University of Basrah
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Study of some immunological parameters with *Helicobacter pylori* infected patients

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿إِنَّمَا يَخْشَى اللَّهَ مِنْ عِبَادِهِ الْعُلَمَاءُ إِنَّ اللَّهَ عَزِيزٌ غَفُورٌ﴾

صَدَقَ اللهُ العَظِيمِ

سورة فاطر ايةَ28

شكر وتقدير

نحمد اللّه عزّا وجل الذي وفقنا في اتمام هذه الدراسة التي ارجوا ان تنال رضاه ، فالحمد للّه حمداً كثيرا .

وكذلك تقدم بجزيل الشكر الى اساتذتي الكرام ورئاسة جامعة البصرة و كلية الصيدلة وكل من ساهم في تعليمي.

كما نشكر كل من ساعدنا من قريب او من بعيد ولو بكلمة او دعوة صالحة .

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Aim of Study

The present study an attempt to clarify the correlation and some statistical analysis among some immune cells with *H. Pylori* infected patients

Abstract

Helicobacter pylori is a gram-negative, spiral-shaped bacterium. The infection is transmitted mainly by the oral-oral, fecal-oral route . It cause mucosal damage and inflammation, It may progress from acute/chronic inflammation, chronic inflammation lead to gastric cancer. Fecal Antigen Test, Serological Test of 35 patients (14 men and 21 women) was studied the peripheral blood leukocyte count and differential. The total number of blood leukocytes and the numbers of neutrophil were significantly increased in *H. pylori*-positive patients (N = 24), as compared with H. pylori-negative ones (N = 11). in the other way the total number of WBC was shown significant different among WBC positive and negative in female while in male was no significant.and neutrophil high significant increase in male and female for positive and negative that is indicator it is possible to depend on neutrophil as prognosis ,diagnosis for infection with H. pylori .While Eosinophil, Lymphocyte ,and other immune cell not correlated with bacterial infection therefor as shown it has not significant variation among immune cells.

Introduction

Helicobacter pylori was the most common human bacterial pathogen in the world [1]. Over third of million people's deaths each year worldwide may be due to this potentially fatal pathogen. *Helicobacter pylori* is a gram negative microaerophilic fastidious bacterium which over centuries has successfully infected around 50 percent of human individuals throughout the world. Very often, infection occurs in childhood and persists lifelong if not treated. This human pathogen is known to induce several gastric disorders, but may also be associated with extra gastric diseases like anemia, dyspepsia, and some immunological disorders [1–2]. Almost all infected subjects develop chronic gastritis, and a considerable percentage of patients further develop ulcer disease or gastric cancer.

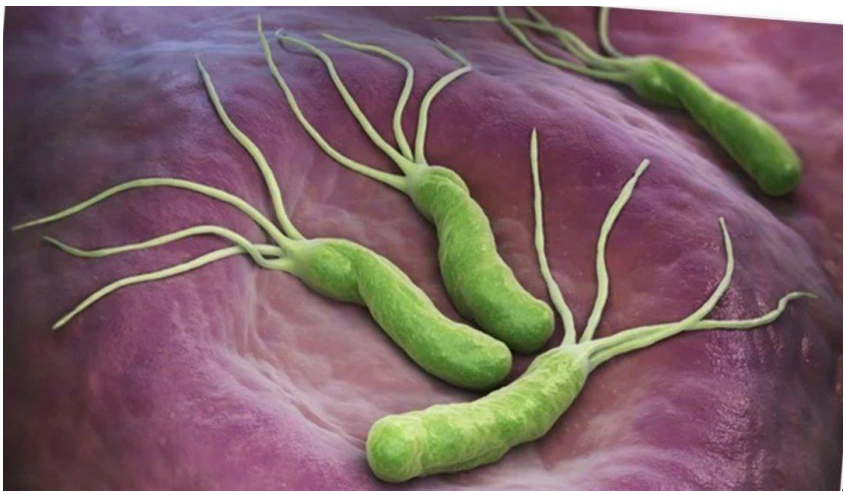

Figure 1. *Helicobacter pylori.*

H. pylori is a gram-negative bacterium with a helical rod shape. It has prominent flagella, facilitating its penetration of the thick mucus layer in the stomach.

Epidemiology & Transmission

Infection with *H. pylori* occurs worldwide, but the prevalence varies greatly among countries and among population groups within the same country.[3] The overall prevalence of *H. pylori* infection is strongly correlated with socioeconomic conditions.[4] The prevalence among middle-aged adults is over 80 percent in many developing countries, as compared with 20 to 50 percent in industrialized countries. The infection is acquired by oral ingestion of the bacterium and is mainly transmitted within families in early childhood.[3,5] It seems likely that in industrialized countries direct transmission from person to person by vomitus, saliva, or feces predominates; additional transmission routes, such as water, may be important in developing countries.[6,7] There is currently no evidence for zoonotic transmission, although *H. pylori* is found in some nonhuman primates and occasionally in other animals.[8,9] *H. pylori* infection in adults is usually chronic and will not heal without specific therapy; on the other hand, spontaneous elimination of the bacterium in childhood is probably relatively common,[10] aided by the administration of antibiotics for other reasons. The prevalence of *Helicobacter pylori* infection in a community is related to three factors: firstly, the rate of acquisition of infection with *H. pylori*-that is, incidence; secondly, the rate of loss of the infection; thirdly, the prolonged persistence of the bacterium in the gastroduodenal mucosa between infection and eradication.

Pathogenicity

Humans ingest many microorganisms each day, but most cannot successfully colonize the stomach. One of the most important antibacterial properties of the human stomach is its acidic pH. Under fasting conditions, the human gastric luminal pH is <2, which prevents the proliferation of bacteria within the gastric lumen. Within the gastric mucus layer overlying gastric epithelial cells, a pH gradient exists, ranging from a pH of about 2 at the luminal surface to a pH of between 5 and 6 at the epithelial cell surface [13,14]. After entering the stomach, *H. pylori* penetrates the gastric mucus layer (15) and thereby encounters a less acidic environment than that which is present within the gastric lumen. *H. pylori* typically does not traverse the epithelial barrier, and it is classified as a noninvasive bacterial organism. Within the gastric mucus layer, most *H. pylori* organisms are free living, but some organisms attach to the apical surface of gastric epithelial cells and may occasionally be internalized by these cells [17,16,18,19] The gastroduodenal response to chronic *Helicobacter pylori* infection is characterized by the infiltration of plasma cells, lymphocytes, neutrophils and monocytes into the mucosa. Eradication studies have shown that this inflammatory response represents a specific reaction to the presence of *H. pylori.* As well as stimulating specific local T and B cell responses and a systemic antibody response, *H. pylori* infection also induces a local pro-inflammatory cytokine response. Interleukin-8 (IL-8), which is expressed and secreted by gastric epithelial cells, may be an important host mediator inducing neutrophil migration and activation. IL-8 mRNA and protein secretion in gastric epithelial cell lines can be up-regulated by the cytokines tumor necrosis factor-α and IL-1 and also by type I strains of H. pylori (expressing the vacillating toxin and cytotoxic-associated protein, CagA). The gastric epithelium thus plays an active role in mucosal defiance. Neutrophil activation and the production of reactive oxygen metabolites will be induced directly by

bacterial factors and indirectly via host-derived cytokines, products of complement activation and bioactive lipids. Strain variation in the induction of both IL-8 from epithelial cells and the oxidative burst in neutrophils may be an important factor determining the extent of mucosal injury.

