Simple Inflammatory Markers for the Early Diagnosis of Celiac Disease

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ABSTRACT

Objective: The aim is to compare the newly diagnosed Celiac and control groups in terms of inflammatory markers such as platelet/lymphocyte ratio (PLR) and neutrophil/lymphocyte ratio (NLR).

Methods: This retrospective study included 68 patients who were newly diagnosed with Celiac Disease (CD) and 30 healthy volunteers. Haemogram tests and C-reactive protein (CRP) measurements were obtained from the hospital records. Then, NLR and PLR were calculated. Kolmogorov-Smirnov test, Student's *t*-test, Mann-Whitney U-test, then Pearson's or Spearman's correlation analysis were used. Receiver operating characteristic (ROC) analyses were performed. Local Ethics Committee for Clinical Research approved this study.

Results: The mean ± SD values of NLR (1.98 ± 1.02, 2.54 ± 1.28), PLR (114.56 ± 40.61, 158.31 ± 62.86), mean platelet volume (MPV) [8.26 ± 0.84, 7.76 ± 1.55], mean corpuscular haemoglobin concentration (MCHC) [34.36 ± 0.68, 33.04 ± 1.63] and red cell distribution width (RDW) [13.42 ± 1.01, 13.25 ± 2.71] were significantly different between healthy and newly diagnosed CD groups ($p < 0.01$). In ROC analysis, NLR ≥ 1.58 had 97.1% sensitivity and 55.2% specificity, PLR ≥ 110.69 had 80% sensitivity and 59% specificity, RDW ≤ 13.25 had 67.6% sensitivity and 56% specificity in predicting newly diagnosed CD.

Conclusion: Neutrophil/lymphocyte ratio, PLR, RDW, MPV and MCHC are significantly different in newly diagnosed CD ($p < 0.01$). Platelet/lymphocyte ratio is correlated positively with NLR and RDW; negatively with, MPV and MCHC ($p < 0.01$). Neutrophil/lymphocyte ratio, PLR and RDW may be early clues to the diagnosis of CD.

Keywords: Celiac disease, mean platelet volume, neutrophils-to-lymphocyte ratio, platelet-to-lymphocyte ratio, red cell distribution width

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INTRODUCTION

Celiac disease (CD) is an immune mediated intestinal inflammatory disorder seen among genetically susceptible individuals and affects more than 1% of the population. Ingestion of gluten containing foods triggers the symptoms (1). Neutrophil/lymphocyte ratio (NLR) is calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. Neutrophil/lymphocyte ratio indicates systemic inflammation and stress in a simple and rapid way (2).

Platelet/lymphocyte ratio (PLR) has been described as a prognostic biomarker for many chronic diseases. Platelets are important coordinators of inflammation (3). Red cell width (RDW), is the measure of variability in the red blood cell size, the higher values reflects heterogeneity in erythrocytes sizes (4), as well as an important inflammatory biomarker (5, 6). Red cell width distribution level might be influenced by inflammation (4).

Mean platelet volume (MPV) is the measurement of the average size of platelets. It reflects the platelet production rate and it's one of the platelet function indices (7). It has been studied as a simple inflammatory marker in several diseases (8).

Mean corpuscular haemoglobin concentration (MCHC) expresses the average concentration of hemoglobin per unit volume of red cell. In a study it was found that, if marrow iron stores were at low levels in patients with Crohn's disease, MCHC values were very low, but in some patients who had iron stores in the marrow, MCHC values were subnormal (9).

C-reactive protein (CRP) is a frequently used inflammation marker. In an inflammatory condition, cytokines IL1β and IL6 increase. This increase positively correlates with circulating CRP levels (10).

The relationship between NLR, PLR, RDW, MPV, MCHC, CRP and CD is unknown. The aim of this retrospective study is to investigate whether simple inflammatory markers are statistically significant in the initial diagnosis of CD.

