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ORIGINAL ARTICLE

Screening of Free Carnitine and Acylcarnitine Status in Children With Familial Mediterranean Fever

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ABSTRACT

Objectives: This study aims to demonstrate the patterns of free carnitine (FC) and acylcarnitine (AC) esters in familial Mediterranean fever (FMF) patients.

Patients and methods: A total of 205 patients (106 males, 99 females; mean age 131.3±52.1 months; range 24 to 254 months) with FMF and 50 healthy controls (27 males, 23 females; mean age 125.7±49.6 months; range 32 to 217 months) were enrolled. Fasting dried blood samples were taken for showing FC and AC ester levels with tandem mass spectrometry from both patients and controls.

Results: Screening of AC profile revealed increased FC, 3-hydroxypalmitoylcarnitine (C16-OH), and 3-Hydroxy octadecanoylcarnitine (C18:2-OH) carnitine levels, while decreased acetyl-carnitine (C2), propionyl-carnitine (C3), butyryl-carnitine (C4), tiglyl-carnitine (C5:1), hexanoyl-carnitine (C6), octanoyl-carnitine (C8), decenoylcarnitine (C10:1), decadienoylcarnitine (C10:2), malonylcarnitine (C3DC), methylmalonylcarnitine (C4DC), glutarylcarnitine (C5DC), hexadecenoylcarnitine (C16:1), 3-Hydroxy butyrylcarnitine (C4-OH), and 3-Hydroxy oleylcarnitine (C18:1-OH) carnitine levels in FMF patients compared to controls. Total AC levels ($p<0.001$) and AC to FC ratio ($p<0.001$) were also lower in FMF patients than the controls.

Conclusion: In this study, we were able to detect some of the AC profile variations in FMF patients; however, usage of carnitine in all patients with FMF is not recommended since we were not able to demonstrate secondary carnitine deficiency in FMF patients of this study.

Keywords: Auto-inflammation; carnitine deficiency; familial Mediterranean fever; fatty acid metabolism.

Familial Mediterranean fever (FMF) is the most common hereditary auto-inflammatory disease, characterized by self-limited fever and serositis.[1] FMF is an autosomal recessive disease mainly affecting Middle-East populations such as Turks, Jews, Arabs, Armenians and other ethnic groups living around the Mediterranean basis like Italians, Greek and Druze. In the Middle-East region, the prevalence of FMF is 1/200-1000 with a high carrier rate of 1/3-5.[2-5] The MEditerranean FeVer gene located on chromosome 16 encodes a protein called 'pyrin'/'marenostin'. Pyrin plays a pivotal role in the regulation of both inflammation and apoptosis.[6,7] FMF is characterized by febrile episodes caused by recurrent auto-inflammation accompanied by signs of serositis such as peritonitis, pleuritis, synovitis and sometimes additional clinical findings of erysipelas-like erythema and acute scrotum attacks. Between these attacks, patients are usually asymptomatic.[1]

L-Carnitine (LC; β-hydroxy-γ-trimethylaminobutyric acid) is an important molecule in human metabolism, especially for the mitochondrial oxidation of fatty acids. Carnitine can be synthesized endogenously from essential amino acids; lysine and methionine.[8] Animal

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products like meat, fish, poultry, and milk are the best sources for dietary carnitine and they provide 75% of the daily requirements.[9]

Carnitine deficiencies are divided into two categories, primary carnitine deficiency and secondary carnitine deficiency (SCD). Primary carnitine deficiency is a rare autosomal recessive inherited disorder of fatty acid oxidation caused by deficiency of plasma membrane carnitine transport resulting from defective plasma membrane carnitine/organic cation transporter 2 activity.[10,11] SCD is related with some inherited metabolic disorders; treatment with some drugs like valproate, zidovudine and pivampicilin.[12-16] SCD could be also associated with chronic conditions including kidney disease, diabetes mellitus, heart failure, cirrhosis, malabsorption, and Alzheimer disease.[17-21] Trauma, sepsis and acute organ failure result in increased needs of carnitine; therefore, may cause SCD.[8,22-24]

To our knowledge, no study has been conducted documenting free carnitine (FC) and acylcarnitine (AC) esters in FMF patients. Our hypothesis was that FMF patients might have lower FC levels than an age and sex matched healthy control group due to increased need of carnitine because of recurrent auto-inflammation. Thus, in this study, we aimed to demonstrate the patterns of FC and AC esters in FMF patients.