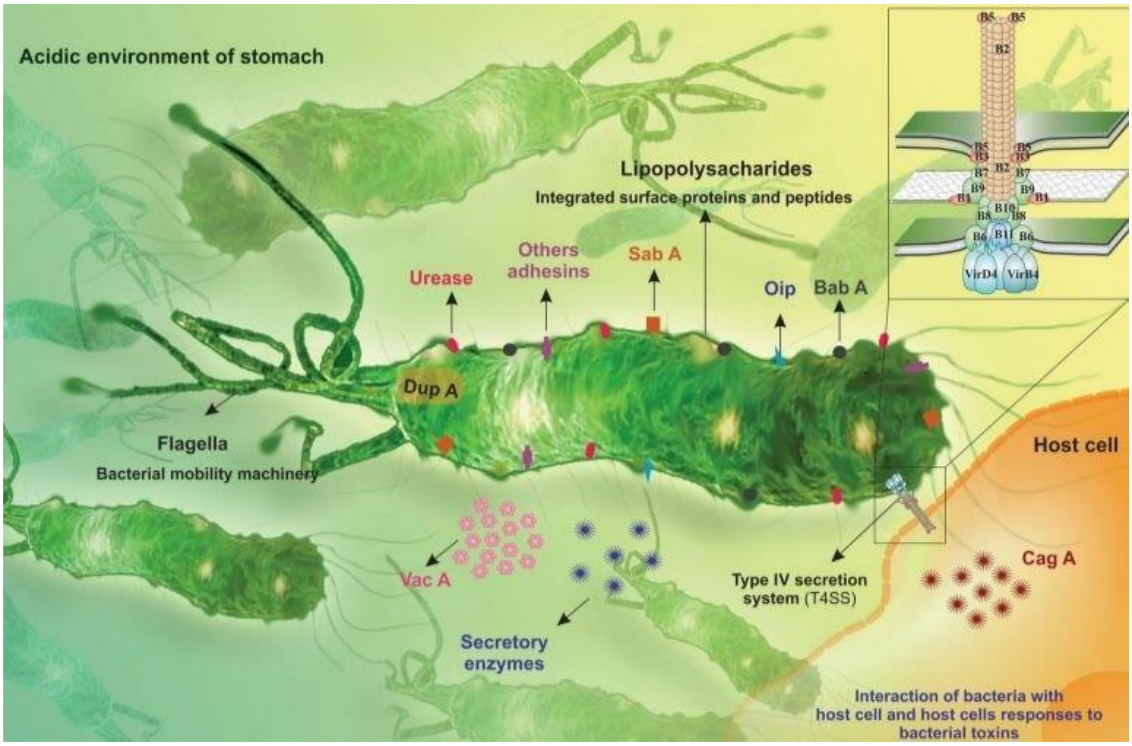

Fig. 2 *Helicobacter pylori* structure and its infection mechanism. Various bacterial entities (e.g., toxins and enzymes) are involved in the interaction of bacteria with the host cells and its evasion from the immune system surveillance. Flagella gives motility and enables the bacterium to grow under the mucosal membrane. LPS lipopoly- saccharides and membrane proteins adhere to the host cell recep- tors. Urease enzyme is used to combat the acidic environment of the stomach by producing ammonia. VacA exotoxin causes injury to the mucosal membrane. T4SS Type IV secretion system that uses a pillus to inject effectors (inset). CagA causes actin remodeling and inhibits apoptosis. Outer proteins (BabA, Oip, SabA, Others adhesins) adhere to the host cells.

Complication of *H. pylori*

H. pylori without treatment for a long time can cause the following:
-gastritis
-gastric ulcers
-duodenal ulcer
-Gastric cancer
-mucosa associated lymphoid tissue (MALT) lymphoma.

