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NEPHROLOGY FORUM

Renal amyloidosis in familial Mediterranean fever

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CASE PRESENTATION

A 14-year-old boy was referred to the Department of Pediatric Nephrology at Hacettepe University Faculty of Medicine because of nephrotic syndrome unresponsive to a 1-month course of oral prednisone. He described attacks of fever and joint complaints since the age of 7 that occasionally were accompanied by chest or abdominal pain. The attacks usually occurred over 1 to 3 days. These joint complaints were either in the form of frank arthritis in the ankle or knee joints, or arthralgia in the same joints. He had been diagnosed as having acute rheumatic fever by the local physician. However, his complaints recurred despite penicillin prophylaxis.

One year ago he was diagnosed as having Henoch-Schönlein purpura because of severe vasculitic rash in the lower exteremities and abdominal pain. He was admitted to the district hospital at that time. His initial urinalysis was said to be normal; however, there was no follow-up after he was discharged. He had no family history of renal disease. The parents were second-degree cousins.

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Key words: Henoch-Schönlein purpura, polyarteritis nodosa, vasculitis, pyrin, TRAPS.

On admission he had a normal blood pressure, marked edema, and a pale, waxy appearance. He had a normal complete blood count and normal serum creatinine, and a serum albumin of 2.1 g/dL. A 24-hour urine collection revealed 8.3 g of protein. His creatinine clearance was 76 mL/min. Serum cholesterol and triglyceride levels were mildly elevated. The acute-phase reactants were elevated, with an erythrocyte sedimentation rate (ESR) of 98 mm/hour, C-reactive protein of 12 mg/dL (normal < 0.5), and serum fibrinogen of 587 mg/dL. Serum complement levels were normal. The antinuclear antibody (ANA), anti-ds-DNA, and antineutrophil cytoplasmic antibody (ANCA) all were negative.

A renal biopsy was performed. Light microscopy showed eosinophlic, amorphous deposits in the mesangial area of the glomeruli. Also visible were the characteristic apple-green birefringence with polarized light and orange-red staining with Congo red, suggesting renal amyloidosis.

Evaluation for mutations in the MEFV (Mediterranean fever) gene showed that the patient was homozygous for the M694V mutation. He was given colchicine, 2 mg/day.

Although his attacks resolved with treatment, he had persistent nephrotic syndrome, and his acute-phase reactants remained high. His renal function started deteriorating in a year. One year later, his serum creatinine was 3.8 mg/dL. He started complaining of diarrhea and had difficulty in taking colchicine at the suggested dose. He started dialysis 18 months after his diagnosis. He continued to have approximately 600 mL of urine output/daily with heavy proteinuria. His blood pressure always was normal and even low, which resulted in occasional problems with his arteriovenous fistula. His serum albumin also remained low, around 2.5 g/dL; his persistent and marked diarrhea exacerbated his malnutrition.

Two years after diagnosis, he presented with fatigue, dyspnea, and cough. He had severe pericardial and pleural effusion and acute pneumonia. He was given intensive antibiotic treatment, and discharged to continue hemodialysis in a local center. Three months later, he was reported to have died with intractable diarrhea.

DISCUSSION

Dr. Seza Ozen (*Professor, Department of Pediatrics, Hacettepe University, Ankara, Turkey*): This patient illustrates a classic example of a misdiagnosis that led to the complication of renal amyloidosis secondary to familial Mediterranean fever (FMF). Familial Mediterranean fever is characterized by recurrent inflammatory attacks [1–5]; its most severe complication is the development of secondary amyloidosis of the kidney. This kidney disease represents the only nephropathy that can be prevented by a very inexpensive drug. Familial Mediterranean fever is the commonest of the autoinflammatory diseases, or the so-called periodic fever syndromes, that have recently gained widespread interest [3, 6–8]. Some of these diseases, like FMF, are characterized by an autosomal-recessive inheritance, whereas others have a dominant transmission. When the FMF gene was identified in 1997, it was thought that the diagnosis would be made in the laboratory [1, 9]. However, subsequent study has confirmed that the diagnosis needs to be based on clinical criteria. Classically, FMF is characterized by self-limited attacks of fever and serositis presenting in the form of abdominal, joint, or chest pain along with an elevation of acute-phase reactants. Although a number of diagnostic criteria have been suggested [10], these criteria need validation in various ethnic and multiethnic groups [10]. At present, the diagnosis of FMF relies on careful follow-up, family history exclusion of other periodic fever syndromes and immune disorders and, finally, the patient's response to colchicine therapy.

The patient presented here had had attacks of fever and self-limited arthritis that occasionally were accompanied by abdominal pain. Episodes of arthritis/arthralgia are a frequent feature of FMF and have been associated with more severe disease in a number of reports [4, 11, 12]. The joints most frequently involved in FMF are the knees and ankles. These patients are sometimes misdiagnosed as having acute rheumatic fever, but their symptoms do not respond to penicillin prophylaxis and these people later present with amyloidosis. When questioned, today's patient also described serositis in the form of abdominal and chest pain. However, these complaints had been ignored by the physicians. The attacks were of a duration typical among periodic fever syndromes.