SUBJECTS AND METHODS

This retrospective study included 68 patients newly diagnosed with CD and 30 healthy volunteers. Informed consent was obtained from the research participants. The patients who were referred to Endocrinology Polyclinic between years 2010─2015 for being underweight and having trouble gaining weight were investigated as possible cases of CD. Their body mass indexes were smaller than 18. Serum samples were found to be positive for anti-gliadin and anti-endomysial antibodies, then duodenal biopsy specimens were taken. There were intraepithelial lymphocytosis, crypt hyperplasia, and villous atrophy. So, a diagnosis of CD was established based on modified Marsh classification (11). Patients were excluded, if: they were taking some drugs (anticoagulant medications, non-steroid anti-inflammatory drugs and oral contraceptives), had a history of acute or chronic infection, haematologic and hepatic disorders, heart failure, peripheral vascular disease and cancer.

The mean age was 23.79 ± 10.23 in the CD study group and 34.96 ± 13.72 in the control group. The two groups were thought to be well matched in terms of sample size and gender distribution.

Measurements

The complete blood count (CBC) analyses were performed by using the same regularly calibrated Haematology Analyser (Beckman/Coulter LH 780 CA, USA). For CBC analyses, obtained blood samples were collected in standard tubes with ethylenediaminetetraacetic acid (EDTA) and they were determined within 2h of collection. By the hemogram analysis, hemoglobin (Hb), white blood cell count (WBC), neutrophil count, lymphocyte count, , platelet count (PLT), red cell distribution width (RDW), platelet distribution width (PDW), mean

platelet volume (MPV) were assessed. Furthermore, the NLR and PLR were calculated. CRP measurements were obtained from the hospital records.

Statistical analyses

In the Kolmogorov-Smirnov test, all variables were checked for normal distribution. Variations between CD and control groups were evaluated by Student's t-test. Mann–Whitney U-test was used if data were not normally distributed. Correlations between variables were performed by Pearson's or Spearman's correlation analysis.

ROC analyses were performed to determine the association of NLR, PLR and RDW with CD. For statistical calculations, Statistical Package for Social Sciences (SPSS) Statistical Software (SPSS for Windows, version 17.0; SPSS Inc. Chicago, IL, USA) was used.

Local Ethics Committee for Clinical Research approved this study. World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects were applied in this study.

RESULTS

The mean age was 23.79 ± 10.23 in the CD study group and 34.96 ± 13.72 in the control group. Differences between the two groups in terms of PLR, NLR, RDW, MPV, MCHC, leucocyte, lymphocit, neutrophil, platelet counts and CRP values were evaluated. The mean±SD values which were significantly different in healthy and newly diagnosed CD groups, respectively, were: NLR 1.98 ± 1.02, 2.54 ± 1.28, PLR 114.56 ± 40.61, 158.31 ± 62.86, MPV 8.26 ± 0.84, 7.76 ± 1.55, MCHC 34.36 ± 0.68, 33.04 ± 1.63 and RDW 13.42 ± 1.01, 13.25 ± 2.71.

In Table-1, age, gender distribution and laboratory measurements of newly diagnosed CD group and controls were shown.

Table 1: Age, gender distribution and laboratory measurements of newly diagnosed CD group and controls.

	Newly Diagnosed CD Group (n = 68)	**Control Group (n = 30)**	***p***
Age	23.79±10.23	34.96±13.72	
Gender(male/female)	19/49	14/16	
NLR	2.5405±1.28095	1.9873±1.02445	0.040*
MPV	7.7690±1.55864	8.2667±0.84785	0.045*
PLR	158.3148±62.86873	114.5637±40.61922	0.001*
RDW	13.2559±2.71774	13.4207±1.01818	0,752
Leucocyte	7.8732±2.32954	7.2517±1.61392	0.194
Neutrophil	4.9562±1.89653	4.2567±1.56463	0.080
Lymphocyte	2.0744±0.63559	2.3200±0.59677	0.076
PLT	307.2794±102.12647	250.4138±57.92453	0.006*
MCHC	33.0485±1.63757	34.3621±0.68734	0.000*
CRP	0.90151±0.69497	0.8700±0.93273	0.956

*($p < 0.05$)

CD = Celiac disease; NLR = neutrophil/lymphocyte ratio; MPV = mean platelet volume; PLR = platelet/lymphocyte ratio; RDW = red cell width distribution; PLT = platelet; MCHC = mean corpuscular haemoglobin concentration; CRP = C-reactive protein.

There was a significantly positive correlation between PLR and NLR (r = 0.540; $p = 0.000$), PLR and RDW (r = 0.258; $p = 0.011$).

