

# Neutrophil-lymphocyte ratio in children with familial Mediterranean fever: Original article

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## Abstract

**Objective:** The aim of present study was (a) to evaluate the relationship between the neutrophil/lymphocyte (N/L) ratio and mutation types of familial Mediterranean fever (FMF) in children and (b) to evaluate the relationship between the N/L ratio and age.

**Material and Methods:** Three hundred forty-three children with familial Mediterranean fever in the attack-free period and 283 healthy control children were included in the study. Patients were divided into subgroups according to mutation types. Neutrophil and lymphocyte counts were retrieved from medical records of patients and the N/L ratio was calculated from these parameters.

**Results:** The N/L ratio of patients was found to be significantly higher than that of controls ( $p < 0.001$ ). Among 343 patients, homozygous, heterozygous, and compound mutations were observed in 39, 253, and 51 patients, respectively. The differences in the N/L ratio among patients with homozygous, heterozygous, and compound mutations were not statistically significant. The most common mutations were M694V ( $n = 126$ ), E148Q ( $n = 70$ ), M680I, ( $n = 33$ ), and V726A ( $n = 28$ ). Significant differences were not observed among these mutations in terms of the N/L ratio ( $p > 0.05$ ). In all subjects, there was a weak but significant relationship between age and the N/L ratio ( $r: 0.215$ ,  $p < 0.001$ ).

**Conclusion:** The N/L ratio, which can be determined by simple methods in routine blood tests, may be used for the follow-up monitoring of chronic inflammation in patients. In addition, the N/L ratio may give an idea to clinicians regarding the early initiation of treatment in patients with typical clinical findings of FMF.

**Keywords:** Children, familial Mediterranean fever, mutation, neutrophil, lymphocyte

## Introduction

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by fever and accompanied by abdominal, articular, and pleural involvement. It commonly affects the populations of Armenians, Arabs, Jews, and Turks commonly (1). FMF gene (Mediterranean fever; MEFV) is located on short arm of chromosome 16 and encodes pyrine/marennosin (2). Pyrine protein plays an important role in inflammation, apoptosis and cytokines. It is expressed in many cells. The function of pyrine is not exactly understood, but it is considered to be involved in the suppression of inflammation (3). If there is a mutation in the pyrine protein, developing inflammation cannot be suppressed (3). During FMF attacks, high levels of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum amyloid A (SAA), fibrinogen, and cytokines such as interleukin (IL) 1 $\beta$ , sIL-2R, and IL-6 (4-6) are observed. These parameters are also indicative of systemic inflammation (7). It was shown that inflammation continues not only during attacks but also during symptom-free periods (8-11). The neutrophil/lymphocyte (N/L) ratio can be easily obtained from the differential white blood cell (WBC) count and has been studied in the context of many diseases (12-17). It is a marker of inflammation and predicts disease prognosis (7,15). The diagnosis of FMF is based on clinical features because there are no disease-specific laboratory findings. Mutation analysis helps and supports the diagnostic evaluations. Therefore, it may be useful to study the N/L ratio, in addition to the levels of acute phase reactants, both for diagnosis and follow-up of FMF.

Some studies regarding the N/L ratio in FMF in adults have been conducted (8,18,19). But according to our knowledge, no such study has been conducted in children with FMF. In current study, we aimed to investigate of the relationship between the N/L ratio and FMF mutations, in addition to the relationship between the N/L ratio and age, in children with FMF.

## Material and Methods

**Study design and patients:** All children with a diagnosis of FMF presenting to the Cumhuriyet University Faculty of Medicine Hospital between the years of 2010 and 2013 were included in the study. The information



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Submitted: 15.09.2014

Accepted: 22.10.2014

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**Table 1.** Demographic characteristics, the N/L ratio and WBC counts of patients and control groups

Parameters	Patients with FMF (n=343)	Control group (n=199)	p
Age (year)	10.54±2.61	10.21±2.98	>0.05
Female (n%)	165 (48)	146 (51)	>0.05
N/L ratio ( $\times 10^3/\text{mm}^3$ )	1.92±1.55	1.08±0.49	<0.0001
WBC ( $\times 10^3/\text{mm}^3$ )	7.56±1.85	6.94±1.89	0.012

FMF: familial Mediterranean fever; N/L: neutrophil/lymphocyte; WBC: white blood cell

about patients was retrospectively evaluated from their medical records. All patients were diagnosed according to the Tel Hashomer criteria (1) and were in the attack-free period. The attack-free period was described as normal physical examination with no clinical symptoms and normal levels of acute phase reactants for at least 2 weeks from the end of an FMF attack period (8). If the patients had any infection, they were excluded from the study. Patients who took other medications and those with pre-existing diseases, such as chronic lung diseases, cardiac diseases, diabetes mellitus, liver diseases, and infectious diseases, were excluded from the study.

