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The evaluation of ENA-78 and fibrinogen levels for the differential diagnosis of familial Mediterranean fever and acute appendicitis

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Abstract

Background: Familial Mediterranean fever (FMF) is a disorder mainly present in the Mediterranean region, characterized by abdominal attacks that mimic acute peritonitis. Therefore, FMF might be confused with other conditions presenting with acute abdominal pain, particularly acute appendicitis (AA). We aimed to evaluate whether epithelial-derived neutrophil-activating peptide-78 (ENA-78) and fibrinogen levels and white blood cell (WBC) counts could be used as potential markers in the differential diagnosis of acute FMF attacks and AA.

Methods: The study involved three groups: patients with AA who underwent surgery (group 1, n=30), patients with FMF attacks (group 2, n=30), and patients with FMF who were attack-free (n=30, controls), which included patients who were diagnosed as having FMF previously but had no attacks for a month.

Results: Significant differences were detected in the ENA-78 and fibrinogen levels between group 1 and group 2. No significant difference was found in the WBC count between group 1 and group 2. It was concluded that the WBC count was more sensitive (66%) for group 1, and fibrinogen showed higher sensitivity (86%) and specificity (96%) for group 2 compared with the other parameters.

Conclusions: We suggest that AA can be distinguished in patients with signs of peritonitis who were diagnosed as having FMF previously with the use of ENA-78 and fibrinogen levels; fibrinogen and ENA-78 might be helpful in discriminating between FMF attacks and AA.

Keywords: appendicitis; epithelial-derived neutrophil-activating peptide-78 (ENA-78); familial Mediterranean fever; fibrinogen; white blood cell.

Introduction

Familial Mediterranean fever (FMF) represents a prototype for episodic febrile conditions, characterized by fever attacks and painful manifestations. It is an autosomal recessive disorder that mainly affects people living in or originating from the Mediterranean basin, including Sephardic Jews, Arabs, Turks, Armenians, and others [1]. Abdominal attacks that mimic acute peritonitis are seen in 95% of patients with FMF [1]. Therefore, FMF might be confused with other conditions that present with acute abdominal pain, particularly appendicitis [2]. Initially, abdominal pain and tenderness may be localized, after which it can become more generalized. Signs of peritonitis, such as guarding, rebound tenderness, rigidity, and a dynamic ileus, are often present because abdominal pain is caused by peritoneal inflammation. These findings can be mistakenly interpreted as an acute surgical abdomen condition, leading to delayed diagnosis or unnecessary appendectomy and laparotomy [3–5].

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FMF attacks are characterized by leukocytosis and elevated acute-phase reactants, such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, haptoglobin, C3, C4, and serum amyloid A (SAA). These inflammatory markers assist physicians in distinguishing FMF attacks from other conditions, such as viral illness, fibromyalgia, functional abdominal pain, and irritable bowel syndrome. Although most acute-phase reactants are typically normalized during the attack-free period, the elevation in these markers can persist in some cases, especially in those with severe or uncontrolled disease [6].

The widely used clinical and laboratory parameters are not specific enough to discriminate between abdominal pain caused by FMF and acute appendicitis (AA). However, diagnostic laboratory tests might decrease unnecessary surgical interventions, perforation rates, and hospitalization time. Epithelial-derived neutrophil-activating peptide-78 (ENA-78) is a member of the CXC chemokine family that is involved in leukocyte recruitment and activation in autoimmune disorders and inflammatory diseases [7–9]. It has been reported that abnormal expression of ENA-78 may be in some cases such as rheumatoid arthritis, psoriasis, autism, and bacterial meningitis. [10–14]. As a potent ELR⁺ CXC chemokine, ENA-78 plays a role in the recruitment and activation of polymorphonuclear leukocytes (PMNLs), which are elevated in patients with infections. PMNLs are among the first cells to exit the peripheral blood and migrate to the inflammatory site [3, 10, 15]. We aimed to evaluate whether ENA-78 level, fibrinogen level, and the white blood cell (WBC) count could be used as potential parameters in the differential diagnosis of recurrent episodes of FMF and AA.

