Predictability of depression by plasma low-grade inflammatory markers in the background of pediatric celiac disease

Pediatrik çölyak hastalığı zemininde plazma düşük dereceli inflamatuvar belirteçlerle depresyonun öngörülebilirliği

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SUMMARY

Objective: Previous hypothesis on the predictability of either psychopathological or chronic metabolic disorders by complete blood count (CBC)-derived, low-grade peripheral inflammatory indicators should be considered with caution given the discrepancies in earlier findings. We aimed to examine the predictability of depression with low-grade inflammatory indices in a background of celiac disease (CD) and the association with gluten-free diet compliance by a case-control study in a pediatric sample.**Method:** A total of 59 children with a biopsy-proven CD were mainly compared with 40 controls in terms of depression and anxiety symptoms, as well as global functionality and CBC-derived indices which the previous studies focused on. Laboratory findings and psychiatric symptoms were examined through subgroups by either depression or gluten-free diet (GFD) compliance. **Results:** Prevalence of depression was 34% in the celiac group and there was a perpetual association of depression with CD. However, none of the CBC-derived indices investigated in earlier studies of either depression or CD was found to be differed by the presence of CD, depression, or status of GFD compliance. **Discussion:** Despite the presence of strong evidence for the role of inflammation on the prevalent comorbidity of depression with CD, the impact of inflammation on the depression-CD relationship was not demonstrated on these subjected markers which have been previously recommended as good indicators of systemic inflammation, however, with a low level of evidence and contradictory findings on predicting inflammation. The predictability of psychiatric and metabolic outcomes based on chronic inflammatory conditions with these CBC-derived indices requires further investigation.

Key Words: celiac disease, depression, neutrophil/lymphocyte ratio, PLR, MPV, inflammatory markers

ÖZET

Amaç: Kronik inflamasyonla seyreden tıbbi durumlara ikincil depresyonda, tam kan sayımına dayalı düşük-dereceli periferik inflamatuvar belirteçlerin tanısal öngörü niteliğine yönelik öncül hipotezleri inceleyen çalışmalarda çelişen bulgular saptanmıştır. Bu doğrultuda, düşük dereceli inflamatuvar belirteçlerin depresyon ve glütenden-kısıtlı diyetle olan ilişkisini çölyak hastalığı zemininde değerlendirerek belirteçlerin öngörülebilirliğinin araştırılması amaçlanmıştır. **Yöntem:** Biyopsi ile kanıtlanmış Çölyak hastalığı tanılı 59 çocuk ve genç, önceki çalışmaların odaklandığı düşük dereceli inflamatuvar indekslerin yanı sıra global işlevsellik düzeyleri, depresyon ve anksiyete semptomları açısından yaş ve cinsiyet bakımından eşleştirilmiş 40 kontrolle vaka-kontrol deseninde alt-grup analizleri ile karşılaştırılmıştır. **Bulgular:** Çölyak grubunda depresyon prevalansı %34'idi. Depresyon veya çölyak hastalığıyla ilgili daha önceki çalışmalarda odaklanılan düşük dereceli inflamatuvar belirteçlerin hiçbirisinin çölyak varlığı ve/veya depresyon komorbiditesi veya glütensiz diyet uyumu açısından değişkenlik göstermediği bulunmuştur. **Sonuç:** Çölyak hastalığı ile depresyonun yaygın komorbiditesi üzerinde inflamasyonun rolüne ilişkin güçlü kanıtlar bulunmasına rağmen, inflamasyonun depresyon-çölyak ilişkisi üzerindeki etkisi, önceki çalışmalarda düşük kanıt düzeyi ve çelişkili bulgulara rağmen sistemik inflamasyonun iyi göstergeleri olarak önerilen bu belirteçler üzerinden güncel bulgularla gösterilememiştir. Bahsi geçen belirteçlerin metabolik hastalıkların psikopatolojik sonuçlarını öngörme potansiyellerine yönelik düşük kanıt düzeyine işaret eden bulgular desteklenmiştir. Düşük dereceli inflamatuvar indekslerin kronik inflamatuvar koşullara dayalı psikiyatrik ve metabolik sonuçları öngörülebilirliğine yönelik daha fazla araştırma gerektiği görülmüştür.

Anahtar Sözcükler: Çölyak hastalığı, depresyon, nötrofil/lenfosit oranı, PLR, MPV, inflamatuvar belirteçler

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INTRODUCTION

Celiac disease (CD) is a chronic inflammatory condition of the small intestine induced by an immune reaction against dietary gluten in individuals with a genetic predisposition. However, it is a multisystem disorder with not only gastrointestinal but also extra-intestinal manifestations such as further hematological, endocrinological, immunological, and several neuropsychiatric consequences. Higher rates of major depression, suicide, anxiety disorders, and eating disorders were reported in earlier studies of either adult or pediatric CD (1-3). The vast majority of present evidence is particularly concentrated on depression and anxiety comorbidities in youth with CD, as well as amelioration with good adherence to the gluten-free diet (GFD) (4, 5). However, previous studies of CD have reported prevalence rates of depression with a wide variability of 6-69% (2, 5). Furthermore, the detection of similar rates of depression and anxiety comorbidities in inflammatory bowel disease as in CD (6) raises a debate as to whether the pathophysiological relationship between different psychiatric disorders and chronic inflammation is nonspecific.

Based on the data regarding chronic stress-associated disturbances in the central nervous system and immune system, an increase in plasma neutrophil-to-lymphocyte ratio (NLR) has been widely recommended as a useful index of ongoing systemic inflammation and relevant immune alterations in recent studies of several chronic metabolic and oncologic disorders, including CD and lymphoma (7, 8). Not only NLR, but also several other complete blood count (CBC)-derived indices such as platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), red blood cell distribution width-to-lymphocyte (RDW/L), etc. have previously been reported in the literature of CD (8, 9). Moreover, some of the subjected CBC-derived indices have also been recommended as potential biologic indicators of the low-grade inflammatory process in several psychiatric conditions such as ADHD, suicidal behavior, schizophrenia, bipolar disorder, and opioid use disorder, as well as major depression and anxiety disorders (10-17).

