

Utility of immature granulocyte in severity of *helicobacter pylori* infection

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ABSTRACT

Introduction: *Helicobacter pylori* infection is a chronic bacterial infection associated with some extragastric diseases as well as gastric involvements that occur most commonly worldwide. In our study, we aimed to investigate the usability of immature granulocytes as a basic indicator that can reflect the severity of *helicobacter pylori* inflammation, to the best of our knowledge, for the first time. **Materials and Methods:** Patients who underwent upper gastrointestinal endoscopy between April 2019 and April 2020 and were diagnosed with antral gastritis were included in this study. The relationship between helicobacter infection and its severity detected in gastric biopsies of patients and immature granulocyte count (IGC), immature granulocyte percentage (IG%), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and mean platelet volume (MPV) were investigated. **Results:** Of the 868 patients, 210 were HP negative, 658 were HP positive (218 mild HP positive, 293 moderate HP positive, and 147 severe HP positive). There were statistically significant differences between the HP negative and HP positive groups in terms of IGC, IG%, NLR, and PLR. However, IG% and IGC were not clinically useful because the median IG% (0.3 vs 0.3) and IGC (0.02 vs 0.02) were the same in the HP negative and total HP positive groups. **Conclusion:** In our study, IGC and IG% were not found useful to detect H. pylori intensity and severity of inflammation.

KEY WORDS: *Helicobacter pylori* infection, immature granulocyte, inflammatory mediators

INTRODUCTION

Helicobacter pylori (HP) is the most common infectious chronic bacterial infection in humans, and more than half of the world's population has had *helicobacter pylori* colonization.^[1] Its prevalence ranges between 20% and 50% in developed countries and reaches 80% in developing countries.^[2] Although HP is the main etiological factor in chronic gastritis, it is asymptomatic in most cases. Furthermore, peptic ulcer is associated with diseases such as gastric carcinoma and mucosa-associated lymphoma (MALT lymphoma).^[3]

Diagnostic tests for HP can be divided into two as invasive or non-invasive depending on the endoscopy requirement. The presence of HP can be determined with anti-HP antibodies or blood urea testing. However, these tests do not give us any information about the density of HP in the gastric mucosa and the severity of acute and chronic inflammation. Gastric biopsy taken at gastrointestinal endoscopy is the gold standard in the study of the etiopathogenesis of gastritis; however, this is an invasive method.^[4]

Some studies have shown an association between inflammatory mediators and acute phase reactants and HP infection. Inflammatory markers such as C-reactive protein and

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Neutrophil-to-lymphocyte ratio (NLR) have been observed to be elevated in HP infections.^[5,6] Immature granulocyte (IG) is a new inflammation marker, which is not sufficiently known by many clinicians. Observation of immature granulocytes in peripheral blood, which is not normally seen in healthy individuals, indicates bone marrow activation and infection.^[7] Owing to technological advances in automated hematological analyzers, IG became easily and quickly measurable with a routine

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complete blood count (CBC).^[8] Some studies have shown that IGs are more effective than markers such as CRP and NLR in determining the severity of infection.^[9]

Numerous studies emphasize that chronic inflammation and platelet activation increase in the presence of HP.^[10] Increased mean platelet volume (MPV), platelet distribution width (PDW), and platelet count are indicators of platelet activation in laboratory tests.^[11]

In this study, we aimed to investigate the relationship between the severity of HP infection and IG. Secondly, we aimed to observe the relationship between inflammation indicators such as NLR, platelet-to-lymphocyte ratio (PLR) and MPV, and HP infection severity about which different results have been reported in the literature.

MATERIALS AND METHODS

The study was approved by the Institute's Ethics Committee (Decree no: 5-3, date: 29.03.2019). In accordance with the study criteria, 868 prospective patients who were admitted to the General Surgery outpatient clinic between April 2019 and April 2020 were included in the study after obtaining their informed written and verbal consent.

