β_2 microglobulin amyloidosis in patients with chronic renal failure undergoing regular haemodialysis

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Abstract

The amyloidosis, caused by β_2 microglobulin (β2M) is a complication of renal substitute therapy, particularly with long term haemodialysis.β_2M the preceding molecule of the amyloid fibrils, accumulates in renal failure due to its reduced excretion; the amount of the referred accumulation seems to depend essentially on the length of time of dialysis and the type of membranes used.

The authors have studied eleven patients undergoing regular haemodialysis, in order to discover the existence of amyloidosis caused by β_2M. Besides the identification of the clinical signs of this entity, namely osteoarticular signs, of which the most characteristic is the carpal tunnel syndrome. The authors systematically biopsied the subcutaneous abdominal fat, made nerve conduction studies, echocardiography, bone x-ray and osteoarticular scintigraphy. The results are presented, comparing the patients with three or less years of haemodialysis with those who have been dialyzed for more than eight years.

Concerning the results, the authors highlight the following: 1) The presence of CTS in 4 patients (36.4 per cent); 2) Cervical spondyloarthropathy was demonstrated in 66.7 percent in long term haemodialysis patients; 3) The existence of cardiac amyloidosis in 20 per cent of patients; 4) the presence of β_2M amyloidosis in 3 patients (27.3 percent) in deep skin biopsies.

Key words: β_2 microglobulin amyloidosis, haemodialysis.

Introduction

Dialysis-associated amyloidosis is a complication that has been recently found in patients with chronic renal failure (CRF) undergoing prolonged haemodialysis.[1,2] In 1980, Assenat and colleagues pointed out the presence of amyloid deposits on the carpal tunnel of patients undergoing haemodialysis when systemic AA or AL amyloidosis was not present.[1,3,4]

β_2 microglobulin (β_2 M) is a precursor molecule of the amyloid fibrils that is specific to CRF.[1,5,6,7] This molecule is a globular, non-glycosylated polypeptide consisted of 99 A.a.[1,8] Its b-pleated structure is pleated and its structural gene is located on the long arm of chromosome 15.[1,4,6,9] Its main biological activity relates to its expression in the class I HLA molecule and to its antigen presentation property.[1,6,8,10] β_2 M is not just a surface protein, but also a secreted protein.[1,10] Several authors suggest that the plasma levels of β_2M depend on hepatocytes, under both normal and pathological conditions.[1] Levels of this polypeptide increase rapidly with age and decreases with renal function.[4,9] Its main site of catabolism is the kidney. [8,11] However, extra--renal catabolism of β_2M raises doubts. It appears to be minimal (3%) under physiological conditions.[1,4,9]

The pathogenesis of β_2 M amyloidosis is not well--known, at present.[6] In order to gain a better understanding of the amyloidogenic process in this setting, several hypothesis have been proposed:

- Concentration hypothesis: the possibility that a slight increase in plasma concentration of β_2 M would be sufficient to cause deposits of this polypeptide due to its spontaneous transformation into amyloid fibrils.[1,12]
- Hypothesis of proteolytic changes: production of limited proteolysis in the aminoterminal end of a polypeptide, resulting in precursor molecules of fibrillogenesis.[1,6,12,13]
- Hypothesis of formation of circulating levels of β_2 complexes: fibrillogenesis would result in the formation of circulating macromolecular β_2 M.[1,6,12]
- Hypothesis of the possible role of the dialysis technique: some retrospective studies would have

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Received for publication on 22nd December 1996

demonstrated that patients treated predominantly or exclusively with cuprophane membrane (bioincompatible) would have a higher complication rate related to β_2 M amyloidosis than patients treated with biocompatible polyacrylonitrile NA69 membrane.[1,6,7,8,9,12] An inflammatory response would probably be triggered due to the contact of the leukocytes with the bioincompatible membrane, resulting in higher formation of amyloids.[1,6,8,12]

• Hypothesis of the local joint mechanisms: the particular tendency of β_2 M amyloidosis to affect joint and periarticular tissues would suggest intervention of local factors.[1,12,14] The possible mechanisms would be local enrichment, failure, degradation site, production of molecules modified by amyloid precursors, and localized synthesis of complete or incomplete β_2 molecules.[1,12,14] The formation of β_2 M and the transformation into amyloid fibrils could then be stimulated by a pre-existing joint pathology.[1,8,12,14]

The onset of clinical and radiological manifestations of β_2 M amyloidosis occurs at a late stage of hemodyalisis.[2] For most patients with this pathology, the average treatment time was 8-12 years.[1,14]

Carpal tunnel syndrome is a complication that is very characteristic of prolonged dialysis and a prominent characteristic of β_2 M amyloidosis.[2,3,5,8]