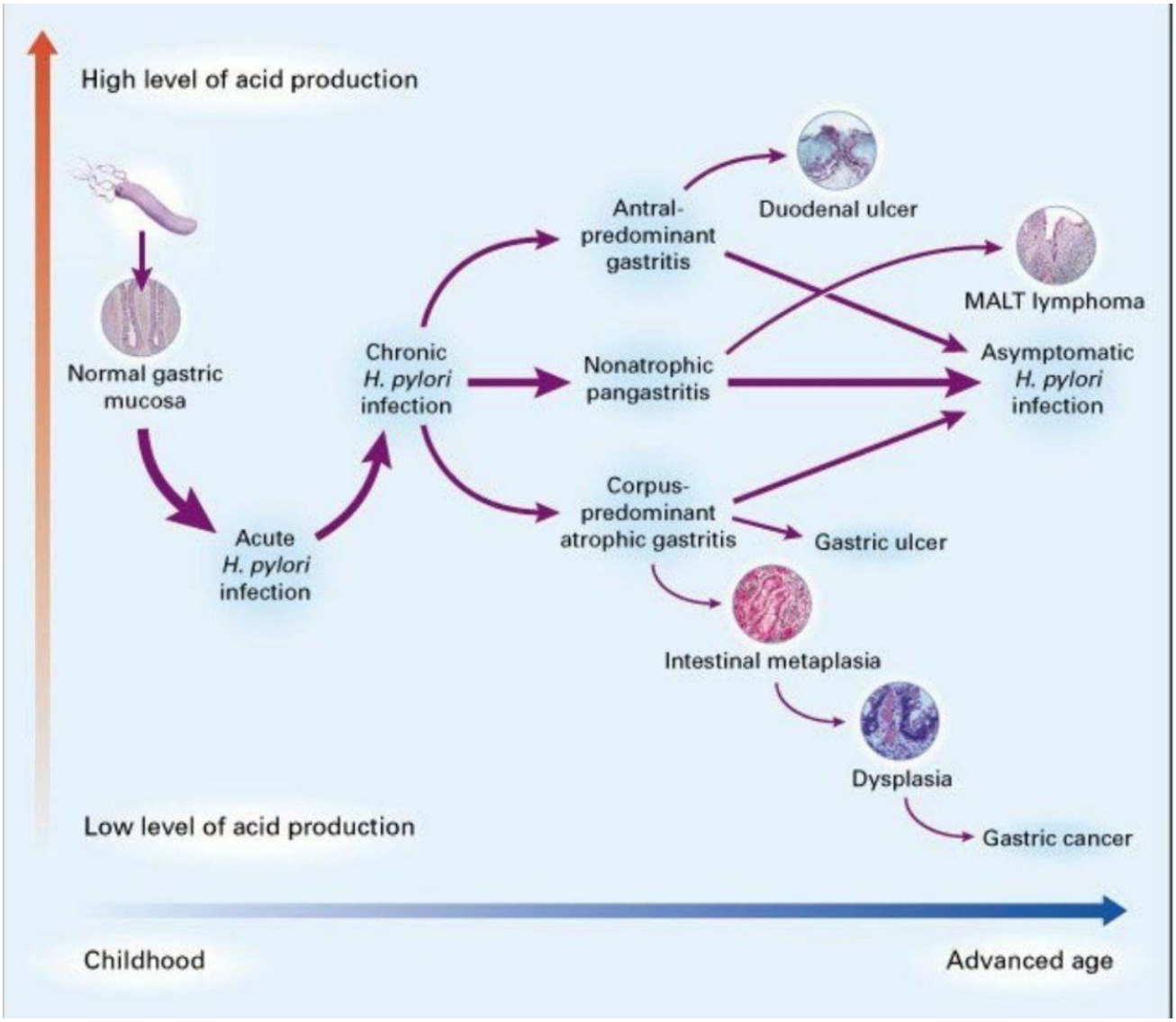

Fig 3 : complication of *H. pylori*

Tests used for examination of *H. pylori*

Since the discovery of *Helicobacter pylori (H. pylori)* in 1983, numerous detection methods for the presence of the bacterium have been developed. Each one of them has been associated with advantages and disadvantages. Noninvasive tests such as serology, 13C urea breath test (UBT) and stool antigen tests are usually preferred by the clinicians.

1-Serological Test

Immune responses against *H. pylori* are utilized to detect infection by analyzing patients' blood or serum for IgG and IgA antibodies. Serology is the only test which is not affected by those local changes in the stomach that could lead to a low bacterial load and to false negative results [20] These non-invasive tests are easy and cheap to perform. The potential for developing a rapid diagnostic test makes serology an interesting option for testing populations in areas with little or no access to medical facilities. Using an automated approach, large cohorts could be tested within a short time, allowing population based studies. they show improved sensitivity and specificity [21,22] This phenomenon could also be utilized as clinical readout to confirm treatment success by analyzing the decline of antibody responses, as shown in different studies.

2-Fecal Antigen Test

Fecal antigen tests detect antigens in stool samples.

The current guideline evaluates the use of the stool antigen test as equivalent to the UBT if a validated laboratory-based monoclonal antibody is used The sensitivity and specificity of the tests analyzed had a high variation between 48.9%–92.2% and 88.9%–94.4%, respectively, depending on the test format. that are fast and easy to use but provide less reliable results [23] available stool antigen tests have been shown to be able to distinguish infected from treated patients enabling the confirmation of treatment. Degradation of antigens in the intestine and consequent disintegration of epitopes might lead to false negative

results. Moreover, the process of sample handling could be fastidious for patients. False negative results may occur when the bacterial load is low, due to proton-pump inhibitors or the recent use of antibiotics or bismuth [24,25].

3-Urea Breath Test

The urea breath test is based on the presence of urease enzyme in live *H pylori* which breaks down urea into ammonia and carbon dioxide After ingestion of urea labelled with either 13C or 14C, breath samples are collected for up to 30 minutes by exhaling into a carbon dioxide-trapping agent The urea breath test is performed by the clinician or the clinician's assistant. been used for diagnosis of H pylori infection. [26,27]

4-Rapid urease Test

The Rapid Urease Test (RUT) is a popular invasive diagnostic *H. pylori* test that is relatively quick, cheap and simple to perform. It detects the presence of urease in or on the gastric mucosa. Best results for RUT are obtained if biopsies are taken from both the antrum and corpus. The biopsy used for RUT can also be used for other tests such as for molecular-based tests of microbial susceptibility. [28,29]

5-PCR

Test use for detection pathogenic genes and antibiotic resistance ,high sensitivity and specificity and disadvantages of it depends on the local availability of the equipment and technical experience ,Time-consuming and have Risk of contamination. [30,31]

6-Histology

The initial way of detecting *H. pylori* infection was the histological exam, and it is still the gold standard for infection detection. Several factors, including the location, size, and quantity of samples, staining procedures, proton pump inhibitor (PPI), antibiotics, all influence the diagnostic accuracy of histology. [32,33]

7- culture

A culture test means that a tissue sample is placed in a special dish or tube containing nutrients normally found in the organism's environment.

If *H. pylori* bacteria are present in the sample, they will grow until they can be seen under a microscope or in a liquid solution. culture is less sensitive but a highly specific method (specificity 100%) for the diagnosis of infections with *H. pylori.* Other benefits of this method include proof of active infection, which is recommended whenever possible in therapy failure. [34,35]

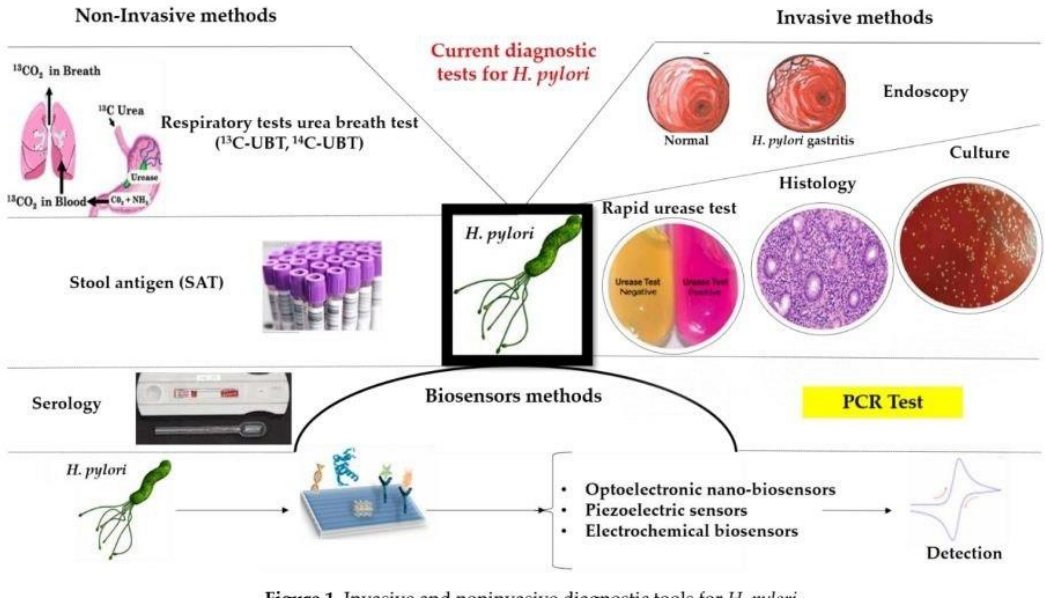

Fig 4 :tests and detection *H. pylori*

Materials and Methods

thirty five patients with PU (subjected with peptic ulcer disease) from Iraq Hospital in Basra . were included in this case-controlled study conducted from December 2022 to March 2023. Inclusion criteria for subjects were symptoms suggestive of peptic disease (burning abdominal pain, chronic vomiting or hematemesis. In the PU

