ASSOCIATION BETWEEN THE SEVERITY OF *Helicobacter pylori* INFECTION AND DIFFERENT HEMATOLOGICAL PARAMETERS

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of MS in Biotechnology

Department of Mathematics and Natural Sciences

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Declaration

It is hereby declared that

1. The thesis submitted is my original work while completing my degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material that has been accepted or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled "Association between the severity of *Helicobacter pylori* infection and different hematological parameters" submitted by Afra Abreshmi (20176004) of Spring, 2020 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Masters of Science in Biotechnology on 15 th January 2023.

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5. All sources used are properly disclosed (correct citation).
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Abstract

Helicobacter pylori infection is a global public health problem that is affecting both developed and developing countries. Approximately 50% (over 3 billion) of the world's population is infected with *Helicobacter pylori*, mainly in developing countries. *H. pylori* is a gram-negative bacterium that is commonly found in the stomach. The vast majority of *H.pylori*-infected people show no symptoms and never develop any problems. However, this bacterium is capable of causing ulcers and less commonly stomach cancer. Recently, its association with some hematological abnormalities has been found. Compared to other developing countries, in Bangladesh, the rate of *H. pylori* infection is particularly high. However, limited studies have been conducted regarding the relationship between hematological parameters with *H. pylori* infection in Bangladesh. Thus, the objective of our study was to determine the correlation between different selected hematological parameters and *H. pylori* infections of different intensities. A total of 697 patients, 290 males, and 407 females, who were suspected *H. pylori* patients, were included in the study. The suspected patients infected with *H. pylori* titer less than 30 U/ml were considered *H. pylori*-negative and more than 30 U/ml were under *H. pylori*-positive group. Patients in *H. pylori*-positive group were divided into a subgroup of severe intensity (titer more than 50 U/ml). Our study found a weak positive correlation between Lymphocyte and *H.pylori* (p= 0.0253*) and between Neutrophil to Platelet ratio (NPR) and *H.pylori* (p=0.008*) in the *H.pylori*-positive group. In the sub-group of severe intensity, a weak positive correlation between Hemoglobin and *H.pylori* (p= 0.01126*) and between NPR and *H.pylori* (p= 0.0362*) was observed. There was a very weak negative correlation found between Platelet and *H.pylori* (p= 0.0005*) and between Platelet to Lymphocyte ratio (PLR) and *H.pylori* (p= 0.0001*) in the *H.pylori*-positive group. In the severe intensity group, a weak negative correlation between Platelet and *H.pylori* (p= 0.01074*) and between PLR and *H.pylori* (p= 0.0034*) was observed.

Keywords: *H. pylori* infection; Hematological parameters; Correlation; Regression Analysis

Dedication

Dedicated to my family.

Acknowledgment

First and foremost, I would like to convey my gratitude to the Almighty for keeping me healthy and giving me patience throughout my thesis work.

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(Afra Abreshmi)

January 2023

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List of Acronyms

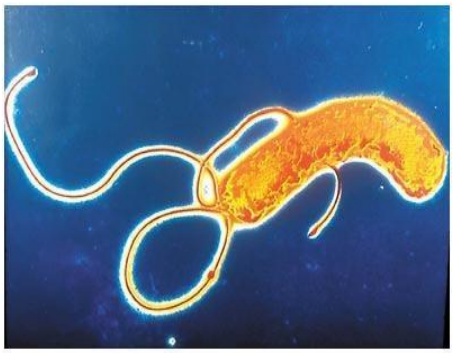
MCV	Mean Corpuscular Volume
MCH	Mean Corpuscular Hemoglobin
PLR	Platelet to Lymphocyte ratio
NLR	Neutrophil to Lymphocyte ratio
NPR	Neutrophil to Platelet ratio
HB	Hemoglobin
RBC	Red Blood Cell
CBC	Complete Blood Count
Fl	Femtoliter
Pg	Picogram

Chapter 1

Introduction

Helicobacter pylori, previously known as *Campylobacter pylori,* is one of the most common infectious agents in the world. It has been estimated that more than 50% of the world's population has *H. pylori* in their upper gastrointestinal tract. However, the prevalence of *H. pylori* infection is much more common in developing countries; including Bangladesh.

The name "Helicobacter" derives from its helical structure. It's a Gram-negative bacterium of about 3 μm long with a diameter of about 0.5 μm. It has four to six flagella at the same location which gives its high motility. According to the World Health Organization, *Helicobacter pylori* have been classified as a carcinogen of class I, (Papagiannakis et al., 2013).



Helicobacter pylori

1.1 *H. pylori* pathogenesis

The understanding of *H. pylori* pathogenesis has been improved by studies that focus on the host and bacterial factors through epidemiology research and molecular mechanism investigations over the past 20 years (Kao et al., 2016). After entering the host cell, *H.pylori* follow four major steps for successful colonization and disease pathogenesis. These steps are as follows:

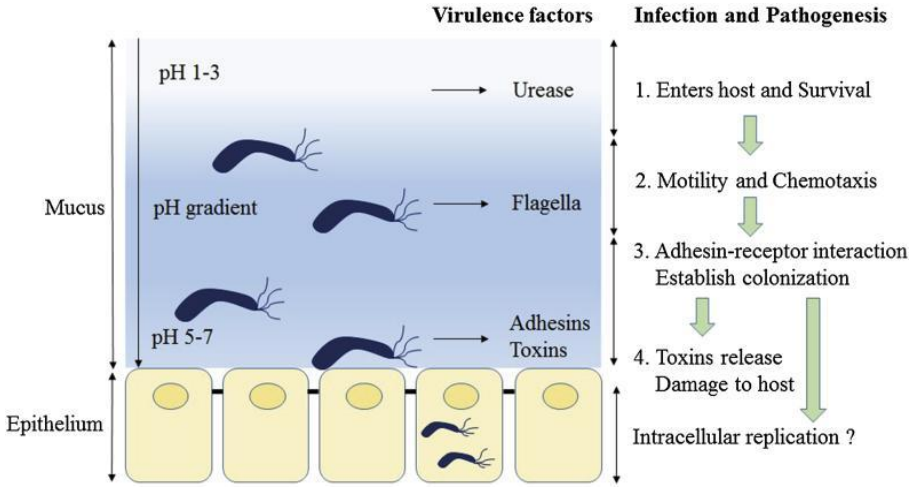
(1) Survival in the acidic stomach

(2) Movement toward epithelium cells by flagella-mediated motility

(3) Attachment to host cells by adhesins/receptors interaction

(4) Causing tissue damage by toxin release.

H.pylori can survive and can move with the help of its urease activity and flagella-mediated motility toward the lower mucus gel above the epithelium. This is then followed by several adhesins, which include blood-antigen binding protein A, sialic acid-binding adhesin, and other outer membrane proteins which interact with receptors on the host epithelium cells. After successful colonization, cytotoxin-associated gene A, and vacuolating cytotoxin A, which are types of toxins, get involved in the damage to host tissue and intracellular replication. (Kao et al., 2016)



Schematic diagram of *Helicobacter pylori* infection and pathogenesis.

1.2 Hematological parameters and *H. pylori* infection

Although *H. pylori* is one of the most common infectious agents in the world, the role of *Helicobacter pylori* infection in hematological system diseases is not well understood (Yan-xu et al., 2017). However, there has been an association found between this microorganism and different hematological parameters. The hematological parameters selected for this study were; Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), RBC, Platelet, Hemoglobin, Neutrophil, Lymphocyte, Neutrophil to Lymphocyte ratio (NLR), Neutrophil to Platelet ratio (NPR), and Platelet to Lymphocyte ratio (PLR).

