Carpal tunnel syndrome caused by amyloid containing β_2 microglobulin: a new amyloid and a complication of long term haemodialysis

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SUMMARY Three patients receiving long term haemodialysis treatment for chronic renal failure due to non-amyloid nephropathy developed the carpal tunnel syndrome requiring decompression surgery. The excised material contained amyloid, which by immunocytochemical techniques was shown to contain β_2 microglobulin. This is, therefore, a new chemical form of amyloid whose deposition is likely to be the cause of osteoarticular and connective tissue disorders, which are being recognised with increasing frequency in patients receiving long term haemodialysis. Raised β_2 microglobulin levels are known to occur in chronic renal failure, and the molecule is unable to cross conventional dialysis membranes. The importance of β_2 microglobulin amyloidosis lies in the threat which it poses to the success of long term haemodialysis.

Key words: renal, failure, connective, tissue, disorder.

Recently there have been reports of bone, joint, and connective tissue problems arising in patients with chronic renal failure (CRF) who have been treated by intermittent haemodialysis for a long period of time. Charra *et al* reported that 38 of 52 patients receiving haemodialysis treatment for more than eight years for CRF not due to amyloid nephropathy developed a carpal tunnel syndrome (CTS).¹ The tissues excised at surgical decompression contained amyloid (shown by Congo red staining but not otherwise categorised). Ninety five per cent of these patients also had shoulder pain which was presumed, but not proved, to be due to amyloid deposition. Bergada *et al* also reported that 15 of 364 patients receiving long term haemodialysis treatment (4%) developed CTS, and 60% of these had histologically proved amyloid.²

In 1985 there were three further publications describing a total of 31 patients, of whom 29 had CTS.^{3–5} These and the remaining three had various combinations of pain, swelling, and effusion, affecting knee, shoulder, elbow, and ankle joints. In many of the patients with CTS decompression

specimens were shown to contain amyloid. Some patients were shown to have amyloid aggregates in synovial fluid, synovial membrane, and joint capsule. There were a number of instances of cystic radiolucencies of bone (cf. carpal bones, humeral and femoral heads, acetabula, and tibial plateaux), and these were again assumed but not proved to be due to the presence of amyloid. In four patients amyloid was also found in rectal biopsy specimens.

All cases occurred in individuals with CRF who had been treated by intermittent haemodialysis for a prolonged period of time, ranging from five to 17 years, with the lowest quoted average value being 8.6 years. In none of the cases was the CRF due to renal amyloidosis. Therefore, the syndrome was seen as a complication of long term haemodialysis and with increasing numbers of patients surviving long term haemodialysis likely to become an increasing problem. The finding of rectal amyloid in some cases is of sinister import, suggesting systemic involvement and the possibility of eventual multiorgan failure. It is, therefore, considered that the elucidation of the mechanism and prevention of amyloid deposition are urgently needed to safeguard the future of haemodialysis therapy.

The presence of amyloid was demonstrated in all cases by positive staining and typical birefringence with Congo red dye, which gave an apple-green

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birefringence when examined in polarised light. In some instances sections were pretreated with potassium permanganate (KMnO_4) and still reacted with Congo red giving a typical birefringence. This suggested the absence of AA amyloid and, at first sight, the presence of AL amyloid. Morita *et al*⁴ performed an immunocytochemical study of formalin fixed, paraffin processed material by the unlabelled antibody peroxidase-antiperoxidase (PAP) method of Sternberger⁶ using anti-AA antibody, anti-P component antibody, several anti-AL antibodies, and anti-prealbumin antibody. Despite the fact that the deposits still reacted with Congo red after treatment with KMnO_4 they did not react with the several anti-AL antibodies used. Morita *et al*, therefore, concluded that the material (which in common with all amyloids contained amyloid P component) also contained a previously undescribed type of amyloid.⁴

During the past several months we have had the opportunity of examining tissues taken at decompression surgery on three patients with chronic renal failure receiving long term haemodialysis treatment. Since amyloid was present in the three specimens we decided to make a detailed immunocytochemical study of the material. We were also aware that Gejyo *et al* in June 1985 had reported the chemical extraction of β_2 microglobulin from amyloid deposits in a typical case,⁷ and therefore we also sought this substance in our specimens. This report describes our findings.

