

Reduced levels of circulating natural killer cells in children with celiac disease

Mehmet Agin¹, Eylem Sevinc², Erkan Dogan³, Nergiz Sevinc⁴

Abstract

Background Celiac disease (CD) is an autoimmune disease characterized by malabsorption. Serologic testing for CD consists of Ig A type of antitissue transglutaminase (tTG), antiendomysium (EMA). These tests are helpful in monitoring adherence to the gluten-free diet (GFD). Natural killer (NK) cell count alterations have been reported in various diseases, such as cancer, Crohn's disease, malnutrition, and autoimmune disorders.

Objective To compare peripheral blood NK cell counts in children with celiac disease (CD) to healthy controls. The second aim was to analyze for possible correlations between NK cells (CD3-/CD16+, CD56+) and tissue transglutaminase (tTG)- IgA and tTG-IgG, as well as endomysial antibody EMA-IgA indicating . gluten sensitivity.

Methods Fifty children with CD were compared to 48 healthy children as controls, with similar age and sex distribution. Peripheral blood NK cell counts were measured by flow cytometry.

Results The median (P25-P75) ages of the 50 celiac patients (23 male; 46%) and 48 controls (21 male; 44%) were 10 (2-17) years and 9 (3-17) years, respectively. Mean follow-up duration was 3 years, ranging from 1-10 years. All CD patients had positive tTG-IgA and EMA-IgA tests, while it was negative in all (100 %) control patients. The absolute number of circulating CD16+ NK cells (259.52 vs. 1404.36 μ /L) and CD56+ NK cells (366.24 vs. 2440.46 μ /L) were significantly lower in the celiac group than the control group ($P < 0.05$ for both). The absolute numbers of circulating white blood cells (7785 vs. 8165 μ /L) and lymphocytes (3106 vs. 3173 μ /L) were not significantly different between the celiac and control groups ($P > 0.05$ for both). Correlation analysis between the absolute number of circulating NK cells and tTG-IgA, tTG-IgG, and EMA-IgA levels in CD patients revealed no significant relationships ($P > 0.05$ for all).

Conclusions Peripheral blood NK cell count are significantly lower in celiac patients than controls, hence, decreased NK cell counts may be an abnormal feature seen in autoimmune diseases. NK cell count in celiac patients has no significant correlations to tTG-IgA, tTG-IgG, or EMA-IgA levels. Therefore, NK cell

count may be inappropriate marker for monitoring compliance to a gluten free diet. [Paediatr Indones. 2020;60:124-9 doi: <http://dx.doi.org/10.14238/pi60.3.2020.124-9>].

Keywords: celiac disease; natural killer cells

Celiac disease (CD), also known as gluten-sensitive enteropathy, is a T cell-dependent chronic inflammatory disease of the proximal small intestine caused by permanent intolerance to gluten.¹ In Western countries, the prevalence of CD in childhood has been estimated to range from 1:80 to 1:300.² Even though CD etiology is still not completely understood, both environmental and genetic factors are believed to be involved in the pathogenesis of CD. When

From the Pediatric Gastroenterology and Hepatology Clinic, Van Training and Education Hospital, Van¹, Pediatric Gastroenterology and Hepatology Clinic² and Pediatric Clinic³, Karabuk Training and Education Hospital, and Department of Public Health, Universitas of Karabuk⁴, Karabuk, Turkey.

Corresponding author: Eylem Sevinc, Karabuk University Faculty of Medicine, Balıklar Kayası Mevkii, Demir Çelik Campus Karabuk, Turkey. Telp. +905058180588; Email: dr.eylemsevinc@gmail.com.

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gluten-containing substances such as wheat, barley, and rye are ingested in HLA-DQ2 and/or HLA-DQ8 positive individuals, T cells are activated to produce cytokines, which destroys both adjacent enterocytes and the structure of the lamina propria by secreting matrix metalloproteinases. Ultimately, increased intraepithelial lymphocytes (IELs) in small intestinal mucosa lead to malabsorption.^{3,4}

Natural killer (NK) cells make up approximately 15% of peripheral lymphocytes and play crucial roles in both innate and adaptive immune responses. They develop in the bone marrow and can migrate to various tissues, such as liver, lung, gastrointestinal tract, and peripheral blood. NK cells are categorized into two subsets according to their surface markers CD16 and CD56, with CD56 as the major subset of NK cells.⁵ Similar to “helper” T cells, NK cells in the gastrointestinal tract are important for protecting against infectious pathogens and preserving the intestinal epithelium.⁶ The activity and number of circulating NK cells may vary in malignancy, malnutrition, autoimmune disease, as well as digestive disorders such as Crohn’s disease and CD.⁷

