Relationship between the severity of *Helicobacter pylori* infection and neutrophil and lymphocyte ratio and mean platelet volume in children

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ABSTRACT

**Introduction:** HP causes local inflammation in the stomach and a systemic humoral immune response. No relationship was found in adult studies investigating the association between HP infection and neutrophil/lymphocyte ratio (NLR) and mean platelet volume (MPV). To our knowledge, there is no study in children. We aimed to evaluate the association between NLR and MPV values with HP infection, severity classification, and pre- and post-treatment status.

**Population and Methods:** Patients with dyspepsia aged between 2-18 years and without any chronic diseases undergoing gastroduodenoscopy were included in the study. Endoscopic mucosal biopsy was performed, HP presence and infection severity were determined according to Sydney Classification.

**Results:** 153 patients with HP positivity and 211 patients with HP negativity were included in the study, the mean age of them was 13.3 ± 3.4 years and 13.1 ± 3.5 years, respectively. No statistically significant difference was found between patients with HP positive and negative patients and also between severity subgroups of HP positive patients in terms of NLR and MPV (p > 0.05).

**Conclusions:** There was no association between NLR and MPV values with HP infection, severity classification, or pre- and post-treatment status.

**Keywords:** child, endoscopy, Helicobacter pylori, mean platelet volume, neutrophil / lymphocyte ratio.

http://dx.doi.org/10.5546/aap.2020.eng.e241

INTRODUCTION

*Helicobacter pylori* (HP) is one of the most common chronic bacterial infections in the world. More than half of the world’s population has HP colonization. The seroprevalence rate of HP was estimated to be 33% in 21 meta-analysis studies performed in children between 2011 and 2016. In addition to that, this rate is reported to be approximately 40% in symptomatic children.

It is a common microorganism found with a frequency of 80% in developing countries. HP causes chronic inflammation in the stomach and elicits immune response. Most of the patients are usually asymptomatic.

HP tends to settle in the antrum which is less acidic. HP attracts neutrophils and lymphocytes. Some substances released from neutrophils and mononuclear cells cause mucosal inflammation and thus cause gastritis. As a result, in addition to many cytokines, neutrophils, macrophages and lymphocytes infiltrate gastric mucosa and subclinical systemic low grade inflammation occurs.

It is the most common cause of chronic gastritis and has a high morbidity. HP is particularly associated with severe gastric diseases such as gastric cancer, gastric infection, peptic ulcer, and chronic gastritis.

The increase in blood leukocytes and especially neutrophils is commonly associated with inflammation. Neutrophil lymphocyte ratio (NLR) is obtained from a simple hemogram and is used as an inflammatory marker in various diseases. NLR has been proposed as an independent predictor.
in various clinical problems from cardiovascular events to cancer.\textsuperscript{7,8} Increased NLR has been shown to be a poor prognosis marker in patients undergoing cardiovascular intervention, and it has been shown to be high in acute coronary syndromes with high NLR.\textsuperscript{9} As a result of HP antibodies cross-reacting with platelet glycoproteins, it has been suggested that there is a decrease in platelet count and consequently immune thrombocytopenia (ITP) develops. Positive response in platelet counts is seen in patients with ITP after HP eradication.\textsuperscript{10,11} The patients with low grade inflammation, such as coronary artery disease, and diabetes mellitus has been shown to have higher MPV values.\textsuperscript{12} There is limited study investigating the association between HP infection and NLR and MPV in adults.\textsuperscript{10,13-15} No significant difference was found between patients with HP positivity and negativity in regards to NLR and MPV in adults with HP infection.\textsuperscript{10,14,15} It has been shown that higher NLR values were associated with severity of HP gastritis in adults.\textsuperscript{13} No relationship was found in adult studies investigating the association between HP infection and neutrophil/lymphocyte ratio (NLR) and mean platelet volume (MPV). To our knowledge, there is no study in children. We aimed to evaluate the association between NLR and MPV values with HP infection, severity classification, and pre- and post-treatment status.