1.3 Selected hematological parameters

A total of ten hematological parameters were selected for the study. The significance of each of the parameters has been discussed.

1.3.1 Mean corpuscular volume (MCV)

MCV blood test measures the average size and volume of the red blood cells. This is often a part of the Complete blood test (Medline, 2019). The results may indicate various types of diseases. For instance; if the cell size is smaller than usual, it may indicate that the person may have certain types of anemia, most commonly iron-deficiency anemia or even thalassemia. On the other hand, if the cell size is larger than usual then it may indicate Pernicious anemia caused by a lack of vitamin B12. A typical adult MCV level is 80–100 femtoliters (fl). If someone's MCV level is below 80 fL, they will likely develop microcytic anemia. Alternatively, if their MCV levels are greater than 100 fL, they could develop macrocytic anemia.

1.3.2 Mean corpuscular hemoglobin (MCH)

MCH refers to the average quantity of hemoglobin present in a single red blood cell. MCH results tend to mirror MCV results. This is because the larger the red blood cell is, the more it contains hemoglobin in it. MCH is also included in the CBC panel. The normal range for MCH is between 27.5 and 33.2 picograms (pg). MCH below this optimum range may indicate iron-deficiency anemia. As iron is important for the production of hemoglobin, if iron is deficient, it results in a lack of hemoglobin. In rare cases, this may also indicate thalassemia. As the production of hemoglobin is limited, this indicates that insufficient RBC is being circulated in the bloodstream. On the other hand, high levels of MCH often indicate anemia caused by vitamin deficiency, particularly B12 and folate.

1.3.3 RBC, Platelet, and Hemoglobin

RBC count is the test that measures the red blood cells in the blood. The optimum range of RBC count in adults is 4.2-5.4 million cells/µL (female adult) and 4.7-6.1 million cells/µL(male adult). RBC lower than the optimum range may indicate several underlying diseases. These include; iron-deficiency anemia, aplastic anemia, hemolysis, rheumatoid arthritis, multiple myeloma, etc. However, an RBC count of more than the normal range may be caused by primary erythrocytosis. This is when the body produces more RBC than normal. One such condition is called polycythemia vera. Secondary erythrocytosis is when the body makes more RBC due to external factors.

Platelet count is the test that measures the platelet in blood. The normal range of platelet count is between 150,000 and 400,000 platelets per microliter. If the platelet is more than the normal range it can put someone at risk of blood clot or stroke. Platelets in less than the normal range may be a sign of cancer, viral or bacterial infections, or other health problems. A low platelet count is referred to as thrombocytopenia and a high platelet count is called thrombocytosis.

The normal range for hemoglobin is 13.2 to 16.6 grams per deciliter (male) and 11.6 to 15 grams per deciliter (female). If the hemoglobin level is lower than the normal range it may indicate anemia caused by several reasons. These include; Iron deficiency, Vitamin B-12 deficiency, Folate deficiency, Bleeding, Kidney disease, Liver disease, Thalassemia, etc. Hemoglobin in more than normal range can be due to polycythemia vera, lung disease, dehydration, etc.

1.3.4 Neutrophils and lymphocytes

Neutrophils are the most common type of white blood cells. They make up around 50% to 80% of all white blood cells in our body. The normal range of neutrophils in a healthy adult is approximately between 2,500 and 7,000 neutrophils per microliter of blood. If the neutrophil count is too low, the condition is called neutropenia. Some of the most common causes of neutropenia can be infections, vitamin D deficiency, autoimmune disease, etc. On the other hand, Neutrophilia or neutrophilic leukocytosis is caused when the neutrophil count is more than the normal range. This is often the result of bacterial infection.

Lymphocytes are another type of immune cell that is composed of T-cells and B-cells. T cells are further divided into cytotoxic T-cells, helper T-cells, and regulatory T-cells. Each of these cells has specific functionality. B-cells are responsible for primary and secondary immune responses in our body. In adults, the normal range of lymphocytes is approximately between 1,000 and 4,800 lymphocytes in every 1 microliter of blood. High levels of lymphocytes are called lymphocytosis, which can be caused as a result of an immune response to infection, or maybe even due to cancer; like lymphoma or leukemia. Lymphopenia or lymphocytopenia is when the lymphocyte is lower than the normal range. This can be caused due to Tuberculosis or typhoid fever,HIV or AIDS, Viral hepatitis, blood diseases such as Hodgkin’s disease, etc.

1.3.5 Neutrophil to lymphocyte ratio (NLR)

NLR is a new addition to the list of inflammatory markers. The N/L ratio is a readily measurable laboratory marker that is used to evaluate systemic inflammation (Balta et al., 2013). The neutrophil to lymphocyte ratio is calculated by dividing the absolute value of neutrophil by the absolute value of lymphocyte. Studies have suggested that the neutrophil-to-lymphocyte ratio (NLR) can be used as an independent prognostic factor in a variety of cancer types. Additionally, NRL is indicative of impaired cell-mediated immunity which is associated with systemic inflammation (Faria et al., 2016). Moreover, NLR is reported as an independent predictor of outcomes in stable coronary artery disease, as well as a predictor of short- and long-term mortality in patients with acute coronary syndromes (Bhat et al., 2014). So, changes in the normal levels of the NLR marker can be an indicator of underlying conditions. However, only the NLR biomarker itself may not give the overall perception of the disease. NLR is also useful in predicting and detecting inflammatory and infectious conditions, and also their postoperative complications. Higher NLR itself is an indicator of mortality in patients undergoing angiography or cardiac revascularization. Increased NLR may also indicate a poor prognosis for various cancers. Recently, it has been found that NLR can be used as a prognostic marker for COVID-19 where a significant difference was found in NLR between those who died and recovered from COVID-19 (Eslamijouybari et al., 2020). Although the Neutrophil-to-lymphocyte ratio (NLR) has proven its prognostic value in cardiovascular diseases, inflammatory diseases infections, and several types of cancers, no cut-off has been proposed as of yet, based on reference values obtained from healthy populations (Forget et al., 2017). However, according to Forget et al, the normal range of NLR in healthy adults has been identified as between 0.78 and 3.53.

1.3.6 Neutrophil to Platelet ratio (NPR)

The neutrophil-to-platelet ratio is a novel inflammatory marker. Studies have shown that the neutrophil-lymphocyte ratio (NLR) and the neutrophil-platelet ratio (NPR) are inflammatory markers involved in the prognosis of certain inflammatory pathologies (Safa et al., 2021). A recent study has found that a high neutrophil-to-platelet ratio (NPR) was associated with long-term poor outcomes in patients with acute ischemic stroke (He et al., 2019). However, the association between NPR and *H.pylori* infection needs further studies.

1.3.7 Platelet to Lymphocyte ratio (PLR)

The platelet-to-lymphocyte ratio (PLR) is a novel inflammatory biomarker that can be applied to many diseases for the prediction of inflammation and mortality. Recently, many studies indicated that in patients with cardiovascular disease, PLR is a strong and independent prognostic factor (Lian ye et al., 2019). It has been found that compared to other prognostic indices that show inflammation, NLR and PLR demonstrate mortality in patients who have gastrointestinal perforation much better (Boyuk et al., 2020).