Chronic arthropathy is also highly common in these patients.[2,5] Chronic arthropathy can be bilateral and affects predominantly shoulders and knees; lytic bone changes, destructive arthropathies and pathological fractures are also relatively common.[2]

In 1987, several studies indicated that β_2 M amyloidosis can also be systemic.[1,2] Thus, deposits have been reported in the systemic vessels, tongue, lung, heart, intestine, vulva and abdominal fat.[1,2,4,10] All deposits were determined by immunochemistry, which confirmed that they were formed by β_2 M.[1,10]

Taking into account the abovementioned aspects, the authors decided to analyze a population of patients with chronic renal failure undergoing regular haemodialysis.

Objectives

The objective was to find whether β_2 M amyloidosis (clinical, laboratory and anatomopathological findings) occurred in patients with chronic renal failure undergoing haemodialysis for three years or less, and its incidence in the two groups of patients with renal failure studied - those undergoing dialysis for three years or less, and for more than eight years, respectively.

Because the number of patients was small, the objective was not towards comprehensively studying a series of β_2 M amyloidosis, rather commenting some aspects and drawing attention to this recently found entity which, in its natural evolution, is almost always accompanied by systemic involvement.

Material and methods

This was a prospective study involving eleven patients with CRF undergoing regular haemodialysis. The patients were having haemodialysis three times a week, and the sessions lasted, on average, three to four hours. In all the patients, the vessels were accessed by an arteriovenous fistula. At this Unit, filters consisting of cuprophane membranes were used for all the patients. The patients were divided into two groups in this study – one group composed of five patients undergoing haemodialysis for less than three years, and one group composed of six patients undergoing haemodialysis for more than eight years.

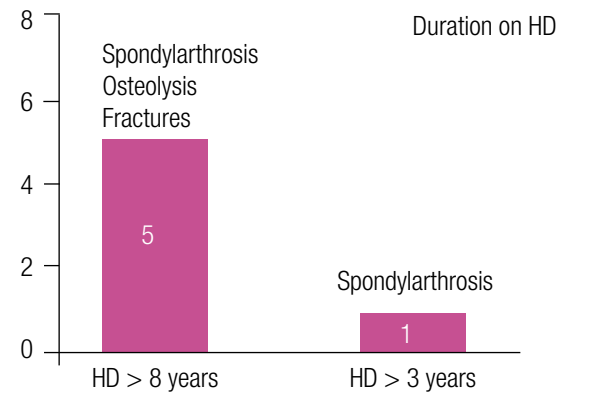
In addition to the clinical evaluation, all the patients had a skeletal X-ray, osteoarticular scintigraphy, M-mode and bi-dimensional echocardiogram, electromyography (EMG), determination of serum β_2 microglobulin (before and after the haemodialysis session) and biopsy of the skin and subcutaneous abdominal fat. Laboratory determinations were also performed for calcium, phosphorus, alkaline phosphatase and parathyroid hormone (PTH) in all the patients.

The presence of sensorial changes (pain, paresthesia due to distribution of median nerve, sometimes aggravated at night) and/or motor changes (affected hand), were considered as clinical evidence of carpal tunnel syndrome (CTS). For the EMG, the confirmation of CTS was based on a suggestive pattern of compression of the median nerve.[1,15]

Evidence of cervical spondyloarthropathy, osteolysis and fractures was based on the skeletal x-rays.

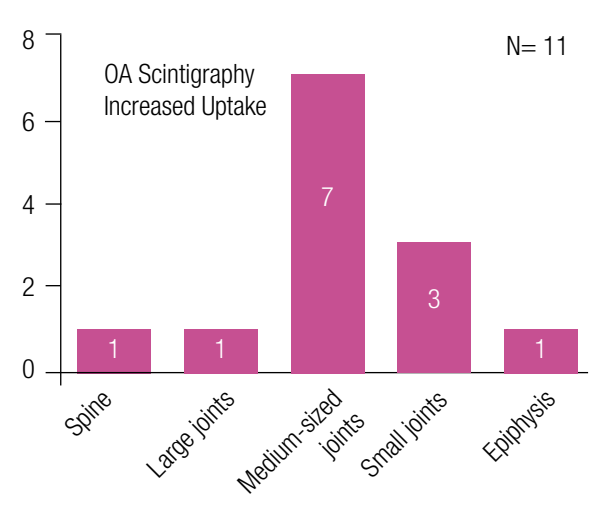
With regard to the osteoarticular 99mTc-MDP scintigraphy, the changes observed were classified according to the main site groups: large, medium-sized and small-sized joints, spine and epiphysis.