This study included 868 patients who had dyspeptic complaints only and were diagnosed with only antral gastritis at the gastroscopic examination. Two biopsies were taken from each of antrum, corpus and incisura angularis endoscopically from all patients included in the study and all biopsies were evaluated histopathologically according to the Sydney classification.^[12] Mucosal biopsies were stained with hematoxylin and eosin which are used for *Helicobacter* organisms. HP density is classified according to the Sydney classification, where mild degree refers to microorganisms that are scarce in number or found with difficulty, moderate degree refers to the more generalized presence of microorganisms with small groups and severe degree refers to when microorganisms form large colonies. All specimens were examined by a single pathologist who was blind to clinical data. Patients with gastritis were divided into four groups according to HP density: HP negative, HP mild, HP moderate, and HP severe. Standard triple therapy (amoxicillin 1 g, clarithromycin 500 mg, proton pump inhibitor) was applied to HP-positive patients with functional dyspepsia. Proton pump inhibitor therapy was given to patients with non-HP dyspepsia.

Patients who have gastroscopic findings other than antral gastritis (gastrointestinal hemorrhage, gastric or duodenal ulcer, etc.), those with the hematological disease, diabetes mellitus, malignancy, cerebrovascular disease, coronary arterial disease, chronic liver, and kidney disease, acute or chronic infection, receiving medications such as nonsteroidal anti-inflammatory drugs, proton pump inhibitors, antiaggregants, anticoagulant, and oral contraceptives were not included in the study.

All patients included in the study underwent a complete blood count. With this simple test, IG count (IGC), IG percentage (IG%),

NLR, PLR, and MPV values were calculated and the relationship between these four groups was compared.

SPSS MacOS-x20.0 (SPSS, Chicago, IL) package program was used for statistical analysis of the data. Descriptive statistics were used in the analysis of qualitative data. Independent sample *t*-test was used for comparison of the groups, and Mann-Whitney U test was used for comparison when data did not approximate a normal distribution. Values with a *P* value less than 0.05 were considered statistically significant.

RESULTS

Five hundred and sixty-eight of the 868 patients included in the study were female (67.6%) and 281 (32.4%) were male. Of these patients, 24.1% (n = 210) were HP negative, 25.1% (n = 218) were HP mild, 33.7% (n = 293) were HP moderate and 16.9% (n = 147) were HP severe. The mean age of HP negative patients was 47.9 ± 18.6 (41 men, 169 women). The mean age of mild HP positive patients was 49.5 ± 14.8 (76 men, 142 women), the mean age of moderate HP positive patients was 49.3 ± 14.2 (98 men, 195 women) and the mean age of severe HP positive patients was 47.1 ± 14.2 (66 males, 81 females). There were statistically significant differences between gender type in both HP negative (females-males, 169:41) and HP positive (females-males, 418:240) groups (*P* = 0.00).

When HP negative and HP positive patients were investigated, the NLR value was statistically higher in HP positive patients (1.92 ± 0.85 vs 2.24 ± 4.42 *P* = 0.02). Again, IGC (0.023 ± 0.014 vs 0.024 ± 0.042 *P* = 0.00) and IGC% (0.31 ± 0.15 vs 0.32 ± 0.31 *P* = 0.03) were also significantly higher in HP positive patients compared to HP negative patients. However, since the mean values were within the normal limits, they were not evaluated as significant (normal immature granulocyte range: 0.0-0.06, normal immature granulocyte percentage: 0.0-0.6). The distribution of parameters comparing *H. pylori*-negative and *H. pylori*-positive patients is shown in Table 1.

The low PLR value in the mild *H. pylori* positive patient group was statistically significant when compared with the HP negative

Table 1: The comparison of *H. Pylori*-negative and *H. Pylori* positive patients

	<i>H. Pylori</i> -negative (n=210)	<i>H. Pylori</i> -positive (n=658)	<i>P</i>
Sex*			
Male	41	240	0,00
Female	169	418	
Age**	47,9±18	48,9±14	0.6
NLR**	1.92±0,85	2.24±4,42	0.02
PLR**	147,4±56,2	138,3±53.1	0,04
MPV (fL)**	10.2±0.8	10,5±3,8	0.17
IGC (103/μL)**	0,023±0,014	0,024±0,042	0.00
IG%**	0,31±0,15	0,32±0,31	0.03

*Chi-square Test. **Mann-Whitney U Test. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MPV: mean platelet volume; IGC: immature granulocyte count; IG% immature granulocyte percentage

patient group (135.8 ± 56.3 vs 147.4 ± 56.2 $P = 0.02$). Mean values of IGC and IG% in the same groups were within normal limits.