The aforementioned indicators have been widely investigated in terms of their nonspecific diagnostic value on systemic inflammation and dietary compliance in CD. However, to the best of our knowledge, there is no data regarding the association of CBC-derived low-grade inflammatory biomarkers such as MPV, NLR, PLR, etc. with celiac-related prevalent psychiatric outcomes, especially such as depression, which may best exhibit their real predictability of either psychiatric or metabolic disturbances, potentially based on ongoing inflammation and relevant immune alterations. In this study conducted with a pediatric sample, we hereby aimed to investigate the aforementioned hypothesis regarding the predictability of psychiatric outcomes of chronic inflammation and relevant immune alterations with those CBC-derived, low-grade peripheral inflammatory indices, via examining the relationship of these biological parameters with depression comorbidity in the background of a chronic inflammatory condition such as CD. Additionally, we further investigated the cross-sectional impact of GFD compliance on both depressive symptoms and CBC-derived indices to examine the previous evidence on the ameliorative potential of psychopathological outcomes with GFD status.

METHOD

Study design and sample collection

A total of 78 children with biopsy-proven CD (8-18 years old) who were newly diagnosed or had been followed up in the pediatric gastroenterology outpatient clinic were referred to child and adolescent psychiatry for psychiatric screening within the last season (during autumn 2021), in line with legal custodian's and self-consent. Diagnosis of CD was verified by duodenal biopsies compatible with Marsh-destructive type (Marsh-type 3) in clients with positive celiac-related autoantibodies. The patients were excluded according to criteria such as having additional medical or psychiatric disorders other than CD and depression; a known previous hematological abnormality in the complete blood count (CBC) like anemia/polycythemia, lymphopenia/lymphocytosis, neutropenia/neutrophilia, or thrombocytopenia/thrombocytosis; any use of medication within last three months

before blood sampling; obesity [body mass index (BMI) > 30 kg/m^2]; and a history of smoking. Some of the patients with CD (n: 19) were excluded from the study after referral to psychiatry for reasons which could affect the laboratory results, such as having additional systemic disorders or previous psychiatric diagnoses and current use of any medication in the last three months. As a result, 59 children with CD were included in the study as the 'celiac group' and compared with opponents (n: 40) in the 'control group', who had neither a systemic nor a psychiatric disorder. The groups were primarily compared in terms of demographic features, scale-scores of depression and anxiety symptoms, as well as the aforementioned hematological indices and relevant ratios being recommended as low-grade inflammatory markers including MPV, NLR, and PLR, as the first step of the analysis (Table 1 and 2).

Psychiatric assessments consisted of a semi-structured clinical interview and child-self-reports. The presence or absence of psychiatric symptoms was hereby verified by a face-to-face clinical interview with both children and their parents to determine each participant's inclusion or exclusion. The psychiatrist then prepared a narrative summary report describing individual psychiatric symptoms and DSM-5 diagnoses with the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version, DSM-5 November 2016, Turkish Version (K-SADS-PL-DSM-5-T). In the second step of the analysis, the patients in the 'celiac group' were allocated according to the existence of depression comorbidity based on the clinical interview. The patients with depression (celiac+depression group) were compared in terms of hematological indices and relevant inflammatory markers with the patients without depression (celiac-depression group) and with the control group (Table 3). In the third step of the analysis, the patients in the 'celiac group' were allocated according to their diet compliance. The patients with higher anti-tissue transglutaminase (anti-tTg) IgA and anti-tTg IgG, as well as those reporting poor adherence to GFD (as if the transgression number was > 2 per month) within the last three months were determined as 'GFD- group', whereas the patients with lower antibodies and good adherence were determined as 'GFD+ group'. In between-group analyses, depression and anxiety scores, as well as inflammatory markers, were compared between each subgroup and the control group (Table 4).

Laboratory procedures

The current hematological and celiac-related nutritional parameters [including complete blood count (CBC), C-reactive protein (CRP), vitamin B12, vitamin D, folate, iron, ferritin, and thyroid function tests (TSH and sT4)] were recorded for all patients as part of their routine clinical management. The acute phase reactants (CRP and ferritin) were especially included in this study to exclude an acute infection that may affect whole blood cell counts. Blood samples were drawn without stasis in the early morning hours, following fasting for more than 12 hours and abstinence of any heavy exercise in the previous 3 days, within last autumn 2021. CBC and other biochemical tests were determined from venous blood samples using a Beckman Coulter Gen S hematology analyzer. CD patients and controls were checked for serum celiac-related autoantibodies, including anti-tTg IgA, anti-tTg IgG, and IgA. As for the verification of CD at the first diagnosis process, the patients with CD also underwent an upper gastrointestinal endoscopy process with multiple biopsies, both from the bulb and distal duodenum. Duodenal lesions were reported according to the Marsh-Oberhuber classification, and a vast majority of the cases had commonly destructive type lesions (Marsh 3b).

Measures

Demographic data, including psychiatric and medical history of both children and parents, were all reported by parents using a form designed by the authors. Socioeconomic status (SES) was determined due to the Hollingshead-Redlich Index (HRI) which allows the social status of each individual to be determined by categorizing their occupation and education into three main categories, such as low (HRI: 0-22), moderate (HRI: 23-44) and high SES (HRI: 45-66).

The K-SADS-PL-DSM-5-T, which is a semi-structured clinical interview, was administered individu-

ally to screen psychiatric diagnosis and comorbidities. The Clinical Global Impression Scale-Severity Score [CGI-S (scoring from '1/normal' to '7/extremelly ill')] and the Children's Global Assessment Scale [C-GAS (scoring from '0/severe impairment' to '100/superior functioning')] were administered by clinicians routinely for a global assessment of the current functioning of the clients.

