Elevated immunoglobulin D levels in children with PFAPA syndrome

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Submitted: 2010-05-02 Accepted: 2010-10-05 Published online: 2011-01-10

Key words: **periodic fever; PFAPA syndrome; immunoglobulin D; HIDS; mevalonate kinase**

Neuroendocrinol Lett 2010; **31**(6):743–746 **PMID:** 21196927 NEL310610A09

Abstract

BACKGROUND: The periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome appears to be more common than generally appreciated and should be differentiated from hereditary periodic fever syndromes, particularly from mevalonate kinase deficiency (MKD).

PATIENTS AND METHODS: 14 unrelated patients (7 males, 7 females) met clinical criteria for both the PFAPA syndrome and MKD. Immunoglobulin D (IgD) levels, mevalonic aciduria and mevalonate kinase (MVK) genotype was determined in all patients.

RESULTS: Children experienced their first febrile episode at the age of 24.5±5.9 months (mean±SD), the clinical diagnosis of PFAPA syndrome was established with delay at 42.7±11.7 months. The duration of febrile episodes was 3.4±0.2 days, the asymptomatic interval between them lasted 5.4±0.9 weeks. Accompanying symptoms included pharyngitis (92.8%), cervical lymphadenitis (85.7%), aphthous stomatitis (21.4%), arthralgia (14.3%) and skin erythema (35.7%). Neither mevalonic aciduria nor MVK gene mutations were found in any of the subjects, however, unexpectedly, increased plasma IgD (322.2±29.2 U/l) levels were detected in all patients.

CONCLUSION: Raised IgD levels may represent a non-specific epiphenomenon, which frequently accompanies PFAPA syndrome as well as MKD. Because of the overlapping clinical and laboratory features, genetic testing of the MVK gene is indicated to differentiate these two conditions, if clinical criteria for both are fulfilled.

ORIGINAL ARTICLE

INTRODUCTION

Fever is a common presenting complaint in children. It consistently causes alarm in parents and accounts for nearly one-third of pediatric outpatient visits (Finkelstein *et al.* 2000). Recurrent fever constitutes a diagnostic challenge for clinicians, due mainly to the intermittent nature of the fever (Scholl 2000; Kovács *et al.* 2003).

The periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome is a clinical entity of unknown etiology first described by Marshall *et al.* (1987). Its true frequency is not known, but it appears to be more common than generally appreciated. This syndrome is characterized by recurrent febrile episodes with head and neck symptoms occurring at regular 1 to 3 month intervals and lasting 3–5 days. Onset of symptoms is usually before the age of 5 years, with increasing age attacks occur less frequently and usually subside completely by the age of 10 years, although affected adult patients have been also described (Padeh *et al.* 2008). Patients are completely well between episodes, experience normal growth and development and never suffer from long-term sequelae (Thomas 1999; Feder & Salazar 2009).

It is now widely recognized that to make the diagnosis of PFAPA syndrome, it is necessary to rule out a group of monogenic periodic fevers (so called auto-inflammatory fever syndromes) that are caused by mutations of the genes involved in the regulation of inflammatory response. These include the familial Mediterranean fever (FMF), the autosomal dominant Hibernian fever (also known as TNF-receptor related periodic syndrome, TRAPS), and the mevalonate kinese deficiency (MKD) caused by mutations in the MVK gene coding mevalonate kinase, an enzyme important for cholesterol synthesis. Elevated imunoglobuline D levels were considered to be a discriminating feature for MKD, which was also baptized as hyper-immunoglobulin D syndrome (HIDS) (van der Meer 1984; Drenth *et al.* 1994; Gattorno *et al.* 2009).

The diagnostic workup is hindered by the fact, that the current PFAPA syndrome diagnostic criteria have very low specificity. A relevant number of patients with monogenic periodic fevers also meet the diagnostic criteria for PFAPA syndrome. On the other hand, PFAPA syndrome may be associated with alterations that are thought to be emblematic for other types of periodic fever. In this paper, we report a cohort of PFAPA patients with significantly increased immunoglobulin D (IgD) levels with no genetic alterations of the MVK gene. We discuss the relevance of IgD in the differential diagnosis of periodic fever syndromes and its (non) specificity for mevalonate kinase deficiency.

PATIENTS AND METHODS

During the years of 2003 to 2007, 214 infants and children were examined for recurrent episodes of fever. Of these, 15 unrelated patients (7 males, 7 females) met the clinical and laboratory criteria for both the PFAPA syndrome and MKD with at least six episodes of periodic fever and were included in this study. Excluded were all patients who had an affected relative, serosal involvement, eye lesions, skin lesions or neutropenia. Excluded was also a patient with PFAPA-like syndrome, who developed Crohn's disease in the course of follow-up. As a part of the diagnostic workup, ESR and CRP values, white blood cell and neutrophil counts as well as immunoglobulin A and G levels were measured during febrile episodes. Culture swabs and hemocultures had to be negative for pathogenic bacteria and fungi.

