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Activities of Cholinesterases, Adenosine Deaminase and Myeloperoxidase in Patients With Familial Mediterrenean Fever

Ailesel Akdeniz Ateşli Hastalarda Kolinesteraz, Adenozin Deaminaz ve Miyeloperoksidaz Aktiviteleri

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Summary

Objective: The aim of this study was to determine Acetylcholinesterase (AChE), Butyrilcholinesterase (BChE), Adenosine Deaminase (ADA) and Myeloperoxidase (MPO) activities in patients with Familial Mediterranean Fever (FMF) during attacks and attack-free periods. Although an inflammatory process is the main pathology in FMF, the activities of AChE, BChE and MPO which might be affected by inflammation has not yet been assessed in FMF.

Materials and Methods: The subjects were divided into 3 groups: Group 1. FMF patients in acute attack period (FMF-AP); Group 2. FMF patients in attack-free period (FMF-AFP); Group 3. Healthy Control subjects (HC). The first two groups consisted of 41 and 35 patients, respectively. Thirty individuals without a history of other potential health problems constituted the healthy control (HC) group. We measured the activities of MPO, ADA, AChE and BChE in whole blood and serum of the study groups. Acute phase reactants (AFP) were also evaluated.

Results: In the current study, while ADA and MPO activities increased, AChE decreased significantly in both whole blood and serum in FMF-AP group, BChE decreased in only whole blood in this group compared with FMF-AFP and HC groups ($p<0.05$). In FMF-AP group, both whole blood and serum ADA ($r=0.313$, $r=0.267$), and MPO ($r=0.341$, $r=0.253$) activities were correlated with C-reactive protein, respectively (all p values <0.001).

Conclusion: Our study demonstrated that there are significant differences between FMF-AP and other groups in terms of AChE, BChE, ADA and MPO activities. Thus, we suggest that elevated ADA, MPO and decreased AChE and BChE activities may be considered as supportive markers to distinguish FMF attacks from attack-free periods. However, further larger-scale studies are needed to validate these results. *Turk J Phys Med Rehab 2012;58:184-8.*

Key Words: Familial mediterranean fever; inflammation; adenosine deaminase; myeloperoxidase; acetylcholinesterase; butyrilcholinesterase

Özet

Amaç: Bu çalışmanın amacı Ailesel Akdeniz Ateşli (AAA) hastalarda atak ve atak olmayan dönemlerde Asetilkolinesteraz (AChE), Bütirilkolinesteraz (BChE), Adenozin Deaminaz (ADA) ve Miyeloperoksidaz (MPO) aktivitelerini belirlemek idi. İnflamatuvar süreç AAA'da başlıca patoloji olmasına rağmen, inflamasyon tarafından etkilenebilen AChE, BChE ve MPO aktiviteleri AAA'da henüz değerlendirilmemiştir.

Gereç ve Yöntem: Olgular 3 gruba bölündüler. Grup 1. Akut atak dönemindeki AAA hastaları (AAA-AD); Grup 2. Atak olmayan dönemdeki AAA hastaları (AAA-AOD); Grup 3. Sağlıklı kontrol grubu (SK). İlk iki grup sırasıyla 41 ve 35 hastadan oluşmaktaydı. Potansiyel olarak herhangi bir sağlık problemi olmayan 30 kişi SK grubunu oluşturdu. Biz çalışma gruplarının tam kan ve serumlarında AChE, BChE, ADA ve MPO aktivitelerini ölçtük. Akut faz reaktanları da ayrıca değerlendirildi..

Bulgular: Bu çalışmada, AAA-AD grubunda, AAA-AOD ve SK grupları ile kıyaslandığında, hem tam kan hem de serum ADA, MPO aktiviteleri anlamlı derecede artmış, AChE düşmüşken, BChE bu grubun yalnızca tam kanlarında düşüktü ($p<0,05$). AAA-AD grubunda hem tam kan hem de serum ADA ($r=0,313$, $r=0,267$) ve MPO ($r= 0,341$, $r= 0,253$) aktiviteleri sırasıyla C-reaktif protein ile korele idi (bütün p değerleri$< 0,001$).