1.4 Objectives

H. pylori is one of the most common infectious agents responsible for causing benign stomach diseases, such as chronic gastritis and duodenal and gastric peptic ulcers. However, it has been reported to cause various hematological disorders, such as iron deficiency anemia, immune thrombocytopenic purpura, and vitamin B12 deficiency. Studies found that *H. pylori* infection was significantly associated with a decrease in serum ferritin, MCV, and MCH. Based on the above findings it can be said that *H. pylori* infection has an association with various hematological parameters. Additionally, the prevalence of *H. pylori* in developing countries is 80%-90. Bangladesh is a developing country, and also has a high prevalence of this infectious agent. However, there has been no significant study on the association between *H. pylori* infection and hematological parameters in Bangladesh. Thus, the main objective of this study was to determine any correlation between *H. pylori* infection of different intensities and selected hematological parameters.

Chapter 2

Materials and method

2.1 Data Collection

The data collection was conducted in a diagnostic facility of a tertiary care hospital. The data collection duration was from January 2022 to September 2022.

2.2 Study participants and inclusion criteria

The study was conducted on a total of 697 patients of which 290 were males, and 407 were females. The subjects were suspected *H. pylori* patients. The suspected patients who were infected with *H. pylori* titer less than 30 U/ml were considered *H. pylori*-negative and more than 30 U/ml were under *H. pylori*-positive group. A total of 402 *H.pylori*-negative and 295 *H.pylori*-positve patients were included in the study. Patients in *H. pylori*-positive group were sub-divided into a group of severe intensity (titer more than 50 U/ml). The *H.pylori*-positive group of severe intensity had 160 patients.

2.3 Statistical analysis

For the statistical study and analysis of the data, programming languages R (version 4.2.1) and RStudio (version 2021.09.2+382) were used. The statistical differences in the means of different parameters amongst the groups were calculated using the two-sided t-test method. The results were presented as mean ± standard deviation (SD). The correlation analysis between different parameters and *H.pylori* infection in *H.pylori*-positive patients was conducted. The correlations were visualized using a scatterplot.

Chapter 3

Results

3.1 Statistical difference in the mean value of the parameters between *H.pylori*-negative and positive patients with a titer of more than 30 U/ml

Amongst 402 *H.pylori*-negative and 295 *H.pylori*-positive patients in the *H.pylori*-positive group, there have been no statistical differences found in terms of NLR, NPR, PLR, MCV, MCH, neutrophil, platelet, lymphocytes, and hemoglobin. However, the RBC in *H.pylori*-positive patients has been found statistically significantly higher than *in H.pylori*-negative patients (4.736±0.607vs 4.586±0.633 respectively, p= 0.00065*) (Table 1).

Table 1: Comparison of *H. pylori*-negative and *H. pylori*-positive patients with a titer of more than 30 U/ml

Parameters	*H.pylori* negative	*H.pylori* positive Titer more than 30 U/ml	p-value
NLR	1.800± 1.08	1.815±1.04	0.773
NPR	0.2073330±0.09	0.2065± 0.09	0.864
PLR	8.7793±5.32	9.1634±4.54	0.132
MCV	84.258±6.72	83.87±7.26	0.309
MCH	27.867±2.76	27.81±3.10	0.723
RBC	4.586±0.633	4.736±0.607	0.00065*
Neutrophil	59.362±10.14	60.6892±10	0.060
Lymphocyte	32.727± 9.42	32.023±8.40	0.2705
Platelet	287.804±87.29	291.52±86.17	0.537
Hemoglobin	12.764±1.83	12.9±1.94	0.167

*Statistically significant ($p<0.05$)

3.2 Statistical difference in the mean value of the parameters between *H.pylori*-negative and positive patients with a titer of more than 50 U/ml

Amongst 402 *H.pylori*-negative and 160 *H.pylori*-positive patients in the severe intensity group, there have been no statistical differences found in terms of NLR, PLR, NPR, neutrophil, lymphocyte, MCH, MCV, platelet, and hemoglobin. However, the RBC in *H.pylori*-positive patients in this group has been found statistically significantly higher than *H.pylori*-negative patients (4.711±0.62 vs 4.586±0.633 respectively, p= 0.0175*) (Table 2).

Table 2: Comparison of *H. pylori*-negative and *H. pylori*-positive patients with a titer of more than 50 U/ml

Parameters	*H.pylori* negative	*H.pylori* positive	p-value
NLR	1.8007± 1.08	1.7297±0.94	0.227
NPR	0.2073330±0.09	0.212053±0.082	0.417
PLR	8.7793±5.32	8.7051±4.29	0.813
MCV	84.2587±6.72	84.0964± 8.05	0.741
MCH	27.867±2.76	27.7216±3.34	0.4638
RBC	4.586±0.633	4.711±0.62	0.0175*
Neutrophil	59.36±10.14	58.930±9.66	0.6268
Lymphocyte	32.72±9.42	32.6±8.572	0.970
Platelet	287.8046±87.29	281.3718±89.003	0.3786
Hemoglobin	12.764±1.83	12.75±1.97	0.977

*Statistically significant ($p<0.05$)

3.3 Correlation analysis

In a comparison of *H.pylori* infection with different parameters in the *H.pylori*-positive group and severe intensity group, there were some correlations observed in terms of NPR, PLR, platelet, hemoglobin, and lymphocytes with *H. pylori* infection. In the *H.pylori* positive group (table 3), a very weak negative correlation was found between platelet and *H.pylori* (-0.201,p=0.0005*). In this group, a very weak negative correlation was found between PLR and *H.pylori* (-0.22, p=0.0001*). A very weak positive correlation was found between Lymphocytes and *H.pylori* (0.130, p=0.0253*). Finally, in this same group, a very weak positive correlation was found between NPR and *H.pylori* (0.15648, p=0.008*).

Table 3: Correlation between different parameters and *H.pylori* infection in *H.pylori*-positive patients (titer more than 30 U/ml)

Parameters	Correlation coefficient	p-value
RBC	0.015961	0.7867
Hemoglobin	0.039	0.5019
Platelet	-0.201	0.0005*
MCV	0.059106	0.337
MCH	0.0808	0.19
Neutrophil	-0.0951	0.11
Lymphocytes	0.130	0.0253*
NLR	-0.105	0.086
NPR	0.15648	0.008*
PLR	-0.22	0.0001*

In the *H.pylori* positive group of severe intensity (table 4), a very weak negative correlation was found between platelet and *H.pylori* (-0.2037,p=0.01074*). In this group, a very weak negative correlation was found between PLR and *H.pylori* (-0.237, p=0.0034*). A very weak positive correlation was found between hemoglobin and *H.pylori* (0.2050, p=0.01126*). Finally, in this same group, a very weak positive correlation was found between NPR and *H.pylori* (0.168,p=0.0362*)

Table 4: Correlation between different parameters and *H.pylori* infection in *H.pylori*-positive patients of severe intensity(titer more than 50 U/ml)

Parameters	Correlation coefficient	p-value
RBC	0.0882	0.2766
Hemoglobin	0.2050	0.01126*
Platelet	-0.2037	0.01074*
MCV	0.127356	0.1309
MCH	0.1262326	0.133
Neutrophil	-0.045	0.567
Lymphocytes	0.128	0.108
NLR	-0.1232	0.1205
NPR	0.168	0.0362*
PLR	-0.237	0.0034*

The following scatterplots show the correlation between the parameters and *H. pylori* infection in each of the groups.

