



Are thiol-disulfide homeostasis & neutrophil-to-lymphocyte ratio useful in the differential diagnosis of acute familial Mediterranean fever attacks & acute appendicitis?

Fatih Tanrıverdi^{1,3}, Çağdaş Yıldırım^{1,3}, Alp Şener^{1,3} & Özcan Erel^{2,4}

Departments of ¹Emergency Medicine & ²Biochemistry, Ankara City Hospital & Departments of ³Emergency Medicine & ⁴Biochemistry, School of Medicine, Ankara Yıldırım Beyazıt University, Ankara, Turkey

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Background & objectives: Continuous subclinical inflammation can be seen in patients with familial Mediterranean fever (FMF) during the attack-free period. The importance of oxidative stress parameters in acute appendicitis (AA) progression has also been shown in previous studies. So, oxidative stress and the oxidant/antioxidant balance may play a role in this persistent subclinical inflammation. With this background the main objective of this study was to investigate the usefulness of combining the thiol-disulfide homeostasis parameters and the neutrophil-to-lymphocyte ratio (NLR) in the differential diagnosis of AA and an acute FMF attack.

Methods: The present study was conducted prospectively with 84 patients who were admitted to the emergency department between May 1, and December 31, 2018. Another 40 healthy individuals were assigned as the control group. The homeostasis parameters of thiol-disulfide were measured by a spectrophotometric method and NLR was measured in the patient and control groups.

Results: Native thiol and total thiol values were lower, while disulfide values were insignificantly higher in patients with AA than in patients with FMF. The white blood cell (WBC), neutrophil and NLR values were significantly higher in the AA group ($P<0.001$, $P<0.001$, $P<0.001$, respectively). When the neutrophil cut-off value for AA was set at 8.55, the calculated sensitivity was 80 per cent, the specificity was 72.2 per cent, and the area under the curve was 0.837.

Interpretation & conclusions: The results of this study suggest that neutrophil, WBC and NLR values can be useful in the differentiation of AA from an acute FMF attack.

Key words Acute appendicitis - familial Mediterranean fever - neutrophil-to-lymphocyte ratio - thiol-disulfide homeostasis

Familial Mediterranean fever (FMF) is an autoinflammatory disease with a periodic fever and recurrent attacks that may include abdominal pain, pleuritis, arthritis, erysipelas and skin lesions¹. Previous studies have shown that continuous

subclinical inflammation can be seen in patients with FMF during the attack-free period. It has also been thought that oxidative stress and the oxidant/antioxidant balance may play a role in this persistent subclinical inflammation. Oxidative stress

increases oxidant levels and produces free radicals. It also reduces antioxidant levels and damages cellular and extracellular structures, causing damage to cellular molecules^{2,3}. Acute appendicitis (AA) is the most common cause of abdominal pain and requires surgical intervention in routine clinical general surgery practices^{4,5}. Although the pathophysiology of AA has been investigated in detail, the presence of oxidative stress been determined to be present in its pathogenesis. The importance of oxidative stress parameters in AA progression has also been shown in some studies⁶⁻⁹. Plasma thiols are physiological free radical scavengers and are known to possess antioxidant effects through various mechanisms. Recent studies have confirmed the relationship between the neutrophil-to-lymphocyte ratio (NLR) and a number of inflammatory markers¹⁰. Previous studies have shown that an acute FMF attack is associated with an increased NLR value compared to patients not experiencing active attacks¹¹. In the differential diagnosis of an acute FMF attack and other low-level abdominal pain with AA, leukocyte and C-reactive protein values do not provide sufficient information¹¹.

Until 2014 the thiol-disulfide balance was measured only one way. However, by using the alternative test methods, one can detect both sides of the balance, and the thiol-disulfide status can be fully evaluated¹².

NLR is an inexpensive and easily obtainable inflammatory response marker. In particular, NLR is a marker that has been studied in many clinical settings from infections to chronic diseases and is used as an indicator for systemic inflammations and infections^{13,14}. It is known that mediators released due to oxidative stress can cause many systemic diseases caused by inflammation. The main objective of this study was to investigate the usefulness of combining thiol-disulfide homeostasis parameters and NLR in the differential diagnosis of AA and an acute FMF attack.

Material & Methods

This open, prospective, controlled study included 84 patients and 40 healthy volunteers. This study was carried out between May 1 and December 31, 2018, in the department of Emergency Medicine, The Training and Research Hospital, Ankara, Turkey, after procuring clearance from the Institutional Ethics Committee. Informed consent forms were read to and signed by all patients and volunteers.