In comparing HP-negative patients with moderate and severe H. pylori-positive patient groups, NLR values were found to be significantly higher in both groups. (1.92 ± 0.85 vs 2.47 ± 6.53 $P = 0.01$ and 1.92 ± 0.85 vs 2.16 ± 0.92 $P = 0.00$). The distribution of parameters comparing H. pylori-negative and mild, moderate, and severe H. pylori-positive patients is shown in Table 2.

There was no statistically significant difference between H. Pylori negative and positive (mild, moderate, severe) groups in terms of MPV.

DISCUSSION

Almost all individuals infected with *Helicobacter Pylori* have gastritis or functional disorders in the stomach. Serious stomach diseases such as peptic ulcer (15-20%), ulcer complications (2-12%) gastric cancer (1-3%), and B-cell lymphoma are observed in infected people.^[2] Furthermore, H. Pylori has been associated with many systemic diseases such as cardiovascular diseases, stroke, anemia, Alzheimer's disease, diabetes, and idiopathic thrombocytopenic purpura.^[13] Many studies have revealed the relationship between gastric and extra-gastric involvements of H.pylori infection and systemic inflammation.^[14-16] Interleukin-6 (IL-6) is a multifunctional cytokine released from a large number of cells, especially monocytes, lymphocytes, mesangial cells, and endothelial cells. A study by Nakagawa et al.^[17] showed that patients with high serum anti-H. pylori levels had significantly elevated serum IL-6 levels. The increase of IL-6 may enhance the severity of extra-gastric diseases such as coronary artery disease by triggering systemic inflammation.^[18] In our study, we investigated the changes of some inflammation markers with the severity of H.pylori infection, which can be determined by an automated complete blood count analysis that is very simple, inexpensive, and rapid.

NLR is a simple and inexpensive biomarker used in the literature to estimate mortality, morbidity, and survival rates in neoplastic diseases such as stomach, thyroid, breast cancer as well as inflammatory conditions.^[19-21] The relationship between HP infection severity and NLR has been studied in several studies in the literature and various results have been observed. In a study, which had a rather small sample size (50 participants)

in 2014, Farah et al.^[22] found that NLR value was higher in HP positive patients compared to HP negative patients. In another study Jafarzadeh et al.^[6] found a higher NLR value in HP positive patients compared to HP negative patients, independent of bacterial cytotoxin-related gene A (CagA) status. In their study involving 1,289 patients in 2019, Kaplan et al.^[11] reported that NLR values were 1.8 ± 0.6 vs 3.2 ± 1.4 ($P < 0.001$) for HP positive and HP negative patients. However, in their study, Guclu et al.^[23] did not find any difference in terms of NLR in HP positive and HP negative patients. In the subgroup analysis of the same study, no difference was observed when compared NLR values in mild and severe HP positive patients, however, only in moderate HP positive patients, the NLR value was lower than that of HP negative patients. In a study investigating 17,028 routine health check-up patients in South Korea, no relation was found between the presence of HP and NLR.^[24] However, in this study HP positivity was measured with serum IgG. In our study, the NLR value was found to be higher in HP-positive patients than in HP-negative patients. When the subgroups of HP positive patients were examined, there was no significant NLR elevation in mild HP positive patients, whereas it was higher in moderate and severe HP positive patients as the severity of infection increased. In moderate HP-positive patients, the NLR value was 2.47, whereas in the severe HP-positive patients, the mean value that we expected to be higher was 2.16. This may be caused by a lower number of cases in the severe patient group. Considering these results, NLR value is thought to be useful in determining HP infection and in monitoring the patients after eradication treatment. However, further studies are needed with larger severe patient groups.