H.pylori-positive patients with a titer of more than 30 U/ml

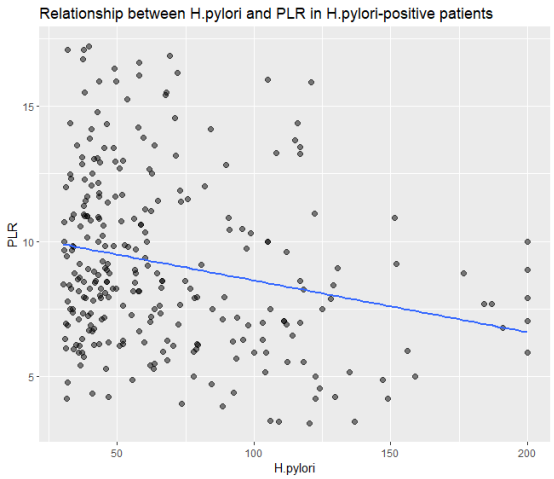


Fig 1: Weak negative correlation between *H.pylori* and PLR in *H.pylori*-positive group (p= 0.0001*)

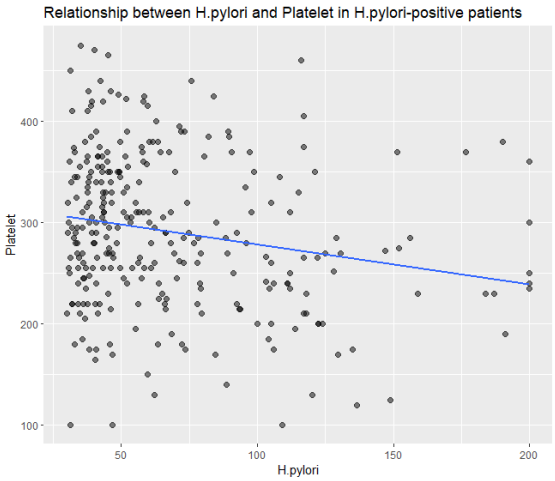


Fig 2: Weak negative correlation between *H.pylori* and platelet in *H.pylori*-positive group (p=0.0005*)

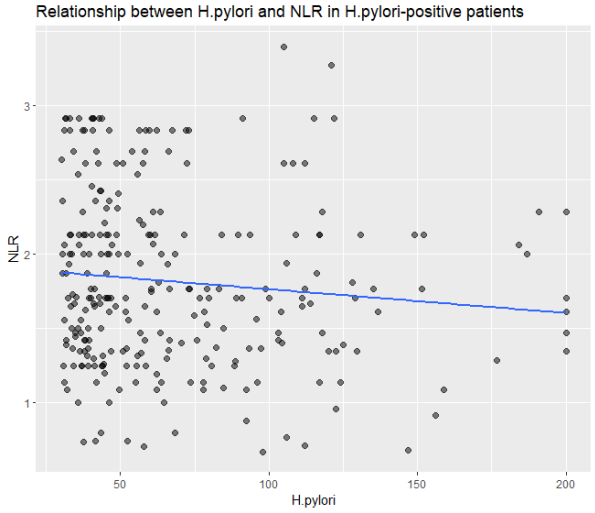


Fig 3: No significant correlation between *H.pylori* and NLR in *H.pylori*-positive group (p= 0. 0.086)

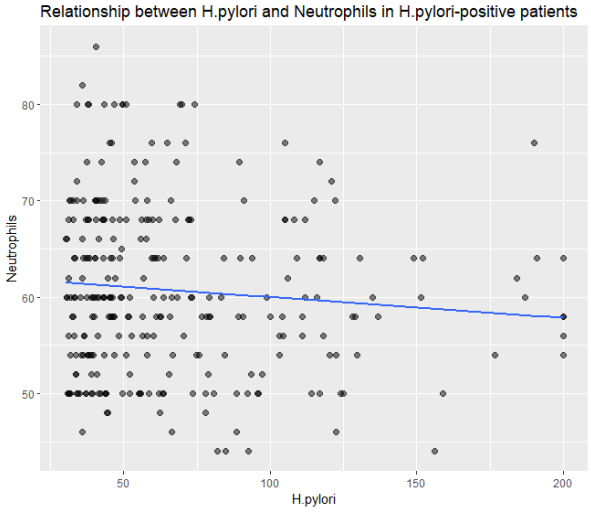


Fig 4: No significant correlation between *H.pylori* and neutrophils in *H.pylori*-positive group (p=0.11)

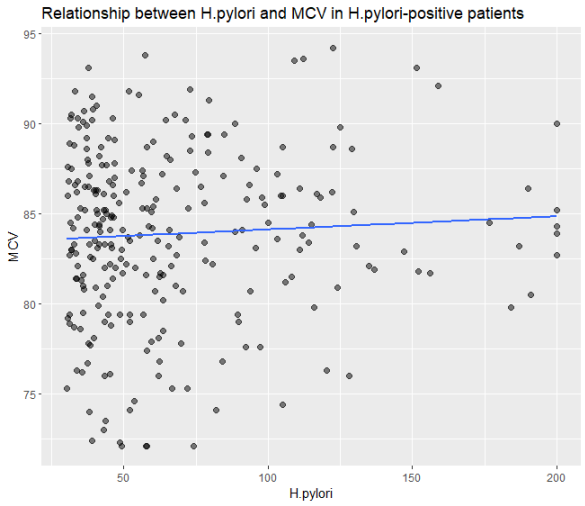


Fig 5: No significant correlation between *H.pylori* and MCV in *H.pylori*-positive group (p= 0.337)

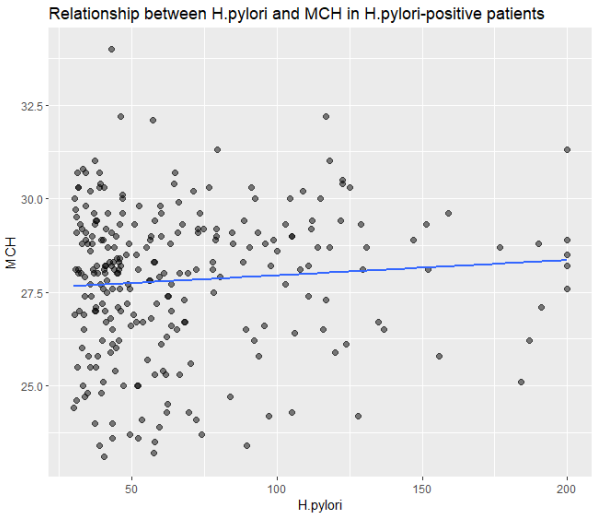


Fig 6: No significant correlation between *H.pylori* and MCH in *H.pylori*-positive group (p=0.19)

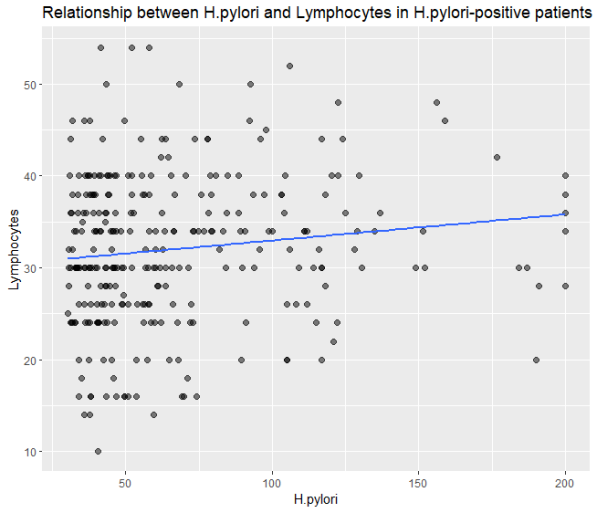


Fig 7: Weak positive correlation between *H.pylori* and lymphocytes in *H.pylori*-positive group (p= 0.0253*)

