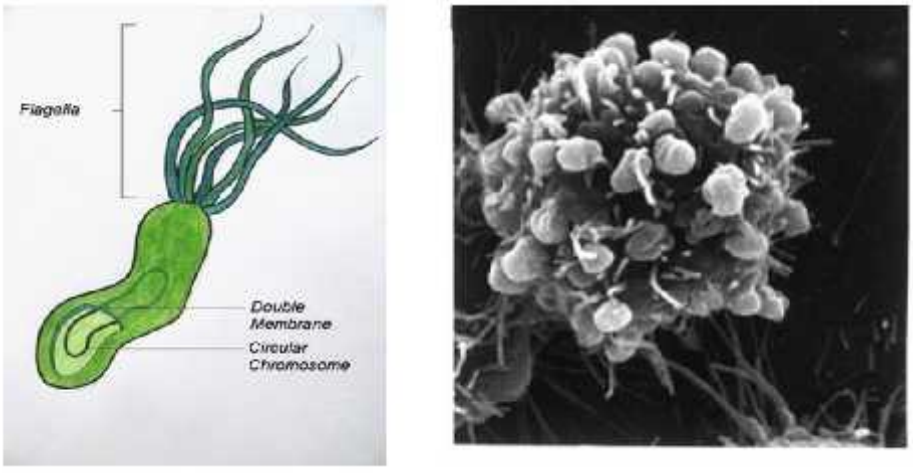

Figure 2.2. a) The spiral and b) coccoid morphology of *H. pylori*. (http://tolweb.org/treehouses/?treehouse_id=4722,https://www.microbiologyresearch.org/docserver/fulltext/jmm/51/4/mjm5104.295.pdf?expires=1612969412&id=id&accname=guest&checksum=1054C5F74569FC6001F526DF42E05F3E,Accession date: 2 March 2021).

Helicobacter pylori; It is an obligatory microaerophilic bacterium showing optimum growth in 3-7 day at 37 ° C and in a humid environment containing 5-10 % O_2 and CO_2, 80-90 % N_2. For its growth, it requires media containing various enriching substances such as blood, hemin, serum, starch or egg yolk, as in the Campylobacter genus. The addition of supplements such as coal or cyclodextrin,

which play a role in removing toxic substances from the medium, contributes to the optimum growth of *H. pylori* (Owen, 2005).

The cell wall of *H. pylori* consists of the inner membrane, peptidoglycan and lipopolysaccharide layers attached to the outer membrane, as in other gram (-) bacteria (Owen, 2005). The biological activity of the membrane of *H. pylori* is different from the biological activity of the membrane of other gram (-) bacteria. This difference is due to the modification in the lipopolysaccharide layer in the cell wall structure. This modification results in the low activity of the *H. pylori* cell wall. The non-high activity of the membrane is responsible for the weakness of the host immune response following infection and thus the escape of the bacteria from the host immune response (Owen, 2005; Muotiala et al., 1992).

The O-polysaccharide side chain of the *H. pylori* lipopolysaccharide is similar to the blood group antigens (Lewis x and y) expressed on the gastric epithelial surface. It is thought that this similarity may be responsible for the escape of the bacteria from the host immune response by hiding in the environment where *H. pylori* lives (Stabile et al., 2005). It is also stated has been reported that anti-Lewis antibodies formed following infection may react with the gastric mucosa and cause an autoimmune response (Appelmelk et al., 1996).

It is estimated that *H. pylori* can encode more than sixty outer-membrane proteins that act as surface antigen. It is thought that the blood group antigen-binding adhesin A and sialic acid–binding adhesin are responsible for binding to gastric epithelial cells. Porin proteins classified as HopA-E (*H. pylori* porins) are thought to play a role in the passive passage of small hydrophilic molecules and nutrients through the outer membrane (Owen, 2005).

2.3. Classification of *H. pylori*

Helicobacter spp. are seen in the gastrointestinal system of animals as well as humans. After the culture of *H. pylori*, the clinical importance of *H. pylori* was better understood. These organisms continue to be of interest for their pathological roles in humans and animals. It has been demonstrated that some newly identified

Helicobacter species also infect humans. The only known genus of Helicobacter family is "Helicobacter". Other bacteria in this genus show host tropism against different animal species. However, tropism is observed against gastrointestinal system tissues and organs in all relationships. Apart from the Twenty-three confirmed *Helicobacter* strains, there are also a few strains awaiting classification. (Table 1.1). Helicobacter genus bacteria can be examined under 2 large groups as gastric and enterohepatic Helicobacter. Among the gastric Helicobacter colonized in the gastric mucosa, *H. pylori* and *H. heilmanii* are the most important infectious species in humans. Among the enterohepatic helicobacter colonized in the intestine and liver, the most important species for human health are *H. cinaedi* and H. *fennelliae* (Owen 2005; Winn et al., 2006).

Table 2.1. Helicobacter spp.
(https://www.ncbi.nlm.nih.gov/books/NBK304349/#!po=1.64835)

Species	
H. pylori	*H. mustelae*
H. acinonychis	*H. nemestrinae*
H. aurati	*H. pametensis*
H. bilis	*H. pullorum*
H. bizzozeronii	*H. rodentium*
H. Canadensis	*H. salomonis*
H. canis	*H. suis*
H. cholecystus	*H. trogontum*
H. cinaedi	*H. typhonicus*
H. felis	*Candidatus H. bovis*
H. fennelliae	*Candidatus H. suis*
H. ganmani	*H. hepaticus*
H. heilmanii	*H. mesocricetorum*
H. macacae	*H. muridarum*

2.4. Localization of *H. pylori*

The natural location of *H. pylori* is the surface of the gastric mucosa. Bacterium; It is localized within the mucus layer covering the gastric mucosa or between the surface epithelium and the mucus layer, where neutral pH prevails. *H. pylori* can colonize any part of the stomach. However, colonization mostly occurs in the antrum epithelium where gastric acidity is relatively low. *H. pylori* can also be colonized in areas with metaplasia in the duodenum (Owen, 2005). In adults, 30% of the proximal duodenum has gastric epithelium to protect against acid secretion to the area. In all these settlements, bacteria are found in clusters.

H. Pylori's tolerance to the acidic pH of the stomach is provided as follows (Figure 2.3.):

1. Urease: Urea $\rightarrow CO_2 + NH_3$, then $NH_3 + H^+ \rightarrow NH_4^+$ acts as a buffer
2. Located in mucus and epithelial cells
3. It has the capacity to create ionic gradients at low pH
4. During early infection, it releases parietal cells that reduce acid release

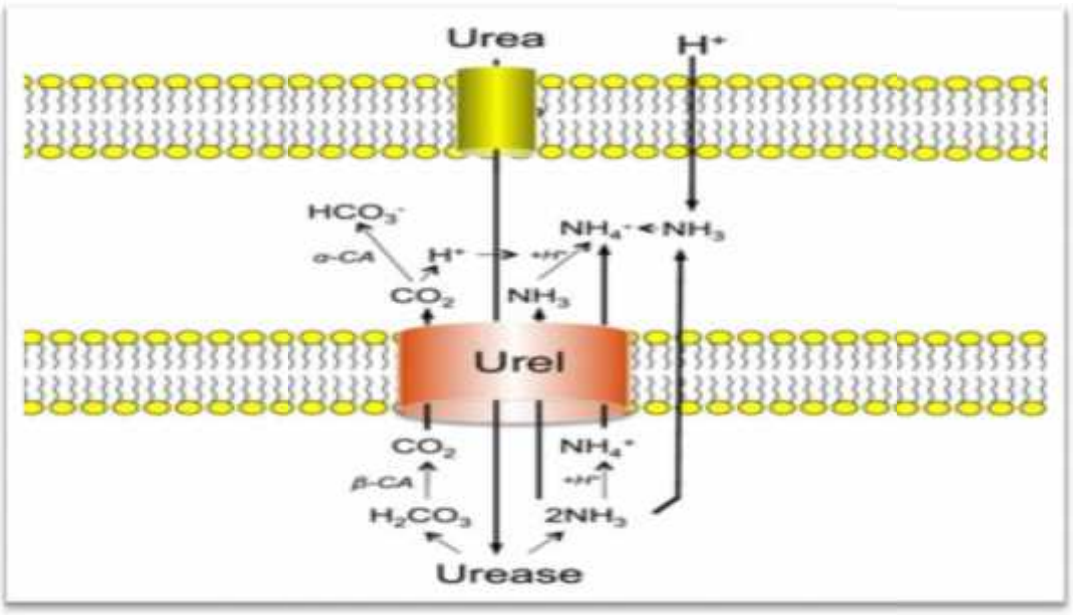

Figure 2.3. *H. Pylori*'s tolerance to the acidic pH of the stomach (https://www.researchgate.net/publication/305452470_The_role_of_acid_inhibition_in_Helicobacter_pylori_eradication/figures?lo=1, Accession date: 2 March 2021).