Echocardiogram was performed in only ten patients (one patient had died) and the results were positive regarding the presence of one of the following: thickening of the left ventricular wall, hyper-refractile



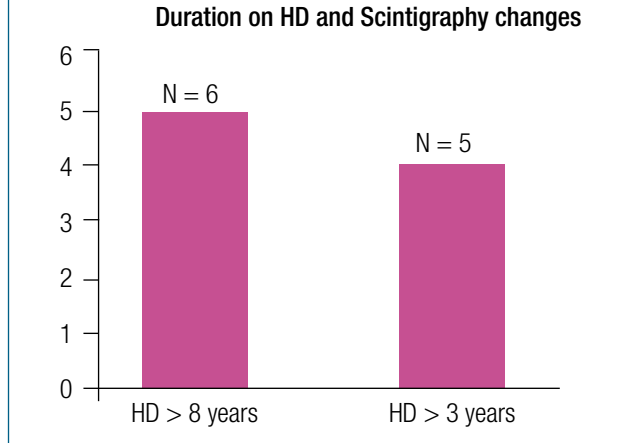
Radiological changes by haemodialysis time.

FIG. 1



Scintigraphy changes according to the site.

FIG. 2



Scintigraphy changes by haemodialysis time.

FIG. 3

myocardium, left ventricular diastolic dysfunction and left atrial dilation.[16]

The investigation of amyloid substance on skin and subcutaneous abdominal fat was performed using Congo Red staining, and β_2 microglobulin was identified using avidin-biotin-peroxidase complex immunohistochemistry.

Results

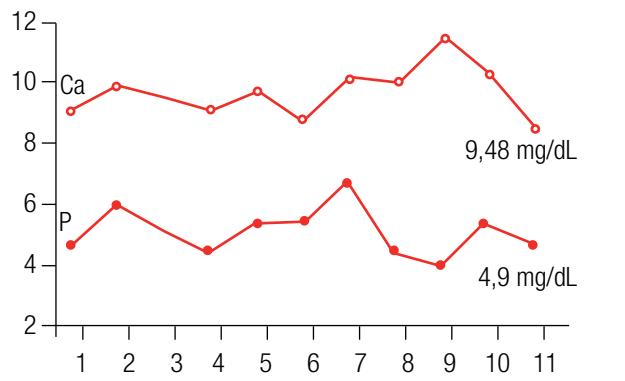
The mean age of all eleven patients with CRF was 56.6 years, ranging from 33 to 79 years; nine patients were male (81.8%) and two were female.

Of the total patients, five had been having dialysis for less than three years (Group I) and six for more than eight years (Group II).

Of the eleven patients, 36.4% (4/11) had clinical symptoms compatible with carpal tunnel syndrome. When Groups I and II were compared, clinical evidence of CTS was observed in 40% (2/5) in Group I (both cases confirmed by EMG) and 33.4% (2/6) in Group II; EMG showed compatible results in four patients (66.7%). We highlight the fact that one of these last patients had undergone a surgical decompression of the median nerve.

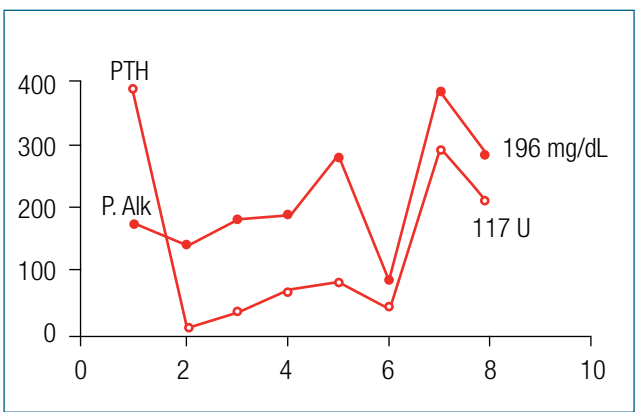
With regard to the skeletal x-ray, radiological changes were observed in 54.5% (6/11) of the patients. In Group II, cervical spondyloarthropathy was observed in four patients (66.7%), osteolysis in one patient (16.7%) and fracture in one patient (16.7%). With regard to duration on dialysis, Group I had a single case of cervical spondyloarthropathy, while radiological changes were observed in five patients (83.3%) of Group II (*Fig. 1*).

The analysis of the osteoarticular scintigraphies showed changes (increased uptake) as follows: small joints - 3 (27.3%), medium-sized joints - 7 (63.6%), large joints - 1 (9%), spinal column - 1 (9%) and epiphysis - 1 (9%) (*Fig. 2*). In relation to dialysis time, scintigraphy changes were observed in four patients (4/5) of Group I and five patients (5/6) of Group II (*Fig. 3*).