Materials and methods

This prospective study was approved by the Ethics Committee of Ondokuz Mayıs University (approval ID: 2013/381). Written informed consent was obtained from all patients before participation in the study. In total, 90 patients were included in the study. The participants were divided into three groups: patients with AA who underwent surgery (n=30, group 1), patients with FMF who presented with acute peritonitis (n=30, group 2), and patients with FMF who were attack-free for a month (n=30, control group). Patients with acute abdomen causes such as acute pancreatitis and acute gastritis were excluded after laboratory and imaging findings. FMF was diagnosed in accordance with the Tel-Hashomer clinical criteria: major criteria: (a) recurrent fever together with serositis, (b) amyloidosis

without any other susceptible agent, and (c) good response to continual treatment with colchicine, and minor criteria: (a) recurrent fever, (b) erysipelas-like erythema, and (c) positive familial background. Two major criteria or two minor criteria along with one major criterion indicate a definite diagnosis of FMF [16]. Patients with a disorder that might cause inflammation and affect the outcomes, those on non-cytokine agents that might influence the cytokine levels, patients with diabetes mellitus or metabolic syndrome, perforated appendicitis, and negative appendectomy, those with an inaccurate diagnosis of FMF, and pregnant patients were excluded. Demographic data and contact information were recorded for all patients.

Biochemical analysis

Serum samples were obtained at admission from patients with FMF attack and before the surgery from those with AA. The blood samples were centrifuged at 3500 rpm for 10 min and plasma was removed and stored in a -70 °C freezer required for analysis. On the day of the laboratory examination, the plasma samples were removed from the freezer and allowed to dissolve. Serum ENA-78 levels were quantified with a sandwich enzyme-linked immunosorbent assay (ELISA) (Human ENA-78 ELISA kits, SunRed, Catalogue No.: 201-12-0132, Shanghai, China), using the Infinite 200 PRO NanoQuant microplate reader (Tecan, Grödig, Austria) for detection. Fibrinogen and WBC counts were evaluated concurrently in the emergency laboratory. The WBC count was obtained using the Beckman Coulter LH 780 hematology analyzer (Brea, CA, USA). Fibrinogen levels were measured using a Sysmex CA-1500 automated blood coagulation analyzer (Sysmex Corporation, Kobe, Japan).

Statistical analysis

Data were analyzed using IBM SPSS version 23.0 software. The Kolmogorov-Smirnov test was used to analyze the data distribution. One-way analysis of variance (ANOVA) was used to compare the findings of the groups. Tukey's honestly significant difference (HSD) and Tamhane's T2 tests were used to perform multiple comparisons. The results are expressed as mean ± standard deviation and frequency (percentage). Spearman's correlation test was used for correlation analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the cut-off values for the fibrinogen, ENA-78 values, and WBC counts in group 1 and group 2. p-Values <0.05 were considered to be statistically significant.

Results

Of the 90 patients included in the study, 54 (60%) were women and 36 (40%) were men. No statistically significant difference was found between the sexes in terms of fibrinogen, ENA-78, and WBC counts ($p=0.283$, $p=0.600$, and $p=0.128$, respectively). The mean ages of the participants were 28.4 ± 10.6 in group 1, 32.3 ± 12.6 years in group 2, and 31.2 ± 10.4 years in the controls. There was no significant difference in the mean ages of the three groups ($p=0.330$). The mean body mass index (BMI) was 24.3 ± 4 kg/m² in group 1, 26.4 ± 4.1 kg/m² in group 2, and 25.6 ± 4 kg/m² in the controls, indicating no significant differences between the groups ($p=0.081$).

Significant differences were found in the mean fibrinogen values between the groups ($p<0.001$). The mean fibrinogen value was 290.3 ± 67.3 mg/dL in group 1, 412 ± 56.5 mg/dL in group 2, and 182.9 ± 43.3 mg/dL in the controls. The highest mean value was detected in group 2, and the lowest was found in the controls.

The mean ENA-78 values differed significantly between the groups ($p<0.001$). The mean ENA-78 value was 15.7 ± 7.3 pg/mL in group 1, 36.1 ± 23.6 pg/mL in group 2, and 5.7 ± 2.7 pg/mL in the controls. The highest ENA-78 value was detected in group 2 and the lowest was in the controls.

Significant differences were found in the mean WBC counts between the groups ($p<0.001$). The mean WBC count was 11.1 ± 2.4 mm³ in group 1, 11.4 ± 2.8 mm³ in group 2, and 7.1 ± 2.1 mm³ in the controls. There was no significant difference between groups 1 and 2, but the mean WBC count was lower among the controls compared with groups 1 and 2 (Table 1). The sensitivity, specificity, and positive and negative predictive values (PPV and NPV) were calculated for group 1 and group 2 (Tables 2 and 3). The WBC count was detected to be more sensitive (66%) in group 1, whereas fibrinogen showed a higher sensitivity (86%) and specificity (96%) for group 2 compared with the other parameters. There was a weak positive relationship between WBC count and ENA-78 in group 2. In group 1, there was a strong positive relationship between WBC count and fibrinogen. There was no significant relationship between the other parameters. When each group was evaluated in itself, no statistically significant relationship was found between fibrinogen and ENA-78 (Table 4). Figures 1 and 2 show ROC curves of fibrinogen level, ENA-78 level, and WBC count in group 1 and group 2. The calculation of area under the curve (AUC) reveals that WBC count in group 1 and fibrinogen level in group 2 are more effective than the other parameters.

