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β_2-Microglobulin-Related Amyloidosis in Chronic Hemodialysis Patients

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Summary. **Amyloidosis, containing β_2-microglobulin (β_2-M), and osteoarthropathy are frequent complications of long-term hemodialysis, but the mechanism of its development is not completely known as yet. β_2-M is an amyloid protein, though other factors including calcium are greatly involved in the development of this disease. Regarding the treatment of dialysis amyloidosis, there has been progress in the development of a β_2-M adsorption column and the successful elimination of β_2-M from blood. Extensive studies should be performed to investigate the effect of antioxidants.**

This paper first reviews the current concepts concerning dialysis amyloidosis, secondly discusses the factors involved in the pathogenesis and finally, attempts to outline new perspectives of treatment for the disease.

INTRODUCTION

Carpal tunnel syndrome (CTS) associated with chronic hemodialysis was first described by Warren and Otieno (1975)[1]. Amyloid is responsible for the development of CTS, as amyloid deposits were recently detected in the synovium of the carpal tunnel[2]. In 1985, our research group[3] identified the principal component of this amyloid as β_2-microglobulin (β_2-M). Later, it was found that this amyloidosis invades mainly the synovial membrane of chronic hemodialysis patients to cause a unique osteoarthropathy. With the currently rising number of long-term hemodialysis patients, dialysis amyloidosis occurs at a noticeably high rate. The present review will deal with the clinical features and current problems of dialysis amyloidosis. Furthermore, the prospects of new therapy for the disease will be discussed.

CARPAL TUNNEL SYNDROME (CTS) AND BONE CYSTS

CTS is a neurological defect caused by compression of the median nerve at the wrist; it has been recognized as a common complication of long-term hemodialysis.

In order to determine the clinical aspect of dialysis amyloidosis, one should first analyze patients with CTS. So far our group[4] has performed operations for CTS in 69 hands of 55 cases among 376 hemodialysis patients and we could confirm amyloid deposition in all cases. Biochemical and immunohistochemical analyses indicated the amyloid as being β_2-M.

Following these findings, Linke et al.[5] analyzed kidney stones and demonstrated the amyloid fraction to be β_2-M and its fragments (Table 1). It has been clearly determined that β_2-M is amyloidogenic in hemodialysis patients.

Recently, the incidence of patients with CTS in bilateral hands is increasing. After the onset of CTS in one hand, it also occurs in the other hand within 3–5 years in most patients. The complication of snap-fingers is also seen. Since β_2-M is a major amyloid component, the most effective therapy is to remove the protein from the blood. A high-flux dialyzer with a high sieving coefficient for β_2-M, such as PMMA or PAN, is used in the treatment of patients with unilateral CTS. However, the onset of CTS in the other hand and the complication of snap-fingers are unavoidable at present.

It is also known that amyloid deposition on the synovial membrane causes the formation of bone cysts in the subchondral bone around the joint. This cyst formation was found to occur at an especially high incidence in the scaphoid and the lunate among the carpal bones. In our study, cystic radiolucencies

Table 1. Biochemical studies on the amyloid fibril proteins in dialysis amyloidosis

Authors (Ref.)	Patients and tissues analyzed	Results
Gejyo et al.[3] Jun. 1985	One patient with CTS; Synovial biopsy specimens	Purification of the protein by HPLC and identification of β_2-**M** with regard to the 16 N-terminal amino acids sequence
Gorevic et al.[6] Dec. 1985	One autopsy case; Bone tumor in left humeral head	Identification of β_2-**M** with regard to 20 and 30 N-terminal amino acid sequences
Linke et al.[5] Apr. 1986	Two patients; Urinary stones	Identification of two fragments homologous to β_2-**M**: one commenced with Ile at position 7 and the other with Ser at position 20, with a cleavage point subsequent to a lysyl residue
Gejyo et al.[7] Sep. 1986	Four cases of CTS; Synovial biopsy specimens	Identification of β_2-**M** with regard to 16, 15, 14 and 11 residues
Gorevic et al.[8] Oct. 1986	One autopsy case; Bone amyloid in humeral head	Identication of monomers, dimers, and higher polymers of intact β_2-**M** by immunoblot analysis
Linke et al.[9] Feb. 1987	One autopsy case; Synovial biopsy specimen	Identification of two major proteins of 12 and 24 K daltons with the same two sequences: one commencing with position 1 and the other with position 7 of β_2-**M**
Argiles et al.[10] May 1987	One patient with CTS; Synovial biopsy specimen	Identification of globin chains as well as β_2-**M**

CTS, carpal tunnel syndrome; HPLC, high pressure liquid chromatography

of the carpal bones were observed on roentgenograms in 86 (22.9%) out of 376 patients[11]. The incidence rose and the size increased for the duration of hemodialysis (Fig 1).