The subjects were categorized into two groups. Group 1 consisted of the patients and Group 2 consisted of healthy controls. Group 1 was divided into 3 subgroups on the basis of mutations: patients with homozygous mutations, those with heterozygous mutations, and those with compound mutations. All patients were examined by the same doctors at the Department of Pediatric Immunology and Allergy. The records of FMF patients were all stored in the same department, and information was retrieved from these records. White blood cell (WBC) counts, neutrophil counts, lymphocyte counts, age, sex, and mutation types of patients were extracted. The N/L ratio was calculated by dividing the absolute neutrophil counts with the absolute lymphocyte counts, obtained from the differential WBC count (12-17).

The N/L ratio and WBC counts were compared between Groups 1 and 2. In addition, the N/L ratio and WBC counts were compared among the mutation-type subgroups of patients. The relationship between the N/L ratio and age was also evaluated.

### Statistical analysis

The statistical evaluation was conducted by using SPSS software (version 15.0; SPSS Inc., Chicago, IL, USA). Categorical data are presented as numbers and percentages, and continuous data are presented as mean±standard deviation. The Kolmogorov-Smirnov test was

applied to check the distribution of parameters. The difference between the categorical variables was determined using the  $\chi^2$  test. For comparisons between Groups 1 and 2, the independent t-test was applied for the continuous variables and the Mann-Whitney U test was applied for nonparametrically distributed variables. Analysis of variance (ANOVA) was used to compare the means of more than two samples and Tukey's honestly significant difference (HSD) test was used for post-hoc analysis. Pearson correlation analysis was used to investigate the relationship between two quantitative continuous variables. A p value of <0.05 was considered to be statistically significant.

### Results

The demographic characteristics of patients and control groups are shown in Table 1. There was no significant differences in age and sex distributions of children between Groups 1 and 2. Groups 1 (patients with FMF in attack-free period) and 2 (healthy control) were composed of 343 and 283 children, respectively. The mean N/L ratios and WBC counts of the two groups shown in Table 1. The N/L ratios and WBC counts in Group 1 were higher than those in Group 2 ( $p<0.001$  for the N/L ratio, and  $p=0.012$  for WBC).

Group 1 was divided into 3 subgroups according to mutation types: homozygous mutations ( $n=39$ ), heterozygous mutations ( $n=253$ ), and compound mutations ( $n=51$ ). The N/L ratios of patients with homozygous mutations were higher than that of patients with heterozygous and compound mutations, but this difference was not significant ( $p=0.061$ ). WBC counts of patients with compound mutations were significantly higher than that of patients with heterozygous mutations ( $p=0.016$ ). However, there was no differences in terms of WBC counts between patients with heterozygous and homozygous mutations as well as between those with homozygous and compound mutations ( $p<0.05$ ).

In current study, the most common mutations were M694V ( $n=126$ ), E148Q ( $n=70$ ), M680I

( $n=33$ ), and V726A ( $n=28$ ). The N/L ratios of patients these mutations were compared among each other. No significant difference in terms of the N/L ratio were observed among patients with these mutations ( $p>0.05$ ).

In addition, relationship of the N/L ratio with age was evaluated. In all subjects, there was a weak but significant relationship between age and the N/L ratio ( $p<0.001$ ). The relationship between the N/L ratio and age in Groups 1 and 2 was evaluated separately; a weak but significant relationship between age and the N/L ratio was also observed within both the groups ( $p<0.013$  for Group 1;  $p<0.001$  for Group 2).

### Discussion

The N/L ratio has been recently reported as an indicator of systemic inflammation with increasing frequency. In current study, the N/L ratio and WBC counts were higher in FMF patients than in control participants. Although the N/L ratio was not different among patients with different mutations, WBC counts of patients with compound mutations were significantly higher than those of patients with heterozygous mutations.

The N/L ratio is a reliable marker for monitoring and evaluating the systemic inflammatory response, and it may have a good prognostic value in the clinical outcome of diseases (7). There have been studies regarding the N/L ratio conducted in children. The N/L ratio was found to be significantly higher in patients with bacterial pneumonia than in those with viral pneumonia (17). In addition, the N/L ratio was considered a useful marker for predicting gastrointestinal bleeding in children with Henoch-Schönlein purpura (16). In children with cystic fibrosis, an elevated N/L ratio was associated with lower body mass index and lower forced expiratory volume in 1 second. It was also implicated as a marker of poorer outcome in these children (15).

There are some studies regarding the N/L ratio in adults with FMF. According to our knowledge, the current study was the first study conducted in children with FMF. In one study that included 62 adult patients with FMF, the N/L ratio of the patients was significantly higher than that of the control group (18). In addition, they reported that patients with the M694V mutations, especially in homozygous form (7 patients), had a significantly higher N/L ratio than those without M694V mutations (18). Such a relationship was not found in the present study. Although the difference was not statistically significant, the N/L ratio of patients with homozygous mutations was higher than

that of other patients, and the p value (0.061) of this analysis was close to being significant.