This boy presented with nephrotic syndrome without any nephritic features. Secondary amyloidosis of FMF can present in a variety of successive forms that reflect the severity of renal involvement [2, 13]: isolated proteinuria, nephrotic syndrome, and renal impairment with nephrotic features. The patient who presents with nephrotic syndrome usually is normotensive, and hematuria is very rare. Often, marked edema and a waxy appearance of the skin are present. These patients are often treated with steroids because of an initial erroneous diagnosis of minimal change disease [14]. The patient, of course, had no response to corticosteroids.

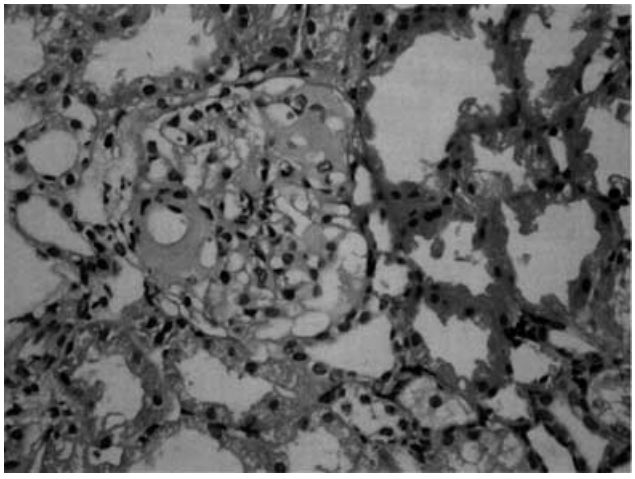

Fig. 1. Light microscopy of renal amyloidosis showing amorphous deposits.

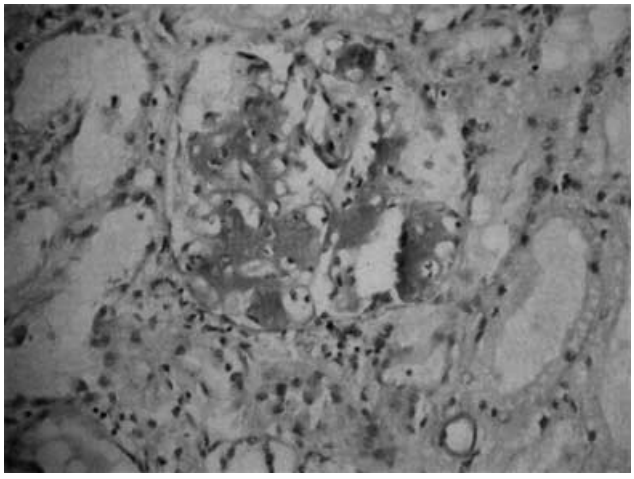

Fig. 2. Characteristic Congo red staining of amyloid deposits.

Diagnosis of amyloidosis is made through renal biopsy. The characteristic finding comprises eosinophilic amorphous deposits in the mesangial matrix of the glomeruli with hematoxylin-eosin staining (Fig. 1). Tissues stained with Congo red have orange-red staining (Fig. 2). Under polarized light, one sees a characteristic apple-green birefringence diagnostic for amyloid deposition. The nature of the amyloid fibrils can be confirmed by immunoperoxidase staining with specific monoclonal antibodies. In FMF the fibrils are of the AA type, secondary to ongoing inflammation. Bone marrow biopsy also is an alternative method for diagnosis. In patients with amyloidosis secondary to FMF, 80% of the marrow biopsies showed amyloidosis [13].

Although FMF is not the only periodic fever disease causing secondary AA amyloidosis, it is the only one responsive to colchicine. One can make a fairly conclusive

diagnosis of FMF if the patient has at least three attacks of fever plus abdominal and/or chest and/or joint pain; and either (*1*) a diagnosis of FMF or amyloidosis has been determined in a family member or is known to have been present in an ancestor, or (*2*) infection is excluded as a cause and the duration of the fever is 6 hours to 6 days. The typical duration is 12 hours to 3 days. Also, FMF can be diagnosed only in the absence of lymphadenopathy, aphthous lesions, and rash (except erysipelas-like erythema) and in the presence of elevated acute-phase reactants. In patients with these criteria, the diagnosis of FMF can be confirmed by a mutation analysis of the Mediterranean fever (MEFV) gene. However, most centers in areas where the disease is common rely on clinical grounds only. In this patient, subsequent mutation analysis of the MEFV gene for FMF confirmed that he had a severe mutation in both alleles, the M694V mutation.

Let's talk for a moment about the pathogenesis of FMF. The gene responsible for FMF was described only in 1997 [1, 9]. Although FMF is an ancient disease, the definition of the gene and its protein has led to new interest in this disease. The commonest of the group of monogenic autoinflammatory diseases, in which increased acute-phase reactants are a feature of the disease per se, FMF has recently emerged as an important topic [3, 6]. Understanding these diseases promises to provide crucial information about the pathway of inflammation in other diseases as well.