In one case, iliac bone grafting was performed as the treatment of the bone cyst in the supraacetabular region. It was found that the contents of the bone cyst were amyloid deposits originating from β_2-M. Similar bone cysts were identified in 6 patients. It is now clear that such bone cysts develop at a high incidence in the supraacetabular region, the head of the humerus and femur, which predispose the patients to spontaneous fractures.

DESTRUCTIVE SPONDYLOARTHROPATHY (DSA)

Recently a new type of spondyloarthropathy has been identified in hemodialysis patients: it is radiologically defined as a narrowing of joint spaces rapidly associated with subchondral bone erosion of adjacent vertebral plates without significant osteophytosis[12].

Our group had 16 patients with cervical vertebral lesions which accords with destructive spondyloarthropathy (DSA) (Table 2).

In magnetic resonance imaging (MRI) T1-weighted imaging showed a decrease in the signal from the vertebral body, while T2-weighted imaging showed a decrease in the signal from the vertebral pulp. Therefore, these findings are deemed useful not only for early diagnosis of the lesion but also for the exclusion of other diseases such as osteomyelitis.

In many cases, DSA appears very closely associated with the development of the β_2-M amyloid. Deposition of the β_2-M amyloid in the intervertebral disks has also been confirmed[13,14]. Even though the role of the amyloid deposits in the pathophysiology of DSA is still uncertain, it has been speculated that it is a clinical sign of dialysis amyloidosis.

DIAGNOSTIC CRITERIA

Originally, the term "amyloidosis" was a diagnostic name established on the basis of pathological findings. Therefore, this diagnostic name can only be used after a tissue specimen has been obtained from the lesion, and identified as Congophilia by Conge red staining, or proven an amyloid fibrils by electron microscopy.

As the clinical features of dialysis amyloidosis have become clearer, it has become obvious that, as shown in Table 3[15], a patient can be fairly definitely diagnosed. However, this diagnostic criteria should be revised in the future as the situation demands.