Children's Depression Inventory (CDI); which is a 27-item, self-rated inventory with each item scored on a 3-point scale (total score: 0-54), was used to determine the severity of depressive symptoms of all participants in this study. The CDI has been designed to measure the assessment of cognitive, affective, and behavioral symptoms of depression in children and adolescents aged between 7 and 17 years. The cut-off score for depression was reported as 19.

Screen for Child Anxiety and Related Disorders (SCARED) – Child Form, which is a 41-item, self-rated inventory with each item scored on a 3-point scale from 0 to 2 (total score: 0-82) and provides a multidimensional assessment for different types of anxiety disorders with a cut-off score of 25 and above, was used to screen the anxiety symptoms in this study. Patients with a diagnosis of any anxiety disorder were excluded from the study according to diagnosis based on clinical interviews.

Current anthropometry with weight and height was evaluated and we obtained the body-mass index (BMI).

Ethics

The research protocol was approved by the local ethics committee (ethics committee decision date: 26.07.2021, decision number: 116/23) and was in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants and their parents/legal custodians prior to the beginning of data collection.

Data Analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS), Version 22.0 (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was performed to assess the normality of the distribution of continuous data. Descriptive statistics were presented as numbers and percentages [n (%)] or mean ± standard deviation, which demonstrated the demographic and clinical characteristics and laboratory findings. A Pearson chi-square test (x^2) was performed on categorical variables, while a Mann-Whitney U-test (Z) or Kruskal-Wallis (KW) test was performed on continuous variables to explore group differences. Post hoc tests were also used to identify differences among multiple groups, along with a Bonferroni correction for each test to reduce type I errors. The Bonferroni adjustments in the between-group analysis were demonstrated as 'p^1, p^2, and p^3' for the analysis according to groups by depression and 'p^a, p^b, and p^c' for the analysis according to groups by GFD compliance (look at footnotes of Table 3 and 4). In the post hoc analysis, the '$p \leq 0.017$' indicates statistical significance after the Bonferroni adjustment. The p-values were based on two-tailed tests with $\alpha = 0.05$.

RESULTS

The findings regarding the comparison of demographic and clinical data between the patients in the celiac group (n: 59) and control group (n: 40) were summarized in Table 1. The mean age of the entire sample was '12.5 ± 0.36 years', with adolescents (≥ 12 years old) constituting the vast majority (approximately 61%). There was no difference between celiac and control groups in terms of age,

Table 1. Demographic data of celiac and control groups.

Variables	Celiac Group (n: 59)	Control Group (n: 40)	Chi-Square (df) or Z	*p*
Age (month)	149.98 – 4.28	151.68 – 4.81	- 0.225	0.822
Age group [Child group (< 12-year-old)]	26 (44.1)	12 (30)	1.99 (1)	0.158
Gender [male]	23 (39)	10 (25)	2.09 (1)	0.148
Education:			0.26 (2)	0.879
-Primary school	10 (16.9)	6 (15)		
-Secondary school	25 (56.8)	19 (47.5)		
-High school	24 (40.7)	15 (37.5)		
Family structure:			0.002 (1)	0.967
-Nuclear family	43 (72.9)	29 (72.5)		
-Others:				
Extended family	12 (20.3)	1 (2.5)		
Separated /Divorced parents	4 (6.8)	4 (10)		
Death parent(s)	0 (0)	6 (15)		
SES (HRS):			12.56 (2)	0.002
-Low (HRS ?22)	34 (57.6)	11 (27.5)		
-Moderate (HRS 23-44)	22 (53.7)	19 (47.5)		
-High (HRS ?45)	3 (5.1)	10 (25)		
Mothers				
Age (year)	38.2 – 0.61	39.8 – 0.77	-1.4	0.164
Education (year)	8.24 – 0.4	12.1 – 0.44	-5.48	<0.001
Fathers				
Age (year)	42.3 – 0.87	43.1 – 0.9	-1.2	0.233
Education (year)	9.8 – 0.4	12.1 – 0.47	-3.9	<0.001
History of parents psychiatric disorders	11 (18.6)	4 (10)	1.38 (1)	0.239
History of parents physical disorders	21 (35.6)	10 (25)	1.24 (1)	0.265

Note: n (%): number of the participants with frequencies in parenthesis, SES: socioeconomic status, HRI: Hollingshead-Redlich Index.
Data presented as mean – SD, or the number of clients along with frequencies. Mann Whitney U (Z) and Chi-Square Tests for comparison of groups. Values in parenthesis indicate degrees of freedom (df).
$p < 0.05$: statistically significant and the significant values are in bold.

age-groups (child or adolescent), gender, education, or family structure. The only notable findings in the demographic data were the differences in SES and the education levels of each parent; all of them were prone to be lower in the celiac group than the controls.

There was no difference between the celiac- and control groups in terms of prominent inflammatory markers such as NLR, PLR, and MPV, as well as most other hematological indices (Table 2). The BMI was lower in the celiac group, without supporting the existence of malnutrition. The data regarding the between-group analysis of the psychiatric scales are summarized in Table 2. The most notable findings were the higher scores of CDI and poor global functioning in the celiac group than opponents.

According to the structured clinical interview, depression was diagnosed in 34% (n: 20) of the celiac group. The comparison of clinical data among three groups, consisting of the CD patients with depression comorbidity, the depression-free CD patients, and the controls, was summarized in Table 3. While there was no difference in the comparison of the ages and age groups, there was a difference in gender distribution (p = 0.03). The female prevalence (80%) was higher in patients with celiac and depression. The groups differed only in TSH (KW: 7.2, df: 2, p = 0.027) and neutrophil levels (KW: 6.1, df: 2, p = 0.047) in terms of laboratory findings. However, the difference between each group disappeared after the Bonferroni adjustment in either TSH (p^2 = 0.095, p^3 = 0.058) or neutrophil levels (p^3 = 0.05). According to the comparison of psychiatric scales, the global functioning was detected as being in a worse state and particularly affected by the presence of depression comorbidity (G-GAS: p^1 = 0.011, p^2 < 0.001, and p^3 < 0.001; CGI-S: p^1 < 0.001, p^2 < 0.001, and p^3 = 0.855). Both of the depression (CDI: p^1 < 0.001, p^2 < 0.001, and p^3 = 1.00) and anxiety (SCARED: p^1 < 0.001, p^2, = 0.002 and p^3 = 1.00) scores were significantly higher in the patients with comorbid depression, even when the patients with any anxiety disorder were excluded.