Helicobacter pylori locates in the human stomach (Figure 2.4.).

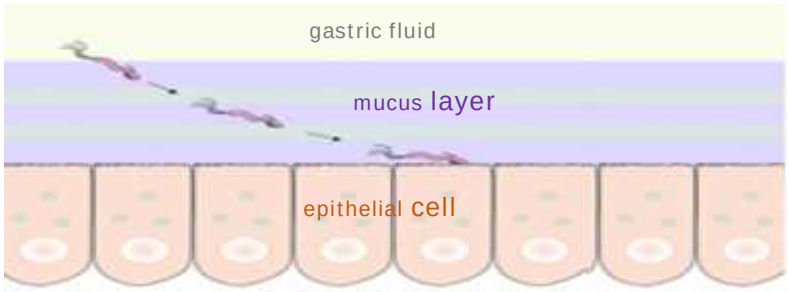

Figure 2.4. Localization of *H. pylori* in human stomach (https://web.stanford.edu/group/parasites/ParaSites2006/Helicobacter/Introduction.html, Accession date: 2 March 2021).

2.5. Epidemiology of *H. pylori*

With a study involving different participants from 62 countries in 2017, the researchers announced that they estimated that one out of every two individuals living in the world was infected with *H. pylori* (Hooi et al., 2017). These results also reveal the prevalence differences between countries. The prevalence of *H. pylori* was reported from highest to lowest as follows: Africa (79.1%), Asia (54.7%), Oceania (24.4%), Latin (63.4%) and North America (37.1%), and one of the striking results of this study is that the prevalence of the disease is increased in newly developing countries compared to industrialized countries. These differences in the prevalence of *H. pylori*-related diseases also reflect differences in urbanization, sanitation, poor hygiene conditions, access to clean water, and socioeconomic status. The study of healthy children under the age of 5 concluded that infection rates are between 20-40% in high-income and 30-50% in upper-middle-income countries. As determined as a result of this study, high infection rates are observed predominantly in low- or low- to middle-income regions or countries. There are studies in the literature reporting significant differences in the prevalence of *H. pylori* even within the same

country. For example, in the USA, the prevalence of *H. pylori* for non-Hispanic whites is between 18.4% -26.2%, while in non-Hispanic whites this rate ranges from 34.5% to 61.6% (Cardenas et al., 2006). However, considering the prevalence of *H. pylori* in developed countries, it is seen that this rate is decreasing in adults, including children (Kotilea et al., 2019). When the cultural results of nearly 9000 children between 2000-2013 in Poland were evaluated, the lowest prevalence of *H. pylori* infection was determined in 2010 (8.9%) (Biernat et al., 2016). In a study of 205 children aged 7-17 years in Iceland, the infection rate was found to be only 3.4% (Asgeirsdottir et al., 2017).

2.5.1. Source and predisposing factors of *H. pylori* infection

Studies have reported that *H. pylori* is obtained through potential sources of infection such as animals and water. Water and animals as potential sources of *H. pylori*: There are many studies to detect *H. pylori* in water (Park et al., 2001; Horiuchi et al., 2001; Eusebi et al., 2014). In three separate studies, *H. pylori* DNA has been shown in one or more water sources using various methods. These; i) well water in Japan (Horiuchi et al. 2001), ii) drinking water from the municipal water distribution system in Scotland (Park et al. 2001), iii) 3 different water distribution systems surrounding Mexico City (Mazari-Hiriart et al., 2001). However, in one study, *H. pylori* culture could be made from water. In this study, *H. pylori* was isolated from the treated wastewater channel in Mexico after long processes. In this study, 37 different *H. pylori* isolates were identified as a result of microscopic examinations, catalase, oxidase and rapid urease tests.

There are many studies showing the relationship between close contact with animals and recovery of infection (Bazolli et al. 2001, Brown et al. 2001, Herbarth et al. 2001). The possibility of *H. pylori* being a zoonosis emerged with two studies reporting a higher prevalence of *H. pylori* infection in meat workers compared to people/subjects who had no involvement in handling animals or animal products in working conditions (Morris et al., 1986; Vaira et al., 1988). On top of this, studies to design the possibility of *H. pylori* being zoonotic have concluded that "the cycle of

H. pylori infection may involve the environment, animals and humans in certain circumstances" and may even be ancestral hosts for these animals (Dore et al., 1999).

Brown et al. (2001) in a large population (n = 3288) study conducted in China, it was observed that feeding pets or working with animals did not increase infection. In the research conducted by Bazzolli et al. (2001), it was shown that exposure to domestic animals had no effect on *H. pylori* infection, so no relationship was found between the two Seroepidemiological studies attempting to reveal the relationship between *H. pylori* prevalence and pet owners generally did not report a positive correlation (Sathar et al., 1997; Webb et al., 1996). However, an increased risk of infection was observed in a study conducted with domesticated hamsters in Germany. here is also a study that indicates the occurrence of high H. pylori in raw milk (Quaglia et al., 2008).

The main predisposing factors associated with transmission and spread of *H. pylori* are of origins and these risk factors are summarized in Figure 2.5.

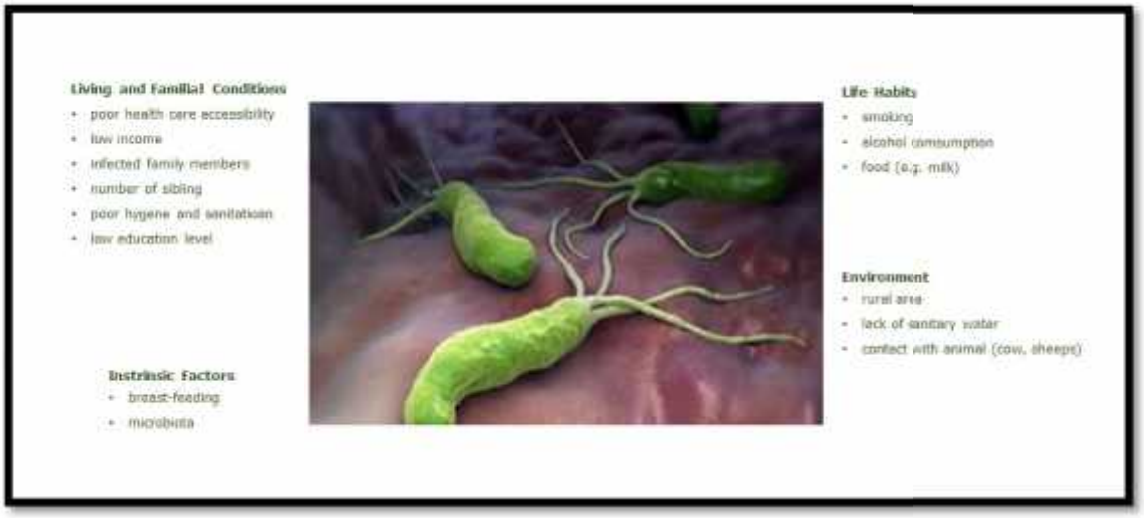

Figure 2.5. The main predisposing factors associated to the transmission and spread of *H. pylori*

2.6. Reservoir and Transmission Path of *H. pylori*

Due to the inability to consistently isolate *H. pylori* from non-human reservoirs, direct person-to-person contact is thought to be the most presumably route

of transmission. The three main transmission mechanisms that can play a role in human-to-human transmission: i) faecal-oral route ii) oral-oral route iii) iatrogenic route. This situation highlights the importance of personal contact for spread (Lambert et al., 1995). These evidences that the appearance of the disease in families of children infected with *H. pylori* increased significantly compared to families of children not infected with *H. pylori* further strengthened the effect of close contact on the appearance of *H. pylori* (Miyaji et al., 2000; Rothenbacher et al., 1999). In another study examining the spread of *H. pylori* by close contact, it was reported that the risk of a child being infected with *H. pylori* was four times higher if the father was infected and eight times higher if the mother was infected (Rothenbacher et al., 1999). In a study done in Japan, it was reported that the risk of infection in children with *H. pylori* (+) mothers was approximately five times higher than children whose mothers were *H. pylori* (-) (Malaty et al., 2000). Apart from close contact, there are studies reporting that family composition is also among the parameters that affect the transmission of *H. pylori*. In the result of this study, it is reported that the possibility of infection increases due to the increase in the number of siblings (Goodman et al., 2000). There is also evidence in the literature that states that children may facilitate the appearance of *H. pylori* in adult family members (Mendall et al., 1992; Webb et al., 1994).