There was a significantly negative correlation between PLR and MPV (r = 0.283; $p = 0.005$), PLR and MCHC (r = 0.361; $p = 0.000$).

There was a significantly positive correlation between RDW and PLT (r = 0.406; $p = 0.000$), RDW and CRP (r = 0.621; $p = 0.000$), RDW and PLR (r = 0.258; $p = 0.011$).

There was a significantly negative correlation between RDW and MCHC (r = 0.376; $p = 0.000$).

Table-2 shows correlations.

Variables		NLR	RDW	PLR	MPV	leucocyte	CRP	neutrophil	lymphocyte	plt	MCHC
NLR	r	1	,002	,540**	-,091	,510**	,113	,762**	-,462**	,140	-,047
	p		,988	,000	,375	,000	,480	,000	,000	,171	,646
RDW	r	,002	**1**	,258*	-,191	,038	,621**	,019	,080	,406**	-,376**
	p	,988		,011	,061	,714	,000	,853	,438	,000	,000
PLR	r	,540**	,258*	1	-,283**	-,077	,139	,141	-,588**	,640**	-,361**
	p	,000	,011		,005	,453	,392	,169	,000	,000	,000
MPV	r	-,091	-,191	-,283**	1	-,136	-,139	-,142	-,033	-,470**	,090
	p	,375	,061	,005		,184	,387	,164	,748	,000	,381
leucocyte	r	,510**	,038	-,077	-,136	1	-,115	,932**	,462**	,264**	-,120
	p	,000	,714	,453	,184		,478	,000	,000	,009	,243
CRP	r	,113	,621**	,139	-,139	-,115	1	-,036	-,238	-,156	-,038
	p	,480	,000	,392	,387	,478		,823	,135	,338	,815
neutrophil	r	,762**	,019	,141	-,142	,932**	-,036	1	,146	,251*	-,093
	p	,000	,853	,169	,164	,000	,823		,153	,013	,365
lymphocyte	r	-,462**	,080	-,588**	-,033	,462**	-,238	,146	1	,126	-,092
	p	,000	,438	,000	,748	,000	,135	,153		,220	,368
plt	r	,140	,406**	,640**	-,470**	,264**	-,156	,251*	,126	**1**	-,493**
	p	,171	,000	,000	,000	,009	,338	,013	,220		,000
MCHC	r	-,047	-,376**	-,361**	,090	-,120	-,038	-,093	-,092	-,493**	**1**
	p	,646	,000	,000	,381	,243	,815	,365	,368	,000	

*($p < 0.05$); **($p < 0.01$)

NLR = Neutrophil/lymphocyte ratio; RDW = Red cell width distribution; PLR = Platelet/lymphocyte ratio; MPV = Mean platelet volume; CRP = C-reactive protein; PLT = Platelet; MCHC = Mean corpuscular haemoglobin concentration.

In ROC analysis, NLR ≥ 1.58 had 97.1% sensitivity and 55.2% specificity, PLR ≥ 110.69 had 80% sensitivity and 59 % specificity, RDW ≤ 13.25 had 67.6% sensitivity and 56% specificity in predicting newly diagnosed CD. ROC curve analysis showed that NLR, PLR and RDW can be used as predictors in newly diagnosed CD.

Figure-1 is ROC Curve for RDW, NLR and PLR.