When the patients with CD were assessed due to their cross-sectional status of GFD compliance based on self-reports, parent-proxy-reports, and current levels of antibodies, two sub-groups consisted of the patients with good adherence to GFD (n: 36) and the others with less adherence to GFD (n: 23). The groups did not differ in terms of age, age groups, and gender (Table 4). The patients without GFD compliance had lower levels of hemoglobin (KW: 8.33, df: 2, p = 0.016) and hematocrit (KW: 8.9, df: 2, p = 0.011). However, the differences between groups disappeared after the Bonferroni correction in post hoc analysis (p^a = 0.019 and p^b = 0.045 for hemoglobin; p^a = 0.011 and p^b = 0.053 for hematocrit). Moreover, the three groups did not differ in terms of iron and fer-

Table 2. Laboratory findings and psychiatric parameters of celiac and control groups

Variables	Celiac Group (n: 59)	Control Group (n: 40)	Chi-Square (df) or Z	*p*
Body mass index (BMI, kg/m2)	19.16 – 0.57	20.82 – 0.62	-2.13	0.033
C-GAS	46.27 – 2.25	79.5 – 1.63	-7.68	<0.001
CGI-S	2.69 – 0.25	1.28 – 0.07	-4.025	<0.001
CDI	14.78 – 1.65	7.78 – 0.91	-2.594	0.009
SCARED	9.5 – 1.25	6.83 – 0.86	-0.934	0.35
Celiac-related autoantibodies				
Anti-Tt IgA (U/mL)	79.5 – 14.5	1.87 – 0.25	- 6.44	<0.001
Anti-Tt IgG (U/mL)	25.1 – 7.65	2.48 – 0.34	- 3.5	<0.001
IgA (U/mL)	134.85 – 8.36	134.68 – 11.95	- 0.66	0.507
Acute-phase reactants				
C-reactive protein (CRP, mg/L)	1.98 – 0.11	1.79 – 0.17	- 1.09	0.274
Ferritin (ng/mL)	27.88 – 8.07	26.96 – 4.2	- 0.82	0.414
Serum vitamin levels				
Vitamin B12 (pg/mL)	384.71 – 16.41	351.63 – 15.24	- 1.39	0.163
Vitamin D (pg/mL)	16.15 – 0.81	18.7 – 1.15	- 1.9	0.057
Folate (pg/mL)	12.8 – 1.4	10.9 – 0.78	-0.67	0.500
Serum iron (mol/l)	75.9 – 4.44	77.17 – 5.86	- 0.09	0.923
Zinc (pg/mL)	755.77 – 31.87	681.24 – 61.51	- 1.47	0.142
Thyroid function tests				
Thyroxine (t4)	1.182 – 0.02	1.18 – 0.021	- 0.12	0.906
TSH	2.44 – 0.13	2.05 – 0.2	- 2.67	0.007
Complete blood count (CBC)				
Hemoglobin (g/dL)	13.58 – 0.15	13.8 – 0.16	- 0.93	0.352
Hematocrit (%)	40.35 – 0.38	41 – 0.46	- 0.72	0.471
White blood cells (/ L)	6.87 – 0.23	7.84 – 0.34	- 2.32	0.02
Neutrophil (/ L)	3.7 – 0.22	4.52 – 0.29	- 2.43	0.015
Lymphocyte (/ L)	2.46 – 0.1	2.6 – 0.13	-0.75	0.454
Platelet (/ L)	297.24 – 10.53	283.55 – 8.79	-1.03	0.304
Mean platelet volume (fL)	11.4 – 1.48	10.16 – 0.14	-0.84	0.399
Basophil (/ L)	0.052 – 0.017	0.041 – 0.001	-2.29	0.022
Eosinophil (/ L)	0.167 – 0.021	0.137 – 0.014	-0.354	0.724
Monocyte (/ L)	0.611 – 0.089	0.541 – 0.029	-0.61	0.524
CBC-related ratios				
NLR (neutrophil / lymphocyte ratio)	1.81 – 0.21	1.92 – 0.17	-1.54	0.123
PLR (platelet / lymphocyte ratio)	133.015 – 8.743	118.65 – 6.25	-0.88	0.377
BLR (basophil / lymphocyte ratio)	0.024 – 0.009	0.017 – 0.001	-1.75	0.08
MLR (monocyte / lymphocyte ratio)	0.267 – 0.034	0.226 – 0.016	-0.25	0.803
ELR (eosinophil / lymphocyte ratio)	0.078 – 0.014	0.053 – 0.004	-0.834	0.404

Note: CGI-S: Clinical Global Impression Scale Severity Scores, C-GAS: Children s Global Assessment Scale, CDI: Children s Depression Inventory, SCARED: Screen for Child Anxiety and Related Disorders. Anti-Tt IgA: Tissue transglutaminase immunoglobulin A antibody level, Anti-Tt IgG: Tissue transglutaminase immunoglobulin G antibody level, Ig A: Immunoglobulin A level, TSH: Thyroid-stimulating hormone.
Data presented as mean – SD. Mann Whitney U (Z) and Chi-Square Tests for comparison of groups. Values in parenthesis indicate degrees of freedom (df).
p < 0.05: statistically significant and the significant values are in bold.

Table 3. Comparison of clinical data, notable laboratory findings, and psychiatric parameters between the celiac+ depression, celiac-depression, and control groups.