patients, *H. pylori* status was determined by fecal antigen test and serological assessments of anti-H. pylori IgG antibodies. Fecal antigen tests detect antigens in stool sample and the serology for anti-*H. pylori* IgG were positive. The control group consisted of 11 asymptomatic patients with negative serology for anti-*H. pylori* IgG. control subjects had not a history of gastrointestinal disease. Individuals with a history of pulmonary disease, cardiovascular disease, diabetes mellitus, hypertension, renal failure, anemia, asthma or neoplasia were excluded from the study. Subjects in the non-infected groups were without illness and did not undergo endoscopy. Serum samples were collected from all subjects.[36]

H. pylori antibody Rapid Test

The *H. pylori* Rapid Test Device (Whole Blood/Serum/Plasma) is a rapid chromatographic immunoassay for the qualitative detection of antibodies to H. pylori in whole blood, serum, or plasma to aid in the diagnosis of *H. pylori* infection in adults 18 years of age and older.

PRINCIPLE

The *H. pylori* Rapid Test Device (Whole Blood/Serum/Plasma) is a qualitative membrane based immunoassay for the detection of *H. pylori* antibodies in whole blood, serum, or plasma. In this test procedure, anti-human IgG is immobilized in the test line region of the test. After specimen is added to the specimen well of the device, it reacts with *H. pylori* antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized anti-human IgG. If the specimen contains H. pylori antibodies, a colored line will appear in the test line region indicating a positive result. If the specimen does not contain *H. pylori* antibodies, a colored line will not appear in this region indicating a negative result. To serve as a procedural control, a colored line will always appear in the

control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred. [37]

Test Procedure

1-Remove the cassette from the sealed pouch. Add 1 drop (25ul) of serum/
plasma/whole blood vertically into the sample hole.

2-Add about 2 drops (80u1-100ul) of sample buffer into the sample hole.

3-Read result within 10 - 20minutes, the result is invalid over 20

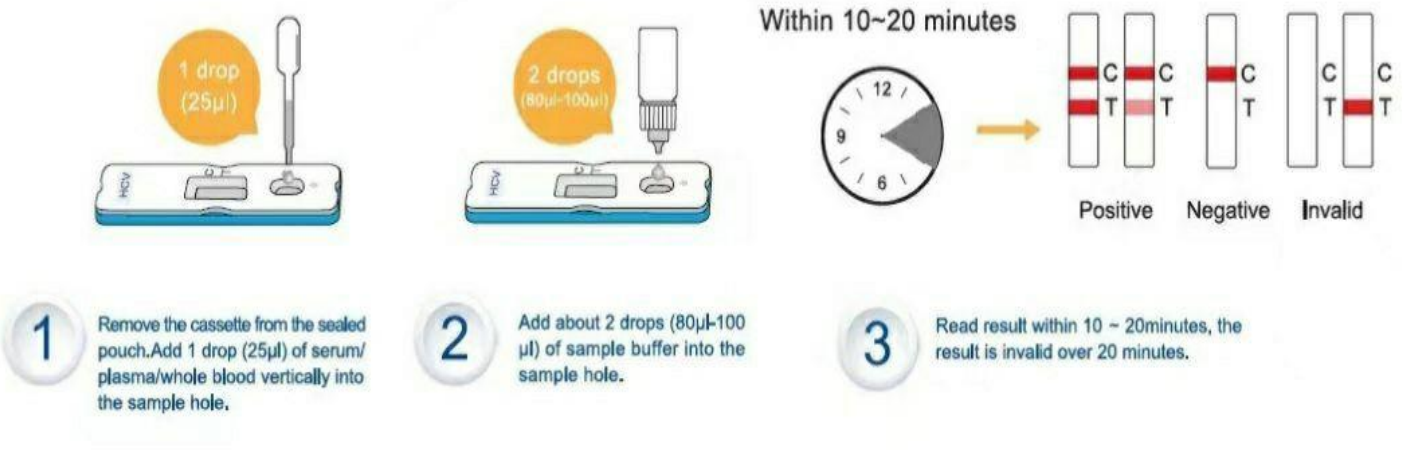

Fig 5 *: H. pylori* antibody Rapid Test procedure

H. pylori Antigen Rapid Test

Cassette is a rapid chromatographic immunoassay for the qualitative detection of *H. pylori* antigens in human faeces specimens to aid in the diagnosis of *H. pylori* infection.

Principle

H. pylori Antigen Rapid Test Cassette is a qualitative, lateral flow immunoassay for the detection of *H. pylori* antigens in human faecal specimens. In this test, the membrane is pre-coated with anti-*H. pylori* antibodies on the test line region of the test. During testing, the specimen reacts with the particles coated with anti-*H. pylori* antibodies. The mixture migrates upward on the membrane by capillary action to react with anti-*H. pylori* antibodies on the membrane and generates a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Storage Conditions is (+2 - 30 °C). [38,39]

Test Procedure

1-Collect the feces

2- Transfer 50mg Feces into the dilution buffer and mix well

3- 2 Minutes Leave the tube alone

4-Unscrew the tip

5-put 2 Drops of Specimen on cassete

6-Read result within 10 minutes, the result is invalid over 20 minutes.

