



Screening Inherited Metabolic Disorders in Patients with Familial Mediterranean Fever

Ailevi Akdeniz Ateşi Ayırıcı Tanısında Doğumsal Metabolik Hastalıkların Değerlendirilmesi

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ABSTRACT

Aim: Familial Mediterranean fever (FMF) is an autosomal recessive auto-inflammatory disease, presenting with recurrent episodes of fever and polyserositis. Mitochondrial fatty acid oxidation disorders and porphyrias can present with periodic abdominal and muscle pain. The aim of this study was to determine the inherited metabolic disorders in differential diagnoses of Turkish pediatric FMF patients.

Materials and Methods: Hundred and seventy-four FMF patients were enrolled in the study. A fasting dry spot blood sample was taken for acyl-carnitine analysis by tandem mass spectrometry from all patients. Fresh, light-protected spot urine test was performed for porphobilinogen screening. Second-tier test with urine organic acid analysis and urine porphyrin metabolites were performed if pathologic findings were detected in acyl-carnitine profile or in porphobilinogen screening, for confirmation. Fifty healthy controls were included.

Results: Of the 174 patients diagnosed with FMF, none of them was diagnosed with porphyria; only one patient with multiple acyl-CoA dehydrogenase deficiency was detected during the study.

Conclusion: Our data revealed that screening for porphobilinogen for pediatric FMF patients is unnecessary, but an investigation of tandem mass spectrometry based acyl-carnitine analysis can be helpful in the differential or additional diagnosis of FMF in developing countries where there is no nationwide expanded newborn screening programme.

Keywords: Familial Mediterranean fever, inherited metabolic disorders, fatty acid oxidation disorders, porphyrias

ÖZ

Amaç: Ailevi Akdeniz ateşi (AAA) tekrarlayan serozit ve ateşle karakterize bir oto-inflamasyon sendromudur. Hastalık klinik bulguları ile genelde kolaylıkla tanınabilmekle birlikte özellikle çocuk yaş grubunda tanı koymakta zorluklar yaşanabilmektedir. Yağ asidi oksidasyon defektleri ve porfiriyalar tekrarlayan kas ve karın ağrısına neden olabilmektedir. Türkiye’de yüksek akraba evliliği nedeniyle hem doğumsal metabolik hastalıklar hem de AAA sık görülmektedir. Çalışmamızda amacımız Türkiye’deki AAA hastalarının ayrıca tanısına girebilecek olan doğumsal metabolik hastalıkların saptanmasıdır

Gereç ve Yöntemler: AAA tanısıyla takip edilen 174 hasta çalışmaya alındı. Tüm hastalardan tandem M/S yöntemiyle asil-karnitin düzeylerinin ölçülmesi için parmaktan kuru kan örneği alındı. Tüm hastalardan taze idrar örneği ışıktan korunarak alındı ve idrarda porfobilinojen tayini yapıldı. İlk testlerinde patolojik bulgu saptanan hastalardan ileri araştırma amaçlı idrar organik asit analizleri veya idrar porfirin metabolitleri çalışıldı. Çalışmamıza kontrol grubu olarak 50 sağlıklı çocuk alındı.

Bulgular: Çalışmaya alınmış 174 hastanın hiçbirisinde porfiri saptanmadı ancak multipl acil-CoA dehidrogenaz eksikliği olan bir hasta saptandı.

Sonuç: Çalışmamız sonucunda çocuk AAA hastalarında rutin porfiri araştırmasına gerek olmadığı ancak ülkemiz gibi genişletilmiş yenidoğan taramasının yapılmadığı ve akraba evliliği oranı yüksek olan ülkelerde tandem kütle spektrometrisi ile asil karnitin analizinin AAA hastalarında ayırıcı tanıda faydalı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: Ailevi Akdeniz ateşi, doğumsal metabolik hastalıklar, yağ asidi oksidasyon defekti, porfiri

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Introduction

Familial Mediterranean fever (FMF) (OMIM: 249100) is an autosomal recessive disease characterized by recurring self-limited fever, abdominal pain and chest pain caused by serositis. Usually patients have additional joint and muscle pains. FMF is the most common hereditary periodic auto-inflammation syndrome (1).