The aim of our study was to investigate the number of peripheral blood NK cells (CD3-/CD16+, CD56+) in children with and without CD and to investigate for potential correlations between NK cells and tissue transglutaminase (tTG) IgA and IgG, as well as endomysial antibody (EMA)-IgA. To our knowledge, this is the first study in children with CD to simultaneously evaluate both the absolute number of circulating NK cells and their possible correlations to tTG-IgA, tTG-IgG, and EMA-IgA.

Methods

This study was carried out at the Department of Pediatric Gastroenterology of Van Education and Training Hospital in Van, Turkey, between January and June 2018. Fifty children with classic CD aged 2-17 years and 48 healthy children (similar age and sex distribution) were included in the study. The diagnosis of CD was done according to the *European Society for Pediatric Gastroenterology, Hepatology, and Nutrition* (ESPGHAN) criteria.⁸ Biopsies of the small intestine were performed for all patients with positive serum tTG-IgA and EMA-IgA tests, and evaluated

according to the modified *Marsh Classification*.⁹ All celiac subjects had enteropathy of Type III-c, according to Marsh's criteria. Celiac patients with comorbid diseases such as diabetes mellitus, IgA deficiency, or Down syndrome were excluded from the study. The study was approved by the Ethics Committee for Non-invasive Clinical Research at Karabuk University. Subjects’ parents provided written informed consent.

For NK cell count measurements, peripheral venous blood specimens were collected in heparin-containing tubes. NK cells were purified from peripheral blood mononuclear cells (PBMC) by using the Human NK Isolation Kit II (Becton, Dickinson and Company BD Biosciences, San Jose, USA). Following isolation, NK cells were phenotypically identified as CD3-/CD16+ or CD56+ cells by flow cytometry.

The data were analyzed with SPSS *version 21.0* software for Windows. Results are expressed as median (P25-P75). Shapiro-Wilk test was used to determine the normality of data distribution. Values of white blood cell count, absolute lymphocyte count, absolute CD16+ NK cell count, absolute CD56+ NK cell count, serum tTG-IgA, tTG-IgG, and EMA-IgA levels had abnormal data distribution, by Shapiro-Wilk test, therefore, median values (interquartile range) between groups were determined and compared using Mann-Whitney U test. Height and body weight values were compared with independent T-test because of normal data distribution between groups. Correlation analyses were evaluated with Spearman’s correlation test. A P value of less than 0.05 was considered to be statistically significant.

Results

The median age of the 50 celiac patients (23 male; 46%) and 48 controls (21 male; 44%) were 10 (P25-P75 2-17) years and 9 (P25-P75 3-17) years, respectively. There were no statistically significant differences between the two groups with respect to age or gender (**Table 1**). Mean follow-up duration of celiac patients was 3 years, ranging from 1-10 years. All CD patients had positive tTGA and EMA-IgA tests. On the other hand, the tTG and EMA-IgA tests of the controls were negative. The absolute number of circulating CD16+ NK cells in the CD

group was significantly lower than that in the control group (259.52 vs. 1404.36 μ /L, respectively; $P < 0.05$) (Figure 1).

Similar to circulating CD16+ NK cells count, the absolute number of circulating CD56+ NK cells in the CD group was significantly lower than that in the control group (366.24 vs. 2,440.46 μ /L, respectively; $P < 0.05$) (Figure 2). There were no statistically significant differences between the two groups with respect to white blood cell (7,785 vs. 8,165 μ /L) and lymphocyte counts (3,106 vs. 3,173

μ /L) ($P > 0.05$) (Table 1). In the CD group, correlation analyses of circulating NK cell count with tTG-IgA, tTG-IgG, and EMA-IgA levels revealed no significant relationships ($P > 0.05$) (Tables 2 and Table 3).