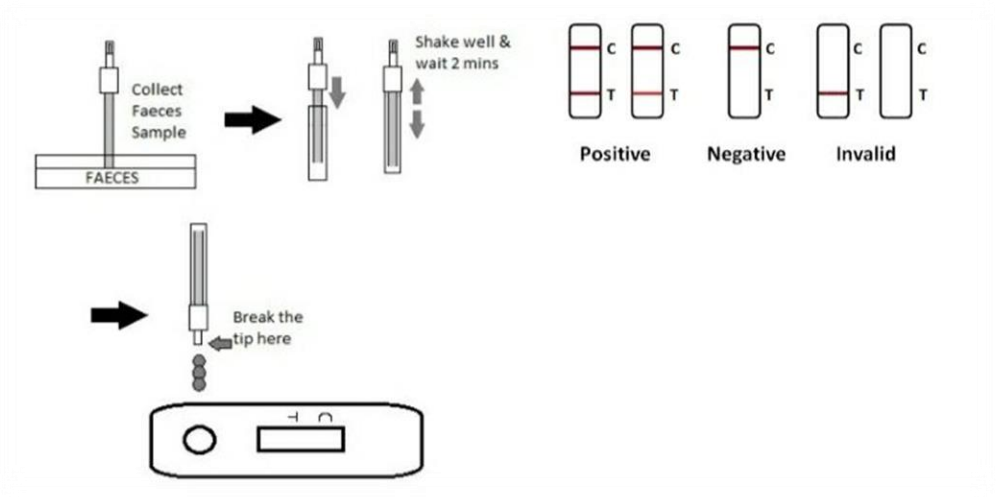

Fig. 6: Procedure of *H. pylori* Antigen Rapid Test

Complete blood count

A CBC measures the amounts of red blood cells, white blood cells, and platelets in a sample of blood. It includes measurements that represent both the actual number of cells as well as the percentage or concentration of each cell type compared to the rest of the blood volume.

Principle of CBC Analyzer The working principle of early hematology analyzers was based on Coulter's Principle. However, they have evolved to encompass numerous techniques such as Flow Cytometry, & Spectrophotometry.

The Procedure of CBC Test:

-Collect about 2 ml of the blood sample in a lavender top tube (EDTA vail).

-Shake the tube softly and make sure the blood is mixed well with EDTA in the vail to avoid clotting.

-Turn "ON" the rotor.

-Put the vail on the rotor to mix the blood sample with the anticoagulant.

-Turn "ON" the hematology analyzer.

-Click on the new sample.

-Click on ID 1, and type the unique ID of the sample and click OK.

-And then, click on ID 2 and type the name of the patient and click OK.

-Then introduce the blood sample to the hematology analyzer (CBC machine).

-After waiting for 1-2 minutes, the result will be displayed on the screen.

-Now print the result, and turn off the machine. [40]

Results and Discussion

Helicobacter pylori causes a chronic infection in gastric mucosa, but its systemic effects are largely unknown. The present study aims to characterize the effect of *H. pylori* infection and gastric mucosal inflammation on the peripheral blood leukocyte count. Fecal Antigen Test ,Serological Test of 35 patients (14 men and 21 women) was studied. The severity of inflammation in antral and body mucosa was estimated. The peripheral blood leukocyte count and differential count were determined by the automatic complete blood count method.

In general the total number of blood leukocytes and the numbers of neutrophil were significantly increased in H. pylori-positive patients (N = 24), as compared with H. pylori-negative ones (N = 11). The total number of blood leukocytes correlated with the numbers of neutrophils in the gastric mucosa. The results show that mucosal inflammation due to H. pylori infection is reflected in the amount of peripheral blood leukocytes. A detailed explanation of this is shown in the figure below (each figure with its details)

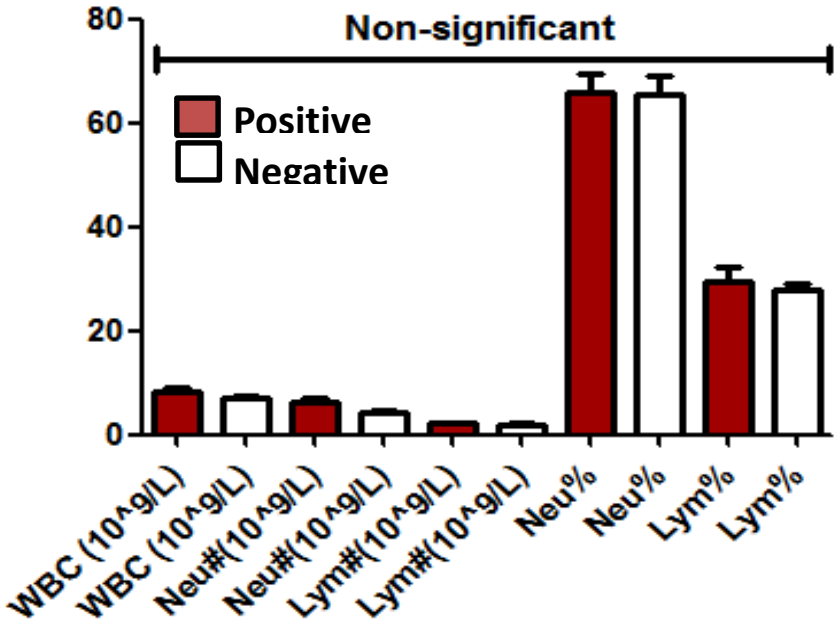

Fig. 7 : Relationship between WBC ,neutrophil ,lymphocyte in meal and female with *H. pylori* infected patient

As shown in the(figure7) that summarized the amount and percentages of different immune cells like WBC, neutrophils, and lymphocytes. The results were non-significant as totally comparison among positive and negative individuals. The small size of sample may be did not explore real effect of *H. pylori* on immune cells as measured by automated CBC. While other studies appeared .

NOTE : The figure above show compares between WBC(neutrophil and lymphocyte) as absolute number while the seventh column on right side show percentage number therefor it had seen defrient in the elevation of column

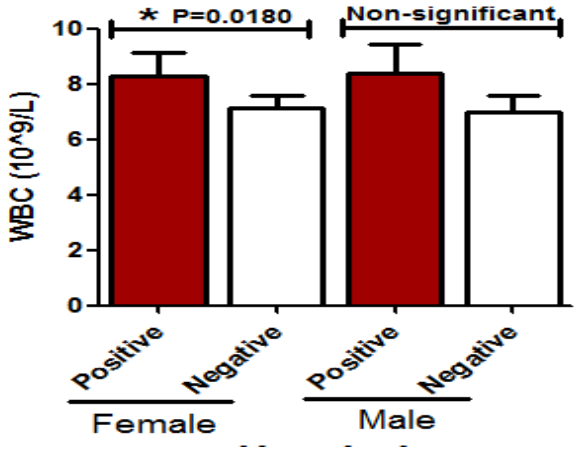

Fig. 8 : Relationship between WBC in meal and female with *H. pylori* infected patient

The comparison the infection between control and infected with *H. pylori* between male and female were shown significant different among WBC positive and negative in female which in male was no significant.

present studies have shown that women have a better immune system against infections than men. This is due to the genetic structural difference. This genetic structure is known as microRNAs. This microRNA is located on the female X chromosome. The prime role of microRNAs (miRNAs) in the immune function includes an innate and adaptive immune response, development and differentiation of immune cells to pathogens, and prevention of developing autoimmunity. Thus these microRNAs give women an immunity advantage over men.