Sonuç: Çalışmamız AAA-AD ve diğer gruplar arasında AChE, BChE, ADA ve MPO aktiviteleri açısından anlamlı farklılıklar olduğunu gösterdi. Bu nedenle biz artmış ADA, MPO ve azalmış AChE ve BChE aktivitelerinin AAA ataklarını atak olmayan dönemlerden ayırmada destekleyici belirteçler olarak düşünülebileceğini önermekteyiz. Bununla birlikte, daha ileri büyük ölçekli çalışmalara bu sonuçların geçerliliği için ihtiyaç vardır. *Türk Fiz Tıp Rehab Derg 2012;58:184-8.*

Anahtar Kelimeler: Ailesel akdeniz ateşi; inflamasyon; adenozin deaminaz; miyeloperoksidaz; asetilkolinesteraz; bütirilkolinesteraz

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Introduction

The important mediators of inflammation are histamine, leukotrienes (LTs), Platelet Activating Factors (PAFs), lysosomal enzymes, prostaglandins (PGs), Reactive Oxygen Species (ROS), and various cytokines. During the inflammatory process, one mediator triggers the generation and release of another mediator. These secondary generated mediators either enhance the action of the initial mediator or paradoxically inhibit its action. In addition, granulocytosis, following injury or infection, is associated with an increased production of proinflammatory and hemopoietic cytokines that are regulated in peripheral tissues by various factors, including Myeloperoxidase (MPO), Adenosine Deaminase (ADA), and cholinergic system such as Acetylcholine (Ach) (1).

Owing to role of various cytokines, ROS, ADA and MPO in inflammation, it is generally accepted that demonstration of their enhanced levels in plasma and tissues indicates the presence of inflammation. ADA has a role for proliferation and differentiation of T lymphocytes. Its levels have been shown to increase in several inflammatory conditions including attack period (AP) of Familial Mediterranean Fever (FMF) (2-6).

MPO is an enzyme released by activated neutrophils and, increased MPO levels have been reported in rheumatologic disorders (7). MPO functions as a cytotoxic and immunoregulatory molecule that induces the production of proinflammatory cytokines in macrophages through activating mannose receptors (8).

In recent years, several studies have focused on the expression and biological role of the neuronal and non-neuronal cholinergic system in inflammatory processes and immune system (9). Cholinergic signaling is notably involved in anti-inflammatory reactions (10). This cholinergic anti-inflammatory pathway mediated by ACh acts by inhibiting the production of pro-inflammatory cytokines, TNF-α, IL-1, IL-6 and IL-18 and suppresses the activation of nuclear factor kappa-B (NF-Kb) expression (11). Circulating ACh may be hydrolyzed by both acetylcholinesterase (AChE) and similar enzyme butyrylcholinesterase (BChE). ACh is cleaved into choline and acetic acid by AChE and BChE (12). Expression of both AChE and BChE has been detected in most non-neuronal cells and organs (12).

FMF is the most common disease of the periodic fever syndromes. It is an autosomal recessive, systemic relapsing autoinflammatory disorder seen in all populations but mainly in Mediterranean populations (13). An inflammatory process is the main pathology in FMF but the factors that affect inflammation in FMF attack and attack-free periods (AFP) are still being investigated. Enhanced levels of inflammatory mediators are found in the plasma of these patients. High generation of ROS were also shown during AP (14,15). There is no specific laboratory test of FMF to distinguish attacks from AFP.

To the best of our knowledge, the status of AChE, BChE and MPO in FMF during AP and AFP has not yet been assessed. The aim of the current trial was to reveal whether AChE, BChE, ADA and MPO would possess a differential diagnostic value between FMF patients with AP and AFP.

Materials and Methods

The study was conducted in Faculty of Medicine, Departments of Medical Biology and Physical Medicine-Rehabilitation-Rheumatology between January 2010 and February 2011. A written consent to participate in the study was obtained from the patients and healthy controls after they were thoroughly informed about the research details. Ethical approval for the study was obtained from the University Ethics Committee.