Patients and methods

Three patients receiving long term haemodialysis treatment (Table 1) for chronic renal failure due to non-amyloid nephropathy developed carpal tunnel syndrome (CTS) requiring surgical decompression. Material from each was available for histopathological study. The tissues were fixed overnight in 10% phosphate buffered formalin, dehydrated in graded alcohols, cleared in xylene, and embedded in paraffin wax. Sections were cut at 6 μm and mounted on slides coated with poly-L-lysine. Initially, sections were stained with haematoxylin and eosin (H & E) and alkaline Congo red,⁸ with and

without pretreatment with potassium permanganate (KMnO_4).⁹

Immunocytochemical studies of sections were also made with the peroxidase-antiperoxidase method (PAP).⁶ The primary antisera used were goat antihuman amyloid A component (Ig fraction) antibody (Calbiochem Ltd) and goat antihuman serum P component antibody, both to dilutions of 1:500 (Atlantic Antibodies Ltd); rabbit antihuman β_2 microglobulin antibody, rabbit antihuman α_2 macroglobulin antibody, and rabbit antihuman prealbumin antibody, all to dilutions of 1:1000 (Dako); rabbit antihuman λ free light chains and rabbit antihuman κ free light chains, both in dilutions of 1:2000 and 1:4000 (Dakopatts); rabbit antihuman IgG, IgM, and IgA with secretory component (Dakopatts). The secondary antisera were rabbit antigoat IgG (Miles-Feda) and swine antirabbit IgG (Dako). The tertiary antisera were goat PAP (Sigma) and rabbit PAP (Dakopatts). For the reactions involving the use of anti- κ and anti- λ light chain antibodies the sections were pretreated with trypsin (0.1% in 0.1% calcium chloride at pH 7.8) for 20 minutes at 37°C. Sections from liver, kidney, and spleen from known cases of primary and secondary amyloidosis and from the heart of a senile cardiac patient with amyloidosis were included in the staining procedures as controls.

Results

The results of these investigations are given in Table 2. In the patients with carpal tunnel syndrome the material available for study consisted of tenosynovium in all cases and, in addition in patient No 3, a portion of collagenous ligament. The deposits observed in all cases were densely eosinophilic and disposed as small rounded and elongated structures in the stroma of the tenosynovium and ligament (Fig. 1). Only in patient No 1 was there a cellular reaction to the deposits; this consisted of macrophages and a few multinucleated giant cells (macrophage polykaryons). All deposits stained with alkaline Congo red (ACR) and all showed apple-green birefringence in polarised light. This combination is denoted as + in the ACR column of Table 2.

Table 1 Details of patients with carpal tunnel syndrome caused by amyloid deposition

Patient No	Age	Gender	Renal disease	Time receiving haemodialysis treatment (years)	Carpal tunnel syndrome
1	32	Male	Alport's syndrome	15	Right side
2	58	Female	Polycystic disease	9	Right side
3	44	Male	Chronic renal failure—cause unknown	17	Left side

Table 2 Histological and immunohistochemical reactions of amyloid deposits in the carpal tunnel decompression material

Patient No	Histology (H & E)	ACR*	KMnO ₄ + ACR*	AA*	P*	β ₂ M*	α ₂ M*	L Chains _λ *	L Chains _κ *	IgA*	IgG*	IgM*
1	Nodule in tenosynovium	+	+	-	+	+	-	-	-	-	-	-
2	Tenosynovium	+	+	-	+	+	-	-	-	-	-	-
3	Tenosynovium	+	+	-	+	+	-	-	-	-	-	-
	Carpal ligament	+	+	-	+	+	-	-	-	-	-	-

ACR=alkaline Congo red; KMnO₄ + ACR=pretreatment of sections with potassium permanganate before staining with ACR; AA=amyloid A; P=P component; β₂M=β₂ microglobulin; α₂M=α₂ macroglobulin; L chains=light chains; IgA, G, M=immunoglobulin classes A, G, and M.

Fig. 1 Amyloid deposits (A) in the tenosynovium of patient No 1. A macrophage polykaryon is arrowed (H & E).

Fig. 2 Positive immunohistochemical reactions for β₂ microglobulin are arrowed. This is material from patient No 2. (PAP).

Fig. 3 Positive immunohistochemical reactions for β₂ microglobulin are arrowed. Material from patient No 3. (PAP).

Positive Congo red staining with apple-green birefringence in polarised light after treatment of the section with KMnO_4 is also denoted as + in the KMnO_4 + ACR column of Table 2.

Positive immunocytochemical reactions were only recorded if they were observed in definitive amyloid foci. In some instances reactions were observed outside such deposits. An example was the α_2 macroglobulin reaction product, which was often observed in the lumina and endothelial lining of blood vessels but never in the amyloid deposits. Obvious prominent reactions are denoted as +. The amyloid deposit in patient No 1 is illustrated in Fig. 1, and the positive reactions for $\beta_2\text{M}$ in patients 2 and 3 are shown in Figs 2 and 3.

In the control sections positive reactions for amyloid A were noted in patients with secondary amyloidosis. The senile cardiac patient with amyloidosis showed a positive reaction for prealbumin. Positive reactions for κ and λ light chains were observed in the myocardium, renal blood vessels, and renal glomeruli of a patient with primary amyloidosis. No other immunoglobulin reactivity was observed. None of the control sections stained for β_2 microglobulin.