The MEFV gene consists of 10 exons. Almost 30 mutations of this gene have been associated with FMF so far [4, 15, 16]. Mutations in the hot spot of exon 10, the region between 680 and 694, seem to be associated with more severe disease, whereas patients with the E148Q mutation can be free of symptoms. Among the patients presenting to our department, M694V is the most frequent mutation, whereas the most frequent mutation among healthy Turkish carriers is E148Q [17].

Expressed mainly in neutrophils and monocytes, MEFV encodes a protein called pyrin (from the Latin word for fever) [1]. Interestingly, most of the autoinflammatory diseases that I will mention also have this pyrin domain in the affected protein [6]. Pyrin is an important element in the body's inflammatory response. Familial Mediterranean fever is a disorder of the innate immune system, that is, the system responsible for quick response to a variety of organisms [8]. A mutated pyrin is associated with uncontrolled inflammation through interleukin β (IL-β) and nuclear factor-kappaB (NF-κB) activation and leads to up-regulation of inflammatory cytokines and pathways. Pyrin interacts with apoptosis-associated speck-like protein (ASC), which contains a caspase recruitment domain that stimulates caspase-1 activation. Caspase-1 activation then leads to IL-1 processing and secretion and raises levels of IL-β [6, 8]. The interaction with ASC also causes NF-κB activation, the

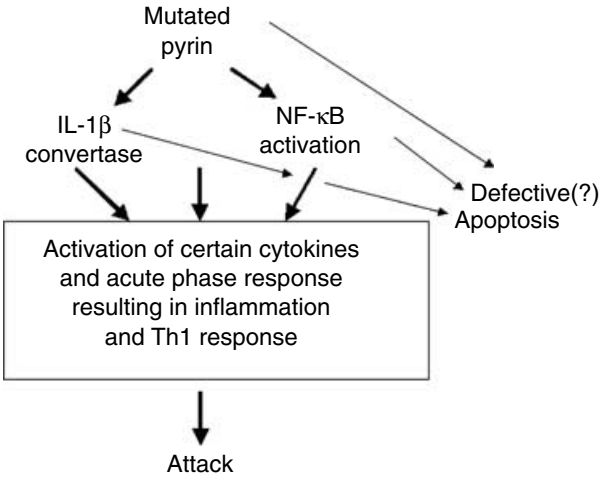

Fig. 3. Suggested pathogenesis of familial Mediterranean fever (FMF). Abbrevations are: IL-1β, interleukin-1β; NF-κB, nuclear factor-kappaB.

phosphorylation of which is associated with increased transcriptional activity of certain inflammatory cytokines (Fig. 3). Apoptosis is also induced through this pathway. However, we are still not sure whether FMF is a proapoptotic or antiapoptotic disease [8].

A mutated pyrin is probably associated with loss of the delicate control of the inflammatory pathways. The inflammatory state of FMF is associated with a Th1 predominance in the inflammatory reponse of the disease, as in Behçet's syndrome and rheumatoid arthritis. This Th1 polarization was suggested by studies from our lab, which showed increased staining of interferon γ (INF-γ) in the peripheral blood samples of patients with FMF [18].

The clinical disease, that is, the phenotype of FMF, is very frequent around the Mediterranean, as is the carrier rate in certain ethnic populations, namely, Arabs, Armenians, non-Ashkenazi Jews, and Turks. The frequency of affected individuals ranges between 1/250 and 1/1000 in these populations [19–22]. Definition of the mutations has led to interesting epidemiologic studies showing that the disease and its carrier frequency are common. In the affected populations, the carrier rate is extremely high: 1 in 3 to 1 in every 6 individuals carries a mutation in the MEFV gene [17, 20, 23]. In fact, the reasons for the frequent selection of such mutations in this gene are currently being debated [24].

Mutation analysis and haplotype associations have helped us trace the ancestry of this disease at least 2500 years, when today's affected populations were living together in the eastern Mediterranean basin [1]. After the spread of MEFV mutations from the eastern Mediterranean in biblical times, many migrations, including those of the 20th century, have resulted in a larger distribution of the disease. Thus the disease is no longer confined to eastern Mediterranean countries. Genetic studies have shown that the disease is present in other European

populations, albeit associated with milder mutations. Indeed, one of the most frequent mutations in the patients of European origin is E148Q, which is thought to be associated with a milder disease and even nonpenetrance [15].

An increasing number of cases has been reported among Askhenazi Jews and people of Italian ancestry as well [20, 25]. Most of these patients have mild disease and less-characteristic features, as they carry mild mutations. Familial Mediterranean fever has even been described in Japanese patients [26]. Furthermore, the carrier rate for a mild mutation in the MEFV gene (E148Q) was detected in 21 of 102 Indians in a study from England [27]. Although the mutations are no longer confined to the original groups, the FMF genetic alteration remains highly selected in eastern Mediterranean populations.

What are the clinical findings in FMF? The mean age of onset and age of diagnosis of FMF in a large Turkish childhood series were 5.5 ± 3.4 years and 9.3 ± 3.7 years, respectively [5]. In a multicenter study representing the largest series of FMF, the mean ages of onset and diagnosis were 9.6 ± 8.6 years and 16.4 ± 11.6 years, respectively (unpublished findings of a national multicenter study in Turkey).

