

The effect of gluten-free diet on mean platelet volume, neutrophil and neutrophil/lymphocyte ratio in children with celiac disease

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Abstract

Aim: Celiac Disease (CD) is a chronic, autoimmune and systemic disease. In the present study, the purpose was to investigate the effect of a gluten-free diet on Mean-Platelet-Volume (MPV), neutrophil and neutrophil/Lymphocyte Ratio (NLR).

Material and Methods: A total of 106 patients who were diagnosed with CD and who were followed-up by the Pediatric Gastroenterology Clinic of the Hospital, and 50 healthy children were included in the study. The demographic data of the patients and the neutrophil, lymphocyte, platelet, NLR were recorded before the diagnosis and one-year after gluten-free diet. The hematologic parameters of the patients were compared with the healthy control group at the diagnosis time. Following a one-year gluten-free diet, the hematologic parameters of the patients were compared with the parameters at the diagnosis time.

Results: The mean age of the patients was 12.1±3.2 years (7-16 years), and the mean age of the control group was 14.5±4.1 years (12-16 years). A total of 48% (51/106) of the patients were female and 34% (17/50) of the control group was female. Hematological parameters of patients compared pre-dietary and post-dietary; lymphocyte and platelet ratios were significantly higher, whereas neutrophils, MPV and NLR were significantly lower. Neutropenia was detected in 15 patients before the diet. It was observed that neutrophil level reached the normal range after gluten-free diet in patients with neutropenia.

Conclusions: The MPV, neutrophil and NLR values, that was low during the diagnosis, may be ameliorative with the gluten-free diet. In addition, CD should be considered in the differential diagnosis of neutropenic patients.

Keywords: Celiac Disease; MPV; NLR; PLR

INTRODUCTION

Celiac Disease (CD) is a systemic disease caused by immune mechanism that is triggered by gluten in cereals like wheat, barley and rye in individuals who have genetic predisposition. The frequency of the disease varies among geographical regions. The highest prevalence is in Turkey and North America which are countries where wheat is an important part of nutrition. In the pathogenesis, several factors such as environmental, immunological and genetic play roles (1-3).

Platelets are specialized blood cells, which play in hemostasis (4). Platelet volume measured to assess platelet size is also important in this regard. Increased MPV is attribute to an increase thrombopoietic stress response (5). Accordingly, platelet activation during

inflammation can be measured with MPV. MPV is applied as an inflammatory indicator in many diseases. The relation between MPV and various diseases was also examined in many previous studies (6-8). Neutrophils initiate the first stage of defense in systemic inflammation, and lymphocytes constitute the protective and regulatory composition of inflammation. High Neutrophil/Lymphocyte Ratio (NLR) is the indicator of an ongoing inflammation.

In previous studies, it was shown that neutrophil and NLR are new biomarkers in detecting systemic inflammatory response, and there is a significant relation between many chronic diseases and NLR (9,10). In our study, we aimed to investigate the relationship between CD and NLR, neutrophil, PLR.

Received: 10.02.2020 **Accepted:** 21.04.2020 **Available online:** 17.06.2020

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MATERIAL and METHODS

Study design

A total of 106 newly-diagnosed patients followed-up by the Pediatric Gastroenterology Clinic between October 2017 and February 2019, and 50 healthy children with irritable bowel syndrome as control group were included in the study. It was paid attention the control group was similar to the patients with celiac disease about age, gender and etc. Our study was designed retrospectively. Those who had systemic diseases, used drugs that affected the leukocyte count and platelet functions, and infectious diseases, were excluded from the study.

Data assessment

The demographic data of all patients (age, gender) and MPV, neutrophil, lymphocyte, platelet, NLR, which are among hemogram parameters, were recorded before the diagnosis and after one-year diet. Endoscopic images and histopathological data of the patients were documented. Concomitant comorbid diseases, medications and previous surgeries were questioned. The hematologic parameters of the patients were compared with the healthy control group at the diagnosis time. After a one-year gluten-free diet, the hematologic parameters of the patients were compared with the parameters at the diagnosis time.