Table 1. Comparison of socio-demographic and laboratory characteristics of the CD to control groups

Variables	CD group (n=50)	Control group (n=48)	P value
Median age (P25-P75), years	10 (2-17)	9 (3-17)	0.674 ^a
Males, n (%)	23 (46)	21(44)	0.710
Mean body height (SD), cm	128.92 (21.59)	131.56 (18.91)	0.632 ^b
Mean body weight (SD), kg (SD)	28.74 (12.62)	31.83 (11.85)	0.597 ^b
White blood cell (P25-P75, μ /L)	7,785 (3200-22790)	8,165 (4280-17920)	0.114 ^a
Lymphocyte (P25-P75, μ /L)	3,106 (1140-5760)	3,173 (763-8140)	0.166 ^a
CD16+ NK cells (P25-P75, μ /L)	259.52 (39.65-965.5)	1,404.36 (79.87-8,505)	0.021 ^a
CD56+ NK cells (P25-P75, μ /L)	366.24 (111.52-1016.32)	2,440.46 (27.66-1,1025)	0.017 ^a
tTG -IgA (P25-P75, RU/mL)	133 (40-300)	N/A	0.001 ^a
tTG -IgG (P25-P75, RU/mL)	90 (24-380)	N/A	0.001 ^a
EMA-IgA(P25-P75, RU/mL)	120 (30-295)	N/A0	0.001 ^a

^aMann-Whitney U test, ^bIndependent sample T-test.

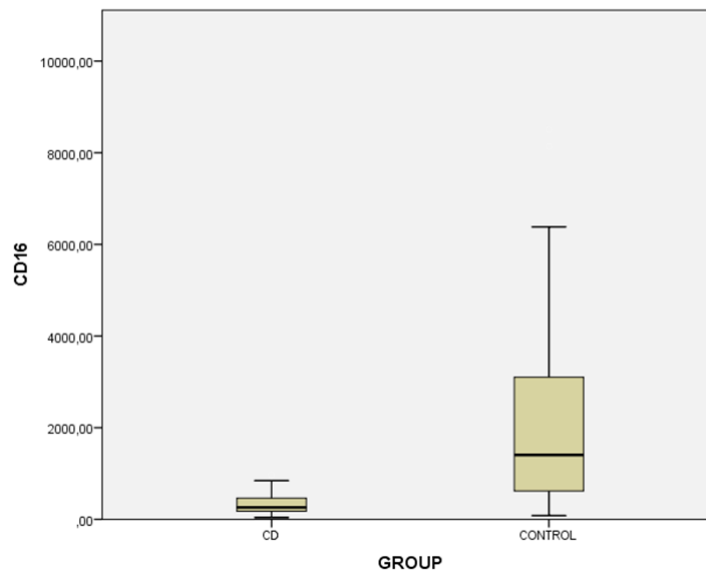


Figure 1. Comparison of median CD16+ NK cell counts in the CD and control groups

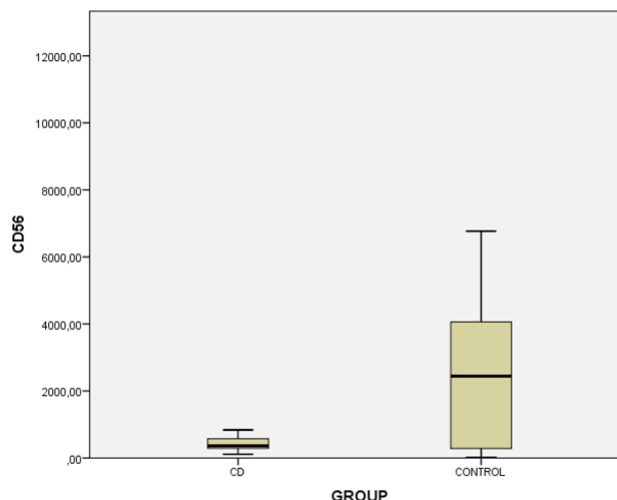


Figure 2. Comparison of median CD56+ NK cell counts in the CD and control groups

Table 2. Correlation between CD16+ NK cell counts with tTG -IgA, tTG-IgG, and EMA-IgA levels

Parameters	r Value ^a	P value
tTG -IgA RU/mL	0.850	0.56
tTG -IgG RU/mL	-0.431	0.76
EMA IgA RU/mL	0.639	0.64

^aSpearman's rank correlation

Table 3. Correlation between CD56+ NK cell count with tTG -IgA, tTG-IgG, and EMA-IgA levels

Parameters	r Value ^a	P value
tTG -IgA RU/mL	0.710	0.62
tTG -IgG RU/mL	-0.820	0.57
EMA IgA RU/mL	-0.176	0.52

^a Spearman's rank correlation

Discussion

In our study, children with CD had lower absolute number of peripheral blood NK cells than controls. In addition, no correlations were observed between NK cell counts and tTG-IgA, tTG-IgG, or EMA-IgA levels. Few studies have evaluated blood NK cell counts in celiac children, so there were a limited number of studies to which we compared our results.