The signs and symptoms of FMF might or might not be related to the attacks. Attack-related findings include [2, 5, 11, 16, 19, 28–30]: fever (in >90%), abdominal pain (in >90%), arthritis or arthralgia (around 50%), chest pain due to pleuritis (in approximately 33% of patients, but much higher in Armenians) or pericarditis, erysipelas-like erythema (varies, up to 20%), myalgia (varies, up to 33%), and scrotal attacks (fewer than 5%). The patient can present with only one of these features during an attack or with a combination of them. Typical attacks last 1 to 3 days. Some features are associated with a longer duration, such as protracted febrile myalgia or, rarely, protracted arthritis that can last for weeks. Signs and symptoms not related to attacks also can occur. Exertional myalgia of the calves and feet is common. Unilateral and bilateral sacroiliitis also can be a feature of the disease.

Renal involvement occurs in a number of forms. First, nonamyloid glomerulopathies have been reported [31–33]. In a nationwide study enrolling 2838 FMF patients from Turkey, 22 had nonamyloid glomerular diseases and displayed various types of glomerulopathies. Second, peristent microscopic hematuria can occur during attacks [2, 30]. Third, several reports have highlighted the frequent association of FMF with certain vasculitides, especially Henoch-Schönlein purpura (HSP) and polyarteritis nodosa (PAN) [2, 34, 35]. These patients may well present with renal vasculitis. Patients with FMF who develop PAN have a younger age at onset as compared with patients without FMF, and the former seem to have some peculiar features such as overlapping features of classic PAN and microscopic polyangiitis as well as overall better prognosis compared to patients without FMF. We had hypothesized that the increase rate of these vasculitides might be due to the heightened inflammatory response in patients with FMF or that vasculitis might be an integral feature of FMF per se [34]. It is interesting that the presented patient had been diagnosed as having HSP 1 year prior to presentation at our department. Renal involvement of HSP might have been considered in the differential diagnosis of this patient, but neither the clinical course nor the biopsy was compatible with HSP nephritis. Finally, if FMF is not treated, amyloid deposition can ensue in several organs.

Renal amyloidosis in FMF and other autoinflammatory syndromes is secondary to the deposition of amyloid A protein in the kidney [5, 30]. The precursor of the deposited amyloid A protein is serum amyloid A (SAA), an acute-phase reactant. Serum amyloid A is produced by inflammatory signals, especially IL-1. Polymerization of SAA into amyloid fibrils requires removal of the C terminal of the AA protein [4]. Amyloid fibrils accumulate in extracellular spaces. The pathologic effect of the deposited AA fibrils occurs because their physical presence disrupts the structures in the kidney.

Many diseases associated with severe inflammation have been associated with amyloidosis of the AA type; among these are rheumatic diseases, such as rheumatoid arthritis, and infectious diseases, such as tuberculosis. Both examples represent chronic diseases in which inflammation persists for a prolonged period. In fact, the persistent augmentation of an inflammatory pathway through the innate immune system might be crucial in the deposition of the amyloid protein.

Newly defined autoinflammatory diseases have been associated with a significant risk of amyloidosis. Among these, the tumor necrosis factor (TNF) receptor–associated periodic fever syndrome (TRAPS), the most common periodic fever disease after FMF, is associated with high levels of circulating TNF [36]. Basically, the genetic defect in the TNF receptor superfamily 1A results in lower shedding, and thus lower levels, of the receptor. The decreased amount of receptor available to bind raises the levels of unbound TNF-α. The two other autoinflammatory syndromes associated with secondary amyloidosis, Muckle-Wells syndrome and chronic infantile neurologic cutaneous articular syndrome, have a pyrin domain that up-regulates IL-1β production [37–39]. Both IL-1β and TNF markedly induce the hepatic synthesis of two major acute-phase proteins, C-reactive protein, and SAA. The level of SAA seems to correlate with the development of amyloidosis [40]. Furthermore, the role of high SAA levels has been further supported by the association of amyloidosis with a certain SAA1 polymorphism that leads to increased SAA levels in the three ethnic populations studied [41–43].

However, why FMF is so frequently associated with renal amyloidosis remains to be clarified. Is it simply because there are increased amounts of acute-phase reactants and especially SAA in the system? Some patients do present with amyloidosis without any prior features of FMF, the so-called "phenotype 2" patients [2, 5, 30]. The lack of clinical symptoms calls into question the presence of acute-phase reactant elevation in these patients. However, we do not know whether these patients had laboratory indices of intense inflammation but somehow lacked clinical manifestations. One wonders whether another genetic factor was involved in these patients.

Perhaps a defective pyrin is causing a lack of suppression of the deposition of amyloid fibrils. On the other hand, since there is an increased risk of amyloidosis in families with a history of amyloidosis, there seems to be a separate genetic factor, or factors that might be segregating within the family, and these factors might or might not be linked to the MEFV gene locus.