Table 1: Comparison of the fibrinogen and ENA-78 values and WBC counts between the three groups.

	Group 1	Group 2	Controls	p-Value
Fibrinogen, mg/dL	290.3 ± 67.3^a	412.3 ± 56.5^b	182.9 ± 43.3^c	<0.001
ENA-78, pg/mL	15.7 ± 7.3^a	36.1 ± 23.6^b	5.7 ± 2.7^c	<0.001
WBC, mm ³	11.1 ± 2.4^a	11.4 ± 2.8^a	7.1 ± 2.1^b	<0.001

^{a,b,c}No statistical difference was found between the groups with the same letter. Bold values were considered statistically significant ($p<0.05$).

Table 2: The fibrinogen, ENA-78, and WBC cut-off levels and the sensitivity, specificity, PPV, and NPV for group 1.

	Cut-off	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %	p-Value
Fibrinogen	>263 mg/dL	0.499	0.381–0.617	51	66	75	40	0.983
ENA-78	>13 pg/mL	0.521	0.404–0.639	50	70	76	41	0.742
WBC	>10,000/mm ³	0.687	0.575–0.798	66	66	80	50	0.004

Bold values were considered statistically significant ($p<0.05$).

Table 3: The fibrinogen, ENA-78, and WBC cut-off levels and the sensitivity, specificity, PPV, and NPV for group 2.

	Cut-off	AUC	95% CI	Sensitivity, %	Specificity, %	PPV, %	NPV, %	p-Value
Fibrinogen	>347 mg/dL	0.958	0.921–0.996	86	96	98	78	<0.001
ENA-78	>19 pg/mL	0.914	0.860–0.969	78	90	94	67	<0.001
WBC	>10,000/mm ³	0.712	0.604–0.819	65	63	78	47	0.001

Bold values were considered statistically significant ($p<0.05$).

Table 4: Correlation of fibrinogen, ENA-78, and WBC count in all groups.

	WBC	ENA-78
FMF-attack		
Fibrinogen		
r	0.215	0.136
p	0.255	0.474
ENA-78		
r	0.382	
p	0.037	
Appendicitis		
Fibrinogen		
r	0.767	0.230
p	<0.001	0.222
ENA-78		
r	0.189	
p	0.317	
FMF-attack free		
Fibrinogen		
r	0.038	−0.056
p	0.841	0.769
ENA-78		
r	−0.008	
p	0.968	

Bold values were considered statistically significant ($p < 0.05$).

Figure 1: The sensitivity and specificity for fibrinogen and ENA-78 levels, and WBC count in group 1 according to the ROC curve.

Discussion

The frequency of appendectomy is 12–25% in the general population and 40% in patients with FMF [17]. In patients with FMF who present with acute peritonitis, laparotomy is the most commonly performed procedure due to AA [18].

Figure 2: The sensitivity and specificity for fibrinogen and ENA-78 levels, and WBC count in group 2 according to the ROC curve.

One of the most common causes of diagnostic uncertainty in patients who present to emergency departments with abdominal pain is that a diagnosis of AA cannot be excluded in a patient with or without FMF who do not undergo an appendectomy [19]. Thus, several biochemical tests are ordered to help diagnose these patients, including WBC counts, and fibrinogen, ESR, and CRP analyses [6].

To date, many clinical findings and biochemical markers have been introduced to effectively differentiate these two conditions [20–25]; however, no single laboratory or clinical factor is able to provide discriminative outcomes. Kisacik et al. [21] stated that serum resistin and visfatin levels failed to distinguish AA from acute FMF attacks. In another study conducted by Kisacik et al. [23], procalcitonin levels were found to be significantly higher in patients with appendicitis than in patients with FMF. However, more comprehensive studies are needed before the implementation routine of procalcitonin testing can be recommended.

Kucuk et al. [25] indicated that the neutrophil-to-lymphocyte (NLR) ratio might be helpful in distinguishing an acute FMF attack from AA because it is a readily available marker. Ugan et al. [24] stated that urinary β_2 -microglobulin (U- β_2 M) assay might be helpful in the differential diagnosis of peritonitis attacks and AA in patients with FMF on colchicine treatment. We planned this study because there is a limited number of studies on this issue and we think that the findings will lead to the use of new biomarkers. We sought to determine whether WBC counts and ENA-78 and fibrinogen would be useful in distinguishing the two diseases.