PATIENTS AND METHODS

This cross-sectional study included 205 FMF patients (106 males, 99 females; mean age 131.3±52.1 months; range 24 to 254 months) who were attending the outpatient Pediatric Rheumatology Clinic of İstanbul University Cerrahpaşa Medical Faculty Children's Hospital between May 2014 and November 2014 and age and sex matched 50 healthy controls (27 males, 23 females; mean age 125.7±49.6 months; range 32 to 217 months). The patients were selected by random sampling and FMF diagnosis was confirmed by a child rheumatologist according to Yalçınkaya criteria. Patients' clinical data, including detailed family history (consanguinity, additional affected individuals, ethnicity, etc.), age, sex, diagnosed age, additional clinical symptoms (fatigue, muscle pains-cramps, psychological symptoms, etc.), medications, drug responses and laboratory data including complete blood count, glucose, transaminases, urea, creatinine, creatine phosphokinase, lipid profile, C-reactive protein, erythrocyte sedimentation ratio, and FMF mutation analyses of FMF were documented. All patients underwent a careful physical examination. Patients with an additional diagnosis (inflammatory bowel disease, etc.) and patients under carnitine treatment were excluded.

A fasting blood sample was taken for FC and AC esters with quadrupole electrospray ionization mass spectrometry-mass spectrometry from children in both groups. Samples consisted of capillary blood were collected on Whatmann S&S 903 filter paper (Sigma-Aldrich, St. Louis MO, USA). The sampling was done by finger prick. Samples were dried in room temperature. Relevant AC butyl esters were extracted and derivatized from 1/3 inch dried blood samples and were analyzed using a protocol described by Schulze et al.[25] previously. AC butyl esters were detected due to mass/ion ratios with electrospray ionization mass spectrometry-mass spectrometry. Agilent 1200 series auto sampler (Agilent, Waldbronn, Germany) and Water Micromass Quattro LC Likrom™ tandem mass spectrometry (Waters, Manchester, England) were used for the analyses. AC was screened by positive ion mode m/z 85 parents screening function. The quantitative values of signal intensities were calculated using MassLynx and NeoLynx softwares (Version 4.1). Both signal intensities and calculated concentrations were exported to spreadsheet software where flagging abnormal concentrations and further evaluations were done.[25,26]

The study was reviewed and approved by the Ethics Committee of İstanbul University Cerrahpaşa Medical Faculty. Signed informed consent was obtained from parents of all patients' who participated in the study. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Statistical analysis

Data were expressed using descriptive statistics such as mean and standard deviation for continuous variables and number and percentage for categorical variables. A one-sample t-test was carried out for the quantitative estimation of AC analyses. Comparison between the groups was

carried out by independent sample t-test. Statistical analyses were done with IBM SPSS Statistics version 21.0 (IBM Corporation, Armonk, NY, USA). *P* value <0.05 was considered statistically significant.

RESULTS

Patients' clinical and demographic characteristics were given in Table 1. AC analyses in spot dried blood samples with electrospray ionization mass spectrometry-mass spectrometry were performed in patient and control groups. Screening of AC profile revealed increased FC (p<0.001), 3-hydroxypalmitoylcarnitine (C16-OH) (p<0.05) and 3-Hydroxy butyrylcarnitine (C4-OH) and 3-Hydroxy octadecanoylcarnitine (C18:2-OH) (p<0.05) carnitine levels, while decreased acetyl-carnitine (C2) (p<0.001), propionyl-carnitine (C3) (p<0.001), butyryl-carnitine (C4) (p<0.001), tiglyl-carnitine (C5:1) (p<0.05), hexanoyl-carnitine (C6) (p<0.001), octanoyl-carnitine (C8) (p<0.05), decenoylcarnitine (C10:1) (p<0.001), decadienoylcarnitine (C10:2) (p<0.05), malonylcarnitine (C3DC) (p<0.05), methylmalonylcarnitine (C4DC) (p<0.001), glutarylcarnitine (C5DC) (p<0.001), hexadecenoylcarnitine (C16:1) (p<0.05), 3-Hydroxy butyrylcarnitine (C4-OH) (p<0.001), 3-Hydroxy oleylcarnitine (C18:1-OH) (p<0.01) carnitine levels in FMF patients than the control group.

Total AC levels (p<0.001) and AC to FC ratio (p<0.001) were lower in FMF patients than the controls. The AC profile of the groups was listed in Table 2.

DISCUSSION

In this study, we evaluated FC and AC levels by using tandem mass spectrometry in FMF patients. To our knowledge, this is the first report regarding the carnitine status of FMF patients.

Cellular energy metabolism is largely based on mitochondrial β-oxidation of fatty acids, especially after prolonged fasting or exercise when carbohydrate stores are depleted. Carnitine plays an important role in esterification of long chain fatty acid and transport through to mitochondria for β-oxidation and ATP generation. Carnitine also stimulates pyruvate dehydrogenase complex activity, the Krebs cycle and increasing branched-chain amino acid oxidation in muscle.[27,28] In addition, neuroprotective and anti-oxidant effects of carnitine have been shown in various studies.[29-31]

Our hypothesis was that individuals with FMF would have lower FC levels than their healthy age and sex matched controls due to increased need of carnitine because of recurrent auto-inflammation; however, neither carnitine deficiency nor decreased FC levels compared to the healthy controls were detected in the study.