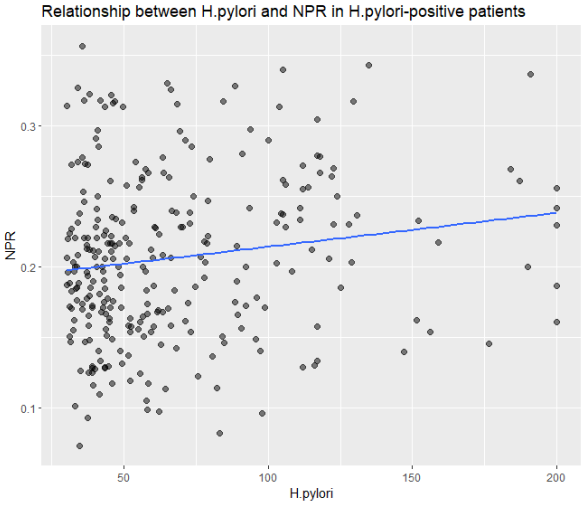


Fig 8: Weak positive correlation between *H.pylori* and NPR in *H.pylori*-positive group (p=0.008*)

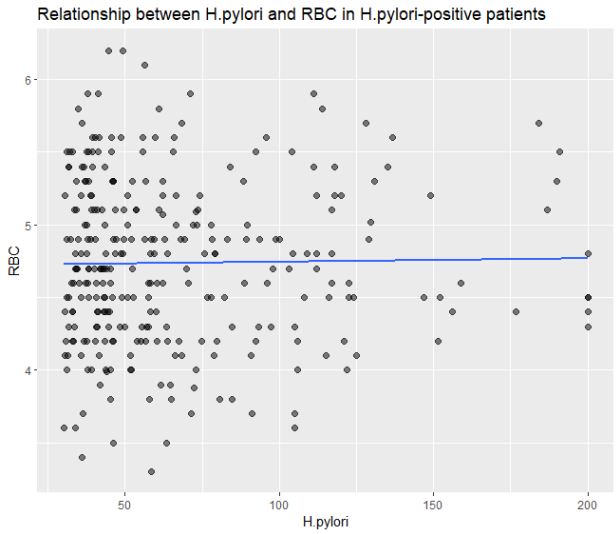


Fig 9: No significant correlation between *H.pylori* and RBC in *H.pylori*-positive group (p= 0.7867)

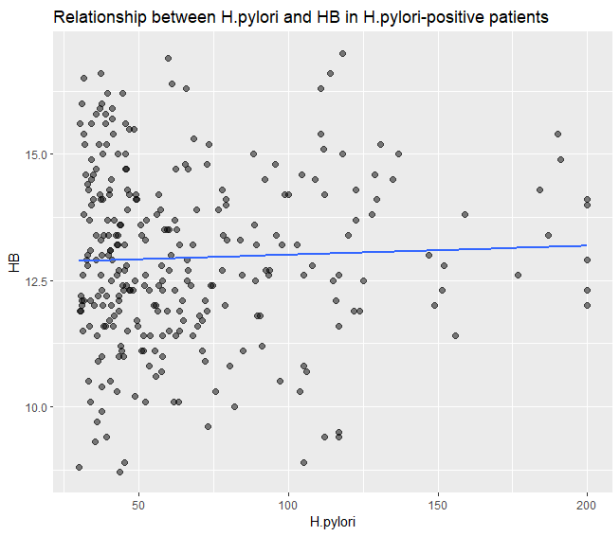


Fig 10: No significant correlation between *H.pylori* and HB in *H.pylori*-positive group (p=0.5019)

<u>*H.pylori*-positive patients of severe intensity with a titer of more than 50 U/ml</u>

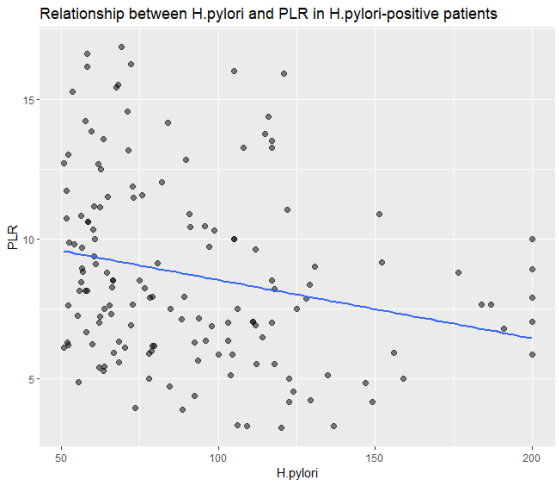


Fig 11: Weak negative correlation between *H.pylori* and PLR in severe intensity group (p= 0.0034*)

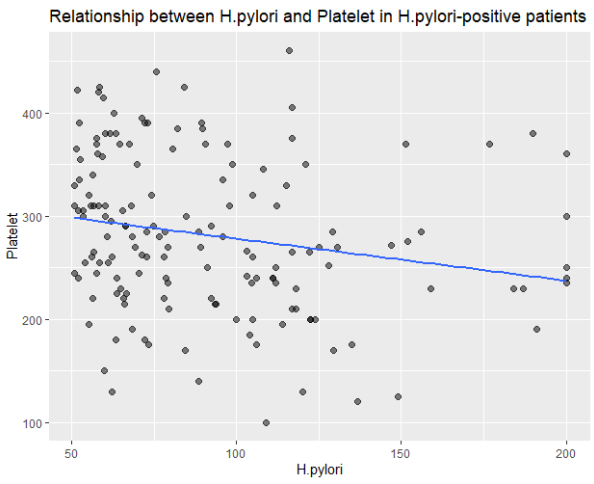


Fig 12: Weak negative correlation between *H.pylori* and platelet in severe intensity group (p=0.01074*)

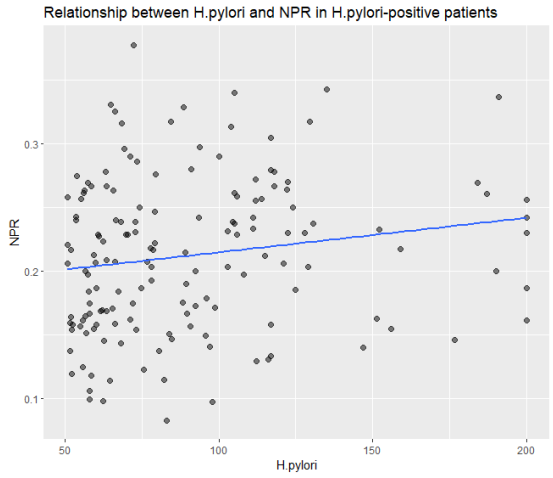


Fig 13: Weak positive correlation between *H.pylori* and NPR in severe intensity group (p= 0.0362*)