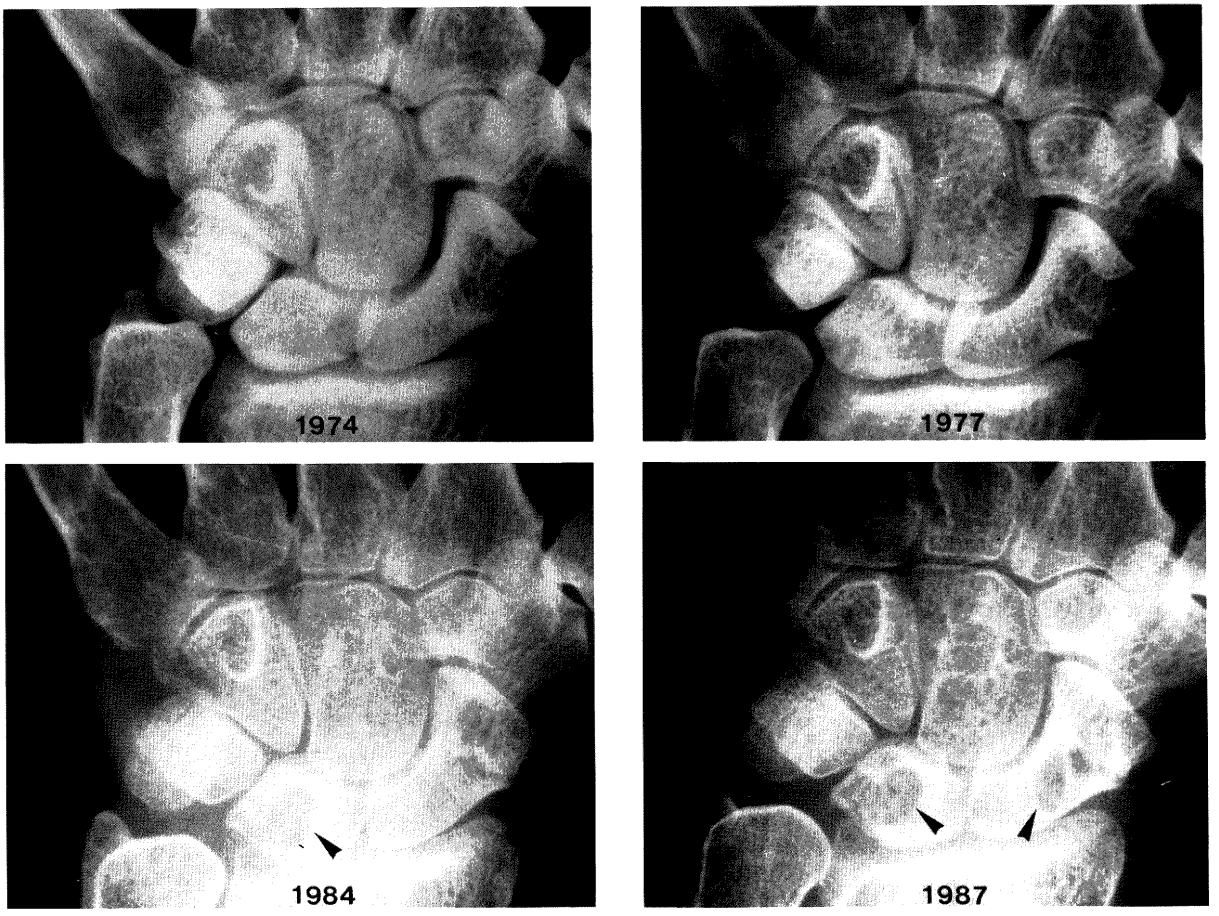

Fig. 1 Amyloid cysts in the carpal bones of a patient treated by hemodialysis. Sequential x-rays show an increase with time of the size and number of radiolucencies.

CONSTITUENTS OF AMYLOID DEPOSITS

Amyloid deposits related to dialysis are mainly composed of an insoluble fibrillar material which contains β_2-M. In addition, amyloid deposits are consistently associated with a glycoprotein known as amyloid P-component (AP), glycosaminoglycans, and some inorgans (Table 4). Recently Argiles et al.[16] have identified α_2-macroglobulin in the fibrillar material and suggested a possible role for α_2-macroglobulin: it may protect β_2-M from proteolytic digestion, leading to its accumulation in an intact form and amyloid fibril formation.

The different types of enzymatic degradation, and the structure of β_2-M fragments, along with the intact β_2-M in amyloid deposits, have been discussed[5-9]. Fig. 2 shows the results of HPLC patterns of β_2-M, the component protein of amyloid. We purified amyloid fibrils by a distilled water extraction method and then separated them using a C_{18}-bonded vinyl alcohol copolymer gel column by means of reverse-phase partition chromatography. The pattern of the amyloid protein prepared from the synovial membrane in carpal tunnel area was exactly in accord with that of native β_2-M, and the other components were few. On the other hand, the amyloid protein in a kidney stone was eluted as broad multipeaks. The amyloid protein of kidney stones was found to consist of more heterogeneous β_2-M than that of the synovial membrane, but the reason for this is unclear.

The mapping analysis of elements contained in amyloid deposits was performed using an electron probe microanalyzer[17]. This analysis revealed a co-deposition of calcium and amyloid in the same site, while neither iron nor aluminum was detected as sharing the same site with amyloid. Thus, a direct co-deposition of these elements can be excluded. There are some clinical cases of dialysis amyloidosis associated with aluminum-related bone disease and

Table 2. Clinical features of 16 HD patients with destructive spondyloarthropathy (DSA)

Cases	Age/Sex	Duration of HD (years)	DSA	CTS	Synovia amyloid	2°HPT*
1	51/F	15.2	C 5-C6	+	+	+
2	48/M	12.5	C 4-C 5	+	+	
3	43/M	7.7	C 5-C 6			
4	49/M	12.6	C 5-C 6			
5	56/M	11.4	C 4-C 5, C 5-C 6	+	+	
6	51/M	9.5	C 4-C 5			+
7	68/M	11.3	C 4-C 5			
8	58/M	9.7	C 5-C 6			+
9	65/F	9.1	C 3-C 4, C4-C 5, C 5-C 6	+	+	
10	31/F	11.3	C 5-C 6, C 6-C 7	+	+	
11	59/M	12.8	C 4-C 5, C 5-C 6, C 6-C 7	+	+	
12	60/F	3.7	C 2-C 3, C 3-C 4			
13	54/M	12.0	C 5-C 6			
14	53/F	6.0	C4-C 5, C 6-C 7			
15	60/F	10.7	C 5-C 6, C 6-C 7			
16	65/M	11.7	C 6-C 7			
Mean	54.4	10.5		38%	38%	19%