Celikbilek et al. (19) investigated the N/L ratio and WBC counts of FMF patients in active and attack-free periods. They reported that the N/L ratio was higher in active FMF than in remission as well as control patients (19). However, they did not find any relationship between active and attack-free periods in terms of WBC counts. In current study, WBC counts were higher only in patients with compound mutations. In addition, Celikbilek et al. (19) reported that the N/L ratio was significantly higher in patients with FMF-related amyloidosis than in patients without FMF-related amyloidosis. Amyloidosis is an important cause of morbidity and mortality in patients with FMF (3). The detection of a higher N/L ratio in FMF patients than healthy subjects indicates that subclinical inflammation in FMF continues in the attack-free periods. So there is a risk for amyloidosis in untreated patients and in patients who do not regularly use the drug colchicine (3). There were no patients in current study with amyloidosis, and all patients were in the attack-free period. Subclinical inflammation and an increase in acute-phase proteins in the symptom-free period has been reported in FMF (9-11). The detection of a high N/L ratio in the current study during the attack-free period supports these findings. This finding is very important for instructing the patients to seek regular treatment even in the attack-free period. In the current study, all patients were young. Thus, it may be said that all patients, especially those with a high N/L ratio, should be closely monitored for the future development of amyloidosis.

Lymphocytopenia is a significant decrease in circulating lymphocyte count that occurs after several events, including systemic inflammation. On the other hand, neutrophilia also develops during systemic inflammation, and it is caused by delayed apoptosis, demargination of neutrophils, and stimulation of stem cells by growth factors (7). From the perspective of immunology, it can be said that neutrophils are responsible for prolonging the inflammation and that lymphocytes have a regulatory role (7). Thus, the N/L ratio shows inflammation (7) and an increase in the N/L ratio may be found in chronic inflammation, especially in FMF patients (18).

The N/L ratio is an indicator of systemic inflammation (7) and many studies have examined the relationship between the N/L ratio and chronic diseases other than FMF. (7,20-22). It was reported that the N/L ratio was a poor prognostic factor in non-small cell lung can-

cer, acute-on-chronic hepatitis B liver failure, advanced pancreatic cancer, and biliary tract cancer (12-14, 23). In patients with idiopathic dilated cardiomyopathy, the N/L ratio is associated with the severity of chronic heart failure (22). This ratio may predict vascular calcification in end-stage renal disease (20), and it may be helpful for the indication of inflammation in chronic kidney disease patients, including those in predialysis and dialysis stages (21).

There was a weak but significant relationship between age and the N/L ratio in all subjects included in the present study. Li et al. (24) studied the relationship of the N/L ratio with age in healthy subjects and observed an increase in the N/L ratio value with increasing age. This situation may be related to an increase in proinflammatory cells in blood with age. Hence, the ratio increases in inflammation-associated diseases (25). Because there was no statistical difference between the patient and control groups in terms of age in current study, the relationship between the N/L ratio and age may not be important.

**Limitation of study:** The patients were evaluated retrospectively and only patients in the attack-free period were included to the current study. Thus, the N/L ratio of these patients could not be compared with those of patients with active FMF. However, the N/L ratio of patients in attack-free period should be compared with that of patients in the acute phase. Apart from the N/L ratio, the relationship between acute phase response parameters (e.g., CRP, ESR, and SAA) and clinical variables should be compared. But they are not routinely requested in patients without any complaints in the attack-free period in our institution. So we could not compare these acute phase parameters with clinical variables. In the future, another study on FMF patients both in active and in attack-free periods together with control participants should be conducted.

In conclusion, this study was conducted on a large group of patients; therefore, its results were accepted as meaningful for showing the relationship between the N/L ratio and FMF in attack-free period. The N/L ratio value can be determined by simple methods in routine blood tests. Therefore, it can be used for the follow-up of patients in terms of chronic inflammation. Amyloidosis is an important cause of morbidity and mortality in patients with FMF. Therefore, even if in attack-free period, patients should be advised to take colchicine treatment regularly for protecting themselves from amyloidosis. In addition, because inflammation is more common in FMF patients, they

should be followed up closely for long-term treatment and for chronic complications of the disease. In addition, mutation types cannot be studied in every hospital or their results may be delayed in most places. So, the N/L ratio may give an idea to clinicians for early initiation of treatment in patients with the typical clinical findings of FMF.

**Ethics Committee Approval:** The current survey was approved by the ethics committee of the Cumhuriyet School of Medicine, Cumhuriyet University.

**Informed Consent:** Written informed consent was obtained from patients or patient's parents who participated in this study.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** All authors participated equally in the study.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study has received no financial support.

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