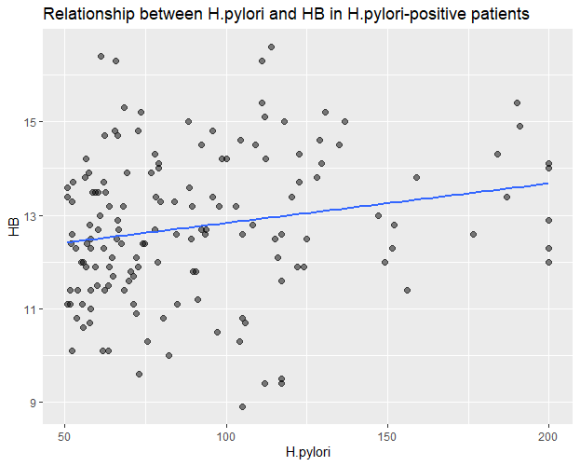


Fig 14: Weak positive correlation between *H.pylori* and HB in severe intensity group (p=0.01126*)

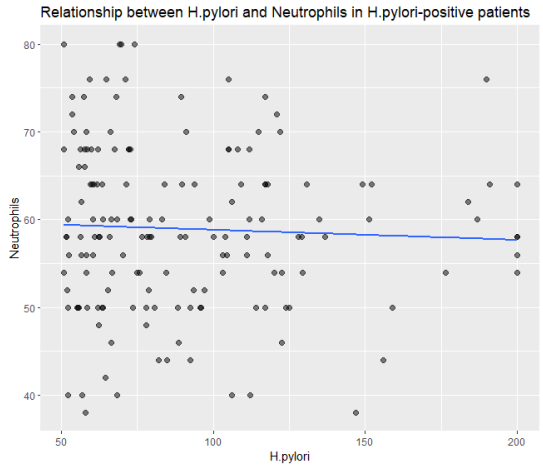


Fig 15: No correlation between *H.pylori* and neutrophils in severe intensity group (p= 0.567)

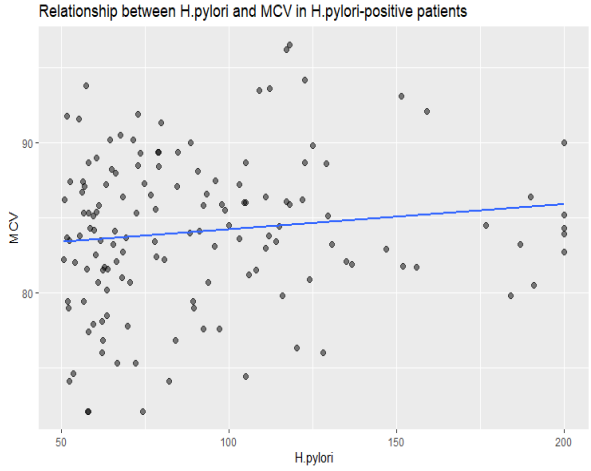


Fig 16: No correlation between *H.pylori* and MCV in severe intensity group (p=0.1309)

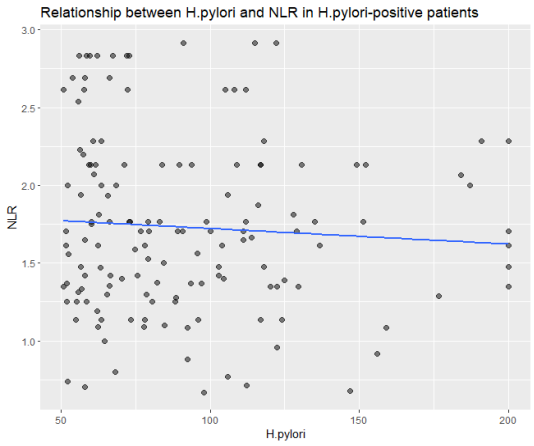


Fig 17: No correlation between *H.pylori* and NLR in severe intensity group (p= 0.1205)

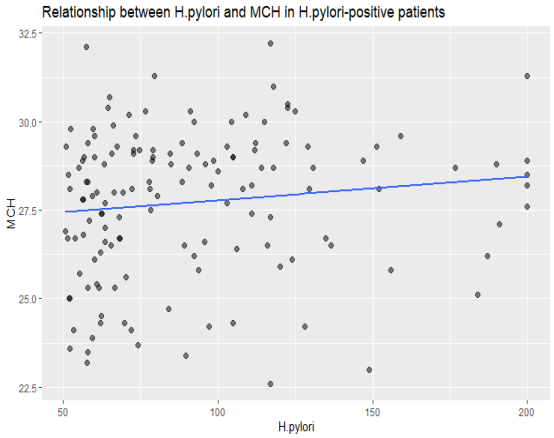


Fig 18: No correlation between *H.pylori* and MCH in severe intensity group (p=0.133)

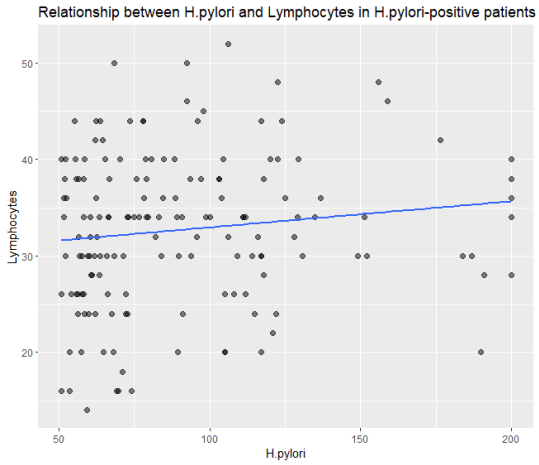


Fig 19: No correlation between *H.pylori* and lymphocytes in severe intensity group (p= 0.108)

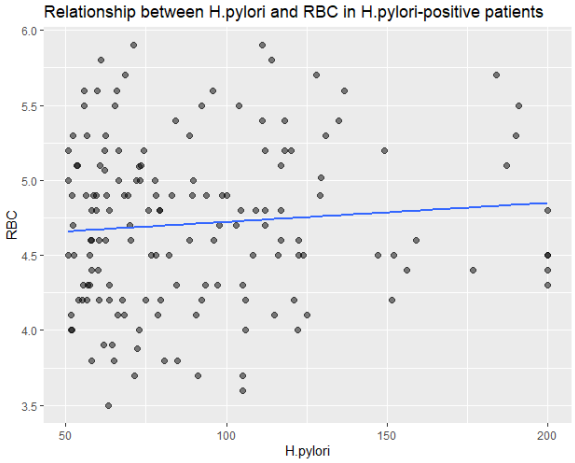


Fig 20: No correlation between *H.pylori* and RBC in severe intensity group (p=0.2766)

Chapter 4

Discussion

From our study, it was found that the mean value of each of the parameters has an increase or decrease from the *H.pylori* negative group. If we consider each of the parameters and compare them with the *H.pylori* negative group, we can see a rise or fall in these parameters from the negative group. However, not all of them have statistical significance.