Patients fulfilling the Tel Hashomer criteria included in the study (16). All of the FMF subjects were under treatment with Colchicine 1.5 gr daily. According to the clinical findings FMF patients were allocated into FMF-AP and FMF-AFP groups. If a patient who had fever and was admitted to the clinic with acute abdominal or pleural or synovial attack within 24 hours was considered as FMF-AP. Patients without an AP for at least 2 months were allocated to FMF-AFP group. All FMF patients and age-/sex-matched healthy controls (HC) were in the age range of 20-40 years old. Patients with other inflammatory, autoimmune, acute or chronic infectious diseases and diabetes mellitus were excluded from the study. The HC group consisted of 30 individuals including 15 males and 15 females without a history of other potential health problems. FMF-AP and FMF-AFP groups were consisted of 41 patients including 20 males and 21 females and 35 patients including 17 males and 18 females, respectively.

Totally, 7 ml sample of venous blood was taken from each patient with FMF attended to the Physical Medicine-Rehabilitation and Rheumatology outpatient clinic during AP or AFP. Two ml of blood was taken in a tube with EDTA (ethylene diamine tetraacetic acid) for whole blood analyses, and the rest in ice-chilled siliconized glass tubes for serum analyses. The blood samples of patients during AP were obtained after 24-48 hours from the acute attack initiation. Blood of HC was obtained same as FMF subjects. Samples were kept in a cool box at +4 °C until they were transferred immediately to the laboratory. The serum samples were obtained by centrifuging blood samples at 3000 rpm for 15 min at 4 °C. They were stored at -20 °C until analysis. With regard to whole blood samples, they were hemolyzed with distilled water and centrifuged at 4000 g force for 10 min. at 4 °C. The clear upper supernatant fluid was taken and ADA, MPO, AChE and BChE activities were measured at this stage. Besides, activities of these enzymes were determined in serum.

Acetylcholinesterase (E.C. 3.1.1.7) and butyrylcholinesterase (E.C. 3.1.1.8) activities were measured by a Shimadzu UV-1201 spectrophotometer using acetylthiocholine and butyrylthiocholine as substrate, respectively, by the method described by Ellman et al. (17). ADA (EC 3.4.5.5) was assayed by the method described by Giusti (18). MPO (EC 1.11.1.7) was measured by the method described by Bradley et al. (19).

Measurement of Acute-phase Reactants

C-reactive Protein (CRP), Erthrocyte Sedimentation Rate (ESR), fibrinogen and White Blood Cell (WBC) count were analyzed on the same day of blood samples were obtained. Serum CRP level was determined by the nephelometric method. ESR was measured by the Westergreen method, and ESR within one hour was recorded. Fibrinogen levels were assessed by a photo-optical method. The total blood count including WBC was measured by autoanalyzer.

All data were expressed as mean+standard deviation (SD). The statistical analyses were made using the Minitab 13 for Windows packet program. Means and standard deviations were calculated according to the standard methods for all parameters. One-way ANOVA test was used for variables which met assumptions (normality test), on the other hand the Kruskal-Wallis test was used for variables that did not meet assumptions. After these tests, the Tukey multiple comparison test was performed to determine different groups. Significance level was accepted at $p<0.05$. Spearman's correlation coefficient was used for assessing correlations.

Results

The mean age of the FMF subjects in FMF-AP and FMF-AFP groups were 26.6±6.8, 27.3±7.5 years, respectively and the mean age of HC group was 27.8±7.6 years. The disease durations were 8.1±7.5 and 9.6±6.3 years for FMF-AFP and FMF-AP groups, respectively. The groups were similar in terms of age and gender (Table 1).

The levels of acute-phase reactants in the FMF patients are shown in Table 2. Fibrinogen, CRP and ESR levels were significantly higher in FMF-AP than in FMF-AFP and HC groups ($p<0.05$). Significant difference in p values was found between the three groups. In post-hoc test, these differences were originated from comparing FMF-AP and HC groups as well as FMF-AP and FMF-AFP groups ($p<0.05$).

As shown in Table 3 and 4, ADA, MPO activities increased and AChE decreased significantly in both whole blood and serum of FMF-AP group, whereas BChE decreased in only whole blood of this group compared with FMF-AFP and HC groups ($p<0.05$). In FMF-AP group, both whole blood and serum ADA ($r=0.313$, $r=0.267$), MPO ($r=0.341$, $r=0.253$) activities were correlated with only C-reactive protein respectively (all P values < 0.001). There were no correlations between all the other parameters.