Variable	Celiac+depression Group (n: 20)	Celiac-depression Group (n: 39)	Control Group (n: 40)	Chi-Square or KW (df)	p
Age (month)	152.35 – 6.9	148.77 – 5.5	151.68 – 4.81	0.31 (2)	0.854
Age group [Child group]	7 (35)	19 (48.7)	12 (30)	3 (2)	0.218
Gender [male]	4 (20)	19 (48.7)	10 (25)	7 (2)	0.03
BMI (kg/m2)	18.34 – 0.78	19.57 – 0.76	20.82 – 0.62	5.45 (2)	0.066
C-GAS	32.75 – 2.75	53.21 – 2.46	79.5 – 1.63	67,4 (2)	<0.001
CGI-S	4.95 – 0.27	1.54 – 0.15	1.28 – 0.07	55.4 (2)	<0.001
CDI	30.55 – 1.8	6.7 – 0.64	7.78 – 0.9	46.4 (2)	<0.001
SCARED	16.8 – 2.7	5.74 – 0.8	6.83 – 0.86	17.4 (2)	<0.001
Anti-Tt IgA (U/mL)	85.5 – 22.9	76.4 – 18.7	1.87 – 0.25	42.7 (2)	<0.001
Anti-Tt IgG (U/mL)	28.1 – 15.2	23.5 – 8.7	2.48 – 0.34	12.48 (2)	0.002
MPV (fL)	14.35 – 4.35	9.89 – 0.15	10.16 – 0.14	1.28 (2)	0.526
NLR	2.02 – 0.51	1.69 – 0.2	1.92 – 0.17	2.4 (2)	0.298
PLR	135.8 – 18.9	131.57 – 9.2	118.65 – 6.25	0.78 (2)	0.676
BLR	0.016 – 0.003	0.027 – 0.014	0.017 – 0.001	3.5 (2)	0.171
MLR	0.275 – 0.049	0.26 – 0.045	0.226 – 0.016	0.16 (2)	0.922
ELR	0.075 – 0.021	0.08 – 0.019	0.053 – 0.004	0.72 (2)	0.697

Note: CGI-S: Clinical Global Impression Scale Severity Scores, C-GAS: Children s Global Assessment Scale, CDI: Children s Depression Inventory, SCARED: Screen for Child Anxiety and Related Disorders, BMI: Body mass index, Anti-Tt IgA: Tissue transglutaminase immunoglobulin A antibody level, Anti-Tt IgG: Tissue transglutaminase immunoglobulin G antibody level, Ig A: Immunoglobulin A level, MPV: Mean platelet volume, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio, BLR: basophil/lymphocyte ratio, MLR: monocyte/lymphocyte ratio, ELR: eosinophil/lymphocyte ratio.
n (%): number of the participants with frequencies in parenthesis. Data presented as mean – SD. Kruskal-Wallis (KW) and Chi-Square Tests for comparison of groups. Values in parenthesis indicate degrees of freedom (df). **p <** 0.05: statistically significant and the significant values are in bold.
When the p-value was found to be smaller than the adjusted p-value (0.017) by the Bonferroni correction in post hoc analysis, it was considered to be significant. p,: p-values of the difference between celiac+depression and celiac-depression groups, p†: p-values of the difference between celiac+depression and control groups, p‡: p-values of the difference between celiac-depression and control groups after the Bonferroni adjustment.

Table 4. Comparison of clinical data, notable laboratory findings, and psychiatric parameters between GFD-, GFD+ and control groups

Variable	GFD- group (n:23)	GFD+ group (n:36)	Control group (n:40)	x^2 or KW (df)	*p*
Age (month)	140.9 ± 7.2	155.8 ± 5.15	151.68 ± 4.81	3.44 (2)	0.178
Age group [Child group]	12 (54.5)	14 (37.8)	12 (30)	3.623 (2)	0.163
Gender [male]	8 (34.8)	15 (41.7)	10 (25)	2.4 (2)	0.302
BMI (kg/m2)	16.8 ± 0.5	20.7 ± 0.8	20.8 ± 0.6	16.9 (2)	<0.001
C-GAS	42.6 ± 3.06	48.6 ± 3.1	79.5 ± 1.63	59.9 (2)	<0.001
CGI-S	2.96 ± 0.38	2.53 ± 0.33	1.28 ± 0.07	18.6 (2)	<0.001
CDI	17.43 ± 3.3	13.08 ± 1.7	7.78 ± 0.91	6.85 (2)	0.033
SCARED	10.52 ± 2.44	8.83 ± 1.35	6.83 ± 0.86	1.3 (2)	0.518
Anti-Tt IgA (U/mL)	195.04 ± 20.6	5.68 ± 0.89	1.87 ± 0.25	65 (2)	<0.001
Anti-Tt IgG (U/mL)	54.14 ± 18.06	6.55 ± 1.7	2.48 ± 0.34	23 (2)	<0.001
MPV (fL)	13.3 ± 3.8	10.2 ± 0.12	10.16 ± 0.14	4.8 (2)	0.091
NLR	1.83 ± 0.44	1.79 ± 0.21	1.91 ± 0.17	2.59 (2)	0.273
PLR	146.7 ± 15.1	124.2 ± 10.5	118.65 ± 6.25	2.15 (2)	0.342
BLR	0.04 ± 0.023	0.013 ± 0.001	0.017 ± 0.001	6.03 (2)	0.049
MLR	0.26 ± 0.041	0.274 ± 0.049	0.226 ± 0.016	0.27 (2)	0.871
ELR	0.075 ± 0.02	0.08 ± 0.02	0.053 ± 0.004	0.8 (2)	0.667

Note: CGI-S: Clinical Global Impression Scale – Severity Scores, C-GAS: Children's Global Assessment Scale, CDI: Children's Depression Inventory, SCARED: Screen for Child Anxiety and Related Disorders, BMI: Body mass index, Anti-Tt IgA: Tissue transglutaminase immunoglobulin A antibody level, Anti-Tt IgG: Tissue transglutaminase immunoglobulin G antibody level, Ig A: Immunoglobulin A level, MPV: Mean platelet volume, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio, BLR: basophil/lymphocyte ratio, MLR: monocyte/lymphocyte ratio, ELR: eosinophil/lymphocyte ratio.
n (%): number of the participants with frequencies in parenthesis. Data presented as mean ± SD. Kruskal-Wallis (KW) and Chi-Square Tests (x^2) for comparison of groups. Values in parenthesis indicate degrees of freedom (df). $p < 0.05$: statistically significant and the significant values are in bold.
When the p-value was found to be smaller than the adjusted p-value (0.017) by the Bonferroni correction in post hoc analysis, it was considered to be significant. p^a: p-values of the difference between GFD- and GFD+ groups, p^b: p-values of the difference between GFD- and control groups, p^c: p-values of the difference between GFD+ and control groups after the Bonferroni adjustment.