FMF mainly affects people of Middle-Eastern origin, including Turkish, Jewish, Armenian and Arab populations. In these ethnic groups, the prevalence of FMF is 1/200-1000 with a high carrier rate of 1/3-5 (2-4).

The Mediterranean fever (MEFV) gene is located on chromosome 16, encodes a protein called 'pyrin'/ 'marennostin'. Pyrin plays a crucial role in the regulation of both inflammation and apoptosis (5,6). Genetic testing for the MEFV gene has only 70-80% positive predictive value, thus the diagnosis of FMF is based on clinical presentation, family history and ethnic origin (7,8).

The diagnosis of FMF may be challenging in pediatric population. Differential diagnosis consists of other auto-inflammatory syndromes (TNF-alpha associated periodic fever syndrome (TRAPS), Hyper IgD syndrome (HIDS), cyclic neutropenia, etc), surgical emergencies (pancreatitis, cholecystitis, etc), Lyme arthritis, inflammatory bowel disease, hereditary angioedema and porphyria (9-13).

Incidence of both FMF and IMD has increased in Turkish patients partially due to high consanguinity rates. FMF and IMD, especially fatty acid oxidation defects like multiple acyl co-A dehydrogenase deficiency, can present with similar symptoms that could result in diagnosing challenges. IMD must be a part of differential diagnosis in countries where expanded newborn screening (ENS) via tandem mass spectrometry is not a part of the nationwide newborn screening programme (14,15).

Detection of MADD deficiency in a male patient, diagnosed with FMF and referred to the pediatric metabolic disease unit, after both his sisters died due to hypoglycemia and hepatocellular insufficiency, led us to investigate IMD in these patients. Both sisters were also followed up after being diagnosed with FMF.

The aim of the present study is to determine the IMDs in differential diagnosis of Turkish pediatric FMF patients.

Materials and Methods

Study Design and Population

174 patients who were diagnosed with FMF were enrolled in the present study. All diagnoses were confirmed by a child rheumatologist according to Yalçinkaya et al. criteria (16). Patients' clinical data, including detailed family history (consanguinity, additional affected individuals, ethnicity, etc.), age, gender, diagnosed age, additional clinical symptoms (fatigue, muscle pains-cramps, psychological symptoms, and dermatitis), medications, drug responses and laboratory data including complete blood count, glucose, transaminases,

urea, creatinine, CPK, lipid profile, C-reactive protein (CRP), erythrocyte sedimentation ratio (ESR) and FMF mutation analyses of FMF were all documented. All patients underwent a careful physical examination. Age and sex matched 50 healthy subjects served as the control group. Patients with an additional diagnosis (inflammatory bowel disease, etc.) and those under carnitine treatment were excluded from the study.

Fasting dried spot blood samples consisted of capillary blood collected on Whatmann S&S 903 filter paper were taken for free carnitine and acyl-carnitine analysis from children in both groups. The sampling was done by finger prick. The samples were dried in room temperature.

Relevant AC butyryl esters were extracted and derivatized from 1/3 inch dried blood samples and were analyzed using a protocol described by Schulze et al. previously (17). AC butyryl esters were detected due to mass/ion ratios via quadripole electrospray tandem mass spectrometry (ESI-MS/MS). Agilent 1200 series auto sampler and Water Micromass Quattro LC Likrom™ tandem mass spectrometry were used for the analyses. AC profile was screened by positive ion mod m/z 85 parents (?) screening function. The quantitative values of signal intensities were calculated using Masslynx and Neolynxsoftwares (Ver 4.1). Both signal intensities and calculated concentrations were exported to spreadsheet software where flagging abnormal concentrations and further evaluations were done (17,18).

Fresh, light-protected spot urine test, described by Watson-Schwartz, was performed for porphobilinogen screening (Watson-Schwartz test; colorless pyrrole porphobilinogen forms a red-violent pigment after reaction with p-dimethylaminobenzaldehyde).

Second-tier test with urine organic acid analysis and urine porphyrin metabolites were performed for confirmation if pathologic findings were detected in acyl-carnitine profile or in porphobilinogen screening.

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

Results

174 patients diagnosed with FMF were enrolled in the study. Patients' clinical and demographic characteristics are shown in Table I.