Discussion

In this study, we demonstrated that FMF patients with acute attacks had higher ADA, MPO and lower AChE activities both in whole blood and serum compared to FMF-AP and HC groups. On the other hand, we determined decreased activities of BChE only in

Table 1. Demographic characteristics of study groups.

Feature	HC (n=30) Mean±SD	FMF-AP (n=41) Mean±SD	FMF-AFP (n=35) Mean±SD	p value
Age (years)	27.8±7.6	26.6±6.8	27.3±7.5	0.555
Duration of disease (years)	---------	8.1±7.5	9.6±6.3	0.259
Gender female (n) / male (n)	15/15	21/20	18/17	

SD: Standard deviation

Table 2. Acute phase reactants of the groups.

Feature	HC (n=30) Mean±SD	FMF-AP (n=41) Mean±SD	FMF-AFP (n=35) Mean±SD	p value
ESR (mm/h)	$10.6±7.9^{b}$	$29.3±19.5^{a}$	$11.9±8.6^{b}$	0.010
CRP (mg/l)	$1.5±3.2^{b}$	$9.5±6.1^{a}$	$1.8±3.8^{b}$	0.041
WBC (/mm^3)	7682±3742	8376±4522	7843±3656	0.222
Fibrinogen (mg/dl)	$285.7±81.8^{b}$	$477.3±168.7^{a}$	$292.9±76.4^{b}$	0.038

SD: Standard deviation, different lower cases represent different group means Namely if one of two means gets different lower case such as "a" and the other gets "b" then there is a significant difference ($p<0.05$) but if both means get the same lower case such as "a" and "a" or "b" and "b" or "a" and "ab" or "b" and "ab" then there is no significant difference between these two means ($p>0.05$).

Table 3. Activities of adenosine deaminase (ADA), myeloperoxidase (MPO), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in whole blood of FMF patients and healthy controls.

Feature	HC (n=30) Mean±SD	FMF-AP (n=41) Mean±SD	FMF-AFP (n=35) Mean±SD	p value
ADA U/g Hb	$1.01±0.33^{b}$	$1.85±0.62^{a}$	$1.16±0.34^{b}$	0.001
MPO U/g Hb	$9.62±3.06^{b}$	$12.52±3.51^{a}$	$9.99±3.20^{b}$	0.001
AChE U/g Hb	$69.22±12.78^{a}$	$62.45±12,04^{b}$	$64.82±13.26^{ab}$	0.046
BChE U/g Hb	$12.40±2.42^{b}$	$10,38±2,20^{a}$	$11.72±2.24^{b}$	0.011

SD: Standard deviation, different lower cases represent different group means. Namely if one of two means gets different lower case such as "a" and the other gets "b" then there is a significant difference ($p<0.05$) but if both means get the same lower case such as "a" and "a" or "b" and "b" or "a" and "ab" or "b" and "ab" then there is no significant difference between these two means ($p>0.05$).

Table 4. Activities of Adenosine deaminase (ADA), myeloperoxidase (MPO), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) in serum of FMF patients and healthy controls.

Feature	HC (n=30) Mean±SD	FMF-AP (n=41) Mean±SD	FMF-AFP (n=35) Mean±SD	p value
ADA U/L	$21.72±6.45^{b}$	$33.41±4.28^{a}$	$23.27±5.76^{b}$	0.001
MPO U/L	$29.67±8.93^{b}$	$41.14±13.65^{a}$	$30.07±7.54^{b}$	0.001
AChE U/ml	$1.49±0.16^{a}$	$1.39±0.11^{b}$	$1.43±0.10^{ab}$	0.004
BChE U/ml	0.29±0.01	0.29±0.04	0.28±0.03	0.178

SD: Standard deviation, different lower cases represent different group means. Namely if one of two means gets different lower case such as "a" and the other gets "b" then there is a significant difference ($p<0.05$) but if both means get the same lower case such as "a" and "a" or "b" and "b" or "a" and "ab" or "b" and "ab" then there is no significant difference between these two means ($p>0.05$).

whole blood of these patients. According to the literature, this is the first study examining the activities of MPO, AChE and BChE in patients with FMF. Results of the current study showed that, ADA, MPO, AChE, and BChE may be used as supportive markers to differentiate FMF attacks from AFP.