In the precolchicine era, as many as 75% of adults with FMF were reported as having amyloidosis [2, 44]. However, one must remember that these figures represented the percentage among symptomatic patients in the late 1960s. In a recent multicenter Turkish survey enrolling 2838 patients, approximately 13% of the FMF patients had amyloidosis. The risk for amyloidosis is much lower among Arab patients with FMF. Thus not all patients with FMF develop amyloidosis.

Many studies have tried to delineate the factors related to the development of amyloidosis. The first suggested as risk factors male gender [41] and the presence of secondary amyloidosis in the family [5]. We had suggested in a small study that one or more modifying hereditary factors might be involved in the segregation of amyloidosis within families [5]. Increased risk to individuals with a family history of amyloidosis was confirmed in larger studies. Many studies have indicted the M694V mutation in association with amyloidosis [4, 11, 12, 15]. However, amyloidosis is not confined to patients with this mutation, and one group in Turkey reported a lack of association of amyloidosis with the M694V mutation [45]. A number of polymorphisms introduce an increased risk of amyloidosis in FMF patients. The first of these is the SAA1 α/α genotype, which introduces a risk of up to sevenfold in patients with FMF [41]. This risk was confirmed in the Armenian, Jewish, and Turkish populations [41–43]. An Ala138Gly alteration in the MEFV gene also has been suggested as a risk factor [46]. However, patients lacking this polymorphism also can develop amyloidosis. Further, studying these polymorphisms is not very easy on practical grounds. In the Turkish study, patients who developed amyloidosis were younger at onset, and the delay in diagnosis was significantly longer ($P = 0.001$; unpublished findings of a nationwide multicenter study in Turkey). However, 5 of the 18 patients who experienced their first attack after the age of 40 developed amyloidosis. Thus the lack of symptoms during childhood does not protect one from amyloidosis. The problem is even more complicated in phenotype 2 patients. Environmental factors also probably play a role, as Armenian patients with FMF who live in the United States do not develop amyloidosis [47].

Our patient had high acute-phase reactants, at least when he was diagnosed, was homozygous for the M694V mutation, and was male. He already had nephrotic syndrome due to renal amyloidosis when he presented to our clinic. At the time we were unable to study the SAA levels or polymorphisms. Clinically, renal amyloidosis generally presents in one of three forms: proteinuric, nephrotic, and renal failure.

Colchicine has been the drug of choice for patients with FMF since the early 1980s. Instituted at a sufficient dose when the patient is diagnosed, colchicine may halt progression of the disease [5, 48]. Furthermore, reports show that proteinuria can remit and even disappear, although the renal amyloid deposition persists [2, 45, 49]. Colchicine treatment also is necessary for protection against amyloid deposition in other organs. In fact, now that patients with renal amyloidosis live longer because of renal replacement therapy, the previously clinically latent involvement of other organs, including the gastrointestinal tract, heart, lungs, thyroid, and adrenal, is becoming evident [2]. In today's patient, gastrointestinal amyloidosis was an important cause of morbidity and mortality. Although we were not able to biopsy his lung, we can speculate that his persistent lung complications were related to amyloid deposition in the lung as well. I do not know whether amyloid deposition in other organs is more resistant to colchicine. Nor is it known whether colchicine is absorbed adequately from the gastrointestinal tract in patients with renal impairment; perhaps renal dysfunction renders them more susceptible to amyloid deposition in other organs as well.

The differential diagnosis of FMF involves a newly recognized group of diseases associated with autoinflammation [6]. These monogenic diseases all affect the IL-1 or TNF pathways, and most of them are associated with a substantial risk of amyloidosis [6]. These diseases were probably under-recognized until recently. The most important feature differentiating FMF from the other diseases is that only FMF responds to colchicine administration. All except FMF seem to be more frequent—at least so far—in people of northwest European ancestry. These diseases, all of which are associated with short episodes of fever and some joint involvement, comprise six syndromes. The first of these, TRAPS [6, 36], is autosomal-dominant, and its typical duration is > 1 week. TRAPS is associated with mutations in the TNF-receptor superfamily 1. Rash is characteristic, with a migratory skin rash that usually comprises erythematous

macules. Amyloidosis is common, and there is an association with cysteine mutations. The differentiating features of TRAPS include myalgia (present in 80% of patients), conjunctivitis and periorbital edema, and the prolonged duration of attacks. Second, the hyper IgD syndrome is autosomal-recessive [50, 51]. It typically lasts for 3 to 7 days. This syndrome arises from a defect in the mevalonate kinase gene, which also affects IL-β secretion. Rash is common, and the lesions can be macules, papules, urticarial lesions, or nodules. Amyloidosis has not been reported. The differentiating features of the hyper IgD syndrome include lymphadenopathy, increased serum IgD (>100 U/mL), and decreased mevalonate kinase in the urine during attacks. Third, the Muckle-Wells syndrome is autosomal-dominant and is distinguished by amyloidosis, progressive sensorineural hearing loss, and febrile urticaria. Fourth, the familial cold autoinflammatory syndrome (FCU), like Muckle-Wells syndrome, is autosomal-dominant [6, 39, 52]. It typically lasts only 1 or 2 days and is diagnosed by urticaria that occurs after cold exposure. The mutations in FCU occur in the CIAS1 gene coding for cryopyrin (contains a pyrin domain in the N-terminal). Amyloidosis is common. Fifth, the syndrome of chronic infantile neurologic cutaneous articular (CINCA) syndrome, or neonatal onset multisystem inflammatory disease (NOMID) is of variable duration and has an autosomal-dominant pattern of transmission [38, 39]. CINCA/NOMID arises from mutations in the CIAS1 gene coding for cryopyrin. Amyloidosis has been reported, and the associated rash is urticaria-like. This syndrome is differentiated by neurologic manifestations ranging from mental retardation and chronic aseptic meningitis to hearing loss. Arthropathy has a characteristic appearance, with osseous overgrowth of the patella and contraction of the knee. Finally, the last of these rare syndromes is characterized by the clinical triad of pyogenic arthritis, pyoderma gangrenosum, and acne.