Inclusion and exclusion criteria: Patients over 18 yr of age who were admitted to the department of

Emergency Medicine, and were diagnosed with an acute FMF attack (had not undergone surgery), were or diagnosed with AA (post-operative pathology results were compatible with AA) were included in this study. Pregnant participants who did not agree to participate in the study and patients with immune suppression were excluded from the study.

Sample collection and study methods: Venous blood samples were collected before the start of any medication in the first hour of admission and then centrifuged. Serum samples were stored at -80°C , then sent to the biochemical laboratory at Ataturk Training and Research Hospital, Ankara, Turkey. Spectrophotometric methods were used to measure the native thiol (NT), total thiol (TT) and disulfide (D) values^{12,14}. Other parameters were studied immediately after blood collection. Biochemical parameters and thiol-disulfide analysis results were compared in patients and healthy volunteers.

Statistical analysis: The SPSS 16.0 (IBM Corp., Chicago, IL, USA) for Windows package programme was used for statistical analyses. Normality analyses of the data were analyzed using Kolmogorov–Smirnov tests. Normally distributed data were expressed as mean \pm SD (standard deviation) and data not showing normal distributions were expressed as median and range (min-max). Kruskal–Wallis test was used for the parameters that were not distributed normally when comparing the three independent groups. The one-way analysis of variance tests was used for normally distributed parameters and Bonferroni test was used as *post hoc* test in two-group comparisons. Receiver operating characteristic (ROC) analyses were used for the predictive value of oxidative stress parameters and NLR. The area under the curve, sensitivity, specificity, negative predictive values and positive predictive values were calculated. Statistical significance was accepted as $P < 0.05$.

Sample size: In the literature, there are no previous studies evaluating the relationships between thiol-disulfide homeostasis parameters and neutrophil and NLR for differentiation between an acute FMF attack and AA. However, in studies with thiol-disulfide homeostasis in patients with ischemic stroke, lung cancer and pulmonary embolism by the same method, it was observed that the NT and TT levels were decreased by 14-18 per cent and 15-18 per cent, respectively, compared to the control group^{15,16}. Bektas *et al*¹⁵

Table I. Demographic features of groups

Demographic parameters	Control (n=40)	FMF (n=43)	AA (n=41)	P
Age, median (minimum-maximum) ^a	37 (18-59)	33 (18-59)	31 (20-51)	0,129
Gender (female/male), n (%) ^b	17/23 (42.50/57.50)	18/25 (41.86/58.13)	15/26 (36.58/63.41)	0.836

^aKruskal–wallis, ^b χ^2 . FMF, familial mediterranean fever; AA, acute appendicitis

calculated that 80 per cent power and five per cent type-1 error for NT parameters showed a significant reduction (10%) between healthy volunteers and a control group. In their study, the sample size required for each group was calculated to be 16 individuals. Similarly, 14 individuals are needed for each group for TT.

Results

Included in this study were 41 patients with acute FMF attacks, 43 patients with AA, and 40 healthy volunteers. There were no significant differences in terms of age and sex distributions between the groups (Table I).

The triple comparisons between NT, TT, D, neutrophil and lymphocyte values were significantly different. However, there was no significant difference between FMF and AA in the bilateral comparisons for NT, TT, D, neutrophil and lymphocyte values. In the comparison between white blood cells (WBCs), NLR and neutrophil pairs, differences were found in each group with the highest values being significantly different and observed in the AA group (Table II).

The ROC analysis was performed for the AA and FMF groups for WBC, neutrophil and NLR. The area under the curve was then calculated. The sensitivity-specificity values for neutrophils were determined where the area under the curve was the highest. According to these analyses, if the neutrophil value was below 4.8, the diagnosis for an acute FMF attack was 100 per cent. At values greater than 4.8, 32.6 per cent were evaluated in favour of AA. However, 80 per cent of the values when the neutrophil count was below 8.55 were not AA. The values above this point should be evaluated in favour of an AA diagnosis with 72.2 per cent (Figure and Table III).

Discussion

In the differential diagnosis of patients presenting with abdominal pain, an acute FMF attack or AA are usually included in the differential

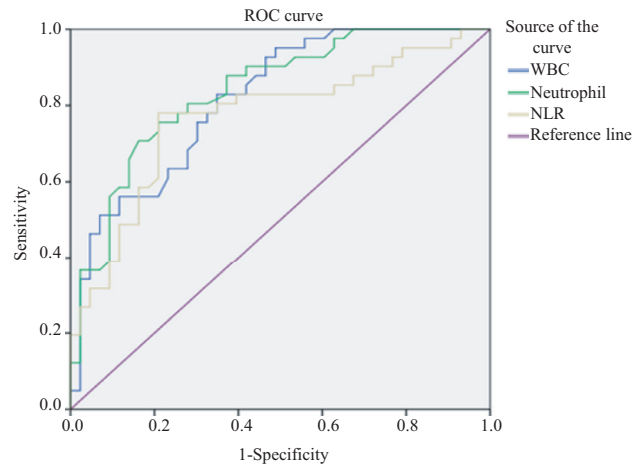


Figure. Receiver operating characteristic analysis and area under the curve (diagnostic evaluation of acute appendicitis).