Discussion

Amyloidosis is a condition characterised by the extracellular deposition of insoluble fibrillar proteins collectively termed amyloid ('starch-like'). This misnomer was originated by Virchow because the staining reaction of amyloid with iodine was similar to that of cellulose. Amyloid is best recognised histologically by its ability to combine with Congo red dye and, in combination, to show an apple-green birefringence when examined in polarised light. Amyloid proteins have a characteristic fibrillar ultrastructure and give a cross β pattern on x ray diffraction. This indicates a characteristic physicochemical structure (β pleated sheeting), which is probably the fundamental feature of amyloid proteins and determines their physicochemical properties. In the present study all the deposits considered to be amyloid stained with Congo red dye and gave the characteristic birefringence.

Early classifications of amyloidosis recognised primary and secondary varieties, with the latter being associated with chronic inflammatory diseases and there being differences in the pattern of organ involvement in what were essentially systemic disorders. A third systemic amyloidosis type is hereditary and found in familial amyloid polynuropathy. In addition to these systemic amyloidoses there are a number of localised forms. Thus amyloid

may be present in medullary carcinoma of the thyroid and in the pancreatic islets of Langerhans. Deposits probably associated with aging are found in the heart and brain, particularly in Alzheimer's type dementia.

Useful as these classifications have been they are not entirely satisfactory and a more useful and rational classification is emerging based on a knowledge of the chemical composition of the amyloid deposits.¹⁰ Thus the primary and myeloma associated forms of amyloid are composed of whole or portions of immunoglobulin light chains and thus are labelled AL. Amyloid secondary to chronic inflammatory or infectious disease contains a protein amyloid A or AA. This is related to a serum protein (SAA), which is an acute phase reactant. Amyloid in the hereditary syndromes has been shown to contain prealbumin,¹¹ as have the deposits in the brain tissue of Alzheimer's presenile dementia and senile dementia of Alzheimer type.¹² Prealbumin is also present in senile cardiac amyloid. Medullary carcinoma of thyroid may have amyloid deposits containing calcitonin or a precalcitonin molecule.¹³

Our studies have shown the presence of an amyloid in the carpal tenosynovium excised from three patients undergoing long term haemodialysis. The observed material reacted with Congo red, both with and without pretreatment with KMnO_4 . The abolition of Congo red staining by KMnO_4 may be interpreted as showing that the amyloid is AA. Failure of KMnO_4 to abolish the reaction is conventionally regarded as a property of AL amyloid. P Component was present in all cases, and this is a frequent feature of amyloid deposits. There was no evidence of immunoglobulin components, either light or heavy chains, and prealbumin was absent. The outstandingly positive results were obtained in sections treated with antibody against β_2 microglobulin.

Since we believe that our results truly indicate the presence of β_2 microglobulin and that this is the major constituent of a new form of amyloid, several points must be considered. Firstly, the PAP method applied to formalin fixed, paraffin embedded tissues has been used previously in the elucidation of the chemical composition of amyloid deposits and is considered to be a sensitive and accurate technique in this context.^{4 14} Secondly, the chemical demonstration of β_2 microglobulin in deposits by Gejyo *et al*⁷ tends to validate our results. Thirdly, our observations on the reactions with KMnO_4 and the absence of demonstrable immunoglobulin light chain products suggest that failure of pretreatment with KMnO_4 to abolish the Congo red staining

reaction may no longer be considered exclusively indicative of the presence of AL amyloid but may also be due to the presence of β_2 microglobulin. Additionally, and very importantly, these observations also indicate that if it is accepted that the β_2 microglobulin is responsible for the Congo red staining and the typical birefringence then it must be in a form which has the physicochemical properties of an amyloid.

It is now appropriate to consider the nature of β_2 microglobulin and whether there are logical reasons for its deposition as an amyloid in the cases which we and others have described. β_2 Microglobulin is a small protein (mol. wt 12 000) which is present in normal biological fluids.¹⁵ Its protein nature would allow it to undergo β pleating to give the appropriate physical properties of an amyloid. It is increased in the serum of patients with diminished renal function. It is particularly increased in the serum of patients undergoing long term haemodialysis, presumably because it will not pass through conventional dialysis membranes.^{16 17}

Therefore there is nothing illogical in β_2 microglobulin forming an amyloid as a consequence of long term haemodialysis. In a true sense it is a new amyloid capable of identification in tissue sections. It seems appropriate that the classification of amyloidosis should now be based on the characterisation of the constituent proteins. Therefore in line with the symbols for other established amyloid proteins we propose that β_2 microglobulin amyloid be designated as amyloid A β_2 M.

The recognition of this new amyloidosis is of academic and immense practical importance. It is a new threatening disease for patients undergoing long term haemodialysis. Treatment should be directed towards reduction of the β_2 microglobulin burden of these patients, and recent studies in our department have shown that this can be achieved by the use of hiflux dialysis membranes.

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