Prior to around 1973, therapy for FMF was limited to symptomatic treatment, but the introduction of colchicine changed the fate of the disease. Effective colchicine therapy prevents secondary renal amyloidosis [5, 48]. Colchicine inhibits leukocyte chemotaxis through a direct effect on cytoplasmic microtubules [4, 28]. Symptomatic pain relievers and nonsteroidal antiinflammatory drugs are given for the acute attacks. Prednisone and immunosuppressive agents can be useful if the patient develops vasculitis.

Unfortunately, all patients with renal impairment and some with nephrotic syndrome will progress to end-stage renal failure in spite of the institution of colchicine therapy, as was the case in our patient. He progressed to end-stage renal failure quite rapidly. His grave course was complicated by uncontrolled inflammation.

Once renal failure occurs, renal replacement treatment should be started, although in FMF patients, therapy is associated with some particular complications [53]. Hemodialysis can pose problems because of the particularly low intravascular volume in these patients. Thrombosis of the vascular access is more frequent in patients with FMF. Our patient experienced problems with his access as well. Continuous ambulatory peritoneal dialysis also can be instituted, but it sometimes increases abdominal febrile attacks [2].

Renal transplantation should be considered as an effective treatment of end-stage renal disease for patients with amyloidosis secondary to FMF. After renal transplantation, colchicine is mandatory [54, 55]. Insufficient doses cause a recurrence of renal amyloidosis in the transplanted kidney. However, cyclosporine induces colchicine toxicity and thus should be monitored appropriately or an alternative immunosuppressive regime should be used [54]. One should lower the dose of cyclosporine A in these patients [2]. In the few series comparing 5-year patient and graft survival rates with those in other nephropathies, the rates were some 5% to 20% lower in amyloidosis patients [56, 57].

Permit me to make some concluding remarks. Physicians practicing in the eastern Mediterranean area have seen many patients with amyloidosis secondary to FMF. With increasing education of general physicians, we've seen a marked decrease in the number of patients developing amyloidosis. On the other hand, improved socioeconomic features might have contributed to this decline, perhaps like the decline claimed in amyloidosis in Armenian patients in the United States. If it is rarer in the United States, why a difference exists in the predisposition to amyloidosis in these patients is an intriguing question. The predilection might be explained by the increased stimuli by certain bacterial antigens to the innate immune system that heightens the inflammatory milieu. Further studies on the interactions of pyrin will shed light on this subject.

QUESTIONS AND ANSWERS

Dr. John T. Harrington (*Division of Nephrology, Tufts-New England Medical Center, Boston, Massachusetts, USA*): What is known about the structure of pyrin, and how does it preferentially increase Th1? Second, does TNF-α have a direct effect in patients with FMF amyloidosis?

Dr. Ozen: Pyrin is a protein that contains a "pyrin" domain, a B-box type zinc finger, a coiled coil, and a B30.2 domain [1, 8]. The "pyrin" domain is responsible for the protein:protein interaction. It interacts with an "apoptosis-associated speck-like protein containing a caspase recruitment domain (CARD)" that is abbreviated as ASC [8]. ASC is an adapter protein involved in apoptosis and inflammation. This interaction likely mediates assembly of large signaling complexes and also

activates caspase-1 [58]. Caspase-1 is then responsible for the secretion of IL-1β and IL-18 [58]. ASC also activates NF-κB, adding to the inflammatory signal. We still are not sure how these events lead to a preferential T-helper 1 activation, but we know that IL-18 is one of the activators of the Th-1 pathway.

As to TNF-α, some authors have reported increased levels in FMF [59]. However, the role of TNF-α in amyloidosis cannot be commented on since the aforementioned studies have enrolled patients without amyloidosis. One study indirectly studied the association by analyzing the TNF-α 308 G-A allele in patients with amyloidosis: this polymorphism is associated with higher plasma levels of TNF-α. However, the authors found no significant difference between the controls and FMF patients with and without amyloidosis for this polymorphism [60]. We have confirmed these data [43].

Dr. Marc de Broe (*Professor, University of Antwerp, Belgium*): FMF is an inflammatory disease, involving neutrophils and macrophages. Acute ischemic/toxic injury is an inflammatory disease, as in acute rejection. Is there information available concerning the incidence and clinical expression of acute renal failure and acute rejection?

