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Is there a link between neutrophil-lymphocyte ratio and patient compliance with gluten free diet in celiac disease?

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ÖZET

Çölyak hastalığında nötrofil-lenfosit oranı glutensiz diyet uyumu ile ilişkili midir?

Bu çalışmanın amacı, çölyak hastalığında (ÇH) diyet uyumu tespit edilmesi için objektif bir belirteç olarak nötrofil lenfosit oranının (NLO) etkisini belirlemektir. Otuz yedi ÇH hastası ve 37 sağlıklı gönüllü çalışmaya dahil edildi. Birinci yılın sonunda, hastalar diyet ile uyumu dikkate alınarak 2 gruba ayrıldı. Glutensiz diyet uyumu (GsDU) olmayan 7 hasta birinci grup içerisine (grup 1), GsDU olan 30 hasta ikinci grup (grup 2) içerisine ve 37 sağlıklı gönüllü kontrol grubu (grup 3) içerisine dahil edildi. NLO hasta grubunda kontrol grubuna kıyasla yüksek bulundu ($p < 0.0001$). Grup 2 hastalar içerisinde tanı anında ve tedavinin 1. yılındaki NLO değerleri arasında anlamlı farklılıklar vardı ($p < 0.0001$). Grup 1 hastalar içerisinde tanı anında ve tedavinin 1. yılında NLO değerleri arasında anlamlı farklılık olmadığı görüldü ($p > 0.05$). NLO tedavinin 1. yılında grup 1 ve grup 2 hastaları arasında ($p = 0.007$), grup 1 ve grup 3 arasında ($p = 0.005$) anlamlı farklılık vardı. NLO'nun Receiver-operating characteristic curve analizi yöntemi ile GsD uyumsuzluğunu değerlendirmekteki perforrnansına bakıldığında kesme değeri 2.51, sensitivitesi % 85, spesifisitesi % 94 (area under curve : 0.819, 95 % confidence interval = 0.589-1.000, $p = 0.009$) olduğu görüldü. NLO, ÇH olan hastalarda hasta uyumunu öngörmede umut vaat eden bir belirteç olabilir.

Anahtar Kelimeler: *nötrofil lenfosit oranı, çölyak hastalığı, inflamasyon, belirteç*

SUMMARY

The aim of the present study is to determine the association of neutrophil-lymphocyte ratio (NLR) as an objective marker for detecting compliance to diet in celiac disease (CD). Thirty-seven patients with CD and 37 healthy volunteers were enrolled to the study. At the end of the first year, the patients were divided into 2 groups considering their compliance with diet. Seven patients, who are not compliant to gluten free diet (GFD), formed the first group (group 1). Thirty patients, who are compliant to GFD, formed the second group (group 2), and 37 healthy volunteers served as the control group (group 3). NLR was significantly higher in the patient group than the controls ($p < 0.0001$). There was a significant difference between the NLR values at the time of initial diagnosis and after a year of treatment in group 2 patients ($p < 0.0001$). However, we obtained no difference in terms of NLR between the initial and the first year of treatment in group 1 patients ($P > 0.05$). At the end of the first year, there were significant differences between group 1 and group 2 ($p = 0.007$) and between group 1 and group 3 in terms of NLR ($p = 0.005$). Receiver-operating characteristic curve analysis suggested the optimum NLR cutoff value for patients with GFD incompatible as 2.51, with a sensitivity and specificity of 85 % and 94 %, respectively (area under curve : 0.819, 95 % confidence interval = 0.589-1.000, $p = 0.009$). NLR may be a promising marker in predicting the patient compliance in patients with CD.

Key words: *neutrophil-lymphocyte ratio, celiac disease, inflammation, marker.*

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Introduction

Celiac disease (CD) is a chronic, autoimmune and inflammatory disease with an incidence of 0.5 to 1% in the general population (1, 2). Patients usually present with gastrointestinal symptoms such as abdominal pain, diarrhea, nausea, and vomiting. However, extraintestinal manifestations such as iron deficiency anemia, vitamin B12 and folic acid deficiencies, osteoporosis and neuropsychiatric symptoms are not rare (2, 3).

