Case Report

Early destructive spondyloarthropathy from combined β_2-microglobulin and transthyretin Met30 amyloidosis in a dialysed patient

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Key words: amyloidosis; β_2-microglobulin; destructive spondyloarthropathy; haemodialysis; transthyretin

Introduction

Dialysis-associated amyloidosis was described in the mid-1980s in patients on dialysis as replacement therapy of end-stage renal failure (ESRF). In 1985 Gejyo *et al.* identified the β_2-microglobulin (β_2M) as the amyloid protein precursor of dialysis-related amyloidosis [1]. Fibril formation in this type of amyloid may be the result of critically high levels of serum β_2M, since this protein is catabolized mainly by the kidneys and amyloid fibrils can be formed from intact β_2M *in vitro* [2].

Familial amyloid polyneuropathy (FAP) type I is an hereditary (autosomal dominant) amyloidosis caused by a single-point mutation in the transthyretin (TTR) gene. In FAP type I, the major component of amyloid fibrils is a variant of transthyretin in which valine has been replaced by methionine at position 30 (TTR Met30) [3]. This disease is characterized by a sensorimotor and autonomic polyneuropathy, ocular involvement, cardiac disease and nephropathy. Dysautonomic features include abnormal gastrointestinal motility, bladder function, impotence, and orthostatic hypotension. The average age at onset of FAP type I is 31.3 ± 6.2 years, but the patients with renal features have a later onset of neuropathy (41.1 ± 13.3 years) [4] and in the families of such patients one finds a higher prevalence of old asymptomatic carriers of the FAP gene. The features of renal involvement range from proteinuria to end-stage renal disease.

Case report

In 1989, a 65-year-old Portuguese woman developed diarrhoea, asthenia, weight loss, orthostatic hypoten-

sion, distal weakness and sensory disturbances in the lower limbs. She also presented proteinuria but normal renal function. Family history of FAP type I was absent and there was no relationship with the large area of the disease in Portugal (Póvoa de Varzim/Vila do Conde). Amyloid deposits were detected in gastric, duodenal and rectal biopsies. There was not any monoclonal peak in serum protein immunoeletrophoresis and the bone marrow aspirate revealed 3% of plasma cells. The radiologic evaluation of skeleton did not revealed any abnormality. Although the late onset and the lack of family history, we search for TTR Met30 mutation, which was positive and TTR related amyloidosis was identified in sural nerve biopsy.

Three years later the patient presented with moderate renal failure (serum creatinine of 2.1 mg/dl) and proteinuria of 6.6 g/24 h. Sudden worsening of renal function necessitated acute institution of haemodialysis. Renal biopsy revealed extensive glomerular, vascular, tubular and interstitial amyloid deposition, tubular atrophy and interstitial fibrosis. She was maintained on haemodialysis, performed thrice weekly (4 h/session) with a polysulphone dialyser. Treatment was well tolerated. Weight loss stopped, as did progression of motor disabling disease.

In March 1995 the patient complained of a lumbar pain and worsening of left lower limb paresis. Fever or other symptoms of infectious disease were absent.

Laboratory data revealed a normocytic anaemia (haematocrit of 31.2%) and normal leukocyte and pletelet counts. Predialytic serum: urea 98 mg/dl, calcium (Ca) 10.4 mg/dl, phosphorus (P) 5.2 mg/dl, alkaline phosphatase 600 U/l, parathyroid hormone 79 pg/ml, ferritin 578 ng/ml, and iron 33 mg/dl. Total serum proteins 6.68 g/dl and albumin 3.11 g/dl and immunoelectrophoresis without any monoclonal band.

Radiographs of the lumbar spine were unremarkable but computed tomography scanning (CT scan) revealed a destructive mass on the fourth lumbar vertebrae (L_4), with incomplete destruction of the vertebral body and preservation of the spinal channel (Figure 1). Magnetic resonance imaging (MRI) confirmed the
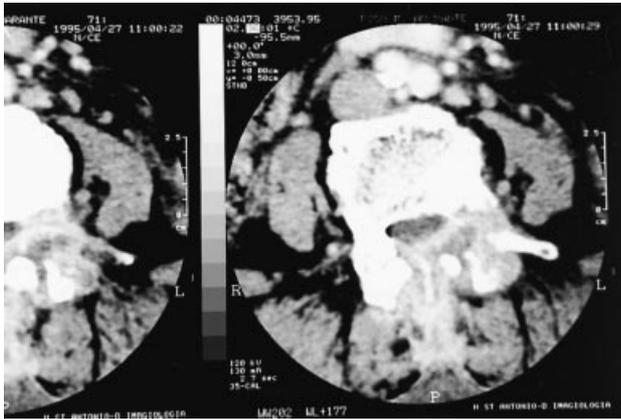
Fig. 1. Vertebral TC scan. Destruction of the four lumbar vertebrae.