The average neutrophil count in the *H.pylori*-positive group was higher than in the negative group but in the severe intensity group, it was less than in the negative group. However, none of the changes had statistical significance (Table 1). Some people may have naturally lower levels of white blood cells and neutrophils than other people. This can be due to a range of factors, including congenital conditions. Not only this but the study conducted by Horrom in 2017 also showed that the neutrophil cells which were exposed to *H. pylori* underwent structural changes (Hypersegmentation) as well. Moreover, the change only happened when neutrophils were directly infected with *H. pylori*, rather than when the bacteria were merely present. However, this sort of change was not seen when neutrophils were exposed to *E. coli*. When eosinophils were exposed to *H. pylori*, no such change was seen. So, *H.pylori* has a direct interaction with neutrophils, through which it changes the cells. Normal neutrophils die as part of a natural process. But infected cells live longer and the altered neutrophils do not kill the bacterium. The number of neutrophils in the blood may increase due to infections by bacteria, viruses, fungi, and parasites (Territo, 2022). However, there are several other reasons to increase the neutrophil count, these include injury, an inflammatory disorder, etc. In the large pool of neutrophils, *H. pylori* can survive with the aid of HP-NAP. *Helicobacter pylori* (*H. pylori*) neutrophil-activating protein (HP-NAP) was originally identified as a virulence factor of *H. pylori* as it has the ability to activate neutrophils for generating respiratory bursts by releasing reactive oxygen species. Later on, HP-NAP was also found to be involved in protecting *H. pylori* from DNA damage and supporting the survival of *H. pylori* under oxidative stress (Wen fu, 2014). *H. pylori* infection usually causes chronic inflammation usually with a neutrophil-rich response from the immune system (Horrom, 2017). *H. pylori*-infected subjects produce greater numbers of neutrophils (Jafarzadeh et al., 2013). A study conducted by Nalbant et al. in 2017 it was found that there was an apparent reduction in the

number of neutrophils, lymphocytes, and NLR in the patients who were *H. pylori* positive where only the reduction in neutrophils was determined to be statistically significant.

In the case of neutrophil to lymphocyte ratio (NLR) we can see no change in the *H.pylori*-positive group to the negative group (Table 1) but decrease in the severe intensity group than the negative group (Table 2). However, none of them had statistical significance. In terms of platelet to lymphocyte ratio (PLR), it was increased in the *H.pylori*-positive group than in the negative group (Table 1). However, in the *H.pylori*-positive group of severe intensity, the PLR value did not change than the negative group (Table 2). However, none of them had statistical significance. A study conducted by Farah et al., in 2014 and 2017, suggests that the NLR and PLR of *H. Pylori*-positive patients are higher than *H. Pylori*-negative patients. However, in our study, we found out that NLR did not change in the *H.pylori*-positive group than in negative patients but in the severe intensity group, the NLR was lower than in the negative group. In the case of PLR, it was higher in the *H.pylori*-positive group than the negative group (Table 1) but did not change in the severe group (Table 2). In a study conducted by Asil et al. in 2016 with 286 patients who had chronic *H. pylori* infection and 130 *H. pylori*-negative control group, it was found that patients with chronic *H. pylori* infection had a higher NLR level than the *H. pylori*-negative group. It was also found that NLR is increased in chronic *H.pylori* infection and returns to normal after successful *H.pylori* eradication treatment. However, in our study, we found no change in NLR level in the *H.pylori-positive* group than in the negative group but decreased in the severe intensity group. However, none of the values had any statistical significance. In a study conducted by Boyuk et al in 2020, no significant difference in platelet, leukocyte, lymphocyte, PLR, and NLR levels between positive/negative *H. pylori* patients was observed. Our study also did not find any statistically significant change in these parameters in the positive groups than the negative group.

On the other hand, the neutrophil to platelet ratio (NPR) did not change in any of the groups of *H.pylori*-positive (Table 1) and severe intensity group (Table 2) than the negative group. An animal study found that after the occlusion of the middle cerebral artery, the depletion of platelets reduced neutrophil recruitment and vascular inflammation (He et al., 2019). In addition, a review of neutrophil-platelet interactions revealed that activated platelets were involved in the release of inflammatory mediators, the increase in vascular permeability, and neutrophil

accumulation(Garcia-Culebras et al., 2018). However, our study found that there has been no significant change in the average value of NPR between the positive and negative groups.

Previous studies conducted by Yan-xu et al. in 2017, revealed significant differences in RBC, MCH, MCV, and MCHC between *H. pylori*-positive and *H. pylori*-negative groups. A study conducted by Kassahun et al. in 2021 also found a significant mean difference in MCH value between *H. pylori*-infected patients and the control group. In another study conducted by Rahman et al. in Dhaka, Bangladesh, a significant ($p<0.001$) lower value of mean MCV and MCH in *H. pylori*-positive patients was revealed. However, Our study found the mean value of MCV was lower in the *H.*pylori-positive group than in the negative group (although not statistically significant) (Table 1). Also, we found no difference in the average MCV in the severe intensity group (Table 2). We did not see any significant difference in terms of MCH in the *H.*pylori-positive group and severe intensity group with the negative group (Table 1 and 2 respectively). However, our study did not correspond with the previous studies in terms of the significant difference in MCV and MCH in the *H.pylori* positive/negative groups, as none of the values had statistically significant differences. Moreover, a study conducted in Palestine and Sudan reported no significant differences were found in the MCH value (Kassahun et al., 2021), which corresponded to our study.

In a previous study, it was suggested that mean hematological parameters and RBC were significantly reduced among *H. pylori*-positive patients than negative patients (Kibru et al., 2014). A study conducted by Kassahun et al. in 2021 found the mean values of RBC count were lower in *H. pylori*-infected patients than in the control group, which was statistically significant. These studies contradicted our study as our study showed that the RBC count was higher in the *H.pylori*-positive group (Table 1) and the severe intensity group (Table 2) than in the negative group. The difference in the mean values of RBC in the positive groups was statistically significant. Red blood cell synthesis is regulated by a number of factors, like folic acid, erythropoietin, iron, vitamin B12, and vitamin C. Iron and vitamin B12 are especially important for hemoglobin synthesis and maturation of red blood cells (Kassahun et al., 2021). According to Kassahun et al., studies have suggested that *H. pylori* infection was independently associated with iron deficiency, vitamin B12 deficiency, and iron-deficiency anemia. So, deficiency in iron and vitamin b12 may result in variations in the size and shape of RBC, and defects in hemoglobin maturation. As *H.pylori* is

associated with vitamin B12 and iron deficiency, there is a strong possibility that it also contributes to the change in RBC and hemoglobin levels. A study conducted by Kassahun et al. suggested that the decrease in RBC count among *H. pylori*-infected patients might be due to blood loss because of chronic erosive gastritis and active bleeding peptic ulcers. Moreover, consumption of iron by *H. pylori* may have decreased iron absorption secondary to chronic gastritis, iron loss due to hemorrhagic gastritis, and active bleeding peptic ulcers, which might contribute to the reduction of blood hemoglobin concentration in *H. pylori*-infected patients (Kassahun et al., 2021). Recent evidence suggests that *H. pylori* infection could cause IDA in the absence of peptic ulcer or other upper gastrointestinal (GI) tract bleeding lesions (Annibale et al., 2000). However, our study did not observe any finding which can suggest a decrement in the RBC count rather there was a slight increase in the average RBC in the *H.pylori*-positive group and the severe group than the negative group.