CD is diagnosed by the evaluation of the segment of the upper intestine by histopathologically in terms of intraepithelial lymphocyte infiltration, crypt hyperplasia and villous atrophy (Marsh classification). The serological tests such as anti-endomysium antibody (EMA), anti-tissue transglutaminase antibody (tTG) and anti-gliadin antibodies (AGA) support the diagnosis (2, 4, 5).

Neutrophil-lymphocyte ratio (NLR) can be obtained with a basic hemogram test. NLR is a simple and inexpensive marker of systemic inflammation. NLR has been associated with some conditions such as nonalcoholic fatty liver, ulcerative colitis, Familial Mediterranean Fever, acute pancreatitis, and it has been suggested that NLR predicts systemic inflammation in these disorders (6–10).

Determining patient compliance to diet is very crucial for the success of treatment in CD. It saves the clinician from unnecessary examinations. Apart from the patient's statements, an objective marker is needed and we aimed to determine whether NLR might be used for this purpose.

Materials and methods

Thirty-seven patients with CD, diagnosed by the histopathological evaluation of the duodenum biopsy together with serological tests at the Gastroenterology Department of Cumhuriyet University (Sivas, Turkey) between January 2009 and June 2012, were evaluated by a retrospective review of patient records. Thirty-seven age- and sex-matched participants were included as controls. The study was approved by the local Ethics Committees and was in accordance with the Declaration of Helsinki. Patients with diabetes mellitus, coronary heart diseases, malignacy, anemia, vitamin B12 and folic acid deficiencies, metabolic syndrome, acute/chronic infection, osteoporosis/osteomalacia, thyroid dysfunction, and history of smoking were excluded.

The Results of patients' hemogram, biochemistry, and acute phase reactants [erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP)] at diagnosis and after first year were obtained from laboratory archieve. And also, the transgression status of the patients during the previous 12 months were

obtained from patient files. The patients with transgression number >2 per month were termed as incompatible with gluten free diet (GFD). So, patients were divided into 2 groups considering their compliance to diet. Seven patients, who are not compatible with GFD, formed the first group (group 1). Thirty patients, who are compatible with GFD, formed the second group (group 2). And 37 healthy controls constituted the third group (group 3).

Blood samples were drawn without stasis at 7-8 a.m. after 20 min of supine rest, following fasting for ≥12 h. The blood was collected in tripotassium EDTA (7.2 mg) tubes. Hematological parameters, including hemoglobin, leucocyte count, platelet count, neutrophil and lymphocyte count, were analyzed by LH 780 analyzer (Beckman Coulter Inc, Miami, Florida). Neutrophil count to lymphocyte count (NLR) was calculated as manually.

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) 14.0 Package (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as arithmetic mean±standard deviation. The significance of the mean differences between groups were assessed by Student's t-test, Also, the nonparametric Wilcoxon Signed Ranks test and Mann Whitney U tests were used to test for differences between related (paired) samples. Relationships between variables were tested using Pearson's correlation analysis. A P-value of <0.05 was considered as statistically significant.

Results

There was not statistical differences between patients with CD and healthy individuals in terms of age (34.3 ± 11.5 versus 31.3 ± 9.1 years, respectively). And also, we did not obtain statistical differences between patients with CD and controls in terms of gender (M/F = 10/27 versus M/F = 12/25, respectively). Abdominal pain, diarrhea, and loss of appetite were the most presenting symptoms (Table 1). The initial laboratory characteristics were summarized in table 2.

