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Localised foot and ankle amyloid deposition

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ABSTRACT

Background: Localised (transthyretin-associated) amyloid is commonly seen in articular/periarticular tissues of elderly individuals. Whether age-associated, amyloid deposition occurs in foot and ankle (F&A) tissues has not previously been investigated. In this study we assessed the nature and frequency of F&A amyloid deposition and determined whether it is associated with age and/or specific articular/periarticular F&A lesions.

Methods: Histological sections of twenty five normal F&A articular/periarticular tissues (16–71 years) and a range of F&A lesions were stained by Congo Red. The amyloid protein was identified by immunohistochemistry and type of matrix glycosaminoglycans determined by Alcian Blue (critical electrolyte concentration) histochemistry.

Results: Amyloid deposits were found in the joint cartilage and capsule of 3/25 normal specimens (57, 62 and 78 years). Amyloid deposits were small, contained transthyretin, and found in areas of matrix degeneration associated with the presence of highly sulphated glycosaminoglycans. In patients older than 47 years, small amyloid deposits were noted in some F&A lesions, including osteoarthritis, Charcot arthropathy, bursa, ganglion, chondrocalcinosis, gout, calcific tendonitis and Achilles tendonitis.

Conclusion: Small localised amyloid deposits in F&A tissues contain transthyretin and occur in areas of matrix degeneration associated with the presence of highly sulphated glycosaminoglycans; these deposits are age-associated and, although seen more commonly in some F&A lesions, are small and unlikely to be of pathogenic significance.

1. Introduction

Amyloid is a fibrillary material which is formed when a number of different proteins adopt a β-pleated sheet structure [1,2]. Amyloid disease is classified on the basis of the type of protein found in the amyloid deposits with over 30 different precursor proteins identified [1]. Abnormal folding of these proteins lead to extracellular tissue deposition of amyloid fibrils which, depending on the nature of the amyloid protein, the tissue location and amount of extracellular amyloid deposited, may or may not result in clinical manifestations.

Localised deposition of amyloid in articular and periarticular tissues of hip, knee, wrist, finger, shoulder, spine and other joints has been recognised by investigators for many years [3–12]. This localised amyloid deposition in joint tissue has been shown to be age-associated and, although disputed by some investigators, has not been directly linked with osteoarthritis (OA) [4,7,9,11,13–15]. It has, however, been noted to be commonly associated with deposits of pyrophosphate crystals in knee articular cartilage and other joint tissues [4,12,16]. Wild-type transthyretin, the amyloid protein found in age-associated (senile) amyloidosis, has been identified in localised amyloid deposits in articular and periarticular tissues of leg, knee, wrist and spinal joints [17–22].

Amyloid deposition has only previously been reported in ankle joint tissues in the context of (AL-associated) systemic and (beta-2 microglobin-associated) dialysis-related amyloidosis as well as (mutant transthyretin-associated) familial amyloid polyneuropathy [23–28]. In this investigation, we have sought to determine whether localised amyloid deposits occurs in foot and ankle (F&A) tissues and whether these deposits are age-associated and present in specific F&A lesions. In addition, we have aimed to determine the nature of the amyloid protein and the type of matrix glycosaminoglycans (GAGs) found in F&A amyloid deposits.

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Table 1
F&A lesions analysed for the presence of amyloid.

F&A lesions	Number of cases analysed	Number of cases with amyloid
Bursa	6	1
Ganglion	5	1
Achilles tendonitis	5	1
Calcific tendonitis	5	1
Gout	6	2
Chondrocalcinosis	6	3
Osteoarthtitis ankle joint	7	1
Hallux valgus	6	1[a]
Hallux rigidus	3	0
Charcot arthropathy	4	1
Rheumatoid arthritis	4	0
Rheumatoid nodule	5	0
Enchondroma	3	0
Periosteal chondroma	2	0
Synovial chondromatosis	2	0
Subungual exostosis	6	0
Morton's neuroma	8	0
Plantar fibromatosis	3	0
Plantar fasciitis	1	0
Pyogenic infection	4	0
Fracture non-union	4	0
Angioleiomyoma	6	0
Tenosynovial giant cell tumour	2	0

[a] This case also showed evidence of osteoarthritis.