Biochemical measurements

The MPV, NLR, platelets, lymphocytes, leukocytes, neutrophils and lymphocytes measurements were made within 30 minutes after the blood samples were collected in tubes that had dipotassium EDTA in them. The absolute neutrophil count being below 1500 mm³ was considered as neutropenia.

Endoscopic evaluation

The endoscopy of the patients was performed with the Fujinon EG530WR Endoscopy Device. Consents were obtained from the participants. All patients starved for 6 hours before the endoscopy, and the endoscopy procedure was performed after the patients were sedated with Midazolam at a rate of 0.1 mg/kg and Ketamine at a rate of 1 mg/kg. The duodenum was examined in detail

during the endoscopy, and multiple biopsies were taken from different parts of the duodenum to diagnose the CD.

Histopathological evaluation

The duodenum biopsies that were taken endoscopically were sent to the pathology laboratory in 10% formaldehyde. The histopathological evaluation was based on Marsh Classification. Characteristic histological findings (increased villous atrophy, intraepithelial lymphocytes, and crypt hyperplasia), was classified with Standard Classification of Marsh (11).

Statistical analysis

The normality of distribution of continuous variables was tested by Shaphiro Wilk test. Mann-Whitney U test (for non-normal data) was used for comparison of two independent groups and Wilcoxon test was used to compare before and after measurements for non-normal data. Chi-square test was used to assess the relation between categorical variables. Statistical analysis was performed with SPSS 24.0; and a p value < 0.05 was accepted as statistically significant.

RESULTS

A total of 156 people were included in the study; 106 of them were patients with CD, and 50 of them constituted the healthy control group. The mean age of the patients with CD who were included in the study was 12.1±3.2 years (range: 7 - 16 years), and the mean age of the control group was 14.5±4.1 years (range: 12 years - 16 years). A total of 48% (51/106) were female, 52% (55/106) were male in the patient group, and 34% (17/50) of the control group were female and 66% (33/50) were male. Significant differences were not detected in the age and gender distribution between each groups (p>0.05). When the two groups were compared in terms of hematologic parameter counts and ratios, it was determined that lymphocyte and platelet counts were significantly higher and neutrophil counts, NLR and MPV were significantly lower in patients with CD than in the healthy control group at the time of diagnosis (p<0.05) (Table 1).

Table 1. Comparison of the patient and control group

Variables	Celiac Patients (n=106)	Control (n=50)	p
Female/Male (n)	51/55	17/33	0.097
	Median±SS [25%-75%]	Median±SS [25%-75%]	
Age (years)	12.1±3.2 [range:7 -16]	14.5±4.1 [range:12 -16]	0.237
Platelet	353±82x10 ³ [296x10 ³ - 434x10 ³]	270±51x10 ³ [232 x10 ³ -313x10 ³]	0.001*
Lymphocyte	3190±512 [2270 -4700]	2535±523 [1970 -3240]	0.006*
Neutrophil	2510±498 [1625 -4220]	4190±975 [2620 -5350]	0.001*
NLR	0.79±0.18 [0.44 -1.38]	1.63±3.1 [1.12 -1.93]	0.001*
MPV	8.4±2.2 [7.3 -9.7]	9.35±3.7 [8.8 -10.1]	0.001*

NLR; Neutrophil / Lymphocyte Ratio, PLR; Platelet/ Lymphocyte Ratio, MPV; Mean Platelet Volume, *Significant at < 0.05; Mann Whitney U-test

Hematological parameters of CD compared pre-dietary and post-dietary; lymphocyte and platelet ratios were significantly higher, whereas neutrophils, MPV and NLR were significantly lower ($p < 0.05$). Platelet and lymphocyte counts decreased significantly and neutrophil counts, MPV and NLR rates increased significantly after diet in patients with CD, ($p < 0.05$). However, it was found that PLR ratio was not significantly affected by diet ($p > 0.05$) (Table 2).