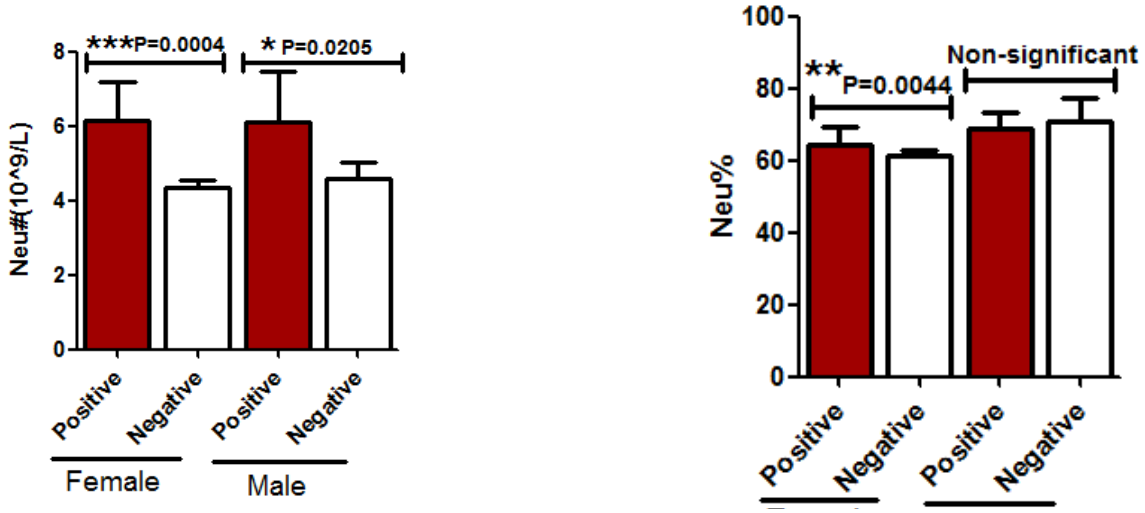

Fig. 9 : Relationship betwen neutrophil in mel and femal with H. pylori infected patient

When each immune cell were comprised as indicator for *H. pylori* infection among healthy and infected patient the figure above show high significant increase of neutrophil in female for positive and negative that is indicator it is possible to depend on neutrophil as prognosis ,diagnosis for infection with *H. pylori* the above explanation could be generalized for male also were show significant variation among positive and negative infection h pylori with p value =0.02