**POPULATION AND METHODS**

The current study was conducted between March 2017 and October 2018.

**Inclusion criteria**

Patients with dyspepsia aged between 2-18 years and without any chronic diseases undergoing gastroduodenoscopy were included in the study.

**Exclusion criteria**

Patients with any organic disease, systemic disease, and infection were excluded from the study because any concomitant disease could change neutrophil and lymphocyte numbers. Patients with intestinal metaplasia or atrophy on endoscopic mucosal biopsies were also excluded from the study. Also, non-compliant patients and patients with treatment failure were excluded from the study.

Of the 390 patients, 364 patients with dyspeptic complaints who were followed up at the Clinics of Pediatric Gastroenterology and underwent gastroduodenoscopy, were included in the study.

At least 4 biopsies including 2 antrum and 2 corpus were obtained from patients. The biopsies were evaluated according to Sydney Classification.\textsuperscript{16,17} All endoscopic mucosal biopsy specimens were stained with hematoxylin and eosin (HE), and HE-stained preparations were evaluated histopathologically and for the presence of *Helicobacter pylori* according to Sydney Classification. Patients with HP positivity were detected and included as patient group in the study. They were divided into three groups as mild, moderate and severe according to Sydney Classification. Patients with gastritis without HP infection, were included as a control group.

Hemogram test was performed from peripheral blood before endoscopy. White blood cell count, neutrophil, lymphocyte and platelet counts were obtained from hemogram. Neutrophil / lymphocyte ratio was calculated by dividing absolute neutrophil count to absolute lymphocyte count. Because this study is retrospective, a written informed consent was not obtained. The Ethics Committee of Mersin University approved the current study (03 October 2018-385).

First-line triple therapy (including PPI, amoxicillin and metronidazole) for 14 days were given to the patients according to the latest ESPGHAN / NASPGHAN guidelines.\textsuperscript{16} Non-compliance is a very important factor in the treatment failure, so non-compliant patients were excluded from the study.\textsuperscript{18} The efficacy of the HP eradication treatment was assessed by a two-step monoclonal stool HP antigen test one month after the end of treatment.

**Statistical analysis**

Statistical analysis was performed using SPSS software version 13.0 (SPAA Inc, Chicago IL, USA). Frequency, percentage, and mean ± standard deviation (SD) were used as descriptive statistics. The Mann-Whitney U test was used to compare groups of numerical variables, and the relationships between categorical variables were analyzed by chi-square test. Comparisons among the groups were performed using Kruskal-Wallis one-way analysis of variance test for five independent groups. \( P \) value was considered statistically significant as a \(< 0.05.\)
RESULTS

During the current study, 840 patients with dyspepsia were followed-up at the Outpatient Clinics of Pediatric Gastroenterology. Gastroduodenoscopy was performed in 390 patients. Of 390 patients, 17 patients did not meet the inclusion criteria: 7 patients with celiac disease, 5 patients with diabetes mellitus, 3 patients with nonsteroidal anti-inflammatory drug gastropathy, one patient with hypothyroidism, and one patient with ataxia telengiectasia. After histopathological examination, 4 patients with intestinal metaplasia were also excluded from the study. These patients were excluded from the study because any concomitant disease and nonsteroidal anti-inflammatory drug use could change neutrophil and lymphocyte numbers. Of 390 patients, 364 patients were included in the study.