In 15 patients with CD, pre-diet neutrophil values were below 1500 / mm³ and these patients were accepted as neutropenia. Folic acid deficiency was detected in 5 patients and vitamin B-12 deficiency was detected in 6 patients. Etiology was not detected in four patients. Neutrophil counts returned to normal at the end of the first year with vitamin supplementation and gluten-free diet in all patients.

Table 2. Comparison of the values of the Patient Group before and after the diet

	Before the diet (n=106) Median±SS [25%-75%]	After the diet (n=106) Median±SS [25%-75%]	P
Platelet	353±82x10 ³ [296x10 ³ - 434x10 ³]	291±72 x10 ³ [256 x10 ³ -344 x10 ³]	0.001*
Lymphocyte	3190±512 [2270 -4700]	2650± 498 [2060 -3560]	0.001*
Neutrophil	2510±498 [1625 -4220]	3470±617 [2620 -4940]	0.001*
NLR	0.79±0.18 [0.44 -1.38]	1.27±0.24 [0.92 -1.9]	0.001*
MPV	8.40±2.2 [7.3 -9.7]	9,25±2.3 [8.1 -9.8]	0.004*

NLR; Neutrophil / Lymphocyte Ratio, PLR; Platelet/ Lymphocyte Ratio, MPV: Mean Platelet Volume, *Significant at < 0.05 ; Wilcoxon test

DISCUSSION

Although platelets play an active role in inflammation, MPV has also been reported to induce inflammation by causing platelet activation (7,8,12). Although there are many studies examining the relationship between MPV and inflammatory diseases, conflicting results have been reported (13,14). While MPV value increased in some diseases, it decreased in some diseases (15-19). When the literature was reviewed, it was detected that there are different results regarding the use of MPV as a biomarker both in patients with CD and in other local or systemic inflammation. In studies that were conducted on adult patients with CD, it was reported that MPV was higher at significant levels at the time of diagnosis, and that MPV decreased at significant levels after a gluten-free diet (20,21). In some studies, it has been reported that there is no significant relationship between dietary compliance and MPV (22). In our study, MPV value was determined to be lower at the time of diagnosis than in the control group. It was also determined that MPV values improved with the gluten-free diet.

White spheres play roles in the pathogenesis of several diseases. In addition to the increases in the number of neutrophils in acute inflammatory processes, the decrease in the number of lymphocytes because of acute stress also reflects the changes in the immune system. The rate of these two subgroups to each other was used as an inflammation marker (23). NLR is an easily accessible and inexpensive inflammatory marker that is calculated by dividing the number of neutrophils by the number of lymphocytes. In the study that was conducted by Temiz et al., it was reported that increased NLR had a strong relation with mortality in cardiovascular diseases

(24). In the study that was conducted by Ghaffari et al., it was reported that increased neutrophil and NLR counts were a predictive factor in terms of heart failure in patients who had myocardial infarction that had ST elevation (25). In the study that was conducted by Markar et al. with patients who had acute appendicitis, they reported that the NLR elevation and clinical findings and symptoms were diagnostic (26). NLR elevation was correlated with the severity of the disease in patients with acute pancreatitis (27). In the study that was conducted by A. Temizi et al. with patients who had acute cholecystitis, it was reported that increased NLR was higher at significant levels in patients who had acute cholecystitis (28). In the study that was conducted by Celikbilek et al., it was reported that NLR was higher at significant levels in patients who had active ulcerative colitis than in the Control Group (29). In the present study of ours, it was found that neutrophil and NLR counts were lower at the time of diagnosis when compared to the Control Group and post-dietary values. Celiac Disease is characterized by a deficiency of trace elements and vitamins, and involves the small intestine. It is known that neutrophil synthesis may be reduced in the absence of vitamins and trace elements like folic acid, Vitamin B-12 deficiency and copper, which are all essential for neutrophil synthesis. A total of 5 patients had folic acid deficiency, and 6 patients had Vitamin B 12 deficiency. No etiological causes were detected in 4 patients. In the follow-ups of the patients, it was determined that neutrophils and NLRs improved with the diet and vitamin support in 11 patients who had vitamin deficiency, and the neutrophil and NLR ratios of the other 4 patients improved at significant levels with only a gluten-free diet. It was observed that there was an improvement in hematological parameters like neutrophil

