

# *Helicobacter pylori* and mean platelet volume: a relation way before immune thrombocytopenia?

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**Abstract. – OBJECTIVE:** *Helicobacter pylori* is associated with chronic immune thrombocytopenia. Eradication of *H. pylori* has been related with a platelet response and this treatment has been included within management strategies for a certain group of patients. However, in patients with normal platelet counts, the effects of *H. pylori* infection on platelet count and mean platelet volume as an important platelet index have not been investigated. In this study, we aimed to assess the relation between platelet indices and *H. pylori* infection in patients with dyspepsia who have otherwise normal platelet counts.

**PATIENTS AND METHODS:** In a retrospective manner, 4823 patients with dyspeptic complaints who have undergone an upper gastrointestinal endoscopy and a rapid urease test were included. Data of whole blood counts before the procedure were recorded from their files. Patients with normal endoscopic findings or simple gastritis were included. Patients with malignancy, GI bleeding, portal hypertension, liver or kidney disease and taking nonsteroidal anti-inflammatory drugs, proton pump inhibitors, cytotoxic medications were excluded.

**RESULTS:** Mean platelet count in *H. pylori* positive and negative patients were  $246381 \pm 92225/\text{mm}^3$  and  $258135 \pm 89912/\text{mm}^3$ , respectively ( $p < 0.001$ ). Mean MPV was higher in *H. pylori* positive group ( $8.9 \pm 1.3$  vs.  $8.23 \pm 0.94$ ,  $p < 0.001$ ). This difference was observed in both genders. MPV was observed to be higher than 10 fL in 20.5% of HP positive patients while in only 2.8% of *H. pylori* negative patients ( $p < 0.0001$ ).

**CONCLUSIONS:** In patients with *H. pylori* infection and normal platelet counts, it may be speculated that an ongoing and compensated platelet destruction-production process may be responsible for the increase in MPV. Likewise, in conditions exclusive for the host or the *H. pylori* strain, platelet destruction may be enhanced leading to immune thrombocytopenia. As our study is the first study to investigate the effect of *H. pylori* in patients with normal platelet counts, our findings may give way to further prospective researches.

*Key Words:*

*H. pylori*, Immune thrombocytopenia, Mean platelet volume.

## Introduction

*H. pylori* is the most frequent chronic bacterial infection worldwide and in individuals of all ages. Infection is more frequent in developing countries<sup>1,2</sup>. *H. pylori* is a Gram-negative microaerophilic bacterium that colonizes the human gastric mucosa. It generally does not invade the gastroduodenal tissue but triggers a host immune response which leads to tissue injury. Although it is a noninvasive organism itself, the antigenic substances it produces such as heat shock protein, urease and lipopolysaccharide activate T-cells. With an enhanced antigen presentation, inflammatory cytokines such as IL-1, IL-6, IL-8, tumor necrosis factor-alpha (TNF-alpha) are released. Besides, both local and systemic B cell response also occurs. Through these local and systemic immune responses, *H. pylori* is known as a causative agent for gastritis, peptic ulcers and probably by a chronic infection and immune stimulation, gastric cancer and mucosa-associated lymphoid tissue lymphoma. It has also been associated with diseases outside the gastrointestinal tract such as coronary heart disease, acne rosacea, chronic idiopathic urticaria, iron deficiency anemia and systemic autoimmune diseases<sup>3</sup>.

Platelets have essential roles in thrombosis and hemostasis. The normal platelet count ranges from  $100 \times 10^9/\text{L}$  to  $450 \times 10^9/\text{L}$ . Circulating lifespan of platelets is 10 days and almost one third of platelets are sequestered in the spleen. Approximately  $100 \times 10^9$  of platelets are released from megakaryocytes every day to maintain the adequate platelet count. Therefore, a regular and continuous balance between production and consumption is required. Generally defined as a

platelet count less than  $150 \times 10^9/L$ , thrombocytopenia is caused by increased destruction or consumption, splenomegaly, and decreased production due to bone marrow suppression or failure. Besides the contribution of genetic factors, age, gender and seasonal variations are known to effect the platelet count.