Spot urine test for porphobilinogen was found positive in 4/174 of FMF patients, while none of these patients had abnormal porphyrin metabolites in their 24 hour-urine samples. None of the children in the control group turned out positive in spot porphobilinogen urine test.

Acyl-carnitine analysis in spot dried blood test using tandem mass spectrometry was performed to all patients. One patient with multiple acyl-CoA dehydrogenase deficiency (MADD) was detected during the study. None of the children in the control group showed pathologic acylcarnitine profile.

Case Report

The patient is a 13-year-old boy with complaints of recurrent abdominal pain, muscle pain and vomiting. His parents were consanguineous (first degree cousins) and had several relatives diagnosed with FMF. He was also diagnosed with FMF and colchicine treatment was started. He didn't use colchicine regularly because his complaints sustained under this treatment. He had several attacks presenting with vomiting, abdominal and muscle pain responding to IV dextrose infusion. His physical examination was normal; he had no mental retardation, no hepatomegaly and no abnormal neurological findings.

His first line investigations revealed a positive urine porphobilinogen test, but urine porphyrin profile was normal. His acyl-carnitine profile revealed high levels of C5-, C8-, C10-, C12-, C5DC-, C14:2-, C14:1- and C14 acyl-carnitine levels (0.620 μ M/L, 1.5 μ M/L, 3.5 μ M/L, 2.55 μ M/L, 1.03 μ M/L, 0.210 μ M/L, 0.960 μ M/L, 1.47 μ M/L respectively). 3-OH-butyric acid, 3-OH-sebacic acid, adipic acid, acetoacetic acid, suberic acid, glutaric acid and 3-OH-isovaleric acid excretion was detected in organic acid analyses. MADD was considered in diagnosis. Riboflavin and coenzyme Q10 therapies along with a frequent feeding to avoid fasting were initiated. Excellent response to treatment was achieved, acyl-carnitine levels were normalized and his clinical complaints were resolved. A homozygous mutation on ETFDH gene (c.1330T>C(p.L377P) was detected, confirming MADD diagnosis.

Discussion

In the present study we screened FMF patients for differential diagnoses of FAODs and porphyrias.

Porphyrias are a group of inherited metabolic disorders of haem biosynthesis (14,19). Clinically they can be differentiated into acute and non-acute porphyrias. Specific patterns of acute accumulation of the haem precursors are associated with clinical features; abdominal, psychiatric and neurological symptoms (20,21). The clinical manifestations

of acute porphyrias are extremely rare before puberty and generally give the first clues in early 30's, maybe with pregnancy (21). Most patients have one or a few attacks and then recover fully for the rest of their lives and less than 10% develop recurrent attacks. Physical examination shows no abnormalities and laboratory investigations are usually remarkable. Porphyrias are often misdiagnosed because of their multiform clinical manifestations, able to mimic other diseases, including FMF. Clinical features alone are not sufficient to confirm the diagnosis; specific laboratory investigations are mandatory for the accurate diagnosis and management of the disease (22,23).

Erhlich's reagent-based tests are simple and rapid assays to test the presence of urinary porphobilinogen. Examination of urine for excess porphobilinogen is the first line test for the suspected acute porphyria attack. During remission, urine, blood or fecal porphyrin concentrations are generally normal in acute porphyrias, making it more difficult to diagnose accurately when the patient is between acute crises (21,24-26). Although screening for urine porphobilinogen revealed 4 patients with a positive screening test, investigation of urine for porphyrin metabolites rendered normal results and no patient was diagnosed with porphyria. One of the limitations that could have influenced our study was that the urine samples were not taken during an acute attack (acute abdomen or muscle weakness). Previous studies have revealed that porphyrin metabolites would usually be within normal limits between acute crises. Also the initial symptom age was not appropriate for the initial acute porphyria attack, and the numbers of male and female patients were equal in the study group while female dominance is expected for porphyria.