and NLR values of non-neutropenic patients after gluten-free diet and it was similar to the healthy control group.

The present study had some limitations and strong points. Rare studies have been conducted with children who have CD. The inclusion of pediatric Celiac patients and comparison of them with the healthy control group are the strengths of our study. In addition, the effect of diet on hematologic parameters was also examined by comparing the data that were obtained both before and after the diet in patients with CD. The fact that our study had retrospective design, and that the trace elements were not examined at the time of diagnosis and in the follow-ups constitutes the limitation of our study.

CONCLUSION

In conclusion it was demonstrated that the neutrophil count is low and lymphocyte count is high in patients with CD compared to the control group. For this reason, we believe that in cases with neutropenia, CD should be kept in mind. In addition, the MPV, neutrophil and NLR values, which are low in cases with CD compared to the healthy control group at diagnosis time, are improved with a gluten-free diet. After the diagnosis, increased MPV, neutrophil and NLR show the compliance to the diet. We believe that hematologic parameters can be used in terms of compliance in the follow-up of patients. New studies that will be conducted with different populations are needed.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: Ethics committee approval for the study was given by the Van Education and Research Hospital Clinical Research Ethics Committee (Van/Turkey, Approval number 01/19, approved on 3 March 2019).

REFERENCES

- Kayar Y, Dertli R. Association of autoimmune diseases with celiac disease and its risk factors. Pak J Med Sci 2019;35:1548-53.
- Dertli R, Kayar Y, Surmeli N, et al. The Relationship Between The Presence of Gastrointestinal Symptoms and Anemia, Thyroid Functions and Bone Mineral Density In Celiac Patients. Eastern J Med 2019;24 :355-60.
- Kayar Y, Surmeli N, Dertli R, et al. Çölyak Hastalarında Demografik, Histopatolojik ve Klinik Özellikler. Van Tıp Derg 2019;26:363-9.
- Both PM, Butterworth RJ. Trombosit size: measurement, physiology and vascular disease. Blood Coagul Fibrinolysis 1996;7:157-61.
- Ceylan Y, Kumanlioglu K, Oral A, et al. The Correlation of Clinicopathological Findings and Neutrophil-to-Lymphocyte and Platelet-to-Lymphocyte Ratios in Papillary Thyroid Carcinoma. Mol Imaging Radionucl Ther 2019;28:15-20.
- Almis H, Bucak IH, Celik V. Mean platelet volume in hepatitis A. European Review for Med Pharma Sci 2016;20:2310-4.
- Beyazit Y, Sayilir A, Torun S, et al. Mean platelet volume as an indicator of disease severity in patients with acute pancreatitis. Clin Res Hepatol Gastroenterol 2012;36:162-8.
- Mete E, Akelma AZ, Cizmeci MN, et al. Decreased mean platelet volume in children with acute rotavirus gastroenteritis. Platelets 2014;25:51-4.
- Sunbul M, Gerin F, Durmus E, et al. Neutrophil to lymphocyte and platelet to lymphocyte ratio in patients with dipper versus non-dipper hypertension. Clin Exp Hypertens 2014;36:217-21.
- Ferroni P, Riondino S, Formica V, et al. Venous thromboembolism risk prediction in ambulatory cancer patients: clinical significance of neutrophil/lymphocyte ratio and platelet/lymphocyte ratio. Int J Cancer 2015;136:1234-40.
- Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiologic approach to the spectrum of gluten sensitivity ('celiacsprue'). Gastroenterology 1992;102:330-54.
- Endler G, Klimesch A, Sunder-Plassmann H, et al. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. Br J Haematol 2002;117:399-404.
- Bath PM, Missouriis CG, Buckenham T, et al. Increased platelet volume and platelet mass in patients with atherosclerotic renal artery stenosis. Clin Sci (Lond) 1994;87:253-7.
- Icli A, Aksoy F, Dogan A, et al. Increased mean platelet volume in hypertrophic cardiomyopathy. Angiology 2014;65:420-4.
- Makay B, Turkyilmaz Z, Unsal E. Mean platelet volume in children with familial Mediterranean fever. Clin Rheumatol 2009;28:975-8.
- Karadag-Oncel E, Ozsurekci Y, Kara A, et al. The value of mean platelet volume in the determination of community acquired pneumonia in children. Ital J Pediatr 2013;39:16.
- Ozturk ZA, Dag MS, Kuyumcu ME, et al. Could platelet indices be new biomarkers for inflammatory bowel diseases? Eur Rev Med Pharmacol Sci. 2013;17:334-41.
- Khawaja IS, Westermeyer JJ, Gajwani P, et al. Depression and coronary artery disease: the association, mechanisms, and therapeutic implications. Psychiatry (Edgmont) 2009;6:38-51.
- Aktas G, Alcelik A, Tekce BK, et al. Red cell distribution width and mean platelet volume in patients with irritable bowel syndrome. Prz Gastroenterol 2014;9:160-3.
- Ramachandran M, Agarwal A, Ravi RNM, et al. Mean platelet volume as short-term follow-up biomarker in children with celiac disease. Indian J Child Health 2017;4:515-7.