Mean platelet volume is a simple marker that indicates systemic inflammation and can be obtained from a routine complete blood count analysis. While some MPV values may be observed in some diseases such as myocardial infarction, acute ischemic stroke, and diabetes mellitus, there are studies reporting low MPV values in several diseases such as active rheumatoid arthritis, ankylosing spondylitis, and inflammatory bowel disease.^[4] Kaplan et al.^[11] and Umit and Umit^[10] have found that MPV value is higher in HP positive patients compared to HP negative patients. In contrast, Guclu and Topal reported no correlation between the severity of inflammation and blood MPV levels in patients with and without H pylori infection^[4,23] and the results of our study were similar to these studies.

The platelet-to-lymphocyte ratio is a novel marker in the literature. It is an easily identified inflammation marker such as NLR. In

Table 2: The comparison of H.Pylori-negative and mild, moderate and severe H.Pylori positive patients

	H.Pylori-negative (n=210)	Mild H.Pylori-positive (n=218)	P	Moderate H.Pylori-positive (n=293)	P	Severe H.Pylori-positive (n=147)	P
Age*	47,9±18	49,5±14	0.4	49,3±14	0.5	47,1±14	0.5
NLR*	1.92±0.85	1.99±1.01	0.64	2.47±6.53	0.01	2.16±0.92	0.00
PLR*	147,4±56.2	135.8±56.3	0.02	138.6±49.0	0.1	141.5±56.1	0.2
MPV (fL)*	10.2±0.8	10.4±0.8	0.07	10.6±5.7	0.4	10.3±0.8	0.3
IGC (10 ³ /μL)*	0,023±0,014	0,02±0,017	0.00	0,024±0,045	0.01	0,032±0,058	0.51
IG%*	0,31±0,15	0,29±0,3	0.00	0,31±0,3	0.09	0,36±0,34	0.5

*Mann-Whitney U test. NLR: neutrophil-to-lymphocyte ratio; PLR: platelet-to-lymphocyte ratio; MPV: mean platelet volume; IGC: immature granulocyte count; IG% immature granulocyte percentage

their study, Pehlivanlı *et al.*^[25] found PLR value to be higher in patients with acute appendicitis. In their study in 2017, Farah *et al.*^[26] reported the PLR value in HP positive patients was higher compared to HP negative patients. Kaplan *et al.*^[11] found that PLR values were higher in HP-positive patients as well. In another study, no relation was found between HP infection and PLR.^[24] In our study, incompatible with the literature, the PLR value was lower in HP-positive patients (147.4 ± 56.2 vs 138 ± 53). Similar results were also found in subgroup analyses (mild, moderate, and severe). These results indicate that more studies are needed on this subject.

Immature granulocyte count has become measurable from complete blood count analysis as a result of advances in automated hematological analyzers technologies. Increased IG count and percentage in peripheral blood indicate an increase in bone marrow activation due to a bacterial infection.^[27] In the literature, IGs have been studied to determine the severity of more localized diseases such as acute appendicitis, as well as systemic infections such as sepsis and SIRS. In their study published in 2020, Karakulak *et al.*^[28] reported that there was a correlation between acute pancreatitis disease severity and IG% on the other hand, Park *et al.*^[29] found that IG% has the insufficient diagnostic ability for appendicitis. In his study conducted on 438 patients with acute appendicitis, Unal found that IGC and IG% were high in both acute appendicitis and complicated appendicitis patients.^[30]

To the best of our knowledge, this is the first study to investigate the relationship between immature granulocytes and HP infection and its severity. In our study, the number of IGC and IG% were significantly higher in HP positive patients compared to HP negative patients. However, since the mean values (IGC 0.02 vs 0.02 and IG% 0.3% vs 0.3%) were overlapped, no specific cutoff value was found. This suggests that HP infection does not affect IG values in peripheral blood.

There are some limitations to our study. The first limitation is that this is a single-center study, and the second is the scarce number of cases in severe HP positive patient group. We believe that comparing control values after eradication treatment will add more objective values.

CONCLUSION

In summary, immature granulocyte count, which is used as an indicator of inflammation, was not found to be an effective biomarker in determining *Helicobacter pylori* infection and severity. Likewise, there is no correlation between HP infection and MPV. NLR can be useful to detect HP infection and severity. More extensive and in-depth studies are needed on this subject.

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Conflicts of Interest

There are no conflicts of interest.

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