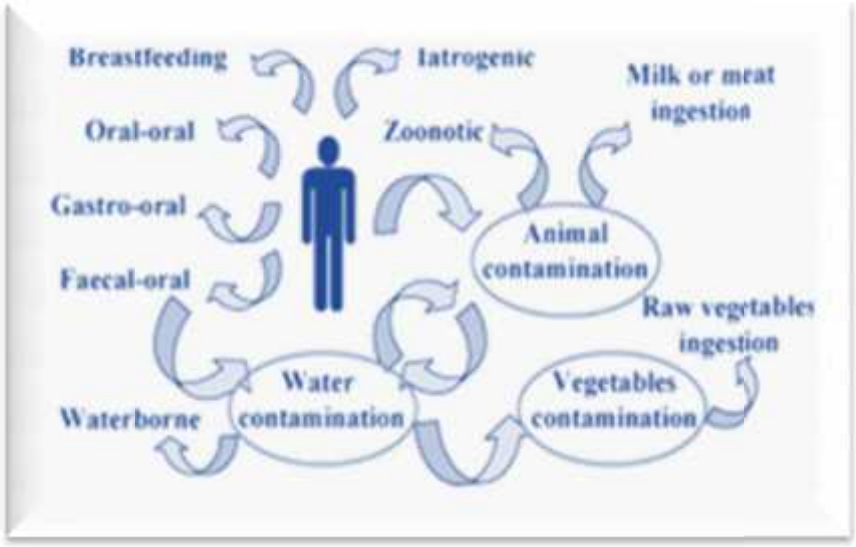

Figure 2.6. Transmission routes for *H. pylori* (Azevedo et al., 2007).

Socio-economic status and genetic disposition should also be listed among the factors affecting the transmission of *H. pylori.* There are many studies emphasizing the inverse relationship between increasing *H. pylori* infection prevalence and low socioeconomic status (Malaty et al., 1994a; Malaty et al., 1998; Murray et al., 1997; Rothenbacher et al., 1997; Van Zanten et a., 1995). Factors such as poorer people of different races living in developed countries, low levels of sanitation, educational level and low socioeconomic status of the person in childhood increase the appearance of *H. pylori* infection. A study of twins concluded that *H. pylori* infection affects acquisition due to greater similarity in monozygotic twins and that sharing the rearing environment contributes to familial tendencies of acquiring *H. pylori* infection (Malaty et al., 1994b).

Un-disinfect endoscopes and other equipment that contact the gastric mucosa cause iatrogenic transition from one individual to another. Those who do endoscopy without gloves are also at high risk. It is known that touching this organism is not a risk, but laboratories still need to be careful about this (Mitchell and Megraud, 2002).

The most important route of transmission is faecal-oral transmission. *H. pylori* can be detected in the faeces of humans infected with *H. pylori*. Faecal water contamination is considered as a source of infection (Lambert et al., 1995).

Malaty et al. (2001) and Kitagawa et al. (2001) investigated the relationship between breastfeeding and horizontal transmission of *H. pylori*. Malaty et al. (2001) showed that breastfeeding plays an important role in contagion, Kitagawa et al. (2001) showed that when breastfeeding is done in accordance with the rules of hygiene (hand and nipples are cleaned), contamination will not occur. However, he showed that horizontal transfer occurs when it is not done in accordance with the cleaning rules. Although there are many studies to cultivate *H. pylori* from the stool and mouth, only a few of them have been successful. In a study conducted by Allaker et al. (2002) with 100 British children, in three children explained that *H. pylori* was cultured in gastric fluid, but not in stool and dental plaque, while the number of children with *H. pylori* DNA in gastric fluid, dental plaque and faces were stated 11, 36 and 8, respectively. A relationship was found between *H. pylori* detected in dental

plaque and gastric fluid with polymerase chain reaction (PCR) and the presence of *H. pylori* in the stomach, but the same relationship was not found for oral and faecal samples. The reason for this was thought to be due to the specificity of the primers used in PCR.

There are many studies showings that *H. pylori* may be transmitted within and between families that named domestic and inter-family transition (Daugle et al. 2001, Taneike et al. 2001, Dore et al. 2001). In a study where infection was ribotyping in all members of a family with two children, it was found that the father and one of the children were infected with the same strain, mother and the other child with different strains. When the treatment was completed after seven weeks, the father and child became negative, but later it was found that the same child was infected with the H. pylori strain, this time in the mother (Taneike et al. 2001).

2.7. Incidence of *H. pylori*

The incidence of *H. pylori* varies between countries and within the country in relation to socioeconomic level and age groups (Mitchell and Megraud, 2002). Malaty et al. (2001), in his study on 224 American children, showed that the *H. pylori* prevalence was 8% at the ages of 1-3, while this rate increased to 24.5% between the ages of 18-23. Accordingly, the incidence is thought to increase with age. The socioeconomic status of the country is of great importance in terms of the prevalence of *H. pylori* infection. The high socioeconomic level in developed countries has led to a decrease in the infection in new generations. In many developed countries, *H. pylori* infection has decreased significantly in recent years. In a study conducted in Finland, a decrease from 38% to 12% was observed in *H. pylori* infection in the period between 1963–1973, 1983–1995.

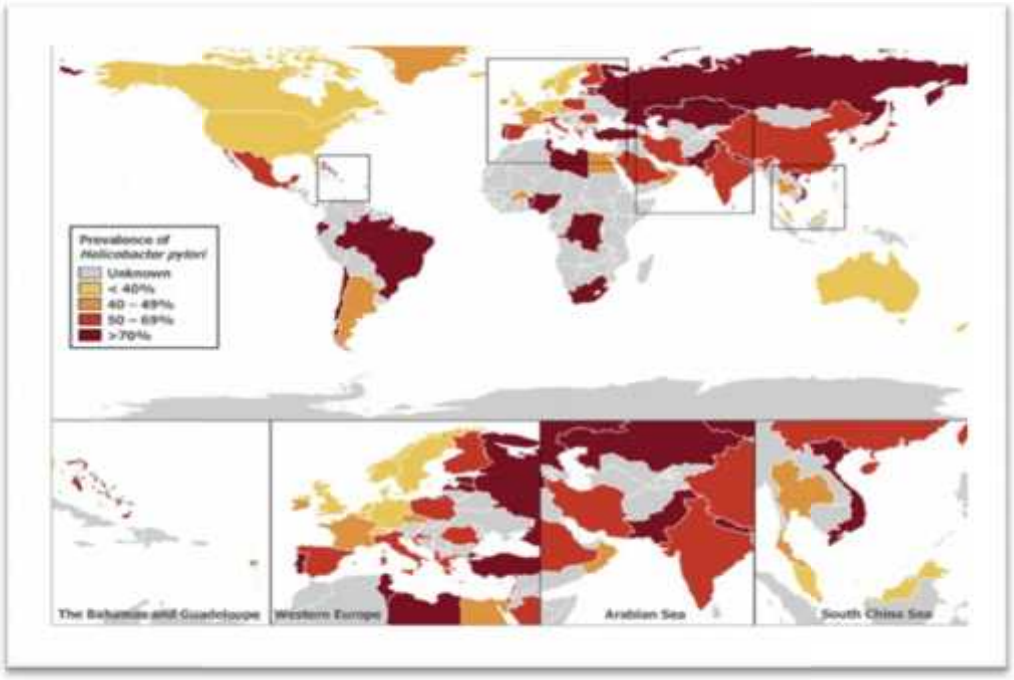

Figure 2.7. Prevalence of *H. pylori* (https://people.ucalgary.ca/~ggkaplan/HP2016.html, Accession date: 2 March 2021).

2.8. Recurrence of *H. pylori* Infection

Following successful *H. pylori* eradication, the recurrence rate of *H. pylori* is low in developed countries, while the opposite tends to occur in underdeveloped countries. In a study of 105 Bangladeshi patients with duodenal ulcers, patients were followed up after successful treatment and the relapse rate was evaluated. Accordingly, 13% repetition is encountered per year, and this rate is higher than developed countries (Honda et al., 2001).

2.9. Pathogenesis of *H. pylori*

Helicobacter pylori is a microorganism well adapted to colonize and survive in the stomach. Factors that play a role in the pathogenesis of *H. pylori* can be grouped under two groups: 1) Colonization factors 2) Virulence factors. While colonization factors enable the bacteria to colonize and survive in the host for a long

time; virulence factors cause three main pathogenic effects: gastric inflammation, disruption of the gastric barrier and alteration of gastric physiology. Many factors belonging to *H. pylori* are thought to play a role both as colonization and virulence factors in in vivo (Dunn et al., 1997). While chronic gastroenteritis is observed in all individuals infected with H. pylori, only a minority of individuals progress peptic ulcer disease or gastric malignancy. Most infected individuals are asymptomatic. Although the reason for the infection to progress asymptomatic in some individuals and with a severe clinical picture in some individuals is not known exactly, it is thought to occur as a result of a combination of various factors belonging to the host, bacteria and the environment (Owen, 2005).