21. Purnak T, Efe C, Yuksel O, et al. Mean platelet volume could be a promising biomarker to monitor dietary compliance in celiac disease. *Ups J Med Sci* 2011;116:208-11.
22. Bolat AD, Koseoglu H, Akin FE, et al. Can serum Mean Platelet Volume be Used as an Inflammatory Marker in Patients with Celiac Disease? *Akademik Gastroenterol Derg* 2018;17:62-5.
23. Gibson PH, Cuthbertson BH, Croal BL, et al. Usefulness of neutrophil/lymphocyte ratio as predictor of new-onset atrial fibrillation after coronary artery bypass grafting. *Am J Cardiol* 2010;105:186-91.
24. Temiz A, Gazi E, Gungor O, et al. Platelet/lymphocyte ratio and risk of in-hospital mortality in patients with ST-elevated myocardial infarction. *Med Sci Monit* 2014; 20:660-5.
25. Ghaffari S, Nadiri M, Pourafkari L, et al. The predictive value of total neutrophil count and neutrophil/lymphocyte ratio in predicting in-hospital mortality and complications after STEMI. *J Cardiovasc Thorac Res* 2014;6:35-41.
26. Markar SR, Karthikesalingam A, Falzon A, et al. The diagnostic value of neutrophil: lymphocyte ratio in adults with suspected acute appendicitis. *Acta Chir Belg* 2010;110:43-7.
27. Suppiah A, Malde D, Arab T, et al. The prognostic value of the neutrophillymphocyte ratio (NLR) in acute pancreatitis: identification of an optimal NLR. *J Gastrointest Surg* 2013;17:675-81.
28. Temizi A, Ozdemir Y, Aslan A, et al. Role of complete blood counts parameters in diagnosis of acute cholecystitis. *Acta Med Mediterr* 2017;33:411-6.
29. Celikbilek M, Dogan S, Ozbakir O, et al. Neutrophil-lymphocyte ratio as a predictor of disease severity in ulcerative colitis. *J Clin Lab Anal* 2013;27:72-6.