ritin levels, as well as other vitamin levels, which may show the absence of iron deficiency or malnutrition in the patients with poor diet adherence. TSH levels tended to be higher in the patients of the GFD- group (KW: 7.6, df: 2, p = 0.022), despite the fact that the clients having thyroid function abnormalities were excluded from the study, however without considering the subclinical thyroid-related disorders. The difference in TSH disappeared after Bonferroni adjustment (p^a = 1.00, p^b = 0.036, p^c = 0.112), as being also in the comparison of platelet levels (KW: 6.6, df: 2, p = 0.036, p^a = 0.041, p^b = 0.055, p^c = 1.00) and basophile levels (KW: 7.8, df: 2, p = 0.02, p^a = 0.322, p^b = 1.00, p^c = 0.017). BMI scores were different between groups even after Bonferroni adjustment (p^a = 0.001, p^b < 0.001, p^c = 1.00).

As for the comparison according to psychiatric scales; CGAS (p^a = 0.955, p^b < 0.001, p^c <0.001) and CGI-S (p^a = 0.371, p^b =0.012, p^c <0.001) levels were differed between groups, however similar between GFD- and GFD+ groups. As for the most notable point in Table 4, CDI (p^a = 1.00, p^b = 0.074, p^c = 0.094) scores differed among the three groups, however, the difference disappeared between the two-group-analysis with Bonferroni correction.

DISCUSSION

The role of inflammation in the etiology of major depression has been investigated in numerous studies conducted in both children and adults, and many inflammation biomarkers were found to be significantly higher in the depressed group, which was also associated with the severity of depression. In this context, NLR, PLR, and MPV were found to be the most studied markers (17-25). These markers have been recommended as useful in the evaluation of inflammation and relevant immune alteration in chronic disorders due to their low cost and high diagnostic power. Therefore, investigating the plausible role of inflammation in the underlying pathogenesis of depression, via an assessment using the subjected peripheral inflammatory markers in the background of a chronic inflammatory condition such as CD in a pediatric sample, was planned with the current study.

The most prominent findings of the intergroup analysis were the perpetual association of depression with having CD, however no association with either CBC-derived, low-grade inflammatory indices or the status of GFD compliance. Depression was found at a rate of 34% in the celiac group with the structured diagnostic interview, and a significant relationship was observed between celiac disease and depression-severity (CDI) scores compared to the controls. However, previous evidence about a triple link mediated by inflammatory/immune processes on the CD-depression association could not be affirmed by investigating the impact of inflammation and related-immune alterations on the common pathogenesis of CD and

depression through the subjected low-grade inflammatory indices. Many other CBC-derived inflammatory parameters examined in previous studies were also included in this study. However, none were found to be changing with the presence of CD, depression, or poor GFD compliance.

The depression scores (CDI) were found to be higher in the group of patients with poor GFD compliance, but intergroup differences disappeared in post hoc analysis. Therefore it can be stated that the GFD compliance status did not affect the celiac-depression relationship. However, several contrary pieces of evidence on the impact of diet adherence were demonstrated in previous studies. In a recent longitudinal examination regarding the impact of GFD on altered inflammatory markers in CD, a significant increase in neutrophil, MPV, and NLR values with GFD after one-year follow-up was reported, and the authors indicated that NLR may be a promising marker in predicting GFD compliance in patients with CD (26). However, the GFD compliance status we examined in the present study was evaluated by a cross-sectional way, and it would be better to look at it with a longitudinal study design to fully understand its clinical and psychological implications.

In a recent clinical survey (nationwide and population-based cohort) on psychiatric disorders in CD (27), it was stated that childhood CD is associated with an increased risk of subsequent psychiatric disorders, which persists into adulthood, and the authors emphasized the importance of the integration of mental health surveillance in the care of CD (27, 28). The authors reported that psychiatric disorders were more common before the diagnosis of CD, and the overall risk for psychiatric disorders was highest in the first year after the diagnosis of CD, which might be associated with excess inflammation (27). Moreover, the majority of the patients presented with psychiatric diagnoses before the diagnosis of CD that might be related to systemic inflammation initiating before CD (1,27,28). Despite the presence of strong evidence for the role of chronic inflammation in the underlying mechanisms of the prevalent comorbidity of depression with CD, we could not affirm the subjected evidence by examining the impact of chronic inflammatory processes and relevant immune alterations through CBC-derived indices such as NLR, PLR, and MPV, which have been persistently suggested as diagnostic or prognostic biological indicators of variable psychiatric and chronic metabolic disorders.

Given the significant evidence from a twin-family study (29), demonstrating the wide variation of NLR and PLR due to the effects of age, sex, and environmental factors such as seasonal conditions and lifestyles, as well as heritability, discrepancies in the findings regarding the association of each marker with medical or psychiatric disorders make sense. On the other hand, in a recent study investigating NLR, MPV, and PLR in adolescents with depression (23), a significantly higher NLR, with no difference in PLR comparison, than those of the controls was demonstrated, even after adjusting for other covariates such as age, sex, BMI, and the severity of depression. However, no findings regarding acute phase reactants were demonstrated in that study, confirming the exclusion of any other systemic inflammation.