destructive spondyloarthropathy of L_4 with low-intensity signal on T1-weighted images and intermediate intensity signal on T2-weighted images. Scintigraphy with ^{99m}Tc-methylene diphosphonate showed radiotracer uptake on the third and fourth lumbar vertebrae. The mass was surgically removed and congophile deposits were identified on cartilage, conjunctive tissue, and nerves. Single antibody immunostainings were all carried out using a streptavidin-biotinylated peroxidase complexes detection system (Amersham Life Sciences, UK) and diaminobenzidine as substrate. Immunostainings with rabbit anti-kappa and anti-lambda free light chains (Dako, Denmark) and mouse monoclonal anti-AA (Dako), all with trypsin pretreatment, were negative. Immunostaining with rabbit anti-TTR (Dako) and the mouse monoclonal antibodies FAP2 (anti-TTR) and 99.2.GB5 (anti-β_2M) were positive. Both monoclonal antibodies were developed in Centro de Estudos de Paramiloidose. Double antibody immunostaining was carried out with rabbit anti-TTR and anti-β_2M in sequential steps: anti-TTR, biotinilated anti-rabbit Ig (Dako), peroxidase complexes, 0.1% biotin (to block the first layer of streptavidin), anti-β_2M, biotinylated anti-mouse IgG$_{2a}$ (Amersham), extravidin–alkaline alkaline phosphatase conjugate (Sigma), diaminobenzidine peroxidase substrate, and finally Fast Red TR/Taphthol AS-MX alkaline phosphatase substrate (Sigma). Non-overlapping, patchy deposits of both TTR and β_2M amyloid were observed, with an apparently random distribution in collageneous areas, except for small-vessel wall infiltration, which, surprisingly, was mostly of β_2M type (Figure 2b).

In November 1995 the patient suffered a fracture of the femur. After surgery she developed pneumonia with sepsis, and died in December 1995.

Discussion

Dialysis amyloidosis usually appears after 5–7 years of chronic dialysis and its clinical presentations are carpal-tunnel syndrome, bone pain, fractures, large
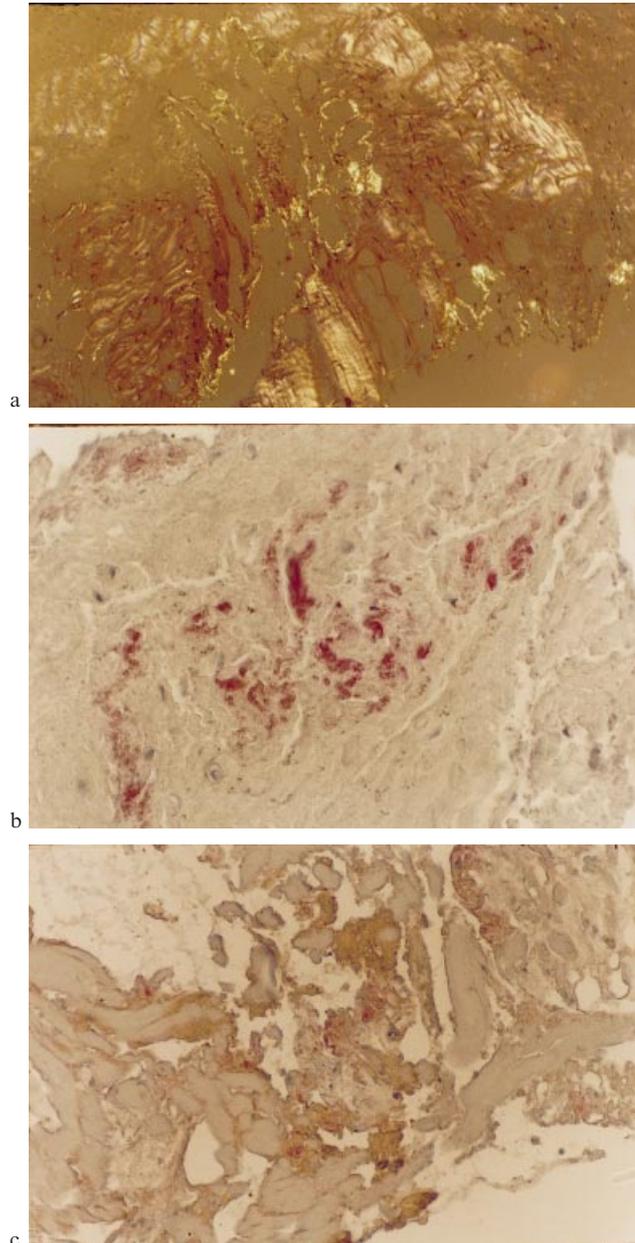
Fig. 2a–c. Photomicrographs ($\times 216$) of the vertebral lesion. (**a**) Apple-green birefringence of amyloid deposits under polarized light. (**b**) Immunostaining for β_2M amyloid (intervertebral disc). (**c**) Immunostaining with both antibodies showing codeposition of ATTR (brown) and Aβ_2M (spongy bone).