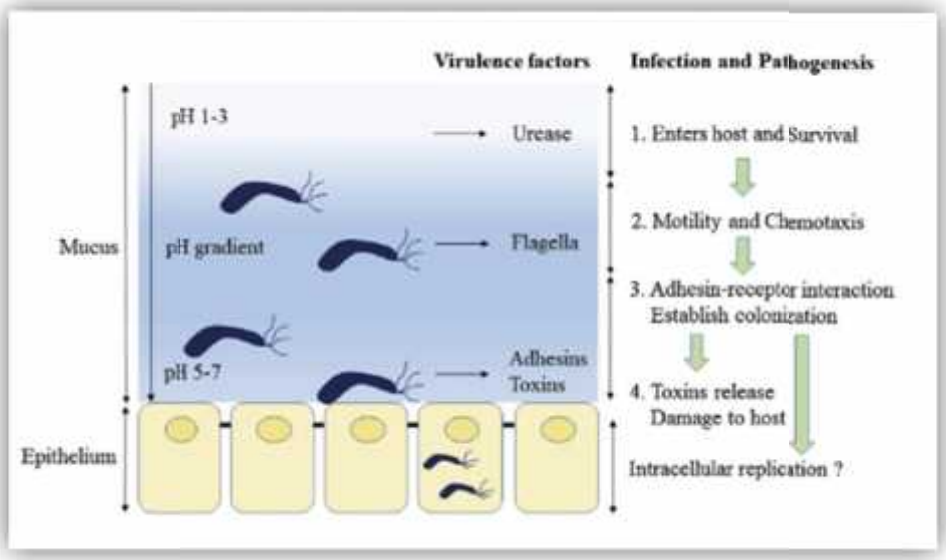

Figure 2.8. *H. pylori* infection and pathogenesis (Kao et al., 2006)

Gram (-) *H.* pylori is spiral and microaerophilic. In addition, this bacterium has urease, catalase and oxidase activity. All these properties of the bacterium provide it with the ability to live in strongly acidic stomach pH. Its urease activity enables the bacteria to neutralize stomach acid and thus convert the acid to ammonia, which helps stimulate protein synthesis. Catalase activity provides the bacteria to get rid of oxidative stress caused by urease activity. In contrast, the inflammatory response damages the lining of the gastric epithelium, allowing *H. pylori* to develop. The flagella-mediated motility of the bacteria allows it to colonize the gastric mucosa and thus facilitate the initiation of the initial infection (Armit et al., 2017).

2.10. *H. pylori* Diagnosis

Although many non- and invasive methods are used in the diagnosis of the infection, each test has its own advantages and disadvantages. More than one test is often used together in the diagnosis of *H. pylori*.

2.10.1. Invasive tests

The basis of invasive tests is based on the investigation of *H. pylori* in biopsy specimen taken during endoscopy. *H. pylori* strain is searched in biopsy samples by histopathological examination, urease test and culture methods.

Histopathological evaluation is very valuable as it enables diagnosis of both gastritis and *H. pylori*. Biopsy material is crushed, spread and stained on the slide. The classic tissue dye is Hematoxylin-eosin dye. Gram staining is an easy, practical and successful method. Tissue and bacteria are seen with Giemsa. The Whartin – Starry silvering method, acridine orange staining is useful when few bacteria are present. The sensitivity of histopathology in the diagnosis of *H. pylori* is 93-99% and the specificity is 95-99% (Erdem, 1999).

Culture is the most reliable method and also allows antibiotic susceptibility tests (Fallone et al. 2016). *H. pylori* is produced in culture media containing antibiotics with samples obtained from biopsy. When the transportation time of biopsies to the laboratory is prolonged, they can only be stored at 70 ° C for 1 day (Miendje Deyi et al., 2011). For this, enriched media are used (Skirrow media and chocolate agar which is a non-selective medium, blood agar with Brain-heart infusion can be used). The material should be incubated at 37 °C for 3-5-7 days. The environment should be humid and 5% oxygen should be available. Commercial ‘gas packs’ can be used for this. The catalase and urease activity of the curved or S-shaped organism indicates that the organism we have is *H. pylori*. It grows hard and is adversely affected by environmental conditions. If there is no reproduction, it is not correct to say ‘there is no *H. pylori’*. Its specificity is 100%, but its sensitivity is 77-92%. The stool sample is also cultured. The chance of isolating *H. pylori* among the fecal flora is low. Specificity is 100%, sensitivity is 30-50% (Erdem, 1999).

Agar dilution assay is recommended for determining the sensitivity of *H. pylori* to antimicrobials. 95% of the strains are resistant to polymyxin B (300 IU disc). 86% of the strains are resistant to nalidixic acid and 92% sensitive to cephalothin (30 mg/disc, for both). This feature is used in the differentiation of Campylobacter and Helicobacter strains. *H. pylori* is sensitive to penicillin, cephalosporins, tetracycline, erythromycin, aminoglycosides and nitrofurantoin. These bacteria are generally sensitive to metronidazole. However, resistance rates reaching 50% have been reported in some regions. It is sensitive to bismuth compounds (Murray et al., 1997).

The rapid urease test (e.g., CLO Campylobacter like organism test) of the biopsy sample is based on the urease produced by *H. pylori* breaking down the urea in the environment and the resulting NH_3 and bicarbonate increasing the pH and showing this with an indicator. Biopsy sample is placed in Christensen medium, Stuart urea test solution, or 10% urea and 1% phenol red solution and colour change is observed. The specificity of rapid urease tests is 98% and sensitivity is 93–97% (Dunn et al., 1997). The test is low with a sensitivity of 76.2% and a specificity of 69.2% in children younger than 6 years (Honar et al., 2016). Confusion can arise in the presence of bacteria with urease such as Yersinia enterocolitica and Proteus vulgaris. However, in the presence of *H. pylori,* the urease test gives a positive result within 1 h, while other bacteria require 12 h (Erdem,1999).

2.10.2. Non-invasive tests

Urea breath test is based on the determination of CO_2 released as a result of the breakdown of orally taken radio-labelled urea by the urease enzyme produced by *H. pylori*. Urea labelled C_{13} and C_{14} is used. The C_{13} urea respiration test is more expensive because it requires mass spectrometry. Sensitivity and specificity over 90% (Dunn et al., 1997).

Serological tests are based on detecting the antibody response against *H. pylori*. Systemic and local immune response develops in which IgG and IgA antibodies participate in infections caused by these bacteria. These antibodies are of diagnostic value rather than protection against infection. Antibodies are determined by hemagglutination, agglutination, complement fixation and indirect

immunofluorescence and ELISA. In ELISA kits, cagA, hspA, hspB, urease subgroups are used as antigens. Sensitivity and specificity vary between 80 and 100% according to the antigen used (Dunn et al., 1997, Vaira et al., 1995). Serological methods are particularly useful in epidemiological studies and in monitoring treatment. While the IgG response decreases in successfully treated cases, it increases in repeats. Pre-treatment antibody titers should be determined and checked six months after treatment. At the end of this period, a decrease in titer by 50% may indicate that the treatment is effective (Erdem, 1999).

There are many molecular methods using DNA amplification and hybridization techniques in *H. pylori* infection studies. These methods are used to rapidly detect *H. pylori* in biopsy samples or to investigate *H. pylori* in non-biopsy samples for epidemiological purposes. These methods are very useful in cases where no definitive results can be obtained with other diagnostic methods and in monitoring the effectiveness of the treatment. Quantitative polymerase change reaction method is also used to detect *H. pylori* in stomach biopsy samples and other biological samples (saliva, dental plaque, stool, etc.). Molecular methods can be used for molecular typing of the microorganism and thus differentiation of recurrence by reinfection can be made by typing *H. pylori* strains obtained from the same patient before and after treatment.

The stool test is based on searching for *H. pylori* antigens in human feaces by ELISA method. This diagnostic method is generally used to evaluate the response of *H. pylori* infection after treatment. Fresh or frozen stool samples are used in this test (Guarner et al., 2010). There is no need for a special transport and storage environment for faecal matter samples. The sample taken should be tested without delay. If the test cannot be done immediately, the stool sample can be stored at 2–8 ° C for 3 days or until studied at -20 ° C to -80 ° C. Samples can be frozen and thawed at most twice. It is an easy to use, inexpensive and fast method. This test is recommended 4 weeks after the completion of eradication therapy. (Nicolas et al., 2001).

2.11. H. pylori Treatment

Helicobacter pylori is a pathogen with an important etiological role in the development of active chronic gastritis, peptic ulcer and gastric cancer. H. pylori also causes MALT (Marshall, 1994). For the first time in 1994, H. pylori was described as a 1.class carcinogen by International Agency for Research on Cancer. For these reasons, H. pylori treatment is important. It has been observed that eradication of H. pylori reduces the rate of peptic ulcer recurrence (NIH Consensus Conference, 1994). The eradication is that the bacteria are not seen with the same diagnostic methods (at least 2 methods) at least 4 weeks after the end of the treatment. Clearance is; It is the failure to show the microorganism histologically or microbiologically immediately after the end of treatment.

Some researchers suggest that all individuals infected with H. pylori should be treated, with or without symptoms. However, only 20% of all people infected with *H. pylori* have clinical symptoms. For this reason, individuals with peptic ulcer disease and gastric MALToma infected with H. pylori have been definitely recommended to be treated, and treatment is recommended for individuals with chronic atrophic gastritis and gastric mucosal dysplasia (NIH Consensus Conference, 1994).