Dr. Ozen: In the reported series of transplanted FMF-amyloidosis patients, the acute rejection episodes are not increased as compared to the other transplanted patients [56, 57, 61]. Although FMF is indeed a neutrophil disease, a specific function is affected, and we can speculate that this gene is not crucial in the immunologic events leading to rejection.

Dr. Mustafa Arici (*Associate Professor, Hacettepe University, Ankara, Turkey*): After 30 years, colchicine remains the sole therapeutic agent. Angtiotensin-converting enzyme (ACE) inhibitors and angiotensin II (Ang II) receptor blockers are effective renoprotective agents in proteinuric renal diseases. Is there any evidence that these drugs are also effective in retarding progression of amyloidosis in FMF patients with severe proteinuria?

Dr. Ozen: So far there is no evidence-based data on the effect of ACE inhibitors or Ang II receptor blockers in the prognosis of amyloidosis secondary to FMF. However, we have recently started using these drugs in our patients with proteinuria. We will have to wait to see the reports on the use of these drugs.

Dr. Bulent Altun (*Associate Professor, Hacettepe University*): Do we have data about the anti-inflammatory cytokine gene polymorphisms or cytokine levels such as IL-10? Also, should we titrate the dose of colchicine in accordance with genotype to prevent amyloidosis?

Dr. Ozen: Actually, in the study I presented from our lab [18], intracellular staining for IL-4, an anti-inflammatory cytokine, was measured by fluorescence-activated cell sorter (FACS) analysis. Using peripheral blood mononuclear cells from FMF patients, we found that IL-4 was not expressed in these samples. In one study, the serum levels of another anti-inflammatory cytokine, IL-10, did not differ from those in controls, whereas the inflammatory cytokines that were studied were increased [59]. Most studies in FMF have measured inflammatory cytokines only and have found them to be increased, as one might expect.

We know that some mutations such as the E148Q are associated with nonpenetrance and a milder phenotype [15, 20, 62]. However, we do not have evidence-based data that the colchicine dose can be titrated according to the mutation. On the other hand, SAA monitoring might be a good alternative for titrating doses of colchicine [62–64].

Dr. Necla Buyan (*Professor, Gazi University Faculty of Medicine, Turkey*): What is the sufficient dosage of colchicine for certain pediatric ages? What can we do for the patients with severe gastrointestinal side effects of colchicine?

Dr. Ozen: The dosage of colchicine in pediatric practice is a matter of debate. One suggestion has been that we use 0.03 mg/kg/day in children [64], while the adult literature suggests that we administer the adult dose to children [2]. However, I do not think a 10 kg child would safely tolerate a 1.5 mg/day dose. In our practice, we give the adult dose only after the child's body surface area exceeds 1 square meter, which is roughly 29 kg. On the other hand, data from the last FMF meeting suggest that the SAA level is a critical factor in defining the risk of amyloidosis. We have shown that the SAA level was the best index for inflammation and that increasing the colchicine dose dramatically decreased SAA levels [64]. Thus we have started to use SAA levels routinely in children to titrate the colchicine dose. We tend to start at an arbitrary dose and titrate it according to the SAA, C-reactive protein levels, symptoms and side effects of colchicine, which mainly is diarrhea.

Severe gastrointestinal involvement of secondary amyloidosis is a major problem. It causes intractable diarrhea and it is very hard to manage. There is some evidence for the use of a lactose-free diet, based on the observation that colchicine might induce lactose deficiency [2]. Antidiarrheal agents are of variable success.

Dr. Sevgi Mir (*Professor, Ege University, Izmir, Turkey*): Does this patient have siblings? If so, what is the treatment policy?

Dr. Ozen: This question reminds us of an important issue. One of our responsibilities as pediatricians is to inquire about the siblings when we diagnose a patient with FMF. Our patient did have a younger sister, who described typical attacks of FMF. She was put on colchicine immediately. We did not perform a genetic analysis on her, because she had the typical phenotype with elevated acute-phase reactants and a brother who already had been clinically and genetically confirmed to have the disease.

Dr. Ferah Sönmez (*Associate Professor, Adnan Menderes University, Aydin, Turkey*): If your patient had a brother 8 years old without any clinical findings of FMF and amyloidosis, and if he were homozygous for the M694V mutation, would you begin colchicine therapy immediately?

Dr. Ozen: There is some controversy about whether asymptomatic individuals should be treated. Some of our colleagues in the United States do not give colchicine to asymptomatic individuals. I would have given colchicine to the asymptomatic brother, because he would have had risk factors for the development of amyloidosis: amyloidosis in the family, male gender, and the M694V hmozygosity, although the risk of this genotype has not been consistent, as we already said. Another reason I would have given him colchicine is that we live in an area where 13% of the patients with FMF also have amyloidosis. I may be biased because of having seen quite a number of children suffering from this complication. For aymptomatic patients, I suggest thoroughly discussing with the family the risk factors that I mentioned.

Dr. Jorge Cannata-Andia (*Professor, University of Oviedo, Oviedo, Spain*): Could you please further comment on the articular and skeletal involvement in FMF, mainly as they compare with other inflammatory diseases?