Conversely; FC, C16:OH and C18:2 levels were higher in children with FMF. In FMF patients; C2, C3, C4, C5:1, C6, C8, C10:1, C10:2, C3DC, C4DC, C5DC, C18:1-OH carnitine levels were lower than controls. Studies suggest that acyl to FC ratio >0.4 might be an evidence for carnitine deficiency. On the other hand, total AC levels and acyl to FC levels were also lower in FMF patients than control group. In our study, neither FMF

Table 1. Demographic and clinic properties of familial Mediterranean fever patients

Variables	FMF patients			Controls		
	n	%	Mean±SD	n	%	Mean±SD
Gender						
Male	106			27		
Female	99			23		
Age (months)			131.3±52.1			125.7±49.6
Mean age of disease onset (months)			59.9±36.6			
Mean age at diagnosis (months)			88.9±44.1			
Clinical findings						
Abdominal pain and fever	201/205	98				
Exertional leg pain	113/205	55.1				
Fatigue	86/205	41.9				

SD: Standard deviation.

Table 2. Free carnitine and acyl-carnitine levels of Turkish familial Mediterranean fever patients and controls

	FMF patients	Controls	
	Mean±SD	Mean±SD	*p*
Free carnitine	46.9893±12.4374	32.6929±7.4647	0.000
Short-chain acyl-carnitines			
C2	4.7080±1.6319	15.3441±4.6601	0.000
C3	0.9259±0.4164	1.8235±0.7870	0.000
C4	0.1523±0.0604	0.2139±0.7600	0.000
C5	0.1152±0.0437	0.1039±0.0298	0.087
C5:1	0.0160±0.0075	0.0196±0.0093	0.006
Medium-chain acyl-carnitines			
C6	0.0446±0.0211	0.0624±0.0216	0.000
C8	0.0730±0.0410	0.0884±0.0431	0.021
C10	0.1160±0.0758	0.1363±0.0700	0.090
C10:1	0.0969±0.0519	0.1296±0.0574	0.000
C10:2	0.0169±0.0103	0.0216±0.0085	0.004
Long-chain acyl-carnitines			
C14	0.1092±0.0817	0.0867±0.0233	0.054
C14:1	0.0701±0.0974	0.0645±0.0277	0.685
C14:2	0.0327±0.0378	0.0327±0.0173	0.986
C16	0.8250±0.2578	0.7963±0.2178	0.472
C16:1	0.0393±0.0147	0.0449±0.0160	0.022
C18	0.5481±0.1791	0.4943±0.1466	0.052
C18:1	0.7519±0.2116	0.7692±0.2131	0.610
C18:2	0.3213±0.1172	0.3324±0.0907	0.538
Acyl-carnitine esters derived from dicarboxylic acids			
C3DC	0.0322±0.0149	0.0398±0.0217	0.005
C4DC	0.2681±0.0845	0.4212±0.1516	0.000
C5DC	0.0287±0.0143	0.0427±0.0186	0.000
Acyl-carnitine esters derived from hydroxylated acids			
C4-OH	0.0351±0.0129	0.0771±0.0501	0.000
C5-OH	0.1977±0.0836	0.2184±0.0807	0.119
C16-OH	0.0210±0.0082	0.0171±0.0067	0.002
C18:1-OH	0.0120±0.0050	0.0149±0.0061	0.001
C18:2-OH	0.0129±0.0074	0.0102±0.0064	0.021
Total acyl-carnitine	4.8622±1.1625	6.0618±1.2849	0.000

SD: Standard deviation.

patients nor controls had high AC/FC ratio. It is interesting that FMF patients had lower total AC levels, as opposed to high FC levels. These results may be an evidence of a possible underlying impairment of peripheral carnitine utilization and mitochondrial energy metabolism in some individuals with FMF.

All of the patients in this study were under treatment of colchicine which is a well-known anti-mitotic drug that decreases leucocyte phagocytosis and motility in inflammatory responses.[32] None of our FMF patients were resistant to colchicine treatment although previous studies suggested that 10% to 15% of FMF patients have resistance to colchicine treatment.[33,34] Well-controlled disease with colchicine treatment might have resulted in suppression of the auto-inflammation, thus the need of carnitine. Also, insidious skeletal muscle damage might have resulted in elevated FC levels in FMF patients.[35]

In conclusion, usage of carnitine in all patients with FMF is not recommended since we were unable to demonstrate SCD in FMF patients in the present study. Further studies screening carnitine status in acute FMF attacks are needed to explore the carnitine profile in FMF patients and highlight the efficacy and necessity of L-carnitine therapies.

Declaration of conflicting interests

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