Immunoglobulin D plasma levels were measured at least two occasions at least one month apart by standard radial immunodiffusion (LC Partingen kit, Dade Behring Marburg, Germany) and an IgD level of more than 100U/ml was considered to be increased. Mevalonic acid in urine was detected by standard chromatography, while organic acids in urine were analyzed by gas chromatography.

Molecular analysis of 10 coding exons (from 2 to 11) of the MVK gene was performed in DNA extracted form peripheral blood lymphocytes by standard methods. DHPLC analyses were conducted in 3500 WAVE® system (Transgenomic, Inc.). Conditions of individual methods were set as proposed by supplier's Navigator® software for each amplicon, with run temperatures corresponding to 80–90% helical fraction in analyzed region of amplicons (exons). To detect homozygous mutations, for each patient and each exon under study, amplification products were mixed with an equal amount of those obtained form a control individual known to carry no variant alleles at the MVK locus, denatured, reannealed and analyzed for heteroduplexes. Finally, the MVK gene of all patients was analyzed by direct sequencing.

Descriptive statistics are reported as mean ± SD. The Ethical Committee formally approved this study, and parents of all patients gave informed consent to the participation in it.

RESULTS

During a five years period, 14 unrelated patients (7 males, 7 females) met the clinical and laboratory criteria for the PFAPA syndrome, namely: 1) regularly recurring fevers with an early age of onset (<5 years of age), 2) presence of at least 1 of the constitutional symptoms with at least one of the three major associated symptoms (aphthous stomatitis, cervical lymphadenitis and pharyngitis), 3) absence of upper respiratory tract infection and cyclic neutropenia, 4) completely asymptomatic interval between episodes with normal growth

and development (Marshall *et al.* 1987 ; Feder & Salazar 2009). Episodes of fever were considered recurrent fever syndrome-related if there were sufficiently documented signs and symptoms during a febrile episode without clinical and laboratory findings confirming infection. None of the patrents' relatives had unexplained febrile episodes.

Children experienced their first febrile episode related to PFAPA syndrome at the age of 24.5 ± 5.9 months (mean ± SD), the clinical diagnosis of PFAPA syndrome, however, was established with a delay of almost two years at the age of 42.7 ± 11.7 months. The mean duration of febrile episodes was 3.4 ± 0.2 days, the interval between individual episodes lasted 5.4 ± 0.9 weeks, during which time the subjects were completely free of any complaints. Of the relevant clinical symptoms, fever episodes were associated with pharyngitis (13/14, 92.8%), cervical lymphadenitis (12/14, 85.7%), aphthous stomatitis (3/14, 21.4%), arthralgia (2/14, 14,3%) and skin exanthema (5/14, 35.7%). Abdominal pain and headache were not observed.

All patients had elevated erythrocyte sedimentation rate (47.27 ± 7.76), increased CRP (82.42 ± 18.95) and leukocytosis (18 360 ± 6 650). Hemoglobin level and thrombocyte counts were normal. All patients had repeatedly negative throat, urine and hemocultures. Test for anti-streptolysin antibody (ASLO) was positive in 2 subjects, however febrile episodes persisted after penicillin treatment and decrease of ASLO to the normal range. Liver and kidney function tests were within the reference range.

IgA concentration in serum was 1.08 ± 0.30. Increased IgD levels were present in all patients (322.2 ± 29.2 U/ml, range: 177–513 U/ml, median: 316 U/ml). Mevalonic aciduria could not be detected by standard chromatography in any of the patients during attacks. Mutational analysis of the MVK gene did not reveal any causative mutation. However, four intronic polymorphisms (IVS1 c.1-118 C→G, IVS6 c.632-18 A→G, IVS8 c.769-38 C→T, IVS9 c.885+24 G→A), and 3 known exonic polymorphisms (Ser52Asn, Ser135Ser, Asp170Asp) have been detected, reaching frequencies from 0.23 to 0.5.

DISCUSSION

Following our earlier report on a gypsy kindred with periodic fever and high IgD levels, in whom the diagnosis of mevalonate kinase deficiency was confirmed by detecting a mutation of the MVK gene (Kovács *et al.* 2003), evaluation of serum IgD levels became a routine part of our diagnostic workup of periodic fever cases. In 14 successive patients who fulfilled the clinical criteria of PFAPA syndrome, we constantly found elevated levels of IgD (322.2 ± 29.2 U/ml). This finding was rather unexpected, as high levels of IgD are by definition regarded as a key laboratory abnormality in mevalonate kinase deficiency (baptized also and more widely known as hyper-IgD syndrome, HIDS). However, genetic testing in none of the subjects revealed any causative MVK gene mutation. Four intronic and three known exonic polymorphisms were detected, these were, however, unrelated to the clinical syndrome.