Serum determinations of calcium and phosphorus.

FIG. 4



Determinations of alkaline phosphatase and parathyroid hormone.

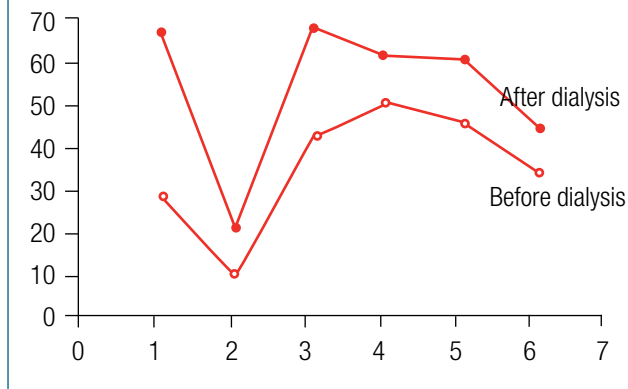
FIG. 5

Mean serum calcium for all patients was 9.48 mg/dl, and phosphorus was 4.9 mg/dl (*Fig. 4*). Mean serum alkaline phosphatase and PTH levels were also analyzed, and were 117 U and 196 mg/dl (*Fig. 5*), respectively.

Echocardiogram revealed the presence of cardiac amyloidosis in two of the ten patients (20%) who had been having haemodialysis for eleven and twelve years.

Mean serum levels of β_2M before dialysis session was 47.6 ± 4.45 mg/dL, compared to 50.5 ± 7.4 mg/dL after dialysis session (*Fig. 6*).

The presence of β_2 M amyloidosis was detected in the deep dermis of three patients (27.3%); two of them had been undergoing haemodialysis for only three years. No case of β_2 M amyloidosis in the abdominal fat was detected.



Determinations of b2 microglobulin before and after dialysis.

FIG. 6

Discussion

Based on the reported results, we would like to make a few comments and draw some conclusions.

The presence of CTS in our small sample showed a significant prevalence (36.4%) even in patients how had only been on dialysis for a short period of time. In fact, one of the cases had surgical decompression of the median nerve. However, no specimen was available for further study, therefore it was not possible to investigate the presence of β_2 M amyloidosis. CTS has been considered one of the major complications of patients with CRF undergoing haemodialysis and a strong relation seems to exist between the incidence of this syndrome and length of time on dialysis.[1,3,15] It is thought that amyloid are mainly deposited on small caliber vessels, however, the deposition is not circumferential, but segmental.[3]

A predominance of cervical spondyloarthropathy was observed in 66.7% of the patients. The prevalence of osteoarticular changes was also higher in patients in haemodialysis for more than eight years (83.3%). The presence of β_2 M amyloid deposits have been reported in some studies on arthropathy in patients in chronic haemodialysis.[17,18] Spondyloarthropathies affect the cervical column frequently, but not exclusively.[1] Multiple lesions may occur and are characterized by narrowing of the intervertebral spaces, and someti-

mes have very rapid evolution.[1] Some histological studies show that amyloids are deposited diffusely on the bone, causing specific areas to become more susceptible and, consequently, more predisposed to fractures.[19]

Osteoarticular scintigraphy revealed a preferential involvement of the small- and medium-sized joints. Scintigraphy has been considered a very useful technique for the diagnosis of dialysis-associated amyloidosis.[1,6] This test reveals a higher number of changes in patients on dialysis for longer time.[6] However, in our case series, no significant changes were observed between the two groups.

The serum determinations of alkaline phosphatase, PTH, calcium and phosphorus revealed levels considered as normal in this type of patients, allowing the exclusion of secondary hyperparathyroidism.

Cardiac amyloidosis was detected in two of the six patients in haemodialysis for more than eight years. Echocardiogram is also a good method of determining cardiac involvement in amyloidosis.[2]

The serum levels of β_2 M observed were far higher than in healthy individuals; a further increase was also observed after dialysis. This condition is similar to some retrospective studies that attribute the increase in the plasma levels of β_2 M to the use of poorly permeable membranes, such as the cuprophane membrane.[1,14,19]

β_2 M amyloidosis in the deep dermis was detected in three patients of our small case series; two of them had been in haemodialysis for three years. This fact is interesting since there seem to be a strong correlation between years on haemodialysis and the presence of β_2 M amyloidosis in the skin, which suggests a continuous deposition over time.[14] Simultaneously, β_2 M amyloidosis was not detected in abdominal fat. This may be justified by the fact that amyloid deposit occurs on interadipose septa and vascular walls, and is usually very hard to detect.[2,15] However, for many authors, this technique represents the best diagnostic method in the evaluation of systemic amyloidosis.[2] ■

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