In H. pylori infection, broad spectrum penicillin derivatives, nitrofuran derivatives, tetracyclines, nitroimidazoles and macrolide. Although there are many antibiotic options, the most commonly used antibiotics are amoxicillin, tetracycline, metronidazole and clarithromycin. When antibiotics are used alone, their eradication rates are very low, so bismuth salts, H_2 antagonists, H^+ pump inhibitors (PPIs) are also added to the treatment.

The addition of PPIs (omeprazole, pantoprazole, lansoprazole) to the treatment of *H. pylori* has contributions such as synergism with antibiotics, increased local antimicrobial concentration, increased pH, strong anti-urease activity, prevention of bacterial metabolism and inhibition of migration. Bismuth salts

(Colloidal Bismuth subsalicylate, Bismuth subsalicylate, Bismuth subnitrate, Bismuth gallate, Bismuth sub-carbonate) block *H. pylori* from adhering to epithelial cells. Bismuth salts accumulate on the inner and outer surfaces of the bacterial membrane, causing the bacteria to separate from mucus and lysis, inhibiting urease. In addition, the polymer glycoprotein complex, which is formed due to the affinity of bismuth salts to mucosal proteins, protects the ulcerated tissue against pepsin activity and increases HCO_3 secretion.

Triple therapy is used due to the failure of monotherapy and dual therapy. Two antibiotics and an anti-ulcer compound are used in triple therapy. The most appropriate treatment, which is valid all over the world today, is triple therapy consisting of amoxicillin, clarithromycin and ranitidine bismuth citrate or PPIs. There are several recommended treatment regimens for *H. pylori* infection. The 2007 American College of Gastroenterology guidelines recommend first-line therapy with 10-14 days of standard triple therapy consisting of a PPI, amoxicillin, and clarithromycin. Recommended treatment options for patients with penicillin allergy include 10-14 days of bismuth quadruple therapy or 10-14 days of bismuth treatment consisting of PPI, bismuth, metronidazole and tetracycline (Chey et al., 2007). However, there is no ideal treatment approved by the FDA (Pounder, 1997). However, many antibiotics *in vitro* suppress the bacteria in the smallest amounts (minimal inhibitor concentration = MIC), but complete eradication cannot be achieved in *in vivo* conditions. The reasons for this can be listed as follows: i) Failure to ensure the stability of antibiotics in different pH's, especially in acidic environments, ii) Antibiotics not being in sufficient concentration and secreted in the mucosa, iii) pH differences on the lumen and epithelial sides of the mucus layer, *H. pylori's* settling in the deep layers of mucus, iv) The transformation of *H. pylori* into coccoid form by changing morphology, metabolism and reproductive characteristics against physical and chemical stresses, v) It is the catalase activity of *H. pylori* and its recovery from phagocytosis (Alarcon et al., 2000).

CHAPTER THREE

3. MATERIAL AND METHOD

3.1. Patient Group

This study is a retrospective study that included 1227 patients who applied to the Near East University (NEU) Hospital with dyspeptic complaints between January 1, 2017 and December 31, 2019 and were examined with a *H. pylori* antigen (Ag) rapid test in the microbiology laboratory. The patients' age, gender, complaints (reasons for admission to hospital) and diagnosis were obtained by retrospective examination of *H. pylori* antigen test in human faecal specimens results and histopathology reports.

3.2. Collection of Samples

3.2.1. Stool samples

In our study were included that patients whose stool samples were examined using the *H. pylori* Ag one-step test (Abon Helicobacter Pylori Ag, LC10951-01, Figure 3.1), which is a rapid stool Ag test.

Figure 3.1. *H. Pylori* Ag one-step test used in human stool samples in the study

The test detects *H. pylori* proteins in stools by rapid qualitative immunochromatographic based immunoassay method. In the test method, anti-human IgG labelled conjugate and monoclonal antibodies are used for *H. pylori* determination. The Ag rapid test procedure includes a plastic tube with a buffer solution and a test card to monitor the reaction. The cassettes and extraction tubes were kept at 2-8 ° C without opening the package. The fresh stool samples were studied in accordance with the package insert instructions of the kit. Samples that could not be studied immediately were stored at 2-8 ° C and studied within 48 h. Samples with damaged packaging were not used in the tests Stool samples taken from the patient were opened from the screwed part of the plastic tube and a lentil-sized amount was taken into the plastic bottle by touching the different parts of the stool. The tube was tightly closed. The extraction buffer in the tube was shaken and vortexed for 15 s to mix with the sample. Cassette test is removed from its pouch. The tip of the tube was broken and 4 drops were dropped into space at the end of the test cassette. Results were read after 5 min.

If only a blue line was observed as a band at the level of the control (C) line, the test was considered negative. The test was considered positive if a pink-red line was observed in addition to the blue line. If no blue line is seen with or without a pink-red line, the test is considered invalid.

3.2.2. Histopathological analyses

Histopathological reports of the presence of *H. pylori* in samples obtained through biopsies from the antrum and / or corpus in 334 of 1227 patients who underwent *H. pylori* Ag rapid test was performed by experienced pathologists (Figure 3.3). For this purpose, 5 µm sized sections were prepared from the biopsy samples taken from the antrum and / or corpus regions, fixed with 10 % formaldehyde and stained with haematoxylin-eosin. The preparations obtained were stained with May-Grunwald Giemsa and the presence of spiral-shaped *H. pylori* attached to the stomach surface was evaluated (Manxhuka-Kerliu et al., 2009). If *H. pylori* was detected on histopathological examination, it was defined as *H. pylori* positive status.

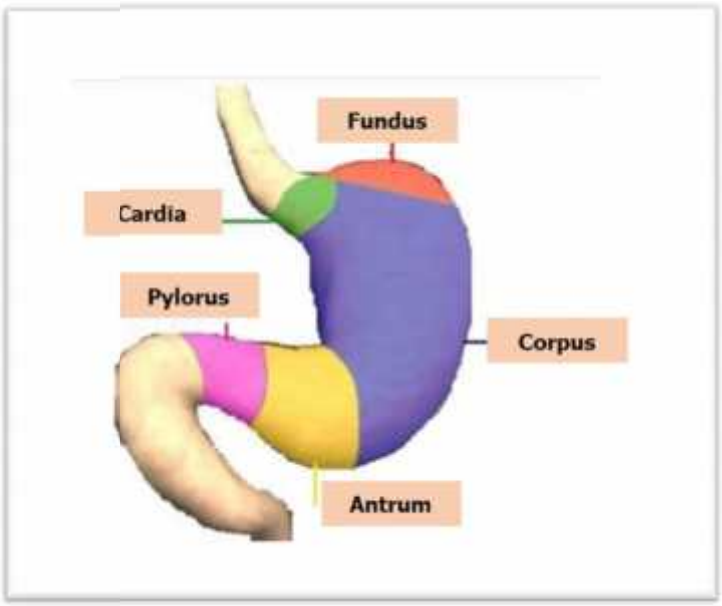

Figure 3.2. Parts of human stomach (https://www.turkcerrahi.com/makaleler/mide/mide-anatomisi/,Accession date: 2 March 2021).

3.3. Statistical Analysis

In this study, data from a total of 1227 patients were used to evaluate the correlation between stool *H. pylori* positivity and demographic characteristics. In the evaluation of the correlation between *H. pylori* positivity and demographic characteristics of the patients who underwent histopathological evaluation, the data of a total of 344 patients were used in the study. Statistical analyses were carried out through the Statistical Package for the Social Sciences (version 20.0, SPSS Inc., Chicago, IL, USA) program.

As descriptive statistics; Number (n) and percentage (%) were used in the evaluation of categorical variables. Statistical power analysis of the sample number was done by Student's t-test (Çapık, 2014). According to the *H. pylori* Ag rapid test or histopathology reports, *H. pylori* positivity, diagnosis, whether histopathology evaluation is performed or not, complaint and diagnosis, gender and age correlation were analysed with the Pearson chi-square test. $p < 0.05$ was considered significant.

Sensitivity [x / (x + z) x 100], specificity [w / (y + w) x 100], positive predictive values (PPV) [x/(x +y) x 100] and negative predictive values (NPV) [w /

(z + w) x 100], were calculated according to the Table 3.1 given below (Mehli et al., 2008).

Table 3.1. Statistical evaluation of two methods (*H. pylori* Ag rapid test and histopathological reports).

	Gold Standard Method		
Method	positive	negative	total
Positive	x	y	x + y
Negative	z	w	z + w
Total	x + z	y + w	x + y + z + w

CHAPTER FOUR

4. RESULTS

4.1. Demographic Data

630 of 1227 patients included in the study were male and 597 were female (Figure 4.1). Male patients were between 17 and 90 years old with a mean age of 35.3, female patients between 19 and 88 years, and their mean age was 33.4.