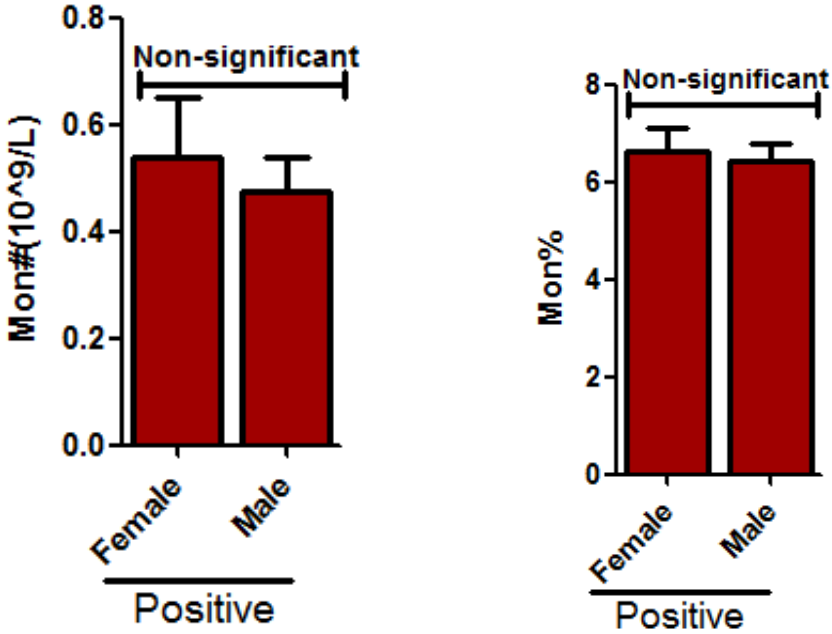

Fig 10 : mon#, mon% in *H. pylori* infected patient

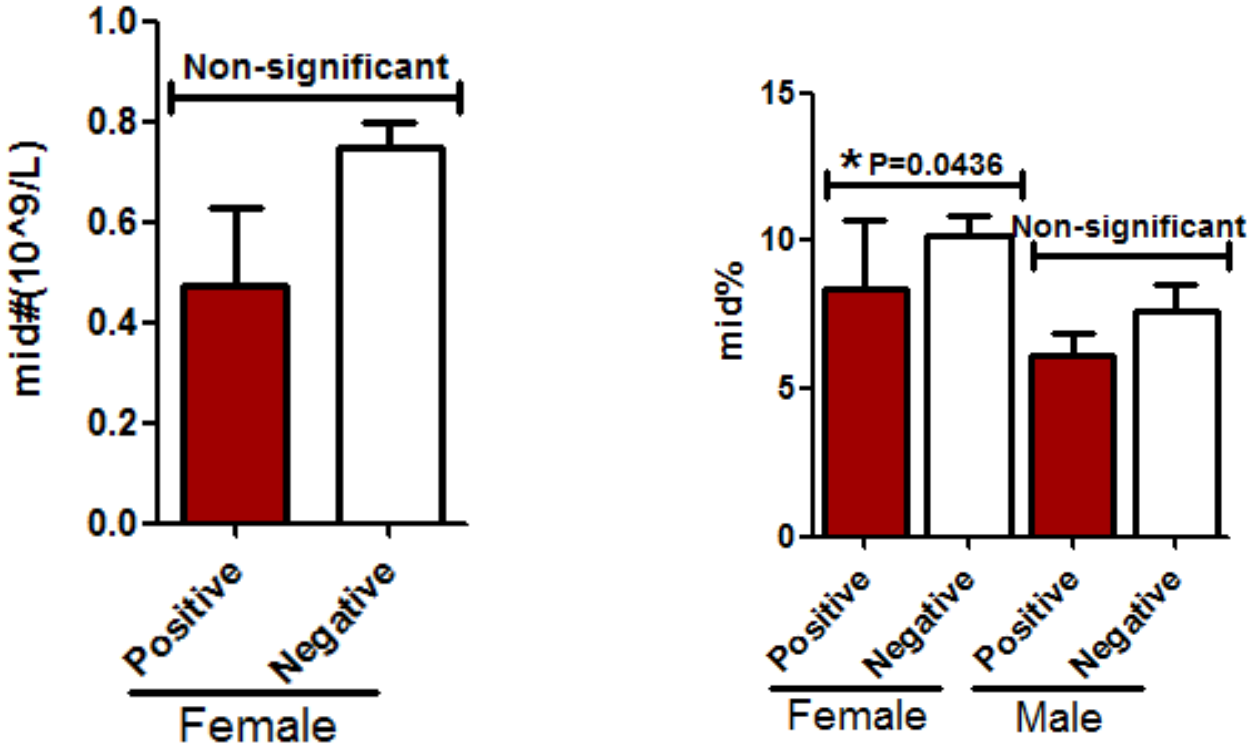

Fig 11 : mid#, mid% in *H. pylori* infected patient

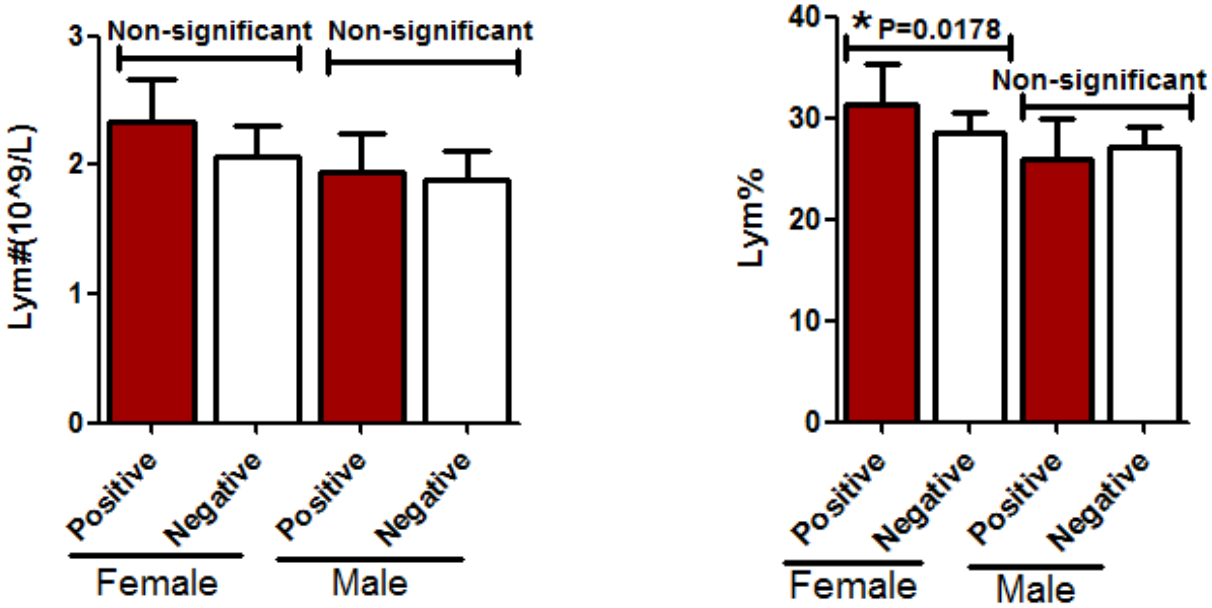

Fig 12 : lym#, lym% in *H. pylori* infected patient

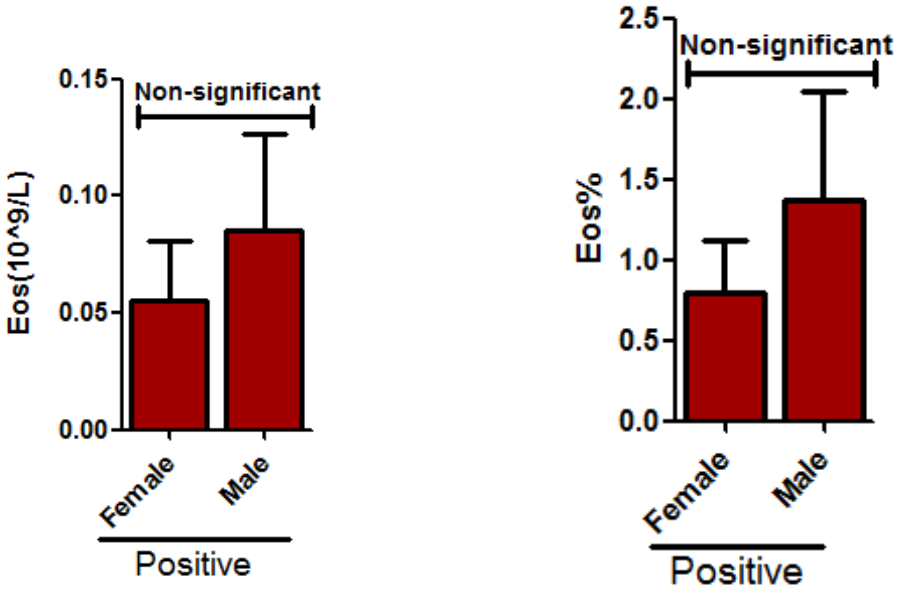

Fig 13 :Eso#, Eso% in *H. pylori* infected patient

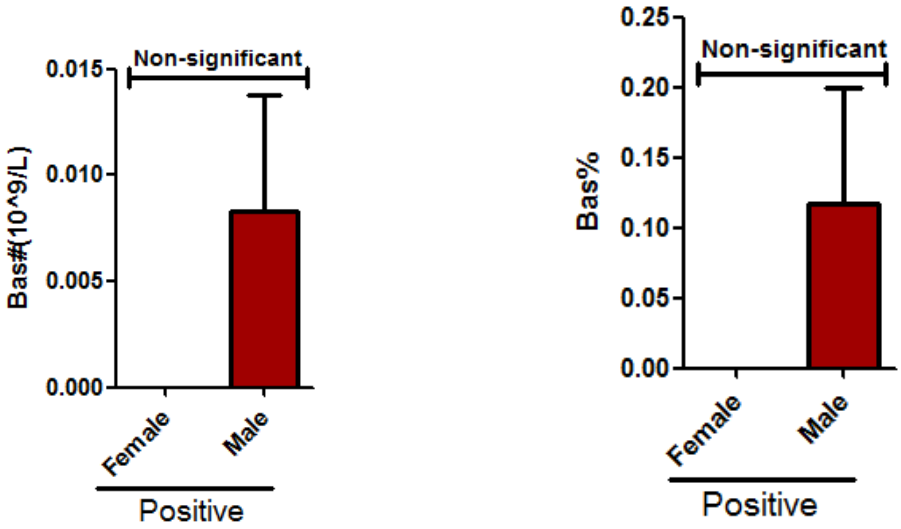

Fig 14 : Bas#, Bas% in *H. pylori* infected patient

The present study agree with scientific approch of immune response regarding Eosinophil, Lymphocyte ,and other immune cell not correlated with bacterial infection therefor as shown it has not significant variation among cell .

while other studies appeared (A Jafarzadeh, V Akbarpoor, M Nabizadeh, M Nemati and MT Rezayati)