The NK cells, physical epithelial barriers, phagocytic leukocytes, as well as dendritic cells are the main components of the innate immune system. An immunoregulatory role of NK cells has been found to be either disease-controlling or promoting disease severity by producing and releasing cytokines and chemokines during activation.¹⁰ CD16+ and CD 56+ NK cells are found predominantly in the peripheral blood.¹¹ In our study, NK cell counts were defined as CD3-/CD16+ or CD56+ lymphocytes. As mentioned previously, both qualitative and quantitative NK cell variations have been reported in various diseases and conditions.¹² A study reported that the number

of CD3-/CD56+ NK T cells in patients with psoriasis were significantly lower than in the controls ($P < 0.05$).¹³ Likewise, in a study evaluating the number of circulating NK cells in Behçet's disease, Hasan MS *et al.*¹⁴ reported a significantly reduced number of CD 56+ NK cells ($P < 0.05$). Recently, Hus *et al.*¹⁵ investigated mean platelet volume (MPV) levels in 28 progressive diffuse large B-cell lymphoma patients and reported that absolute counts of CD3-/CD16+, CD56+ NK cells were significantly lower than that of controls ($P < 0.05$). Consistent with previous studies, Ichikawa *et al.*¹⁶ found that the number of circulating NK cells was fewer in patients with Sjögren's syndrome than in the healthy controls ($P < 0.05$). In addition, Zheng *et al.*¹⁷ reported that patients with active Crohn's disease had a higher number of peripheral NK cells than patients with active ulcerative colitis ($P < 0.01$). All of the studies mentioned including ours suggest that the number of circulating NK cells may vary according to disease.

Environmental, genetic, and immunological factors are believed to affect the etiopathogenesis

of CD.¹⁸ As in other autoimmune diseases, the role of NK cells in CD has been investigated, but most of these studies were performed in adults.^{19,20} A study reported that in adult patients with CD, the mean absolute number of NK CD3-/CD56+ cells in peripheral blood [340 (SD 50) cells/mL] was significantly lower than in the controls [360 (SD 80) cells/mL].⁴ In addition, another study found that a gluten-free diet in celiac patients led to an increase in NK cells.²¹ Consistent with those results, a previous study claimed that deficiency of NK cells may cause the loss of immunological tolerance in patients with CD.²² On the other hand, Dunne *et al.*²³ reported that circulating innate lymphocyte populations, including NK CD56+ cells and invariant NK T cells, were significantly decreased in patients with CD, but not in celiac children. Our study results were inconsistent with those of a previous study, with a possible reason may have been their small number of subjects (n=22).²³

The reduction of absolute number of NK cells in circulation is generally parallel to a reduction in NK cell cytotoxicity. Low NK cells and cytotoxicity could lead to defective control of ongoing autoimmune responses.^{24,25} Few studies have investigated the relationship between NK cells and cancer in celiac disease. A previous study reported that the decrease of NK cells may increase the prevalence of malignancy in untreated celiac adult patients.²⁶ Although celiac children in our study did not comply with the gluten free diet (GFD), no malignant disease was encountered. However, it is necessary to keep this risk in mind in untreated celiac patients.

For evaluating compliance to GFD, tTG-IgA and EMA-IgA levels have been widely used in the celiac patients.² We found no correlations between NK cell counts and tTG-IgA, tTG-IgG, or EMA-IgA levels. Hence, these markers may be inappropriate for monitoring compliance to the GFD, especially in children. We could not find a correlation study between these parameters in the pediatric medical literature.

There were some limitations in this study. As there have been relatively few studies evaluating the number of peripheral blood NK cells in children with CD, we compared our findings to only a small number of studies. A second limitation was that we did not evaluate NK cell activity in the intestinal tissue.

In conclusion, to date, few researchers have focused on absolute numbers of circulating NK cells in children with celiac disease. In our study, celiac patients have significantly lower absolute numbers of circulating NK cells than controls. More studies will be required on the role of NK cells in order to elucidate the pathogenesis of celiac disease.

Conflict of Interest

None declared.

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