*2°HT: secondary hyperparathyroidism, C: cervical vertebra

Table 3. Diagnostic criteria for dialysis amyloidosis

Chief symptoms and signs

1) Carpal tunnel syndrome.
2) Trigger finger.
3) Bone cyst, juxta-articular cysts, cystic radiolucency on X-ray.
4) Destructive spondyloarthropaty.
5) Articular symptoms other than the above, eg. shoulder pain.
6) Pathological fracture.
7) Ischemic enterocolitis.
8) Others, eg. urinary stones, subcutaneous tumors.

Pathological findings

1) Congo red staining of tissue from the lesions is positive, or amyloid fibrils are revealed by electron microscopy, and amyloid deposition is confirmed.
2) Immunohistochemical coincidence of localization between amyloid and β_2-**M.**

Matters of reference

−Long dialysis history (more than 5 years)
−High levels of serum β_2-**M**
−For osteoarthropathic symptoms, diagnosis by exclusion of secondary hyperparathyroidism and aluminum-related bone disease.

Diagnostic criteria

"Probable" dialysis amyloidosis

1) Cases having more than two of the chief symptoms and signs 1)—5).
2) Cases having one of the chief symptoms and signs 1)—8) and the pathological finding 1).

"Definite" dialysis amyloidosis

1) A probable case 1) with the pathological findings 1) or 2).
2) A probable case 2) with the pathological finding 2).

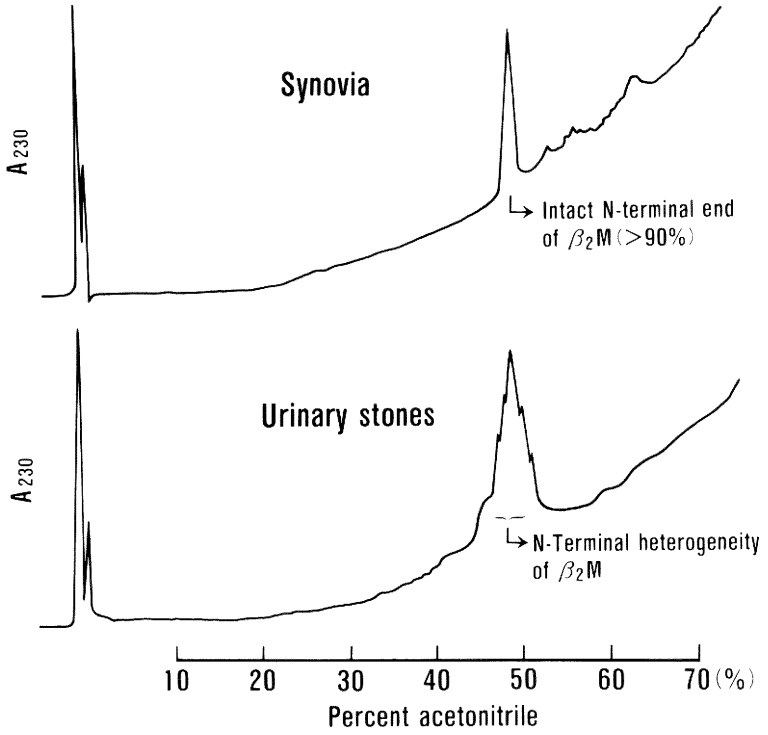

Fig. 2 Reverse-phase HPLC profiles of β_2-M amyloid fiblil proteins from synovia and urinary stones.

iron overload. In a mapping of an amyloid urinary stone, calcium was also present in the amyloid in layers around the central calcium oxalate. Accordingly, it is obvious that calcium is a principal factor associated with the amyloid.

At present it is not clearly known why hemodialysis patients develop amyloidosis. it would seem that the accumulation of β_2-M is the key point, and that amyloid deposition *in vivo* is a complex process involving many factors (Fig. 3).