2. Materials and methods

2.1. Cases studied and specimen analysed

Samples of histologically normal articular cartilage and capsule/synovium of the ankle, and first metatarsophalangeal joint, A

chilles tendon, and plantar fascia were obtained from ten above-knee amputations undertaken for bone /soft tissue neoplasia (6 females and 4 males; age range 16–78 years). In addition, samples of normal ankle joint capsule derived from a further 15 above-knee amputations cases (age range 19–52 years) were analysed for the presence of amyloid. A wide range of F&A lesions (Table 1), obtained from the files of the Nuffield Orthopaedic Centre, Histopathology Department, Oxford were also assessed for the presence of amyloid. The age of the patients at the time of specimen sampling was noted. None of the patients from which normal or lesional F&A tissue was obtained had a family history of amyloid disease or clinically showed features of systemic amyloid disease or familial amyloid polyneuropathy/cardiomyopathy.

2.2. Congo red staining

Specimens were fixed in 10% formalin overnight, routinely processed and 5 µm sections of paraffin-embedded tissue stained with alkaline Congo red to determine the presence of amyloid [4,29]. This involved: taking the sections to water, staining the nuclei in with Mayer's haematoxylin (Pyramid Innovation, UK), and then differentiating in 2% sodium hydrogen carbonate (VWR, UK). The sections were then treated with a stock alkaline chloride solution (VWR, UK) for 20 min, stained with alkaline Congo red (VWR, UK) for 20 min, before dehydration in alcohol, clearing in xylene and mounting on glass slides.

2.3. Immunohistochemistry

In cases where amyloid was identified by Congo Red staining, the nature of the amyloid protein component in the deposits was determined by immunohistochemistry with polyclonal antibodies directed against the amyloid-associated proteins -2 microglobulin, immunoglobin light chains, transthyretin, and P component, and with a monoclonal antibody (MC1) directed against amyloid A protein. Details of antibodies used are shown in Table 2. Sections were incubated at 37 °C for at least 24 h to improve tissue adhesion before immunohistochemistry. Tissue sections were dewaxed in xylene and rehydrated by successive immersion in graded alcohol and water. Endogenous peroxidase was blocked by treating the sections with 0.2% hydrogen peroxide (VWR, UK) in 80% alcohol for 30 min. Details of antigen retrieval for antibodies used in this study are shown in Table 2. Immunohistochemistry was performed using an indirect immunoperoxidase technique with 3, 3-diaminobenzidine chromogen (ChemMate Envision, Dako, UK). Sections were incubated with primary antibodies for 30 min at room temperature with labelled polymer and 10 min in 3,3-diaminobenzidene. The sections were then counterstained using Mayers haematoxylin for 3 min and blued in 2% hydrogen sodium carbonate (VWR, UK).

2.4. Mucin immunohistochemistry

The Alcian blue (pH5.8), magnesium chloride (MgCl2) critical electrolyte concentration (CEC) technique was employed to indicate the presence and nature of matrix glycosaminoglycans (GAGs) present in the tissues examined [3,6,29–31]. Sections (5 m) were stained for 18 h in solutions of 0.1% Alcian blue 8GX in 0.25 M acetate buffer (pH 5.8) with MgCl2 added at the following concentrations: 0.06 M, 0.3 M and 0.7 M. In general, both unsulphated GAGs and polycarboxylated GAGs do not bind Alcian blue in situ. At concentrations, higher than 0.2 M MgCl2 there is persistent staining for sulphated GAGs and at 0.7 M MgCl2 only highly sulphated GAGs, such as heparan sulphate and keratan sulphate, are stained.

3. Results

3.1. Age distribution and nature of amyloid deposits in F&A tissues

Localised amyloid deposition, as revealed by Congo red staining (with apple-green birefringence on polarisation microscopy), was identified in articular/periarticular F&A tissues derived from amputation specimens of three cases who were aged 57, 62 and 78 years (Figs. 1a, b and 2a, b). Focal amyloid deposition was seen in collagenous connective tissue of the first metatarsophalangeal joint capsule in one case and hyaline articular cartilage and capsular tissue of the ankle joint in two cases. In these cases, the amyloid deposits were small and not associated with an inflammatory reaction in oedematous, partly degenerate connective tissue. Other sampled tissues in these cases were negative for amyloid. Amyloid was not identified in F&A tissues from

Table 2
Details of anti-amyloid protein antibodies used in this study.

Antibody specificity	Supplier	Dilution	Pre-treatment	Incubation Time
Transthyretin	Leica (UK)	1:200	Trypsin	60 min
Amyloid A	Dako/Aligent (UK)	1:400	None	30 min
Amyloid P component	Dako/Aligent (UK)	1:700	Trypsin	30 min
Beta-2 microglobulin	Dako/Aligent (UK)	1:4000	None	30 min
Kappa light chains	Dako/Aligent (UK)	1:20,000	Microwave [(Tris buffer EDTA (pH 8.5)]	30 min
Lambda light chains	Dako/Aligent (UK)	1:20,000	Microwave [(Tris buffer EDTA (pH 8.5)]	30 min