A widely used marker of platelet function, mean platelet volume (MPV), is affected from the production step through activation and finally sequestration. In patients with thrombocytopenia, platelet volume analysis may be practical in differential diagnosis. Platelet size does not depend on its age. It is rather determined as they are produced from the megakaryocyte as pre-platelets. Like their size and shape, their cytoplasmic characteristics are determined by the megakaryocytes from which they arise. Large and big platelets are more active and have a tendency to aggregate. This type of platelets contains denser granules, secretes more serotonin and  $\beta$ -thromboglobulin and produces more thromboxane A<sub>2</sub> compared to small platelets<sup>4,5</sup>.

In thrombocytopenia, platelet size may be suggestive of a specific etiology. Large platelets suggest either a process of platelet destruction leading to the production of younger and larger platelets or a possible congenital condition. Another possible explanation for increased MPV is the role of platelets as cells of immunity like polymorph nuclear leukocytes; the increased size of platelets may be due to activation. In thrombocytopenic patients, normal MPV may indicate an impaired thrombopoiesis.

Besides thrombocytopenic conditions, changes in MPV have been studied in several diseases<sup>6,7</sup>. Platelet volume is reported to increase in conditions and diseases such as impairment of tissue oxygenation and nutrition, diabetes mellitus<sup>8</sup>, myocardial infarct<sup>9</sup>, ischemic shock<sup>10</sup>, renal artery stenosis<sup>11</sup> and hypertrophic dilated cardiomyopathy<sup>12</sup>. In a study by Ozaydin et al<sup>13</sup>, MPV values of simple febrile convulsion patients were found to be higher than those of complex febrile convulsion patients. Confusingly, MPV values have been reported to decrease in diseases such as inflammatory bowel disease<sup>14</sup>, pneumonia<sup>7</sup>, Kawasaki disease<sup>15</sup> and acute phase Familial Mediterranean Fever<sup>6</sup>.

Immune thrombocytopenia (ITP) is a benign autoimmune hematological disorder characterized by auto-antibody mediated platelet destruction. With an unknown specific etiology, autoreactive B lymphocytes and abnormal antibody

production as well as other abnormalities in certain cellular immunity levels were suggested to contribute to chronic ITP<sup>16</sup>. Associations with bacterial or viral infections are also suggested. Gasbarrini et al, for first time, has observed increased platelet counts after *H. pylori* eradication in ITP patients in 1998<sup>17</sup>. Recently with numerous studies, improvement of platelet counts in ITP patients with the treatment *H. pylori* has been demonstrated<sup>18</sup>. While in certain studies, no favorable effect was observed<sup>19</sup>. The contrariety may be due to different strains of *H. pylori* in different geographic regions.

Although *H. pylori* -ITP association is well documented and eradication treatment has its place in certain group of ITP patients, the possible association of MPV and *H. pylori* alone in normal platelet counts have been studied with small group of patients. In the study of Topal et al<sup>20</sup>, they have investigated in 114 patients, a hypothesis as MPV being used as a non-invasive indicator of the intensity of *H. pylori* related gastritis, though no relation was observed. The effect of *H. pylori* over MPV has not been assessed in this study.

In this study, we aimed to investigate a relation between platelet indices and *H. pylori* infection in patients with dyspepsia who have otherwise normal platelet counts.

## Patients and Methods

### Patients

Patients with dyspeptic complaints who have undergone upper gastrointestinal endoscopy with normal endoscopic findings or simple gastritis between 2004 and 2014 were enrolled in our study in a retrospective manner. In our unit, over 2000 procedures were performed per year. Rapid urease test is performed on every biopsy specimen. Exclusion criteria were; 1) gastrointestinal bleeding, 2) gastric or duodenal ulcers, 3) gastrointestinal malignancy, 4) portal hypertension, 5) chronic liver disease, 6) chronic kidney disease, 7) patients on nonsteroidal anti-inflammatory drugs, proton pump inhibitors or any cytotoxic treatment. Patient characteristics were summarized in Table I. Whole blood counts were determined by Sysmex and Beckman Coulter LH 780 autoanalyzers within the period of our cohort. In our hospital, blood samples are collected in EDTA containing tubes for whole blood count and reached to the laboratory in less than 2 hours.