As for more evidence regarding depression-related alterations in the peripheral inflammatory process and cellular immunity, a significant relationship (even a positive correlation in some) between depression severity and different types of subclinical inflammatory markers such as NLR, PLR, MLR, MPV, etc. was reported in several recent studies (17-25). Other psychiatric disorders such as obsessive-compulsive disorder (19,30), bipolar disorder (25,31), anxiety disorders (32), etc. are other psychiatric conditions where the aforementioned low-grade inflammatory markers have been investigated. On the other hand, the wide range of associations between these markers and a variety of psychiatric conditions may also demonstrate the non-specificity of those relationships. It should be noted that the acute-phase reactants were not included in the majority of the studies on the subject; simply a statement about the exclusion of the inflammatory diseases was instead reported in most of their methods. Additionally, there were some other limitations in previous investigations, such as not controlling results for covariates that could influence inflammatory status, such as menstrual cycle, eating habits, physical activity, etc. The positive relationship between the inflammatory markers

indicated in these studies and the lack of these parameters makes it hard to be sure that systemic inflammation from other causes has been properly ruled out.

Besides all these, the CD is defined as a common cause of various hematological disorders such as anemia, including either iron, folate, or pernicious types in children. Hematological abnormalities may be associated with CD-based nutritional deficiency of folate, vitamin B12, and copper (33). These hematological changes, which have been explained by a variety of factors related to the natural course of the disease in CD, make it difficult to investigate the effects of inflammation-related mechanisms in the psychopathological tendencies of the celiac background and may explain the discrepancies in the literature. All these differences in our findings may be due to sample composition. Current findings may contribute a shed of distinct evidence to the literature regarding the conflicting place of the qualitative use of these CBC-derived low-grade inflammatory indices in demonstration of the impact of systemic inflammation and related immune alterations on the underlying mechanisms of psychopathologies in chronic systemic disorders.

Besides several beneficial contributions and strong sides of the study design, such as verification of psychiatric diagnosis by structural interview, the inclusion of acute-phase reactants for demonstration of proper exclusion of any acute inflammation based on other sources; controlling the covariates which would potentially influence the findings of indices such as age and sex match between study groups; collecting blood samples by fixing several individuals (starving or exercise status) and conditional (in the same season and similar early morning times); as well as high results in power analysis (G-power), the findings should be considered in the context of the following limitations. First, the inclusion of only clinically referred clients with CD limited the generalizability of the interpretation of the findings. Second, the cross-sectional design is limited to evaluating the real implications of diet-compliance on depressive symptoms, so further longitudinal evaluation on the alterations in depressive symptoms and severity could be better for investigating the real influences of GFD status. And last, another limitation is that ancillary markers to examine the role of inflammation were not included.

CONCLUSION

Despite the presence of strong evidence for the role of inflammation in the prevalent comorbidity of depression with CD, we could not confirm the impact of inflammation on the depression-CD relationship through those markers which have been widely recommended as good indicators of systemic inflammation, however, with a low level of evidence and contradictory findings on predicting inflammation. Further investigations, including other more proven biomarkers of chronic inflammation such as cytokines, etc., would be beneficial to compare with the findings relevant to subjected CBC-derived indices.

Conflicts of interest: The authors declare that they have no conflict of interest.

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REFERENCES

1. Butwicka A, Lichtenstein P, Frisén L, Almqvist C, Larsson H, Ludvigsson JF. Celiac Disease Is Associated with Childhood Psychiatric Disorders: A Population-Based Study. The Journal of Pediatrics. 2017;184:87-93.e1.

2. Ludvigsson JF, Reutfors J, Ösby U, Ekbom A, Montgomery SM. Coeliac disease and risk of mood disorders — A general population-based cohort study. Journal of Affective Disorders. 2007;99(1):117-26.

3. Simsek S, Baysoy G, Gencoglan S, Uluca U. Effects of Gluten-Free Diet on Quality of Life and Depression in Children With Celiac Disease. Journal of Pediatric Gastroenterology and Nutrition. 2015;61(3).

4. Simsek S, Baysoy G, Gencoglan S, Uluca U. Effects of Gluten-Free Diet on Quality of Life and Depression in Children With Celiac Disease. Journal of Pediatric Gastroenterology and Nutrition. 2015;61(3):303-6.

5. Pynnönen PA, Isometsä ET, Aronen ET, Verkasalo MA, Savilahti E, Aalberg VA. Mental Disorders in Adolescents With Celiac Disease. Psychosomatics. 2004;45(4):325-35.

6. Engström I. Mental Health and Psychological Functioning in Children and Adolescents with Inflammatory Bowel Disease: a Comparison with Children having Other Chronic Illnesses and with Healthy Children. Journal of Child Psychology and Psychiatry. 1992;33(3):563-82.

7. Gheith RE, El Gazzar II, Bahgat DMR, Nour El-Din AM. Elevated tissue transglutaminase antibodies in juvenile idiopathic arthritis children: Relation to neutrophil-to-lymphocyte ratio and disease activity. The Egyptian Rheumatologist. 2017;39(4):233-7.

8. Sarikaya M, Dogan Z, Ergul B, Filik L. Neutrophil-to-lymphocyte ratio as a sensitive marker in diagnosis of celiac disease. Annals of gastroenterology. 2014:431-.

9. Sarikaya M, Dogan Z, Ergul B, Filik L. Platelet-to-lymphocyte ratio for early diagnosis of celiac disease. Indian Journal of Gastroenterology. 2015;34(2):182-3.

10. Avcil S. Evaluation of the neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and mean platelet volume as inflammatory markers in children with attention-deficit hyperactivity disorder. Psychiatry and clinical neurosciences. 2018;72(7):522-30.

11. Inanli I, Aydin M, Çaliskan AM, Eren I. Neutrophil/lymphocyte ratio, monocyte/lymphocyte ratio, and mean platelet volume as systemic inflammatory markers in different states of bipolar disorder. Nordic journal of psychiatry. 2019;73(6):372-9.

12. Velasco Á, Rodríguez-Revuelta J, Olié E, Abad I, Fernández-Peláez A, Cazals A, Guillaume S, de la Fuente-Tomás L, Jiménez-Treviño L, Gutiérrez L, García-Portilla P, Bobes J, Courtet P, Sáiz PA. Neutrophil-to-lymphocyte ratio: A potential new peripheral biomarker of suicidal behavior. Eur Psychiatry. 2020 Feb 17;63(1):e14.