Figure 4.1. Distribution of patients who applied to the NEU Hospital between January 1, 2017 and December 31, 2019 and whose stool samples were sent to the microbiology laboratory for *H. pylori* Ag rapid test by gender.

When the age distribution of the patients is analysed; The number of patients in the range of 1-20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 and over 61, respectively 192 (15.6%), 485 (39.5%), 162 (13.2%), 138 (11.2%), 121 (9.9%) and 129 (10.5%) (Figure 4.2.).

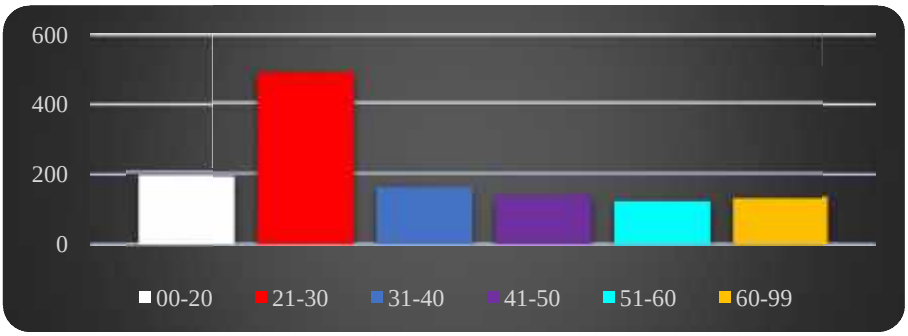

Figure 4.2. Distribution of patients who applied to the NEU Hospital between January 1, 2017 and December 31, 2019 and whose stool samples were sent to the microbiology laboratory for *H. pylori* Ag rapid test by age.

It was determined that the patients applied to the hospital with dyspeptic complaints such as 1106 (90.1%) abdominal pain, 22 (1.8%) nausea and 99 (8.1%) retrosternal burning (Figure 4.3).

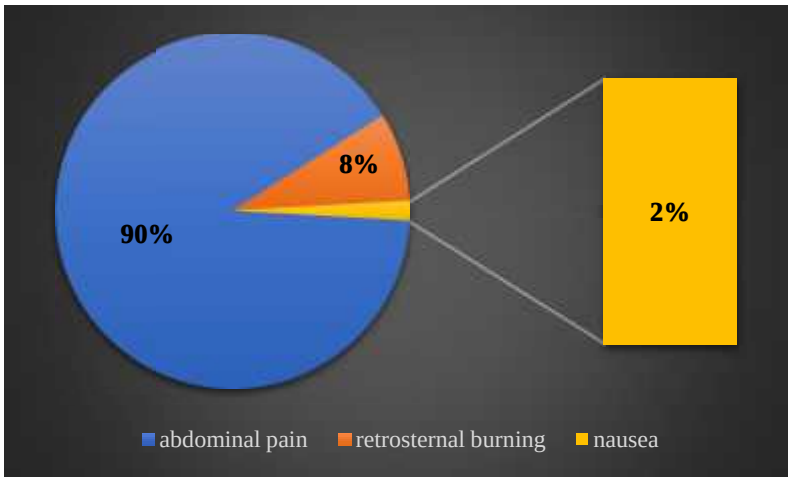

Figure 4.3. Distribution of patients who applied to the NEU Hospital between January 1, 2017 and December 31, 2019 and whose stool samples were sent to the microbiology laboratory for *H. pylori* Antigen rapid test by their complaints.

The demographic characteristics of the patients included in the study are shown in Table 4.1.

Table 4.1. Demographic characterizations of patients who applied to the NEU Hospital January 1, 2017 and December 31, 2019 and whose stool samples were sent to the microbiology laboratory for *H. pylori* Ag rapid testing.

		n	%
Age	≤ 20	192	15.6
	21-30	485	39.5
	31-40	162	13.2
	41-50	138	11.2
	51-60	121	9.9
	61 ≤	129	10.5
Genders	male	630	51.3
	female	597	48.7
Complaint	abdominal pain	1106	90.1
	nausea	22	1.8
	retrosternal burning	99	8.1
Total		1227	100

4.2. The Results of *H. Pylori* Antigen Rapid Test and Histopathological Reports

It was determined that 67 (5.5%) of 1227 patients whose *H. pylori* Ag rapid test in stool were examined had *H. pylori* Ag tests positive (Figure 4.4). It was determined that 344 (28%) of the patients were biopsied and according to histopathology reports, *H. pylori* was found positive (Figure 4.5.) in the antrum and/or corpus samples in 84 (24.4%) of the patients who underwent biopsy (Table 4.2).

Figure 4.4. Sample Ag rapid test image of *H. pylori* in human faecal specimens

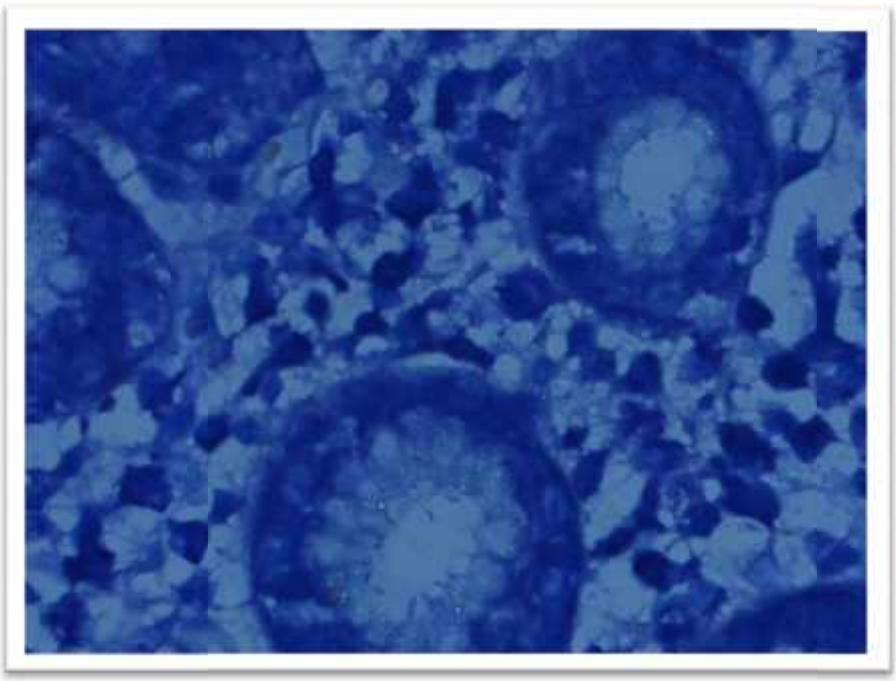

Figure 4.5. Sample histopathology image of *H. pylori*

Table 4.2. Detection rates of *H. pylori* presence according to the *H. pylori* antigen rapid test and histopathology reports of the patients who applied to the NEU Hospital January 1, 2017 and December 31, 2019.

		n	%
Antigen test	**HpSA (+)**	67	5.5
	HpSA (-)	1160	94.5
	Total	1227	100.0
Histopathology	***H. pylori* (+)**	84	24.4
	***H. pylori* (-)**	260	75.6
Total		344	100.0

The presence of *H. pylori* in 344 samples examined by both histopathological report and Ag methods in faecal specimen was determined as 67 by *H. pylori* Ag rapid test and 84 according to the histopathology report (Table 4.3).

Table 4.3. The result and percentage of *H. pylori* in samples examined by both Ag rapid test method in faecal specimens and *H. pylori* histopathology reports in samples obtained through biopsies from the antrum and/or corpus.

	Method n (%)	
Results	**Stool antigen**	**Histopathological**
(+)	67 (19.5)	84 (24.4)
(-)	277 (80.5)	260 (75.6)
Total	344 (100)	344 (100)

The power analysis, which evaluated the correlation between *H. pylori* positivity and demographic characteristics, was determined to be 0.83 at the 0.05 significance level and 95% confidence interval (n=344). This is another sign that the sample size is adequate.

4.3. Evaluation of Sensitivity and Specificity of Antigen Test Compared to Histopathology Reports

Although *H. pylori* was positive in the histopathology report of 84 patients, it was determined that 17 of them were negative with the *H. pylori* Ag rapid test in faecal specimens (Table 4.4.). The sensitivity of *H. pylori* Ag rapid test in faecal specimens was 79.7% and the specificity was 100%. PPV and NPV were found 100% and 93.8% respectively.

Table 4.4. Comparison of *H. pylori* antigen rapid test in stool results with *H. pylori* histopathology reports in samples obtained through biopsies from the antrum and/or corpus.

Ag rapid test	Histopathology (+)	Histopathology (-)	Total
HpSA (+)	67	0	67
HpSA (-)	17	260	277
Total	84	260	344

HpSA: antigen for stool
Ag: Antigen

4.4. Diagnostic Results Obtained from Histopathology Reports

According to histopathology reports, 321 (93.3%) of the patients were diagnosed with gastritis and 23 (6.7%) of them were diagnosed with gastroesophageal reflux disease (Table 4.5).