**Table I.** Patients characteristics and *H. pylori* positivity.

	<i>H. pylori</i> positive n = 1701	<i>H. pylori</i> negative n = 3122	<i>p</i>
Age (mean ± SD)	60.1 ± 14.9	60.1 ± 15.5	0.92
Sex % (F/M)	44.3/55.7	47.4/52.6	0.04
Mean Platelet count	246381 ± 92225	258135 ± 89912	<0.001
Mean Platelet Volume	8.9 ± 1.3	8.23 ± 0.94	<0.001

*P*: Level of significance (<0.05).

Our study was approved for ethics by our local Ethics Committee.

### Statistical Analysis

Continuous variables such as age, platelet count and mean platelet volume were analyzed by Kolmogorov Smirnov test for normality. All continuous variables were not normally distributed. These variables were compared in *H. pylori* positive and negative group by Mann Whitney U test. Categorical variables were analyzed with chi square test. *p* values less than <0.05 were accepted as statistically significant.

### Results

4823 patients with a mean age 60 (18-80) were enrolled in the study. 46.3% were female and 53.7% were male. Mean age was similar in *H. pylori* positive and negative group. In *H. pylori* positive group, 44.3% of the patients were female and 55.7% were male. In *H. pylori* negative group, 47.4% of the patients were female and 52.6% were male.

In *H. pylori* positive and negative patients, platelet counts and MPV values were not normally distributed and were analyzed by nonparametric Mann-Whitney U test. In *H. pylori* positive patients, mean platelet count was 246381±92225/mm<sup>3</sup>, while

258135±89912/mm<sup>3</sup> in *H. pylori* negative patients. The difference between two groups was statistically significant. Mean MPV value was 8.9± 1.3 in *H. pylori* positive patients while 8.23± 0.94 in *H. pylori* negative patients with a statistical significance (Table I).

Since gender distribution was dissimilar in *H. pylori* positive and negative groups, platelet count and MPV were analyzed separately for each gender. Platelet counts were lower and MPV values were higher for both genders with *H. pylori* positivity (Table II).

In respect of higher MPV values, 2.8% of *H. pylori* negative patients and 20.5% of *H. pylori* positive patients were observed to have MPV higher than 10 fL (Figure 1).

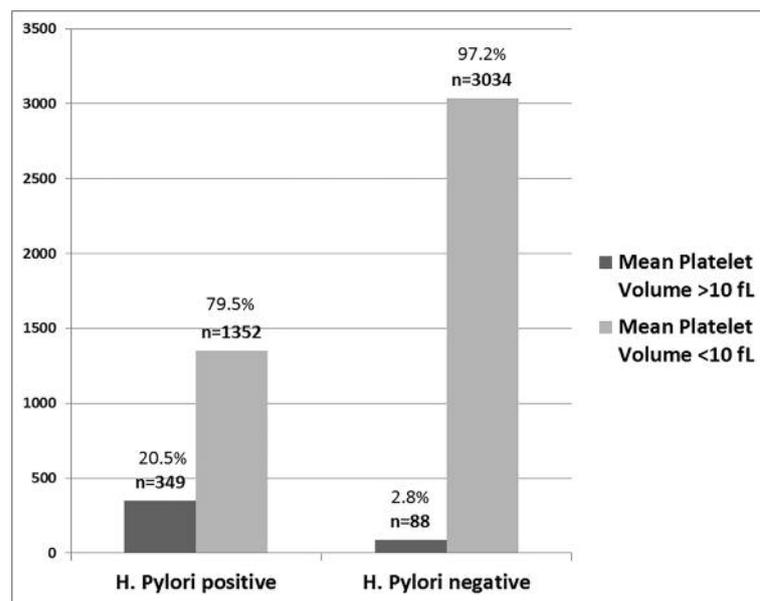
### Discussion

We evaluated platelet counts and MPV values in *H. pylori* positive and negative patients determined by urease test. Urease test is a reliable method for the detection of *H. pylori* with a sensitivity of 80-100% and specificity of 97-99%<sup>21</sup>.