Table I. The baseline clinical characteristics of the patients

	Patients (n=37)
Median age at diagnosis, years (range)	32.0 (18-72)
Compliance with diet, (n,%)	30 (81.1)
Loss of appetite, (n,%)	29 (78.4)
Vomiting, (n,%)	8 (21.6)
Abdominal pain, (n,%)	29 (78.4)
Diarrhea, (n,%)	26 (70.3)
Weight loss, (n,%)	3 (8.1)
Constipation, (n,%)	3 (8.1)
Arthritis, (n,%)	5 (13.5)
Dermatitis herpetiformis, (n,%)	2 (5.4)
Neuropsychiatric symptoms, (n,%)	3 (8.1)

There was a significant difference between patients and the healthy controls in terms of NLR at diagnosis (3.26 ± 1.31 vs. 1.72 ± 0.45, p < 0.0001) (Table 3). There was significant difference in terms of NLR in group 2 patients between diagnosis and the first year of treatment (3.29 ± 1.42 vs. 1.83 ± 0.82, p < 0.0001), in the contrary, in group 1 patients we obtained no difference in terms of NLR between diagnosis and the first year of treatment (3.13 ± 0.67 vs. 2.99 ± 1.12, p > 0.05) (Table 4). At the end of the first year, there were significant differences between group 1 and group 2, and between group 1 and group 3 in terms of NLR, (p1 = 0.007, p2 = 0.005, respectively) (Table 5, figure 1). Another important finding of the study was the demonstration of a significant difference between group 2 and group 3 (p3 = 0.517). The detailed Results are summarized in table 5 and figure 1.

Table II. The baseline laboratory characteristics of the patients

	Patients (n=37)
Albumin (g/dL)*	4.26 ± 0.40
Calcium (mg/dL)*	9.41 ± 0.39
Phosphorus (mg/dL)*	3.42 ± 0.75
Alkaline phosphatase (IU/L)*	69.80 ± 29.55
ALT (IU/L)*	19.23 ± 10.44
AST (IU/L)*	22.15 ± 7.53
EMA positive (n,%)	31 (83.7)
Elevated tTG (n, %)	31 (83.7)
Elevated CRP (n,%)	4 (10.8)
Elevated ESR (n,%)	3 (8.1)
Histological classification (n,%)	
Marsh I	17 (45.9)
Marsh II	8 (21.6)
Marsh III	12(32.4)

*mean±SD, EMA: anti-endomysium antibody, tTG: anti-tissue transglutaminase antibody, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein, ALT: alanine transaminase, AST: aspartate transaminase

Table III. Comparison of laboratory features of patients and healthy controls at diagnosis

	Patients (n=37)	Controls (n=37)	*p* value
Hemoglobin, g/dL	13.44 ± 1.21	14.41 ± 1.53	0.004
Platelet, $\times 10^9$/L	302.56 ± 84.07	272.24 ± 64.08	0.086
CRP, mg/L	4.58 ± 4.92	2.69 ± 1.58	0.103
ESR, mm/h	9.02 ± 5.61	6.71 ± 4.49	0.153
Leucocyte, $\times 10^9$/L	8.17 ± 3.91	6.71 ± 1.23	0.037
Neutrophil, $\times 10^9$/L	5.64 ± 2.91	3.77 ± 0.94	<0.0001
Lymphocyte, $\times 10^9$/L	1.79 ± 0.72	2.20 ± 0.53	0.007
NLR, %	3.26 ± 1.31	1.72 ± 0.45	<0.0001

NLR: neutrophil-lymphocyte ratio, ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

Table IV. NLR at diagnosis and at first year of the treatment in patient population

	NLR at diagnosis	NLR at first year of the treatment	p value
Group 1 (n=7)	3.13 ± 0.67	2.99 ± 1.12	>0.05
Group 2 (n=30)	3.29 ± 1.42	1.83 ± 0.82	<0.0001

NLR: neutrophil-lymphocyte ratio, Group 1: GFD incompatible patients, Group 2: GFD compatible patients

Tablo V. The comparison of groups at the end of the first year of gluten free diet (GFD) treatment