Table 4.5. Diagnosis determined by histopathology reports

Diagnosis	n	%
gastritis	321	93.3
gastroesophageal reflux	23	6.7
Total	344	100.0

4.5. Comparison of Results with Gender

When the distribution of *H. pylori* presence by gender was examined with *H. pylori* Ag rapid test, it was found that 21 (3.5%) of 597 women and 46 (7.3%) of 630 men were *H. pylori* positive. The distribution of H. *pylori* presence in stool by gender was statistically significant ($p < 0.05$). According to the *H. pylori* Ag rapid test results, *H. pylori* risk in male was determined to be 36 times higher than female (Odds ratio: 36, Table 4.6).

Table 4.6. Distribution of *H. pylori* presence in human faecal specimens obtained from *H. pylori* antigen rapid test results by gender.

	HpSA (stool)		Total	O.R	P
	(+)	(-)			
female	21 (3.5%)	576 (96.5%)	597	1	
male	46 (7.3%)	584 (92.7%)	630	36	0.004
Total	67 (5.5%)	1160 (94.5%)	1227		

O.R: odds ratio

When the distribution of *H. pylori* presence by gender was examined with the histopathology reports, it was found that 16 (6.4%) of 250 women and 68 (72.3 %) of 94 men were *H. pylori* positive. The distribution of H. *pylori* presence obtained from the histopathology reports result by gender was statistically significant ($p < 0.05$). According to the histopathology reports results, *H. pylori* risk in men was determined to be 43 times higher than women (Odds ratio: 43, Table 4.7.).

Table 4.7. Distribution of *H. pylori* presence obtained from the histopathological reports by gender.

	HpSA (Histopathology)		Total	Odds ratio	p
	(+)	(-)			
female	16 (6.4%)	234 (93.6%)	250	1	
male	68 (72.3%)	26 (27.7%)	94	43	0.000
Total	84 (24.4%)	260 (75.6%)	344		

O.R: odds ratio

When the distribution of histopathological evaluation by gender was examined, it was determined that 250 (41.9%) out of 597 women and 94 (14.9%) out of 630 men were made histopathological evaluation. It was determined that histopathological evaluations in in samples obtained through biopsies from the antrum and/or corpus were 4.2 times in women more than men (Table 4.8.).

Table 4.8. Distribution of whether the make histopathological evaluation was performed or not by gender.

	Make histopathology				
	(+)	**(-)**	**Total**	**O.R**	**P**
female	250 (41.9%)	347 (58.1%)	597	4.2	
male	94 (14.9%)	536 (85.1%)	630	1	0.001
Total	344 (28.0%)	883 (72.0%)	1227		

O.R: odds ratio

Examining the distribution of patients who applied to the hospital with abdominal pain complaints by gender, it was found that 510 (85.4%) out of 597 women and 596 (94.6%) out of 630 men had abdominal pain. It was determined that abdominal pain complaints were 2.9 times in males more than females (Table 4.9.).

Table 4.9. Distribution of applied to the hospital with abdominal pain by gender.

	Abdominal pain				
	(+)	**(-)**	**Total**	**O.R**	**P**
female	510 (85.4%)	87 (14.6%)	597	1	
male	596 (94.6%)	34 (5.4%)	630	2.9	0.000
Total	1106 (90.1%)	121 (9.9%)	1227		

O.R: odds ratio

According to histopathology reports, when the distribution of patients diagnosed with gastritis by gender was examined, it was found that 242 (94.2%) out of 257 women and 79 (90.8%) out of 87 men had gastritis diagnosis. It was found that females were diagnosed with gastritis 1.6 times as often as males (Table 4.10).

Table 4.10. Distribution of patients diagnosed with gastritis according to the pathology report by gender

	Gastritis		Total	O.R	P
	(+)	(-)			
female	242 (94.2%)	15 (5.8%)	257	1.6	
male	79 (90.8%)	8 (9.2%)	87	1	0.000
Total	321 (90.1%)	23 (6.7%)	344		

O.R: odds ratio

4.6. Comparison of Results with Age Groups

When the distribution of *H. pylori* presence by age was examined with the *H. pylori* Ag rapid test, it was found that 4 (2.1%) of 188 patients the ages of ≤ 20 years, 44 (9.1%) of 485 patients between the age of 21-30, 12 (7.4%) of 162 patients between the age of 31-40, 3 (2.2%) of 138 patients between the age of 41-50, 4 (3.3%) of 121 patients between the age of 51-60 and 0 (0%) of 129 patients the age group of 61 and over were *H. pylori* positive. The distribution of H. *pylori* presence in stool by age was statistically significant ($p < 0.05$). According to the *H. pylori* Ag rapid test results, *H. pylori* risk in patients between the age of 31-40 was determined to be 8 times higher than patients between the age of 21-30 (Table 4.11).

Table 4.11. According to the *H. pylori* Ag rapid test results, distribution of *H. pylori* presence by age

	HpSA (stool)		Total	O.R	p
	(+)	(-)			
20 ≥	4 (2.1%)	188 (97.9%)	192	2	
21-30	44 (9.1%)	441 (90.9%)	485	1	
31-40	12 (7.4%)	150 (92.6%)	162	8	
41-50	3 (2.2%)	135 (97.8%)	138	2	
51-60	4 (3.3%)	117 (96.7%)	121	3	
61 ≤	0 (0%)	129 (100%)	129	0	0.005
Total	67 (5.5%)	1160 (94.5%)	1227		

O.R: odds ratio

When the distribution of *H. pylori* presence by age was examined with the histopathology reports, it was found that 10 (2.7%) of 37 patients the ages of ≤ 20 years, 45 (27.7%) of 162 patients between the age of 21-30, 13 (24.5%) of 53 patients between the age of 31-40, 6 (13.9%) of 43 patients between the age of 41-50, 10 (2.1%) of 47 patients between the age of 51-60 and 0 (0%) of 2 patients the age group of 61 and over were *H. pylori* positive. The distribution of H. *pylori* presence obtained from the histopathology reports result by age was statistically significant ($p < 0.05$). According to the histopathology reports results, *H. pylori* risk in patients between the age of 21-30 was determined to be 2.4 times higher than patients between the age of 41-50 (Table 4.12.).

Table 4.12. According to the histopathology reports, distribution of *H. pylori* presence by age group.

	HpSA (Histopathology)		Total	O.R	p
	(+)	(-)			
20 ≥	10 (27.7%)	27 (%)	37	2.3	
21-30	45 (27.8%)	117 (%)	162	2.4	
31-40	13 (24.5%)	40 (%)	53	2.1	
41-50	6 (14.0%)	37 (%)	43	1	
51-60	10 (21.3%)	37 (%)	47	1.7	
61 ≤	0 (%)	2 (%)	2	0	0.005
Total	84 (24.4%)	260 (75.6%)	344		

O.R: odds ratio

When the distribution of histopathological evaluation by age was examined, it was found that 37 (19.2%) of 192 patients the ages of ≤ 20 years, 162 (33.4%) of 485 patients between the age of 21-30, 53 (32.7%) of 162 patients between the age of 31-40, 43 (31.2%) of 138 patients between the age of 41-50, 47 (38.1%) of 121 patients between the age of 51-60 and 2 (1.5%) of 129 patients the age group of 61 and over were histopathological evaluation for *H. pylori* positive. It was determined that the age group for which histopathological evaluation was made the least was those over 61 years old and those under 20 years old. It was determined that histopathological evaluations were 41.2 times in patients between the age of 51-60 more than patients between the age of 61≤ (Table 4.13.).

Table 4.13. Distribution of whether the make histopathological evaluation was performed or not by age.

	Make histopathology		Total	O.R	p
	(+)	(-)			
20 ≥	37 (19.2%)	155 (80.8%)	192	15	
21-30	162 (33.4%)	323 (66.6%)	485	31.2	
31-40	53 (32.7%)	109 (67.3%)	162	30	
41-50	43 (31.2%)	95 (68.8%)	138	28.1	
51-60	47 (38.1%)	74 (61.9%)	121	41.2	
61 ≤	2 (1.6%)	127 (98.4%)	129	1	0.000
Total	84 (24.4%)	260 (75.6%)	344		

O.R: odds ratio

Examining the distribution of patients who applied to the hospital with “abdominal pain” complaints by age, it was found that 173 (90.1%) of 192 patients the ages of ≤ 20 years, 435 (89.7%) of 485 patients between the age of 21-30, 145 (89.5%) of 162 patients between the age of 31-40, 129 (93.5%) of 138 patients between the age of 41-50, 10 (86.7%) of 121 patients between the age of 51-60 and 119 (92.2%) of 129 patients the age group of 61 and over had abdominal pain. There was no statistically difference between abdominal pain and age groups.