diagnosis. In fact, in patients with an FMF diagnosis, AA may be misdiagnosed as an acute FMF attack¹⁷. In this case, a delay in the diagnosis may cause serious morbidity and mortality. FMF is endemic in Turkey and it is important to distinguish AA from an acute FMF attack in order to prevent unnecessary operations¹⁸.

The plasma thiol pool is one of the main antioxidant mechanisms. Oxidants oxidize thiols and form the D bridges. These bridges however, can also be reduced back to the thiol groups. Homeostasis of thiol-disulfide has some significance in the antioxidant defense^{12,19}. Erel and Neselioglu¹² were the first to measure the thiol-disulfide homeostasis with an automatic colorimetric method and found that plasma D levels were increased in smokers, diabetics, obesity, and patients with pneumonia, while they were decreased in some forms of cancer¹². In the present study, the parameters for thiol-disulfide homeostasis were found to be significantly different between an acute FMF attack and AA, compared with healthy volunteers. However, there was no significant difference between AA and an acute FMF attack. In addition, patients with AA had decreased NT and TT values compared to patients with acute FMF attacks. D values were also higher in patients with AA than in patients with

Table II. Thiol/disulphide homeostasis parameters in control, familial mediterranean fever, acute appendicitis groups

Parameters	n	Mean±SD	SE	95% CI for mean		Median (min-max)
				Lower bound	Upper bound	
Native thiol*						
FMF	43	402.10±98.65	15.04	371.74	432.46	423.50 (122.10-597.30)
Control	40	447.73±48.71	7.70	432.16	463.31	445.60 (310.40-550.00)
AA	41	421.40±79.38	12.40	396.35	446.46	437.10 (191.60-574.40)
Total	124	423.20±80.48	7.23	408.90	437.51	436.40 (122.10-597.30)
Total thiol*						
FMF	43	440.14±87.49	13.34	413.22	467.07	446.90 (281.70-670.40)
Control	40	490.49±57.07	9.02	472.24	508.75	480.50 (344.40-619.70)
AA	41	476.05±74.11	11.57	452.65	499.44	484.00 (279.30-611.20)
Total	124	468.26±76.75	6.89	454.61	481.90	475.60 (279.30-670.40)
WBC*						
FMF	43	9.67±3.60	0.55	8.56	10.78	8.80 (4.50-20.40)
Control	40	7.89±1.69	0.27	7.35	8.43	8.00 (5.20-13.00)
AA	41	14.48±3.73	0.58	13.30	15.65	14.30 (7.90-21.50)
Total	124	10.69±4.19	0.37	9.94	11.43	9.20 (4.50-21.50)
Disulphide						
FMF	43	18.89±14.82	2.26	14.33	23.45	17.90 (0.05-79.80)
Control	40	13.63±7.67	1.21	11.18	16.08	12.25 (2.00-40.50)
AA	41	26.40±14.16	2.21	21.93	30.87	24.10 (0.01-81.40)
Total	124	19.68±13.64	1.22	17.25	22.10	17.63 (0.01-81.40)
Neutrophil						
FMF	43	6.99±3.17	0.48	6.01	7.96	3.20 (2.50-16.50)
Control	40	4.16±1.21	0.19	3.78	4.55	1.63 (2.20-8.20)
AA	41	11.61±3.60	0.56	10.48	12.75	6.17 (4.90-18.60)
Total	124	7.61±4.18	0.38	6.86	8.35	3.00 (2.20-18.60)
Lymphocyte						
FMF	43	2.04±0.71	0.11	1.82	2.26	2.00 (0.50-3.90)
Control	40	2.37±0.59	0.09	2.18	2.56	2.40 (1.10-3.60)
AA	41	1.97±0.75	0.12	1.73	2.21	1.90 (0.90-4.00)
Total	124	2.13±0.70	0.06	2.00	2.25	2.10 (0.50-4.00)
NLR						
FMF	43	3.82±2.13	0.32	3.17	4.48	3.20 (0.76-9.90)
Control	40	1.90±0.83	0.13	1.63	2.16	1.63 (0.92-4.27)
AA	41	6.91±3.53	0.55	5.80	8.03	6.17 (1.40-17.00)
Total	124	4.22±3.17	0.28	3.66	4.79	3.00 (0.76-17.00)

*Bonferroni test was used as One way-ANOVA (analysis of variance) ve *post-hoc* test. Others: Kruskal–Wallis. FMF, familial mediterranean fever; AA, acute appendicitis; WBC, white blood cell; NLR, neutrophil lymphocyte ratio; SD, standard deviation; CI, confidence interval

acute FMF attacks. This decrease in NT and TT levels could be the result of an oxidative load increase. Thus, an increase in D emerges, which may be due to the reduction in thiols.