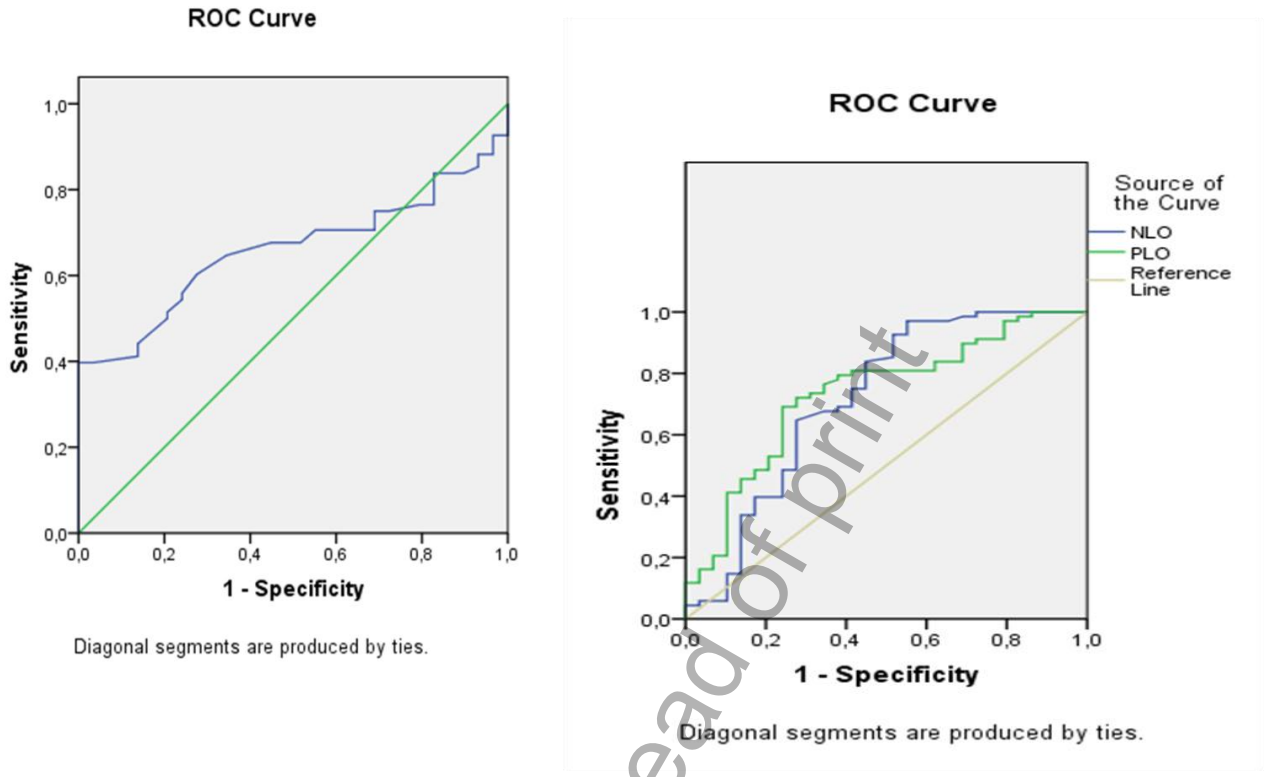

Fig. 1: RDW (red cell width distribution) showed an area under the curve (AUC) of 0.659 (95% CI, 0.555-0.764) [$p < 0.013$]. NLR (neutrophil/lymphocyte ratio) showed an AUC of 0.719 (95% CI, 0.593- 0.869) [$p < 0.001$] and PLR (platelet/lymphocyte ratio) showed an AUC 0.734 (95% CI, 0.624-0.844) [$p < 0.000$].

DISCUSSION

Celiac disease (CD) is a chronic inflammatory condition of the small intestine. Both, genetic and environmental factors take place. There is permanent intolerance to gluten/gliadin [prolamin] (12, 13).

The main result of our study was determining the best marker for subclinical inflammation in the early diagnosis of celiac disease by comparing NLR, PLR, MPV and RDW.

When the two groups were evaluated in the terms of PLR, NLR, RDW, MPV, MCHC, leucocyte, lymphocit, neutrophil, platelet counts and CRP values; NLR and PLR were significantly higher; MPV, MCHC and RDW were significantly lower in newly diagnosed CD

than in healthy group. In ROC analysis, NLR ≥ 1.58 had 97.1% sensitivity and 55.2% specificity, PLR ≥ 110.69 had 80% sensitivity and 59 % specificity, RDW ≤ 13.25 had 67.6% sensitivity and 56% specificity in predicting newly diagnosed CD. We suggest that NLR, PLR and RDW may be useful as a strong predictor of early diagnosis of CD.

Celiac Disease is a chronic inflammatory condition of the small intestine. In the diagnosis, blood tests such as antigliadin and antiendomysial antibodies and upper gastrointestinal endoscopy are used and small intestinal biopsy is taken. In this study, anti-gliadin and anti-endomysial antibodies were found positive, then duodenal biopsy was performed and it represented intraepithelial lymphocytosis, crypt hyperplasia and villous atrophy. So, a diagnosis of CD was established based on modified Marsh classification.