ENA-78 is a chemokine that is a potent stimulator of neutrophils; it induces a variety of biologic responses, such as chemotaxis, enzyme release, the upregulation of surface receptors, and intracellular calcium mobilization [26–28]. The production of ENA-78 and other chemokines can result in a chemotactic gradient that has the capacity to increase the migration of granulocytes and monocytes/macrophages from the bloodstream through the endothelium, resulting in a marked increase in chronic inflammation [28, 29]. In rheumatoid arthritis, it was reported that local ENA-78 production occurred in the joints [30, 31]. The predominance of several chemokines in other collagen diseases, such as systemic lupus erythematosus and systemic sclerosis, has also been described [29, 32]. Thus, we aimed to evaluate whether ENA-78 level could be used as a potential parameter for use in the differential diagnosis of recurrent episodes of FMF and AA.

When we compared the ENA-78 and fibrinogen values and the WBC counts in our study, we found significant differences in ENA-78 and fibrinogen values, but not in WBC counts between group 1 and group 2 (Table 1). When the sensitivity and specificity of the evaluated parameters were assessed, the sensitivity of the WBC count was higher for group 1, whereas fibrinogen sensitivity was higher for group 2.

The WBC count is a laboratory parameter widely used in both FMF and AA in emergency departments [6]. However, in the context of current information, there are no data showing that the WBC count can be used alone to differentiate between FMF attacks and AA. In a review encompassing studies published between 2010 and 2015, Acharya et al. [33] found that the sensitivity and specificity of the WBC count were 79% (95% confidence interval [CI]: 0.78–0.81) and 55% (95% CI: 0.54–0.57), respectively. Using this approach, the accuracy of a diagnosis of appendicitis can be enhanced, reducing the number of negative appendectomies performed, thereby reducing the implied adverse impact to patients and treatment costs to hospitals [34]. In our study, it was found that there were significant differences in WBC counts between the controls and the other two groups; however, this finding had no effect on discriminating between AA (group 1) and FMF attacks (group 2) (Table 1). The sensitivity and specificity of the WBC counts were 66% in the AA group and 65% in the FMF attack group (Tables 2 and 3). Although the WBC count appeared to be a marker in the diagnosis of AA, it seems that it cannot distinguish FMF attacks from AA.

In emergency departments, fibrinogen analysis is a commonly ordered test to diagnose acute FMF attacks. Fibrinogen accumulation is an expected feature in damaged tissues and inflammatory foci. *In vitro* studies

have shown that fibrinogen can deeply alter WBC function, leading to changes in cell migration, phagocytosis, chemokine and cytokine production, degranulation, and other processes. Many of the effects of fibrinogen on leukocyte activity appear to be mediated by a specific receptor on leukocytes, the integrin receptor $\alpha M\beta 2$ [34, 35]. In this respect, studies have been performed to determine if fibrinogen may be used as a biomarker to reduce negative laparotomies in suspected AA. Menteş et al. [36] indicated that serum values of fibrinogen could be used as a new acute-phase reactant in the diagnosis of AA.

Nyuwi et al. reported that fibrinogen had a sensitivity of 88% and a specificity of 55% in the diagnosis of AA. They found that the use of fibrinogen serum levels in the diagnosis of AA might reduce the rate of negative appendectomies [37]. In our study, there were significant differences in the fibrinogen values between the groups (Table 1). In addition, the sensitivity and specificity of fibrinogen were 51% and 66% in group 1 and 86% and 96% in group 2, respectively.

There are some limitations to our study. First, the number of participants in each group was limited; therefore, it limits the power to detect changes in markers as variable as ENA-78, fibrinogen, and WBC count. Second, it would be better to include a group of healthy volunteers with no FMF, especially in view of the baseline values for ENA-78. Third, in patients with FMF, ENA-78 and fibrinogen may increase in the presence of conditions that cause peritonitis. These biomarkers are useful in distinguishing FMF from AA if the cause of peritonitis is AA. No comparison was made with other diseases that may be the cause of peritonitis. Finally, the study could be supported by CRP, ESR, haptoglobin, C3, C4 and other acute-phase reactants. Extensive haptoglobin and complement factor measurements were beyond the scope of this study and were therefore not performed.

Conclusions

Fibrinogen and ENA-78 might be helpful in discriminating FMF attacks from AA. We recommend routinely studying ENA-78 and fibrinogen in patients with FMF who present with peritonitis in order to distinguish between an FMF attack and AA.

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