Of the 364 patients, the mean age of the 153 patients (103 girls) in the patient group was 13.3 ± 3.4 years and the mean age of the 211 patients in the control group (157 girls) was 13.1 ± 3.5 years (Table 1). There was no significant

| Table 1. Demographic and laboratory characteristics of patients and controls |
|-----------------------------|-----------------------------|-------|
|                            | Patients (n = 153) | Controls (n = 211) | p |
| Age (yrs)*                 | 13.30 ± 3.46 | 13.12 ± 3.52 | 0.627 |
| WBC*                       | 7.47 ± 2.00 | 7.67 ± 2.40 | 0.390 |
| Hemoglobine (g/dL)*        | 12.87 ± 2.37 | 12.79 ± 1.25 | 0.702 |
| MPV*                       | 7.87 ± 0.73 | 7.87 ± 0.66 | 0.995 |
| Neutrophil count†          | 4.24 ± 1.61 | 4.39 ± 2.09 | 0.452 |
| Lymphocyte count†          | 2.36 ± 0.71 | 2.34 ± 0.79 | 0.812 |
| NLR‡                       | 1.76 (1.12) | 1.76 (1.22) | 0.093 |

WBC: white blood cells; MV: mean platelet volume; NLR: neutrophil lymphocytes ratio.
*Data are presented as mean ± standard deviation.
†Data are presented as median (interquartile range).

| Table 2. Pre and post-treatment values of patients with HP positivity |
|-----------------------------|-----------------------------|-------|
|                            | Pre-treatment (n = 88) | Post-treatment (n = 88) | p |
| WBC*                       | 7.74 ± 2.15 | 7.16 ± 2.08 | 0.013 |
| MPV*                       | 7.81 ± 0.72 | 7.87 ± 0.79 | 0.380 |
| Neutrophil count†          | 4.44 ± 1.71 | 4.01 ± 1.90 | 0.064 |
| Lymphocyte count†          | 2.40 ± 0.68 | 2.32 ± 0.61 | 0.195 |
| NLR‡                       | 1.83 (1.26) | 1.60 (0.98) | 0.342 |

WBC: white blood cells; MV: mean platelet volume; NLR: neutrophil lymphocytes ratio.
*Data are presented as mean ± standard deviation.
†Data are presented as median (interquartile range).

| Table 3. Comparison of HP subgroups in regards to degree of HP severity |
|-----------------------------|-----------------------------|-------|
|                            | Mild form (n=73) | Moderate form (n=55) | Severe form (n=25) | p |
| WBC*                       | 7.21 ± 2.19 | 7.69 ± 1.76 | 7.73 ± 1.91 | 0.315 |
| MPV*                       | 7.82 ± 0.69 | 7.95 ± 0.83 | 7.81 ± 0.64 | 0.551 |
| Neutrophil count†          | 4.01 ± 1.75 | 4.46 ± 1.35 | 4.44 ± 1.69 | 0.234 |
| Lymphocyte count†          | 2.35 ± 0.74 | 2.35 ± 0.68 | 2.42 ± 0.69 | 0.913 |
| NLR‡                       | 1.62 (1.17) | 2.00 (0.98) | 1.95 (1.17) | 0.732 |

WBC: white blood cells; MV: mean platelet volume; NLR: neutrophil lymphocytes ratio.
*Data are presented as mean ± standard deviation.
†Data are presented as median (interquartile range).
difference in terms of age and gender between the
two groups (p > 0.05). No statistically significant
difference was found between patients with HP
positive and negative patients in terms of NLR
and MPV (p > 0.05) (Table 1). When pre and
post treatment values of NLR ratio and MPV of
88 patients treated were compared, we did not
find any significant difference between them
(p > 0.05) (Table 2). There was no statistically
significant difference between subgroups of HP
positive patients (mild, moderate and severe) in
terms of MPV and NLR rates (p > 0.05) (Table 3).

DISCUSSION

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 socio-
economic 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 al15 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 post-
treatment 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.

Limitations

First, since the present study is a cross-
sectional study, we could not detect a causal
relationship between NLR, MPV and HP. Second
limitation is that the absence of long-term follow-
up after treatment to control treatment response,
and the lack of monitoring of this marker over
time. NLR and MPV were measured only once
after HP eradication treatment.
In conclusion, there was no association between NLR and MPV values with HP infection, severity classification, or pre- and post-treatment status.

REFERENCES