In the case of platelet count, there was an increase in the *H.pylori*-positive group than the negative group (Table 1). In the severe intensity group, there has been a reduction in the average platelet count than the negative group (Table 2). However, none of the values had statistical significance. In a study conducted by Umit et al. in 2015, it was found that the patients with *H. Pylori* had lower platelet counts compared to patients who were *H. Pylori* negative, even though these patients had platelet counts within the normal range. It also showed that, since they had a lower platelet count compared to the *H. pylori* negatives, *H. pylori* reduces the platelet values before immune cytopenia affects the platelets. In correspondence to this study, our study showed that the average platelet count was below the normal range in each of the groups. Platelets are known to respond to bacterial invasion. Researchers found that by bridging between innate and adaptive immune mechanisms, platelets and anti-PF4/polyanion antibodies cooperate with each other in an antibacterial host response (Palankar et al., 2018). In recent years platelets have gained recognition for their inflammatory functions, which then modulate the immune response during infectious diseases. Similar to innate immune cells, platelets contain PRRs (pattern recognition receptors), which recognize different components that increase during infection (Portier et al., 2020). During an infection, platelets sense pathogens through their receptors and can respond through the secretion and expression of antimicrobial proteins, cytokines, and adhesion molecules. As a part of the innate immune system, platelets can recognize pathogens from all major classes of microorganisms. According to Mark A. Marinella, any inflammatory process such as bacterial infection that elevates

serum IL levels (especially IL-6), may also increase the circulating platelet count. In our study, we have found a rise in platelet count in the *H.pylori*-positive group than in the negative group (although it was not statistically significant). In terms of the severe intensity group, there was a drop in the average platelet count. Thrombocytopenia, or low platelet counts, is common in acute infections and can correlate with disease severity (Portier et al., 2020). There is growing evidence that the eradication of *Helicobacter pylori* effectively increases platelet count in Immune thrombocytopenia patients who are infected with this bacterium (Kuwana, 2014). Based on some recent systematic reviews, more than half of the patients have successfully recovered platelet counts after an *H. pylori* eradication treatment (Lee, 2020).

A significant decrement in mean hemoglobin concentration ($p<0.001$) of *H. pylori*-infected patients as compared to the control was found in a study conducted by Kassahun et al. in 2021. In another study conducted by Nasif et al. (2021), a significant difference in hemoglobin concentrations between patients with positive *H. pylori* with IDA and the control subjects ($p<0.0001$) was found. A meta-analysis of randomized control trials of *H. pylori* eradication has indicated that eradication of this infection can increase hemoglobin levels. An association between *H. pylori* infection and hemoglobin level exists. However, the underlying reason for this still needs further research (Yan-xu et al., 2017). Our study showed there were no statistically significant differences between positive and negative patients in terms of average hemoglobin (Table 1). However, in the severe intensity group, a very weak positive correlation was found (Table 4). This indicates that with an increase in the *H.pylori* infection, hemoglobin also increases.

In previously conducted studies, a significant difference was not observed in leukocyte, lymphocyte, platelet, PLR, and NLR levels between positive and negative *H. pylori* patients (Boyuk et al., 2020). Our study also showed no statistically significant difference in lymphocyte, platelet, MCV, MCH, NPR, and PLR between *H.pylori* positive and negative groups. However, according to a study conducted by Karttunen et al., it was found that the total number of blood leukocytes and the numbers of lymphocytes and basophils were significantly increased in *H. pylori*-positive patients (N = 58), as compared with *H. pylori*-negative ones (N = 38) which contradicted our study as our study showed the lymphocyte count had no statistically significant change in the *H.pylori*-positive group (Table 1) and the severe intensity group (Table 2) than the negative group.

In previous studies it has been shown that the association between the PLR marker and *H.pylori* infection can be useful for detecting *H.pylori* infection and severity, deciding about treatment time and follow-up to ensure eradication of the infection (Farah et al., 2018). Our study showed a very weak negative correlation between the platelet-to-lymphocyte ratio (PLR) and *H.pylori* infection (p=0.0034*) and a very weak positive correlation between neutrophil-to-lymphocyte ratio (NPR) and *H.pylori* infection (p=0.0362*)in the *H.pylori*-positive group (figure 01 and 08 respectively). In the case of the severe intensity group, a very weak negative correlation between PLR and *H.pyolri* (p=0.0034*) and a very weak positive correlation between NPR and *H.pylori*(p=0.0362*) was found (figure 11 and 13 respectively). In a study conducted by Boyuk et al. in 2020, a correlation between the presence of *H. pylori* infection and PLR was not found which contradicted our study as we found a weak correlation between *H.pylori* infection and PLR.

From our study, a very weak negative correlation was found between the platelet count and *H.pylori* infection (p=0.0005*) in the *H.pylori*-positive group which showed with an increase in *H.pylori* infection, the platelet count decreased (figure 2). In the case of the severe intensity group, a weak negative correlation was also found between platelet count and *H.pylori* infection (p=0.01074*) (figure 12).

On the other hand, a very weak positive correlation was found between lymphocyte count and *H.pylori* infection (p=0.0253*) in the positive group (figure 7). *H.pylori* has a distinctive niche, which is hostile to most microorganisms. It can survive in an extremely acidic environment. Moreover, *H. pylori* have an arsenal of mechanisms that enable the successful evasion of innate and adaptive immunity in order to persist within the human gastric mucosa (Reyes et al., 2019). Although *H. pylori* is not invasive, the bacterium and its products can come in contact with cells in the lamina propria. As a result, the infected gastric mucosa has a significant influx of immune cells, including DCs, macrophages, and lymphocytes (Reyes et al., 2019). So, in the case of bacterial infection, an increase in blood lymphocytes can be considered a normal phenomenon. Our studies showed that with an increase in *H.pylori* infection, the lymphocyte count also increased (although with a very weak positive correlation). However, in the severe intensity group, no statistically significant correlation was observed between lymphocyte count and an increase in *H.pylori* titer. This may suggest that the correlation between lymphocytes and *H.pylori* persist under 50 U/ml *H.pylori* titer. Studies found that the lymphocyte count in the

peripheral blood increases significantly as the intensity of *H. pylori* increases (Guclu et al., 2017). Our study corresponds with this study in terms of an increase in the lymphocyte count with an increase in *H.pylori* titer. However, with an increase in *H.pylori* infection in the positive group and positive group of severe intensity, the neutrophil count did not have any statistically significant correlation with *H.pylori*. In terms of the severe intensity group, we found a very weak positive correlation between hemoglobin and *H.pylori* infection (p=0.01126*) (figure 14). This may suggest that, hemoglobin inceases with the increase in *Hpylori* infection when the *H.pylori* titer is above 50U/ml.

Our study showed that the subjects in the negative and the positive groups have an average age of 40 to 42. So, the subjects are mostly in their middle age. Studies show that the prevalence of *H.pylori* among middle-aged adults is over 80 percent in many developing countries, compared to 20 to 50 percent in industrialized countries (Suerbaum, 2002). Our study also corresponded with this finding. However, the prevalence of *H. pylori* infection increases with age. About 50% of the population is infected at ages above 60, and around 10% are infected between 18 and 30 (Hou et al., 2019). According to Boyuk et al. (2020), *H. pylori*-positive patients are younger when compared to *H. pylori*-negative patients. This shows that *H. pylori* infection is acquired at an early age.

Conclusion

Our study only found a statistically significant increase in average RBC count in the *H.pylori*-positive group and the severe intensity group than the negative group. Apart from this parameter, there were no statistically significant changes from the negative group. In terms of correlation analysis, our study found several correlations between some of the parameters and *H.pylori* infection. There has been an association between *H.pylori* infection and NPR, PLR, platelet, hemoglobin, and lymphocytes in the *H.pylori*-positive group, although the correlations were very weak. No other parameters had any statistically significant correlation with *H.pylori* infection.

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