Fatty acid oxidation plays a major role in energy production especially in catabolic stages. FAODs are a group of disorders of energy metabolism with a high prevalence in Europe. The toxic effects of accumulated acyl-carnitines and acyl-CoA species and energy deficiency due to impaired fatty acid and ketone body formation are responsible for the clinical manifestations (27). The clinical findings and symptoms are very heterogeneous. The classical presentation in the newborn is acute hypoketotic hypoglycemia, encephalopathy and liver dysfunction. Severe cardiomyopathy, arrhythmias or conduction defects are some of the other leading symptoms. Older children and adults may present with exercise-illness induced myopathy, muscle weakness, with pain or with acute rhabdomyolysis or cyclic vomiting (28). Each FAODs have a specific acyl-carnitine profile but a genotypic correlation is warranted for accurate diagnosis.

In the present study, we investigated FAODs in Turkish FMF patients and we found one patient with MADD. MADD results from defects of electron transfer flavoprotein (ETF) or ETF ubiquinone oxidoreductase (ETFQO). ETY and ETFQO carry electrons to the respiratory chain from multiple dehydrogenases linked to flavin adenine dinucleotide (FAD). The clinical picture is diverse ranging from a fatal disease in the newborn with hypoglycemia, hyperammonemia and

Variables	FMF patients	Control group
Sex (Male/Female)	92/82	27/23
Age (Months)	131.28±52.05	132.11±52.3
Initial complain starting age (Months)	59.89±36.64	
Diagnosing age (Months)	88.92±44.12	
Clinical characteristics		
Abdominal pain and fever	168/174 (96.5%)	
Musculoskeletal findings	98/174 (56.3%)	
Fatigue	80/174 (45.9%)	
Skin lesions	12/174 (8%)	

FMF: Familial Mediterranean fever

acidosis accompanied by hepatomegaly to muscle weakness, recurrent bouts of vomiting in later childhood or adulthood. The riboflavin responsive phenotype has a good prognosis (29). Our patient is a 13-year-old boy with complaints of cyclic attacks of abdominal pain, muscle pain and vomiting. The diagnosis of MADD was confirmed by mutation analysis, and treatment with riboflavin and a high calorie diet with frequent feeding to avoid fasting resulted in an excellent response. Overall positive family history, positive mutation on MEFV gene and symptoms resembling acute FMF attack can easily lead FAODs to be misdiagnosed.

Countries like Turkey with high consanguinity rates have an increased incidence risk of inherited disorders like FMF or IMDs. In the present study parental consanguinity was found in 29.9% of the patients while the reported consanguinity rate in Turkey was 23.3% (30). As the consanguinity ratio in Turkish FMF patients was higher than the normal population, our study group consisted of patients with a high risk for inborn errors of metabolism that are usually autosomal recessively inherited. FAODs can be easily diagnosed in the newborn in countries where ENS with tandem mass spectrometry is a part of the nationwide screening. Unfortunately ENS with MS/MS is not a part of the nationwide newborn screening programme and the prevalence of FAODs is still unknown.

The present study is the first to investigate IMDs in the differential diagnosis of FMF. Our data revealed that screening for porphobilinogen for pediatric FMF patients is unnecessary, but an investigation of tandem mass based acyl-carnitine analysis can be helpful for the differential or additional diagnosis of FMF in developing countries where there is no nationwide ENS.

Authorship Contributions

Ethics Committee Approval: The study was reviewed and approved by the Ethics Committee of Cerrahpaşa Medical Faculty, Istanbul University, Informed Consent: Signed informed consent was obtained from all patients who participated in the study, Concept: Ertuğrul Kıyıkım, Mehmet Şerif Cansever, Kenan Barut, Design: Ertuğrul Kıyıkım, Tanyel Zubarioğlu, Ayşe Çiğdem Aktuğlu Zeybek, Özgür Kasapçopur, Data Collection or Processing: Ertuğrul Kıyıkım, Mehmet Şerif Cansever, Ayşe Çiğdem Aktuğlu Zeybek, Kenan Barut, Analysis or Interpretation: Ertuğrul Kıyıkım, Mehmet Şerif Cansever, Ayşe Çiğdem Aktuğlu Zeybek, Literature Search: Ertuğrul Kıyıkım, Ayşe Çiğdem Aktuğlu Zeybek, Tanyel Zubarioğlu, Writing: Ertuğrul Kıyıkım, Tanyel Zubarioğlu, Kenan Barut, Özgür Kasapçopur, Peer-review: Externally peer-reviewed, 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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