*H. pylori* is linked to ITP with an unclear mechanism. One probable explanation is molecular mimicry; an antibody induced by *H. pylori* may cross react with platelet glycoprotein antigens. CagA positive strains are also suggested as pathogen-

**Table II.** *H. pylori* positivity and platelet indices for both genders.

	Female		<i>p</i>	Male		<i>p</i>
	<i>H. pylori</i> positive (n= 754)	<i>H. pylori</i> negative (n=1481)		<i>H. pylori</i> positive (n=947)	<i>H. pylori</i> negative (n=1641)	
Platelet count (/mm <sup>3</sup> )	253167 ± 102523	269963 ± 93101	<0.001	240978 ± 82777	247731 ± 85640	0.03
Mean platelet Volume (fL)	9.21 ± 1.25	8.19 ± 0.93	<0.001	8.79 ± 1.29	8.28 ± 0.95	<0.001



**Figure 1.** MPV values >10 fL for *H. pylori* positive and negative patients (chi square test,  $p < 0.0001$ ).

ic causatives for ITP. One other probability is the enhancing effect of *H. pylori* on already present antiplatelet antibodies and platelet phagocytosis. Genetic factors of the host are also reported to provide a susceptibility for *H. pylori* related platelet destruction<sup>22</sup>. Eradication of *H. pylori* has been associated with a platelet response, to a degree and in certain group of patients. Though still bearing controversy, this treatment is mentioned in ITP management guidelines<sup>23</sup>.

On the other hand, besides the platelet counts, MPV which is increased in patients with ITP has its own issues. From the perspective of thrombocytes as components of the immune system, MPV has been the subject of many studies demonstrating a link with inflammatory conditions. Platelets with higher MPVs are suggested to be used as indicators of inflammation since they are more active and inclined to aggregate. At the same time, MPV may indicate a rapid thrombopoiesis and may increase in states of platelet destruction.

Although the relation of *H. pylori* and ITP is thoroughly investigated, MPV has been the subject of only 2 small studies. These studies focused on the practicability of MPV in the prediction of *H. pylori* infection intensity and observed no correlation. However, they have not referred to the effect of *H. pylori* infection on platelet counts or MPV<sup>20,24</sup>.

Our study is the first study to demonstrate the increase in MPV in patients infected with *H. py-*

*lori*. In retrospective studies, the nature of confounding factors which may effect the main finding is not easily managed. For this reason, as in all retrospective studies, our study bears the risk of having a type I statistical error. Thus,  $p$  values of both comparisons with MPV and platelet counts being less than 0.0001, suggest that the value of alpha, which is related to the level of significance that has a direct connection on type I errors is also quite low. We also tried to eliminate almost all confounding factors with our detailed patient record system.

Another probable problem for studies on MPV is the lack of standardization and the specificity of MPV determination for the autoanalyzer. Additionally, the period from the collection of the blood to an EDTA containing tube and storage until analysis may affect MPV values<sup>25,26</sup>. Sysmex and Beckman coulter LH 780 autoanalyzer have been used within the period of our cohort using EDTA containing tubes. Since we do not aim to manifest the normal values of MPV, and just compare two groups we believe that this may have an unessential effect on our results.

There is no definitive clinically significant cut-off value for MPV. There is not a plain relation between a MPV value and a significant platelet activation. However, certain MPV cut-off values have been attributed as predictive factors for certain inflammatory diseases. 9.25 fL was suggest-

ed as a predictive factor for major cardiac event in patients who have underwent percutaneous coronary intervention while in another study, values over 9.7 fL were suggested to show a relation with clopidogrel ineffectiveness in patients with unstable angina pectoris<sup>27,28</sup>. On the other hand, no definitive cut-off value has been proposed to indicate an increased trombopoiesis. In a study from Turkey, mean MPV values in healthy subjects were determined as  $8.9 \pm 1.4$  fL<sup>29</sup>. From this perspective, we accepted 10 fL as our cut-off value and observed that MPV over 10 fL is more frequently observed in *H. pylori* positive group.

### Conclusions

In patients with *H. pylori* infection and normal platelet counts, it may be speculated that an ongoing and compensated platelet destruction-production may be responsible for the increase in MPV.

Likewise, in conditions exclusive for the host or the *H. pylori* strain, platelet destruction may be enhanced leading to immune thrombocytopenia. As our study is the first study to investigate the effect of *H. pylori* in patients with normal platelet counts, our findings may give way to further prospective researches.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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