In a study by Omma *et al*²⁰, NT and TT levels were found to be significantly lower in acute FMF attacks than in the control group, and D levels were found to be higher. Similar conclusion was achieved when the

Table III. Receiver operating characteristics analysis

Variable (s)	Area	SE ^a	Asymptotic 95% CI	
			Lower bound	Upper bound
WBC	0.82	0.045	0.73	0.91
Neutrophil	0.84	0.043	0.75	0.92
NLR	0.77	0.053	0.67	0.87
Cut-off values for neutrophil (diagnostic evaluation for acute appendicitis)				
Neutrophil				
Cut-off	Sensitivity	Specificity	PPV	NPV
4.80	1.00	0.33	0.59	1.00
6.45	0.93	0.47	0.62	0.87
7.35	0.88	0.63	0.69	0.84
8.55	0.80	0.72	0.73	0.80
9.55	0.76	0.79	0.78	0.77
10.35	0.58	0.88	0.83	0.69
10.45	0.56	0.91	0.85	0.68
13.80	0.37	0.98	0.94	0.62
ROC, receiver operating characteristics; PPV, positive predictive value; NPV, negative predictive value; SE, standard error				

groups with and without acute FMF episodes were compared. In our study, similar results were obtained when the acute FMF attack and control groups were compared. However, no significant difference was found between the AA and acute FMF attack groups.

In a study conducted by Ozyazici *et al*²¹, NT and TT levels were lower while D levels were higher in patients with AA compared to the control group. In other words, the thiol-disulfide homeostasis was observed to shift towards the D direction. In our study, NT and TT levels were significantly decreased and D levels were increased in patients with AA compared to the control group. When patients with AA or an acute FMF attack were compared, there was a decrease in NT and TT values and an increase in D values in patients with AA, but these changes were not significant.

NLR is an easily accessible and novel inflammatory marker, which can be easily calculated from a complete blood count. Some studies have shown that NLR is closely linked to other inflammation markers^{22,23}. Several biochemical parameters have been investigated to differentiate AA from acute FMF attacks. Procalcitonin has been found to be significantly higher

in patients with AA compared to patients with acute FMF attacks²⁴. However, since procalcitonin is not routinely studied, and is expensive, its use is limited.

Yazici *et al*²⁵ found that NLR was higher in 90.2 per cent of patients with AA than in other causes of abdominal pain in their study. In our study also, WBC, neutrophil and NLR values were significantly higher in the AA group than in patients with acute FMF attacks.

In another study performed by Ishizuka *et al*²⁶, patients undergoing an appendectomy with an NLR >8 were associated with gangrenous appendicitis. Furthermore, in a study conducted by Kucuk *et al*²⁷, NLR values of patients with acute FMF attacks and AA were significantly higher in the AA group. As a result of their study, when the NLR cut-off value was 4.3 for AA, there was a 78 per cent sensitivity, a 62 per cent specificity, and the area under the curve was 0.76. Similarly, in our study, NLR was higher in the AA group. In contrast, the ROC analysis showed that the neutrophil ratio was more distinctive than in other studies. When the neutrophil cut-off for AA was 8.55, the diagnosis had an 80 per cent sensitivity, a 72.2 per cent specificity, and the area under the curve was 0.837.

The main limitation of our study was the small sample size. Acute FMF attacks and AA differentiation require not only a biochemical test but also its correlation with clinical and other radiological examinations. Hence, it may be of value to evaluate thiol-disulfide homeostasis parameters, neutrophil, and NLR along with other clinical parameters.

Overall, we believe that these biomarkers can be helpful in a differential diagnosis by obtaining a wide range of studies with an easy, inexpensive, and automatic technique. It is important that NLR and neutrophil values are determined in a simple and cost-effective manner. It is hence suggested that NLR and neutrophil values can be used to make differential diagnoses in studies to be performed with homogenous patient groups, and these results may prevent unnecessary surgeries. Studies with larger sample sizes are, however, required to support the results of our study.

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Conflicts of Interest: None.

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