It was determined that abdominal pain complaints were 2.1 times in patients between the age of 41-50 more than patients between the age of 51-60 (Table 4.14)

Table 4.14. Distribution of applied to the hospital with abdominal pain by age.

	Abdominal pain		**Total**	**O.R**	**p**
	(+)	**(-)**			
20 ≥	1 73 (90.1%)	19 (9.9%)	192	1.4	
21-30	435 (89.7%)	50 (10.3%)	485	1.3	
31-40	145 (89.5%)	17 (10.5%)	162	1.2	
41-50	129 (93.5%)	9 (6.5%)	138	2.1	
51-60	105 (86.7%)	16 (13.3%)	121	1	
61 ≤	119 (92.2%)	10 (7.8%)	129	1.8	0.36
Total	1106 (90.1%)	121 (9.9%)	1227		

O.R: odds ratio

According to histopathology reports, when the distribution of patients diagnosed with “gastritis” by age was examined, it was found that 34 (94.4%) of 36 patients the ages of ≤ 20 years, 115 (89.8%) of 128 patients between the age of 21-30, 52 (89.1%) of 53 patients between the age of 31-40, 40 (95.2%) of 42 patients between the age of 41-50, 46 (97.8%) of 47 patients between the age of 51-60 and 38 (89.5%) of 129 patients the age group of 61 and over had gastritis diagnosis. It was found that patients between the age of 31-40 were diagnosed with gastritis 6.1 times as often as patients between the age of 61 ≤ (Table 4.15).

Table 4.15. Distribution of patients diagnosed with gastritis according to the pathology report by age.

	Gastritis		Total	O.R	p
	(+)	(-)			
20 ≥	34 (94.4%)	2 (5.6%)	36	2	
21-30	115 (89.8%)	13 (10.2%)	128	5.2	
31-40	52 (89.1%)	1 (10.1%)	53	6.1	
41-50	40 (95.2%)	2 (4.8%)	42	2.4	
51-60	46 (97.9%)	1 (2.1%)	47	5.4	
61 ≤	34 (89.5%)	4 (10.5%)	38	1	0.004
Total	321 (90.1%)	23 (6.7%)	344		

O.R: odds ratio

CHAPTER FIVE

5. DISCUSSION

Many methods such as serological techniques, culture, stool antigen test, urease test, histopathological examination, urea breath test, and polymerase change reaction are used in the clinical diagnosis of helicobacter infections. There is still no consensus on what or what is the gold standard method in the diagnosis of *H. pylori.* In addition to recommending the use of more than one method together to increase the accuracy of the diagnosis, which methods will be used in the diagnosis; It is also recommended to make a decision considering the price, accessibility of the method used, sensitivity and specificity, the current condition of the patient and the age of the patient (Altındiş and Özdemir, 2003; Usta and Özen, 2007; Tünger, 2008).

In the literature, there are studies reporting that the stool *H. pylori* Antigen test is more appropriate to evaluate the treatment or use it for scientific purposes (Tringali et al., 2017). According to the results of the research, the sensitivity of the antigen test varies between 21.4 - 94% and the specificity between 87.5-100% (Gisbert et al., 2006; Silva et al., 2010; Ceken et a., 2011, Güven et al., 2019). In our study, the sensitivity of the *H. pylori* antigen test was determined as 79.9% and the specificity as 100%. In our study results, we think of different reasons affecting the sensitivity of the stool antigen test compared with the histopathology report. For example, watery or amorphous stool may give false results as the *H. pylori* antigen is more diluted and the bacterial concentration will be low. In addition, heat, the time between working and working, and the recent use of H^+ pump inhibitors or antibiotics may cause false results (Shimoyama, 2013).

The presence of *H. pylori* in 56 patients was investigated by histopathology, stool antigen, urease and culture test in China. They used the following as evaluation criteria: The patient was accepted as *H. pylori* positive if the culture alone or with histology was positive, and *H. pylori* negative if all tests were negative. They determined the sensitivity of the stool test as 92.6%, specificity 88.5%, PPV 89.3%

and NPV 92%. As a result, they suggested that the stool antigen test can be used as a simple, non-invasive test that gives accurate results for the diagnosis of *H. pylori* (Li et al., 2004).

The diagnostic power of histopathological methods is closely related to the number of biopsies taken, the bacterial load of the sampled area, the staining method and / or the knowledge and skills of the pathologist making the diagnosis (Aydın et al., 2003; Wang et al., 2015). In a study conducted by Khalifehgholi et al. (2013) biopsy, blood and stool samples of 91 patients were examined in terms of *H. pylori* and the highest sensitivity compared to polymerase change reaction was obtained with histopathological analysis and rapid urease test with a rate of 95.6% (Khalifehgholi et al., 2013). In another study by Pacheco et al. (2001) biopsy samples taken from patients with dyspepsia were examined by serological, histopathological, rapid urease test and polymerase change reaction, and the highest positivity (86%) was obtained by histopathological method (Pacheco et al., 2001).

In this study, while 67 (5.5%) of them were found to be *H. pylori* (+) with the stool Ag rapid test result, 84 (24.4%) individuals were diagnosed with *H. pylori* as a result of the histopathological examination of gastric biopsy samples taken from 344 people. There were no significant differences between methods, and the overall agreement between the stool Ag test and histopathological analysis was found to be quite high.

In the study conducted by Mete et al., the appearance of *H. pylori* was reported as 73.7% as a result of histopathological evaluations of 797 patients who underwent gastroscopic investigation (Mete et al., 2014). In another study comparing the urea breath test with the *H. pylori* stool Ag test, 627 *H. pylori* positive specimens were also found to be positive for *H. pylori* stool Ag test. 23 samples with the negative urea-breath test, was negative for *H. pylori* stool Ag and 45 samples with the (+) urea breath test were found positive for *H. pylori* stool Ag test. For the diagnosis of *H. pylori* infection, the rate of compliance between urea breath test and stool Ag test was reported to be 94.9% (Queiroz et al., 2013).

In the study conducted by Mărginean et al., It was stated that patients with pylori infection were most frequently admitted with abdominal pain (Mărginean et al., 2013). This is in parallel with our research.

In epidemiological studies conducted on populations, there is a general opinion that *H. pylori* infection is more common in males than female, however, there is also researches that states not related to gender (de Martel et al., 2006; Yu et al., 2015). Although the distribution of the infection by gender is not fully explained, it may be interpreted as the fact that men are more intense in unhygienic environments and more risky consumption trends such as smoking and alcohol. In this study, 3.5% of the stool samples belonging to women and 7.3% of the samples belonging to men were found to be positive for H. pylori, while the distribution of *H. pylori* positivity by gender was found to be statistically significant (p=0.004). In addition, similar to other studies, it was determined that the risk of *H. pylori* in men is higher than in women (odds ratio: 36). In this study, 6.4% of the histopathology report belonging to women and 72.3% of the samples belonging to men were found to be positive for *H. pylori*, while the distribution of *H. pylori* positivity by gender was found to be statistically significant (p=0.000). In addition, similar to other studies, it was determined that the risk of *H. pylori* in men is higher than in women (odds ratio: 11.3).

Although *H. pylori* infection is seen in almost every age group of people, the majority of the cases (80%) are encountered in the adult age group before the age of 50. Although it is thought that the increase in the prevalence of infection in parallel with age will be due to the possibility of constant contact with the bacteria throughout the life of the people, on the contrary, there are also studies reporting the presence of infection in young children (Ozbey et al., 2015; Nagy et al., 2016).

In our study, it was determined that the ages of patients tested for *H. pylori* Ag rapid test in stool ranged from 17 to 90 years. *H. pylori* positivity varies according to age groups, the most common positivity rate was found to be between the ages of 31-40 with the Ag rapid test in stool and between the ages of 21-30 with histopathology reports. The distribution of *H. pylori* positivity by age was found to be statistically significant ($p < 0.001$), while it was determined that the risk of *H.*

pylori was higher in all other age group individuals compared to the age group of 61 and over (Odds rates vary up to 8 times). When *H. pylori* risk was examined in more detail among other age groups other than the 61 and above group, it was determined that the risk of *H. pylori* was much lower in patients between the ages of 21-30 (with Ag rapid test) and 41-50 (with histopathology reports) age groups according to other age group. Among the reasons for higher *H. pylori* positivity in the 21-30 and 31-40 age groups, it may be considered that the patients are comprised of individuals of active working age and thus the exposure to factors causing infection. In addition to these reasons, the development of atrophied stomach structure and the increase in antibiotic and antisecretory treatment attempts at later ages decrease the number of detectable microorganisms (Salles-Montaudon et al., 2002). In addition, socio-economic status, genetic factors and level of education are thought to contribute positively to factor colonization in these age groups (Peterson et al., 2004).

In our study, in which the results were evaluated retrospectively, it was concluded that the *H. pylori* stool Ag test may use in the detection of *H. pylori* and infection because it was invasive, cheap, easily applicable and gave positive results close to histopathological results.

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