Formerly, systemic inflammation couldn't be shown in CD. In a study in 2004, proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, INF-γ and tumor necrosis factor-α (TNF-α) levels in plasma were found significantly higher in CD, when compared to control group (14).

To determine systemic inflammation, various haematological and biochemical markers can be measured. In different chronic conditions, total leukocyte count and CRP found to be increased (15). Leucopenia has been reported in some children with celiac disease (16). In our study, we could neither found a significant difference in leucocyte counts nor in CRP levels between CD group and healthy group.

Haematologic abnormalities that may be associated with CD include leucopenia, anaemia, thrombocytosis or thrombocytopenia. We excluded iron deficiency anaemia or functional hyposplenia. Our patients had normal levels of ferritin, vitamin B12 and vitamin D when they were diagnosed.

Thrombocytosis was reported in up to 60% of patients with CD (17). It is more common than thrombocytopenia (16, 18). In our study there was a significant difference in the platelet count between CD and healthy group ($p < 0.05$).

The NLR and MPV are markers of inflammation and endothelial dysfunction, respectively. NLR, has become a biomarker of numerous chronic inflammatory diseases (19, 20). In our study, NLR and PLR were significantly higher; MPV, MCHC and RDW were significantly lower in newly diagnosed CD than in healthy group.

Neutrophilia or lymphopaenia results in high NLR while lymphocytosis or neutropaenia results in low NLR. In our study there wasn't a significant difference between CD group and healthy group in the neutrophil and lymphocyte counts.

In a study (21), NLR was found significantly higher in patients with CD (2.42 ± 1.24) when compared to control group [1.92 ± 0.58] ($p < 0.019$) and in ROC analysis NLR was statistically remarkable in the diagnose. In our study, NLR was significantly higher in CD than in healthy group ($p < 0.05$).

Another inflammatory marker is PLR. In various diseases like end-stage renal failure, and ovarian epithelial carcinoma, PLR value has been shown to correlate with poor prognosis (22, 23). In our study, PLR is significantly higher in CD than in healthy group ($p < 0.05$).
In Crohn patients NLR values were found to be correlated with WBC and CRP levels (24). In our study, NLR is correlated with PLR, lymphocyte and neutrophil.

Red cell distribution width correlates with the degree of anisocytosis or variation in red blood cell width. In a study, an elevated RDW with normal haemoglobin concentration found to be a reliable predictor of CD (25). In our study, we found a significant difference in RDW in newly diagnosed CD than in healthy group ($p < 0.05$).

In our study, ROC curve analysis showed that NLR, PLR and RDW are significantly different in newly diagnosed CD than in control group.

Large platelets are likely more reactive, thus, MPV could be thought to reflect platelet function. In the bone marrow, cytokines and growth factors may elicit the production of more reactive and larger platelets (26). MPV is a marker of the functional status of platelets (27) and has been studied as a simple inflammatory marker in several diseases. In our study, we found a significant difference in MPV in CD than in healthy group ($p < 0.05$).

Mean corpuscular haemoglobin concentration is the average concentration of hemoglobin inside an erythrocyte. We found a significant difference in MCHC between CD and healthy group.

Rapid and certain diagnosis in CD by serological tests and duodenal biopsy is important. To find out some simple inflammatory markers in CD, can guide us to predict the diagnose and prognose.

Limitation of our study is to be a small sample sized retrospective study. The future studies with larger number of patients can provide further data on these simple inflammatory markers.

CONCLUSION

Neutrophil/lymphocyte ratio, PLR, RDW, MPV and MCHC values are significantly different in newly diagnosed CD than in control group ($p < 0.01$). These inflammatory markers are cheap and can be measured easily and quickly. NLR, PLR and RDW may be early clues to the diagnosis of CD.

Author Contributions

A Arslan, A Carlioglu conceived paper, participated in study design, oversaw data collection, interpretation of data, conducted data analysis, wrote manuscript and approved final version. A Carlioglu critically revised manuscript, provided oversight to study. M Filiz, E Bayraktar, I Baydar, A Turhan, F Sonmez conceived paper, oversaw data collection, interpretation of data, conducted data analysis, approved final version. The authors declare that they have no conflicts of interest.

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