	Group 1 (n=7) (mean±SD)	Group 2 (n=30) (mean±SD)	Group 3 (n=37) (mean±SD)	P_1	P_2	P_3
Leucocyte, $\times 10^9$/L	8.61 ± 1.45	6.95 ± 1.72	6.71 ±1.23	*0.026*	*0.013*	0.537
Neutrophil, $\times 10^9$/L	6.00 ± 1.13	3.91 ± 1.23	3.77 ±0.94	*0.002*	*0.002*	0.611
Lymphocyte, $\times 10^9$/L	2.00 ± 0.42	2.36 ± 0.92	2.20 ±0.53	0.145	0.305	0.418
NLR, %	3.13 ± 0.67	1.83 ± 0.82	1.72 ±0.45	*0.007*	*0.005*	0.517

NLR: neutrophil-lymphocyte ratio,
P_1: p value of comparison between group 1 (GFD incompatible group) and group 2 (GFD compatible group)
P_2: p value of comparison between group 1 (GFD incompatible group) and group 3 (healthy controls)
P_3: p value of comparison between group 2 (GFD compatible group) and group 3 (healthy controls)

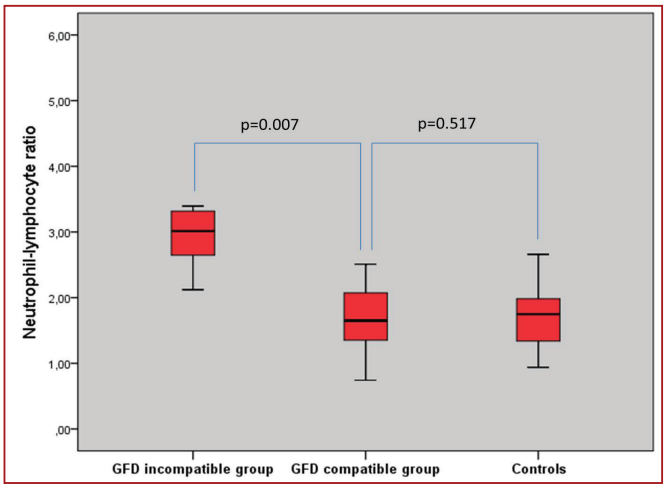

Figure 1. The comparison of groups at the end of the first year of gluten free diet treatment.
GFD, Gluten free diet

We obtained no correlation between NLR and age, gender, duration of illness, acute phase proteins (ESR and CRP) and histological classification (Marsh classification). Receiver-operating characteristic (ROC) curve analysis suggested the optimum NLR cutoff value for patients with GFD incompatible as 2.51, with a sensitivity and specificity of 85% and 94%, respectively (area under curve: 0.819, 95 % confidence interval = 0.589-1.000, p = 0.009) (Figure 2).

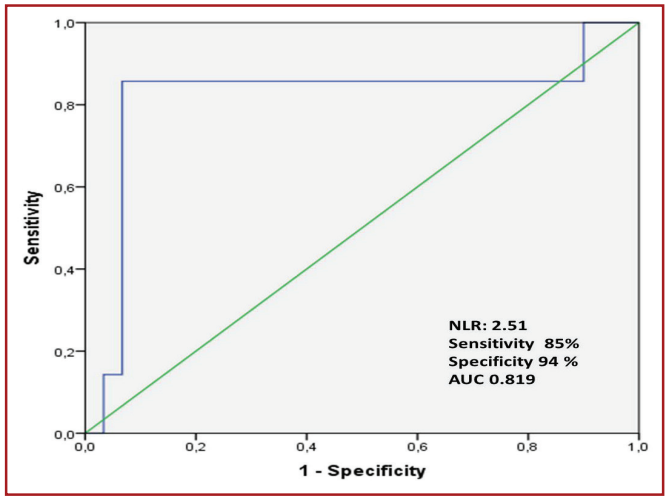

Figure 2. Receiver-operating characteristic (ROC) curve of the neutrophil-lymphocyte ratio (NLR) to predict patients with gluten free diet incompatible.