Immunoglobulin D (IgD) being discovered in 1965, is a unique immunoglobulin with a concentration in serum far below those of IgG, IgA, and IgM but much higher than that of IgE. Despite studies extending for more than four decades, a specific role for serum IgD has not been defined, while for IgD bound to the membrane of many B lymphocytes, several functions have been proposed (Simon *et al.* 2001). The amount of serum IgD is measured routinely in some clinical laboratories, almost always together with the concentrations of IgG, IgA, and IgM, although the clinical relevance of increased or decreased serum IgD values (in comparison to the reference interval or so-called normal range) has not been proven. The serendipitous discovery of an increased serum IgD concentration during attacks of some patients with periodic episodes of fever (Simon *et al.* 2001) led to a renewed search for a specific role of serum IgD. It is still not clear how IgD is involved in the pathogenesis of MKD. Incubation of neutrophils with IgD in vitro leads to increased secretion of interleukin 1 and tumor necrosis factor (Drenth *et al.* 1996). However, IgD levels do not correlate with disease severity, mevalonate kinase enzyme activity, or genotype and the clinical manifestation of MKD may antedate serum IgD elevation (Simon *et al.* 2001; D'Osualdo *et al.* 2005). Elevated IgD has been recorded also in other clinical conditions including diabetes, pregnancy, AIDS and Hodgkin disease (Vladutiu 2000). More importantly, there are reports on elevated IgD levels in individual patients with other recurrent fever syndromes, including PFAPA syndrome, in those cases, however, no attempt was made to rule out MKD by genetic testing (Padeh *et al.* 1999; Medlej-Hashim *et al.* 2001).

By filling this gap, our data provide new evidence, that raised IgD may be a common attribute of PFAPA syndrome, as well. Thus, it is suggested, that, IgD elevation should be viewed as an epiphenomenon reflecting inflammatory activation, rather than a specific marker for any certain disease condition. There is, therefore, sufficient reason to abandon the historical name of "hyperIgD syndrome" and replace it by a more pathogenetic term, such as mevalovate kinase deficiency.

Our recent results emphasize the difficulties to clinically differentiate the PFAPA syndrome from other periodic fever syndromes, specifically from mevalonate kinese deficiency (Ataş *et al.* 2003; Gattorno M *et al.* 2009). Only two clinical signs could possibly help differentiate between PFAPA and MKD – the periodicity of febrile episodes and the presence of aphthous stomatitis. Although intervals between episodes tend to be variable in MKD, clockwork periodicity characteristic for PFAPA syndrome does not necessarily exclude this entity (Thomas 1999; Padeh *et al.* 2008; Feder & Sala-

zar 2009). Similarly, absence of aphthous ulcers does not exclude PFAPA syndrome, as 29 to 67% of subjects in large PFAPA syndrome series also lacked this type of oral lesions (Thomas 1999; Simon *et al.* 2001; Garavello *et al.* 2009). Even the dramatic resolution of fever after a single dose of corticosteroid (Feder & Salazar 2009), which was observed also in three of our patients, should not be regarded as unique to PFAPA syndrome. Fever attacks in MKD are associated with activation of a common inflammatory pathway and increased production of cytokines (Stojanov *et al.* 2006; Førsvoll & Oymar 2007), therefore prednisone might exert an unspecific anti-inflammatory effect in this entity as well.

It was suggested, that periodic fever cases with elevated IgD levels and MKD-like phenotype, but without mutation of MVK gene, may represent a variant-type, which may occur in as much as 24% of subjects with alleged MKD (Simon *et al.* 2001; D'Osualdo *et al.* 2005). However, because of the overlapping clinical sings, this assumption based entirely on elevated IgD levels may not be true. From our recent results it appears that IgD elevation is not specific for MKD and seems to be a common feature of PFAPA syndrome, as well. Hence, definite diagnosis of MKD should be only made on the basis of positive genetic testing. This is of overall importance, as exclusion of MKD and the correct diagnosis of PFAPA syndrome may enable patients to undergo treatment, which are thought to be effective in the reduction of the frequency of febrile episodes (Feder 1992; Garavello *et al.* 2009; Feder & Salazar 2009).

The results of our study are limited by the relatively low number of patients and the fact that genetic testing for other periodic fever syndromes could not be carried out. Nevertheless, all patients that might be suffering from familial Mediterranean fever and systemic-onset juvenile chronic arthritis (serosal involvement) or Behçet's disease (eye lesions, recurrent genital ulcers) were excluded on clinical grounds. Also, none of our patients had an affected relative as would be expected in MKD, FMF, TRAPS or other known hereditary periodic fever syndromes. Even in the improbable (but due to the lack of genetic testing still possible) case, that any of the patients considered by us to have PFAPA syndrome is a carrier of a mutation for any autoinflammatory syndromes other than MKD, the main message of this work remains unaffected: elevated IgD without mevalonic aciduria and MVK gene mutations is a general finding in PFAPA syndrome and possibly other recurrent fever syndromes other than MKD and thus due to its low specificity, is not an appropriate marker for MKD.

ACKNOWLEDGEMENTS

This work was supported by grant 1/4314/07 by the Slovak Academic Grant Agency, VEGA.

Conflict of interest declaration

The authors declare no conflict of interest.

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