joint arthropathy and spondyloarthropathy, tendon rupture and contracture, subcutaneous masses, and renal calculi. A close relationship has been documented between time of dialysis and prevalence of amyloid deposits in synovial and juxta-articular bone [5]. Cervical or lumbar pain in a dialysis patient, specially with dialysis for more than 5 years, may correspond to Aβ_2M destructive spondyloarthropathy, but other causes of the destructive arthropathies must be ruled out. These causes are infections discitis, osteomyelitis, neoplasm, brown tumours due to hyperparathyroidism, calcium pyrophosphate dihydrate (CPPD) crystal

deposition disease, and AL amyloidosis. CT scan and MRI are very useful to differentiate amyloid deposits from infectious discitis or osteomyelitis. MRI of amyloid destructive spondyloarthropathy demonstrates low to intermediate signal intensity in lesions on both T1- and T2-weighted images while MRI of infectious discitis demonstrates low signal intensity on T1-weighted images and high signal intensity on T2-weighted images. In vertebral osteomyelitis the prevertebral soft tissue mass is often visible [6]. Parathyroid hormone levels and serum Ca × P product are elevated in hyperparathyroidism, but usually normal in β_2M amyloidosis. CPPD crystal deposits are usually associated with chondrocalcinosis of peripheral joints, which seems to be rare in patients receiving maintenance haemodialysis. Histological identification by Congo red and immunohistochemical staining remains the gold standard for diagnosis of β_2M amyloidosis.

Risks factors for development of Aβ_2M include age and time of dialysis. Destructive spondyloarthropathy usually affects patients older than 50 years, and is very rare in children, even after 5 years of regular dialysis. The high prevalence in the elderly may be explained by low metabolic activity of chondrocytes, fibroblasts, and probably osteoblasts, so that they are unable to repair articular lesions [7].

The main factor for development of Aβ_2M seems to be the increased serum level of β_2M. Advanced glycation end-products (AGE), elevated levels of cytokines and impaired bone metabolism may promote β_2M amyloid deposition [8]. AGE-modified proteins exhibit enhanced binding to collagen and are less susceptible to degradation to proteases [9].

Substances other than β_2M have been described in amyloid deposits, probably trapped in the cross-linked AGE-β_2M and AGE-modified collagen. These constituents are amyloid P component, ubiquitin, apolipoprotein E, proteoglycans, and glycosaminoglycans.

Some of them, ubiquitin and apolipoprotein E, have amyloid-enhancing factor activity and promote the formation of amyloid.

Simultaneous presence of AL and β_2-microglobulin (β_2M) in amyloid deposits of the same patient had been well described [10], but there are no reports of ATTR and Aβ_2M codeposition. The presence of unusually severe lesions of amyloidosis in a patient with dialysis for less than 3 years may be due to accelerated amyloid formation caused by codeposition of β_2M- and TTR-derived amyloid fibrils.

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