Dr. Ozen: The articular manifestation of FMF is typically a monoarthritis affecting the knees and ankles [2, 62]. It is nonerosive and remits spontaneously. The other autoinflammatory diseases occasionally mimic these articular attacks. However, they have their own distinguishing features and do not respond to colchicine [50, 52, 62].

I must add that a "protracted arthritis" has been described in FMF, even progressing to joint replacement [2]. Since the disease is so frequent in this part of the world and is associated with an increased inflammatory milieu, it is tempting to speculate that these patients have another chronic disease superimposed on FMF.

Dr. Ruhan Duşunsel (*Professor, Erciyes University, Kayseri, Turkey*): What is your recommendation about immunosuppression therapy (time, dosage, etc.) in patients with amyloidosis? Can their relatives be living donors?

Dr. Ozen: It is widely recommended that one decrease the dose of cyclosporine A in FMF patients [54, 55]. The administration of a full dose of colchicine and cyclosporine A is associated with increased side effects of both drugs, such as severe myoneuropathy [65].

Relatives can and have been used as donors [54–57, 61]. However, one has to make sure that they are free of symptoms related to FMF. A genetic analysis would be appropriate when possible.

Dr. John Dirks (*Professor, Chair, ISN COMGAN, Massey College, Toronto, Canada*): Colchicine has been a considerable advance in FMF but it has limitations. What would be the alternatives? What do you postulate as molecular sites for possible therapeutic interventions?

Dr. Ozen: Actually, I believe colchicine is a very safe drug at the doses we use. Concerns about fertility are not supported in the recent literature [28, 66, 67]. The drug's low cost is another advantage. Interferon alpha has been suggested as an alternative treatment in colchicine-resistant patients [68], but one has to recognize its side effects when deciding whether to use this drug.

In the future, stem cell treatment might be an alternative if we become more competent in the field. This procedure can provide healthy leukocytes without the genetic defect. Milledge et al [69] have reported a patient who was cured of FMF when he underwent bone marrow transplantation for his primary disease. However, it is not ethical to perform a bone marrow transplant in a patient who has FMF only; for the time being, colchicine is a much safer and effective option.

Dr. Harrington: What do we know about the effect of colchicine on neutrophils at the molecular level?

Dr. Ozen: Colchicine inhibits leukocyte chemotaxis and is mainly concentrated in neutrophils. The affinity of colchicine for neutrophils might be due to the absence of the P-glycoprotein efflux pump on their membranes [67] and might explain how the high concentration of colchicine in these cells exerts its intracellular effect. Although we don't know its exact mode of action, colchicine is thought to act on the microtubuli to effect chemotaxis. The drug also can reduce the expression of adhesion molecules, thus interfering with leukocyte transfer to the inflammatory site [4].

We do not know whether colchicine administration has a direct inhibitory effect on the formation of amyloidosis as well, other than through its anti-inflammatory effect. However, in a mouse model, it suppressed amyloidogenesis at a late stage [70].

We also use colchicine in another neutrophil disease, Behçet's disease, where it is effective for at least some of the clinical features of the disease.

Dr. Mehmet Sukru Sever (*Professor, Istanbul University School of Medicine, Turkey*): Have any studies described the permeability and survival of the peritoneal membrane in patients who receive continuous ambulatory peritoneal dialysis as renal replacement therapy?

Dr. Ozen: I've seen no published data on this subject. Theoretically, I would expect peritoneal survival in FMF to be shorter because of all the inflammatory cytokines in the peritoneum at the time of the attacks. Some of these cytokines have a potential for producing fibrosis.

Dr. Nilgun Cakar (*Associate Professor, Social Security Children's Hospital, Ankara, Turkey*): What are the suggestions for medical care during pregnancy in patients with FMF?

Dr. Ozen: During pregnancy, FMF attacks can lead to miscarriage and other complications. We currently suggest that women continue to take colchicine during pregnancy [28, 62, 66]. We also recommend amniocentesis during gestation to detect chromosomal abnormalities [66]. In patients with renal amyloidosis, renal function can deteriorate during pregnancy [66].

Dr. Cem Sungar (*Professor, Bayindir Hospital, Ankara, Turkey*): What are the consequences of colchicine toxicity?

Dr. Ozen: Colchicine toxicity is a very severe clinical entity with a high mortality rate. It can cause bone marrow depression, acute renal failure, hepatic toxicity, and disseminated intravascular coagulopathy [71]. Unfortunately, the drug is not dialyzable. Thus precautions should be taken to keep the drug away from young children.

Dr. Gultekin Suleymanlar (*Professor, Akdeniz University, Antalya, Turkey*): Colchicine can interact not just with cyclosporine but with other drugs as well. We have observed severe rhabdomyolysis along with acute renal failure in a patient who was receiving colchicine plus cyclosporine and a statin. We also have experience with mycophenolate mofetil (MMF) and colchicine; the combination was associated with an increased severity and rate of the gastrointestinal side effects of MMF [72] in patients with renal amyloidosis secondary to FMF. Thus, the dose of MMF should be reduced, or azathioprine should be substituted for MMF.

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