Case report

Synovial amyloid in chronic haemodialysis contains β_2 microglobulin

F E BRUCKNER,¹ MARGARET BURKE,² R S PEREIRA,³ A J EISINGER,⁴ M BENDING,⁴ J KWAN,⁴ A K OSMAN,¹ AND B WATSON³

From the ¹Department of Rheumatology, St George's Hospital, London; the Departments of ²Histopathology and ³Immunology, St George's Hospital Medical School, London; and the ⁴Renal Unit, St Helier Hospital, Carshalton, Surrey

SUMMARY A case of amyloid arthropathy occurring in a patient receiving long term chronic haemodialysis treatment is reported. He was found to have raised serum β_2 microglobulin (β_2 M), and β_2 M was detected in the synovial amyloid deposits.

One of the complications of long term chronic haemodialysis is amyloid arthropathy.¹⁻³ We describe a patient, in whom β_2 microglobulin (β_2 M) was found in the synovial amyloid deposits.

Case report

The patient was a 38 year old man who was in chronic renal failure from chronic glomerulonephritis of unknown cause. He had received regular haemodialysis treatment for 18 years using a cuprophane haemodialysis membrane. He had had four unsuccessful renal transplants during that time. A total parathyroidectomy was performed in 1982. He had a left total hip replacement in 1980 and a right total hip replacement in 1981. Both carpal tunnels were decompressed in 1985 for carpal tunnel syndrome, but no histology was performed. Over the last 18 months he gradually developed pain, swelling, and stiffness of the shoulders, wrists, left thumb, knees, and left ankle.

Clinically there was swelling, pain, and restriction of all these joints with effusions in the knees and shoulders. There were no rheumatoid nodules or gouty tophi. The erythrocyte sedimentation rate was 10 mm/h, and tests for rheumatoid factor and antinuclear antibodies were negative. Serum uric acid, calcium, and phosphate were normal. Serum β_2 microglobulin on two occasions was 44 and 46 mg/l (Pharmacia RIA — normal up to 3 mg/l).

Synovial fluid from the right knee contained scanty cells and no crystals. An x ray examination showed a cyst 1 cm in diameter in the head of the right fibula suggestive of amyloid osteoarthropathy.⁴ At arthroscopy of the right knee, large fronds were seen and these areas were biopsied.

Materials and methods

METHODS

The tissue was fixed in 10% phosphate buffered formalin, processed, and embedded in paraffin wax. Sections 5 μ m thick were stained with Mayer's haematoxylin-eosin and with Congo red, the latter with and without pretreatment of the sections with potassium permanganate.⁵

Immunohistochemical staining was performed on paraffin sections using the peroxidase-antiperoxidase method of Sternberger⁶ for polyclonal antibodies and with an indirect immunoperoxidase method for monoclonal antibodies.⁷ The reaction was developed using diaminobenzidine and hydrogen peroxide. Polyclonal antibodies to β_2 M (Dacopatts Ltd) were diluted 1:200 in TRIS buffered saline (pH 7.4). Monoclonal antibodies to κ and λ light chains (Unipath Ltd) were used at a dilution of 1:1000. All sections were incubated with the primary antisera for one hour at room temperature.

CONTROLS

Sections of tonsil were used as positive controls. Specificity of staining was checked by omitting the primary antibody in both test and control sections.

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Correspondence to Dr F E Bruckner, Department of Rheumatology, St George's Hospital, Blackshaw Road, London SW17 0QT.

Fig. 1 Deposition of amyloid beneath the synovium (a) showing red-green birefringence on polarisation (b). This was reduced by prior treatment of the tissue with potassium permanganate. (Congo red).

and by screening cases of amyloid deposition in the kidney in myeloma and in rheumatoid arthritis, and also in Crohn's disease of the large intestine and medullary carcinoma of the thyroid.

ELECTRON MICROSCOPY

Tissue was fixed in 4% glutaraldehyde/I M cacodylate buffer, with secondary fixation in 1% osmium tetroxide, followed by dehydration and embedding in Araldite. Thin sections (0.5 μ m) were examined on a Zeiss 109 transmission electron microscope.

Results

The synovium contained focal stromal amyloid, as demonstrated by congophilia with red-green birefringence under polarised light (Fig. 1). Immunohistochemical staining for β_2 M was positive in the test section (Fig. 2) and negative in all the other cases of amyloid. Immunoglobulin light chains were not detected in any of the specimens. Amyloid deposition was confirmed ultrastructurally by the presence of rigid non-branching fibrils 10–12 nm in diameter (Fig. 3).

Discussion

Our findings, using immunohistochemical staining, confirm those of Gejyo *et al*⁸ and Gorevic *et al*,⁹ who isolated β_2 M biochemically from amyloid in their two patients with amyloid arthropathy complicating chronic haemodialysis. Similar findings have also been reported by Casey *et al*¹⁰ in a further two patients receiving long term haemodialysis who developed β_2 M-rich amyloid deposits in bone. Ultrastructurally, both Gorevic *et al*⁹ and Casey *et al*¹⁰ observed that the amyloid fibrils had an unusual curvilinear configuration. This was not seen in our case, however, which conforms to the conventional ultrastructural appearances of amyloid, described as 'fine, non-branching fibrils 8–10 nm in diameter and up to 1 μ m in length, arranged randomly'.¹¹ Further evidence for the amyloidogenic properties of β_2 M has been illustrated by the in vitro formation of amyloid from intact β_2 M,¹² which perhaps is not surprising in view of its biochemical similarity to the constant regions of immunoglobulin light and heavy chains.^{13 14}

There have been several reports of raised levels of

Fig. 2 Immunoperoxidase staining of synovium showing that the stromal deposit of amyloid contains β_2 microglobulin (arrow), which is not present in adjacent tissue. (Peroxidase-antiperoxidase).

Fig. 3 Ultrastructural appearances of amyloid showing straight non-branching fibrils approximately 10–12 nm in diameter.

serum β_2 M in patients receiving long term haemodialysis.^{15–17} This appears to correlate with the use of cuprophane dialysis membranes, and β_2 M levels fall during polyacrylonitrile dialysis. Similar high levels of serum β_2 M have been found in patients receiving chronic ambulatory peritoneal dialysis treatment.¹⁷ Amyloid arthropathy has not, to date, been reported in these cases, though this may merely reflect the shorter duration of their treatment. It remains to be seen if lowering serum β_2 M alone, by changing the dialysis technique, is sufficient to prevent amyloid deposition.

We have demonstrated β_2 M in synovial amyloid as opposed to amyloid in tissues removed at the time of carpal tunnel decompression. Its immunohistochemical detection in paraffin sections is simple, reproducible, and specific, with the additional advantage that retrospective studies on stored material may be performed.

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