ADA has a role in purine metabolism that converts adenosine and deoxyadenosine to inosine and deoxyinosine by irreversible deamination (20). ADA exists in all nucleated cells, but its concentration varies in different tissues (20). ADA is required for lymphocyte proliferation, maturation and differentiation, particularly in T cells. Determination of ADA activity in different tissues may help identification of immune system activation in inflammatory conditions. ADA activity increases in inflammatory diseases characterized by T-cell activation and proliferation. Escalated ADA activity has been shown in serum and plasma of the patients with rheumatic diseases such as rheumatoid arthritis and Behcet's disease (21,22). FMF attacks are characterized by serosal inflammation rich in Polymorphonuclear Leukocytes (PMNL). Elevated concentrations of ADA degradation products might contribute to increased numbers of PMNL infiltration into the serosal membranes during FMF attacks (13,23). In accordance with our results, Kisacik et al. (6) found significantly higher plasma levels of ADA in FMF-AP compared to FMF-AFP and HC groups. Our results suggest that ADA may contribute maintaining of inflammatory cascade in FMF during acute attacks.

MPO, which is a neutrophil-specific enzyme, represents neutrophil activation directly (24). It induces the production of proinflammatory cytokines in macrophages. It is also the major enzymatic source of leukocyte-generated oxidants. Elevated MPO activity plays a crucial role in chronic and rheumatic inflammatory processes such as rheumatoid arthritis, Systemic Lupus Erythematosus (SLE), ankylosing spondilitis and Behçet's disease (25-27). Interestingly, to our knowledge, the role of MPO in FMF which is characterized by serosal inflammation rich in Polymorphonuclear Leukocytes (PMNL) has not yet been assessed up to this study. We determined that FMF-AP patients presented higher whole blood and serum MPO activities than those of FMF-AFP and HC subjects. Furthermore, we observed positive correlations of whole blood and serum ADA, MPO activities with CRP levels in FMF-AP group.

In the current study, we evaluated whole blood and serum AChE, BChE activities in FMF patients for the first time. We found decreased activity of these enzymes in whole blood of FMF-AP patients. Apart from that, only decreased AChE activity was determined in serum of these patients as well. In accordance with our results, Davis et al. (28) reported decreased AChE activity in the rat jejunum during inflammation induced by Trichinella spiralis infection. In addition, plasma choline esterase activity in advanced rheumatoid arthritis was found to be lower than in less advanced cases (29). In an earlier study conducted on a geriatric population, negative correlations of IL-6 and TNF-α with choline esterases were reported (30). In the same study, frailty was also associated with high inflammatory markers and low choline esterase activities (30).

ACh is an anti-inflammatory molecule. Neuronal and non-neuronal ACh is related to cholinergic anti-inflammatory pathway to suppress peripheral inflammation. Some clinical and experimental studies indicated that the cholinergic antiinflammatory reflex was impaired during endotoxemia and cytokine-mediated diseases such as FMF (1,11,31). Nazarov et al. (32) reported inhibitory effect of CRP on the breakdown of ACh by AChE. This influence of CRP was most likely a result of the higher binding capacity of CRP to ACh than AChE. Hence, we can conclude that in an acute FMF attack, escalated concentrations of CRP lead to reduced levels of free ACh that may result in increase in levels of IL-1 and TNF-alpha. Low activities of AChE and BChE determined in FMF-AP group in this study might also be related to diminished availability of their substrate, ACh. We can suggest that in acute FMF attacks, acetylcholinesterase inhibitor could be taken to enhance free ACh level to suppress abnormal inflammation for therapeutic purpose.

In conclusion; this is the first report investigating the activities of MPO, AChE and BChE in FMF patients. ADA, MPO activities increased and AChE decreased significantly in both whole blood and serum of FMF-AP group, whereas BChE decreased in only whole blood of this group compared with FMF-AFP and HC groups. Increased ADA, MPO and decreased AChE, BChE activities may be used as supportive markers to differentiate FMF attacks from attack-free periods. Further larger-scale studies are needed to support these results. Apart from that, taking an AChE inhibitor in FMF acute attack may enhance free ACh level to suppress abnormal inflammation for therapeutic goals in these patients.

Conflict of Interest:

Authors reported no conflicts of interest.

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