Among controls the total WBC counts ranged from 5,000 to 10,000 cell/mm3. Leukocytosis (WBC >10,000 cells/mm3) can be attributed to infection, inflammation, tissue damage, burns, dehydration, thyroid storm, leukemia, stress or steroids (George and Panos, 2005). In this study a higher total WBC count, neutrophil count and NLCR were seen among *H. pylori*-infectedPU patients and in the AS group compared to the control group. A study from Japan found *H. pylori* eradication decreases blood neutrophil and monocyte counts (Kondo et al, 2004). The mechanism causing a higher total WBC count and neutrophil count in H. pylori infections remains to be determined. The generation of leukocytes from bone marrow stem cells is potentiated by proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1 and IL-6 (Hawley et al, 1991). An increased production of TNF, IL-1, IL-6 and IL-8 has been reported among *H. pylori*-infected individuals (Romero-Adrian et al, 2010). These cytokines may enhance the differentiation of WBC during H. pylori infection. In the present study, a higher NLCR was observed among subjects infected *with H. pylori*; this reflects an increased neutrophil count without a change in lymphocyte count. These observations suggest.

H. pylori-infected subjects produce greater numbers of neutrophils. The proinflammatory cytokine IL-17A, the signature cytokine of TH17 cells, has been shown to increase neutrophil counts via induction of G-CSF (von Vietinghoff and Ley, 2009). IL-17A expression is also increased in *H. pylori*-infected individuals (Jafarzadeh et al, 2009b; Kimang'a et al, 2010). Interestingly, it has been demonstrated *H. pylori*-derived neutrophil-activating protein (HP-NAP) increases the lifespan of neutrophils (Cappon et al, 2010). The results of the present study show the mean total WBC count and the mean neutrophil were significantly higher in the AS group compared to the control group. These

observations are consistent with our previous findings regarding differences in CRP, a sensitive inflammatory marker, among AS and *H. pylori*-negative control groups (Jafarzadeh et al, 2009a). The higher total WBC and neutrophil counts among the AS group may be due to the induction of subclinical microinflamatory reactions caused by H. pylori. We also studied the association among CagA antibodies and total WBC counts and neutrophil counts among *H. pylori* infected subjects. The results show no differences in WBC or neutrophil counts among PU and AS groups in relationship to cagA+ *H. pylori* strains. In our previous study, we found CRP levels were not influenced by the expression of bacterial CagA virulence factor (Jafarzadeh et al, 2009a). It has been reported cagA+ H. pylori strains cause more serious gastric inflammation than cagA-negative strains and are associated with a higher risk for PU disease and gastric cancer (Costa et al, 2009).Some studies, failed to find an also

citation between inflammatory cytokines, such as IL-6 and TNF-a, and virulence factors, such as CagA (Kim et al, 2000). Polymorphism among genes encoding for cytokines, such as IL-1, TNF-a and IFN-g, has been associated with *H. pylori* induced gastric adenocarcinoma and peptic ulcers (Basso and Plebani, 2004). Major variations in cagA+ genotype (Lopez-Vidal et al, 2008). Both host and bacterial factors should be considered in understanding *H. pylori*-associated inflammatory responses, such as elevated WBC counts. The present study showed no significant differences between CagA-positive and CagA-negative strains regarding the total WBC counts and total neutrophil counts. Total WBC and neutrophil counts were independent of the CagA status among H. pylori strains. Therefore, the total WBC and neutrophil counts were not suitable markers to determine *H. pylori* strains. Monitoring the total WBC and neutrophil counts and NLCR prior to, during and after treatment of PU disease may improve predictive or prognostic values. Our results suggest further studies are needed in this field. In

conclusion, the results of the present study show higher total WBC counts and NLCR among PU and AS groups compared to controls. However, these parameters were not impacted by bacterial CagA status. [41,42,43,45]

while other studies appeared (Associate Professora , Yasin Sahin, M.D.a , Ozlem Gubur, M.D.b and Emine Tekingunduz, M.D.b)

More than half of the world's population is still infected with HP. Approximately 4.4 billion people were reported to be infected worldwide in 2015.1 The prevalence of HP is high in developing countries and is often associated with socioeconomic level and hygiene situation.1 The leukocytes and its subgroups, and NLR have been shown to be indicators of systemic inflammation in previous study.19 There is limited study investigating the association between HP infection and NLR and MPV.10,13-15 Guclu M et al.15 did not find any significant difference between patients with HP positivity and negativity in regards to NLR and MPV. The lymphocyte and thrombocyte values were within the normal range in patients with HP positivity, but were significantly higher than in patients with HP negativity. The reason for this increase is probably the increase in absolute lymphocyte levels. Also, the number of cases with severe HP gastritis was low. Authors suggested that studies with higher number of severe HP gastritis are needed. As compatible with other study, there was no any changes in MPV values in HP positive patients.10 In a study including 50 HP positive and 50 HP negative patients, the leukocyte, lymphocyte and neutrophil counts were found higher in HP positive patients than HP negative patients.13 Higher NLR values were also detected in HP positive patients. In addition, higher NLR values were associated with severity of gastritis and increased symptoms. HP negative patients had significantly lower NLR levels. It has been shown that higher NLR values returned to normal levels after successful treatment and eradication. The authors suggested that NLR can be used in the follow-up of patients after

successful treatment. In contrast to this study, although we have 3 times higher patients than Farah et al.13 study, we did not detect higher NLR in patients with HP positive. As compatible with this study, we found a decrease in NLR after HP eradication treatment, but this is not statistically significant. Further studies with more HP infected patients are needed to be confirmed. In Jakarzadeh et al.14 study, mean leukocyte count, neutrophil count and NLR were significantly higher in patients with HP positivity and asymptomatic group than control group.14 The mean leukocyte count, neutrophil count and NLR were significantly different between the asymptomatic group and HP positive group. In addition to that, no difference was found between the 3 groups in terms of lymphocyte counts. The authors suggested that higher leukocyte and neutrophil counts in the asymptomatic group are probably due to subclinical microinflammatory reactions caused by HP. In the current study, no significant difference was detected between patients with HP positivity and negativity in regards to NLR and MPV. There was no statistically significant difference between subgroups of HP positive patients (mild, moderate and severe) in regards to MPV and NLR rates ($p > 0.05$). When pre and posttreatment values of NLR and MPV of 88 patients who received HP eradication treatment were compared, we also did not find any significant difference between them ($p > 0.05$). It has been shown a decrease in neutrophil counts after HP eradication in a study conducted in Japan.20 As consistent with this study, we also detected a decrease in neutrophil counts, but these differences were not statistically significant. As compatible with previous two studies, we found that NLR and MPV values did not correlate with the severity of HP infection in children.10,15 The reason why we could not detect any relationship may be that there is a small number of patients with severe HP infection in the current study. Because of that, further studies with more patients with severe HP infection may be needed.

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