Discussion

Neutrophil-lymphocyte ratio (NLR) is measured by dividing neutrophil count to lymphocyte count with a basic hemogram test. NLR is a simple and inexpensive marker that can be used as an indicator of systemic inflammation. The value of NLR as a marker of systemic inflammation has recently been invastigated in various diseases such as ulcerative colitis, nonalcoholic fatty liver disease, familial Mediterranean fever (FMF), and acute pancreatitis (6–10). Celikbilek et al. (6) have shown that NLR was significantly higher in active ulcerative colitis patients than those of inactive patients and control group. Torun et al. (7) demonstrated that NLR is increased in active ulcerative colitis and peripheral blood NLR can reflect disease activity and can be used as an additional marker for estimating intestinal inflammation. Ahsen et al. (9) showed that NLR values were significantly higher in FMF patients than the controls, and it may be used in FMF patients as an indicator of the subclinical inflammation. Suppiah et al. (10) have shown that elevation of the NLR during the first 48 h of admission is significantly associated with severe acute pancreatitis and is an independent negative prognostic marker in acute pancreatitis.

Celiac disease (CD) is a chronic systemic disease characterized by small intestinal inflammation and villous atrophy after the ingestion of gluten-containing nutrients such as barley, wheat, and oats by genetically susceptible individuals (11). Gluten proteins contain large quantities of glutamine. After digestion, peptides are transported into the mucosa, where key glutamine residues are deamidated by tissue transglutaminase (tTG). Deamidation Results in a negative charge, and subsequently the deamidated epitopes are more efficiently bound to the specific HLA DQ2 or DQ8 receptors on the surface of antigen presenting cells which are positively charged (11–14). Intestinal DQ2- or DQ8-restricted CD4+ T cells then recognise the deamidated gliadin peptides and produce inflammatory cytokines such as interferon-gamma, IL-4, IL-5, IL-6, IL-8 and tumor necrosis factor alpha. It is known that these inflammatory cytokines could be higher in patients with CD before the development histologic changes in villouses (12, 14). Romaldini et al. (14) have compared the levels of IL-6 and TNF-alpha in pre-treatment and under treatment patients with CD with the control group. They concluded that IL-6 levels were significantly increased in untreated patients compared with treated and controls. And also, they suggested that IL-6 levels may be used as a noninvasive measure of CD activity and response to treatment. Street et al. (15) have evaluated the levels of IL-6 ve TNF-alpha in patients with CD. At diagnosis, IL-6 and TNF alpha were significantly higher than the controls. They showed that GFD have decreased the level of IL-6. Since Manavalan et al. (16) have compared the inflammatory cytokines in patients with active CD, they found that proinflammatory cytokines were higher in patients on GFD for less than 1 year than the patients on GFD for more than 1 year.

Despite the presence of systemic inflammation in patients with CD, acute phase reactant such as white blood cell count, CRP, and ESR are usually normal. Fernandez et al. (17) reported that CRP was higher in 16 % and ESR was higher in 9 % of 68 patients with CD. In our study, we have found elevated CRP in 10.8 % and elevated ESR in 8.1 % of the patients. The predictive value of CRP and ESR in demonstrating the disease activity is limited, so showing histological changes by endoscopic investigation and by serological tests are being used (2, 18).

The treatment of CD is the removal of gluten from diet lifetime. The symptoms and the complications in the clinical course of the disease can be controlled with diet therapy (18). However, in some of the patients, symptoms and histological changes continue despite a 6 to 12 months of complete diet compliance. However, the relevant studies have stated that compliance with diet range from 17 % to 90 % (19). Compliance with diet was 81.1 % in our patient population.

Limitations of the study were as follows: a. the study has a retrospective design, b. it is a single-center study with a relatively small sample size, which might underestimate or overestimate the Results. So, more specifically designed prospective studies are needed to externally cross-validate our findings in

a larger cohort of CD patients.

In conclusion, NLR is an important measure of systemic inflammation as it is cost effective and can be calculated easily. To our knowledge, this is the first study demonstrating a correlation between NLR and the compliance with GFD in CD. Our Results suggest that NLR is strongly associated with diet compliance and may predict GFD incompatibility in patients with CD.

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