13. Yu Q, Weng W, Zhou H, Tang Y, Ding S, Huang K, Liu Y. Elevated Platelet Parameter in First-Episode Schizophrenia Patients: A Cross-Sectional Study. J Interferon Cytokine Res. 2020 Nov;40(11):524-529.

14. Fusar-Poli L, Natale A, Amerio A, Cimpoesu P, Grimaldi Filioli P, Aguglia E, Amore M, Serafini G, Aguglia A. Neutrophil-to-Lymphocyte, Platelet-to-Lymphocyte and Monocyte-to-Lymphocyte Ratio in Bipolar Disorder. Brain Sci. 2021 Jan 6;11(1):58.

15. Dionisie V, Filip GA, Manea MC, Movileanu RC, Moisa E, Manea M, Riga S, Ciobanu AM. Neutrophil-to-Lymphocyte Ratio, a Novel Inflammatory Marker, as a Predictor of Bipolar Type in Depressed Patients: A Quest for Biological Markers. J Clin Med. 2021 Apr 29;10(9):1924.

16. Zhu X, Zhou J, Zhu Y, Yan F, Han X, Tan Y, Li R. Neutrophil/lymphocyte, platelet/lymphocyte and monocyte/lymphocyte ratios in schizophrenia. Australasian Psychiatry. 2021:10398562211022753.

17. Suarez E, Sundy J. Novel markers of inflammation and their relevance to depression: the unique relation of the neutrophil: lymphocyte ratio (NLR) and the cortisol: C-reactive protein (CORT/CRP) ratio to an intermediate phenotype of major depressive disorders (MDD). Brain, Behavior, and Immunity. 2017;66:e11-e2.

18. Adhikari A, Dikshit R, Karia S, Sonavane S, Shah N, De Sousa A. Neutrophil-lymphocyte ratio and C-reactive protein level in patients with major depressive disorder before and after pharmacotherapy. East Asian Archives of Psychiatry. 2018;28(2):53-8.

19. Bulut NS, Yorguner N, Çarkaxhiu Bulut G. The severity of inflammation in major neuropsychiatric disorders: comparison of neutrophil–lymphocyte and platelet–lymphocyte ratios between schizophrenia, bipolar mania, bipolar depression, major depressive disorder, and obsessive compulsive disorder. Nordic Journal of Psychiatry. 2021:1-9.

20. Canan F, Dikici S, Kutlucan A, Celbek G, Coskun H, Gungor A, Aydin Y, Kocaman G. Association of mean platelet volume with DSM-IV major depression in a large community-based population: the MELEN study. J Psychiatr Res. 2012 Mar;46(3):298-302.

21. Mazza MG, Lucchi S, Tringali AGM, Rossetti A, Botti ER, Clerici M. Neutrophil/lymphocyte ratio and platelet/lymphocyte ratio in mood disorders: A meta-analysis. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2018;84:229-36.

22. Meydaneri GG, Meydaneri S. Can neutrophil lymphocyte ratio predict the likelihood of suicide in patients with major depression? Cureus. 2018;10(4).

23. Özyurt G, Binici NC. Increased neutrophil-lymphocyte ratios in depressive adolescents is correlated with the severity of depression. Psychiatry research. 2018;268:426-31.

24. Puangsri P, Ninla-aesong P. Potential usefulness of complete blood count parameters and inflammatory ratios as simple biomarkers of depression and suicide risk in drug-naive, adolescents with major depressive disorder. Psychiatry Research. 2021;305:114216.

25. Usta MB, Aral A, Bozkurt A, Sahin B, Karabekiroglu K. Examination of neutrophil, platelet, and monocyte-lymphocyte ratios in adolescents with bipolar disorder-manic episode and

depression. Dusunen Adam. 2019;32(4):328-33.

26. Uslu AU, Korkmaz S, Yonem O, Aydin B, Uncu T, Sekerci A, Topal F, Sencan M. Is there a link between neutrophil-lymphocyte ratio and patient compliance with gluten free diet in celiac disease? Gulhane Medical Journal. 2016;58(4):353-356.

27. Lebwohl B, Haggård L, Emilsson L, Söderling J, Roelstraete B, Butwicka A, Green PHR, Ludvigsson JF. Psychiatric Disorders in Patients With a Diagnosis of Celiac Disease During Childhood From 1973 to 2016. Clin Gastroenterol Hepatol. 2021 Oct;19(10):2093-2101.e13.

28. Coburn SS, Puppa EL, Blanchard S. Psychological Comorbidities in Childhood Celiac Disease: A Systematic Review. Journal of Pediatric Gastroenterology and Nutrition. 2019;69(2):e25-e33.

29. Lin BD, Hottenga JJ, Abdellaoui A, Dolan CV, de Geus EJC, Kluft C, Boomsma DI, Willemsen G. Causes of variation in the neutrophil-lymphocyte and platelet-lymphocyte ratios: a twin-family study. Biomark Med. 2016 Oct;10(10):1061-1072.

30. Özyurt G, Binici NC. The neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in adolescent obsessive-compulsive disorder: Does comorbid anxiety disorder affect inflammatory response? Psychiatry research. 2019;272:311-5.

31. Sanchez-Autet M, Arranz B, Sierra P, Safont G, Garcia-Blanco A, de la Fuente L, Garriga M, Marín L, García-Portilla MP. Association between neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, and C-reactive protein levels and metabolic status in patients with a bipolar disorder. World J Biol Psychiatry. 2022 Jul;23(6):464-474.

32. Uzun N, Akıncı MA. Hemogram parameters in childhood anxiety disorders: Could anxiety disorders be related with inflammation? Medical Hypotheses. 2021;146:110440.

33. Baydoun A, Maakaron JE, Halawi H, Abou Rahal J, Taher AT. Hematological manifestations of celiac disease. Scandinavian Journal of Gastroenterology. 2012;47(12):1401-11.