NEW THERAPEUTIC APPROACHES

In the treatment of CTS, surgical decompression of the carpal tunnel, in which the flexor retinaculum is dissected, should be performed in the early stage of the disease. In the treatment of patients with severe arthritis, microscopic synovectomy is effective in alleviating pain.

At present it cannot be said that any basic therapy has been established. However, we believe it is advantageous to eliminate β_2-M as much as possible from blood (Table 5). Owing to the development of dialyzer membranes with excellent β_2-M-eliminating capacity, it has become possible to eliminate β_2-M by diffusion without depending on convection. A dialyzer which is capable of adsorbing β_2-M is also effective in eliminating β_2-M from the blood.

In addition to the development of these dialysis membranes, it has long been considered desirous to develop a column which is capable of selectively adsorbing only β_2-M. Recent progress in developing such columns is quite promising.

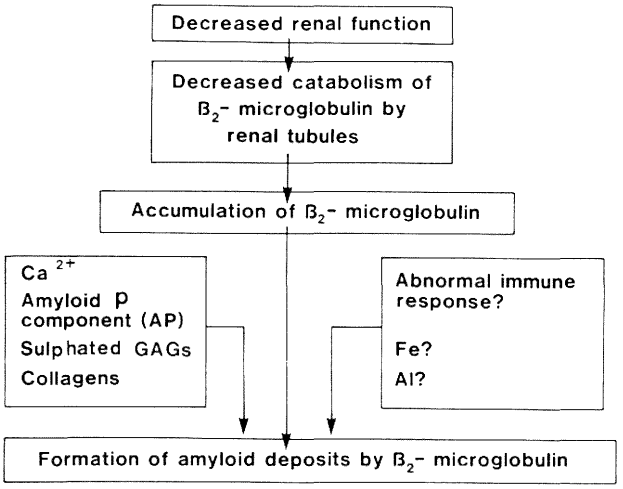

Fig. 3 Pathogenic mechanism of amyloid deposits by β_2-M in hemodialysis patients.

Table 4. Constituents of β_2-M-amyloid deposits

Amyloid fibril protein: β_2M
P-Component (glycoprotein) AP
Glycosaminoglycans: Heparan sulfate
Other mucopolysaccharides
Inorganics: calcium
aluminum?
iron?
α_2-Macroglobulin? (Argiles, A. et al.)[16]

Table 5. New methods for β_2-M removal

1. Highly permeable membranes
2. Hemoperfusion:
   - Antibody column
   - Adsorption column:
     - activated charcoal
     - MMA-DVB adsorption column[18]
     - PSt-MA adsorption column[19]
     - organic alkyl compound-ligand[20]

Recently Yokoyama et al.[19] estimated the molecular surface three-dimensional structure of β_2-M by means of computer graphics. They demonstrated that hydrophobic amino acids are gathered in the center of the molecule, and their hydrophobic residues are surrounded by basic residues to form domains.

Judging from this three-dimensional structure, the ligand which binds β_2-M was surmised to be a substance having hydrophobic acidic domains. On the basis of the theoretical background described above, they developed a new adsorbent in which polystyrene maleic acids are immobilized in porous particles. This adsorbent can adsorb 2.5 mg of β_2-M per milliliter. However, clinical applications have not yet been reported.

On the other hand, Furuyoshi's group[20] developed a new adsorbent which has an organic compound as the ligand: that is, they introduced alkyl chains on the surface of porous cellulose beads. Their adsorbent is reported to be able to adsorb 2.1mg of β_2-M per milliliter.

Using a combination of a high-flux membrane dialyzer and an adsorption column, it is now possible to eliminate β_2-M efficiently. Since we are in the preliminary stages, more time is necessary before we can draw any conclusion regarding the clinical efficacy of this system over long-term use.

Finaly let us refer to be the problem of free radicals in relation to treatment of CTS. Ishizaki et al .[21] proved that the ratio of serum methylguanidine to serum creatinine is high in patients with dialysis amyloidosis, and that their blood is in the state of advanced hyperoxidation. It was also found that administration of an antioxdant, such as alpha-tocopherol nicotinate and camostat mesilate, alleviates symptoms of the joints. In the future, antioxidants may be used as new supportive drugs in the treatment of dialysis amyloidosis.

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