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Amyloid Deposits in Senile Vertebral Arteries, Immunohistological and Ultrastructural Findings

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Abstract: In a study on amyloid deposits in vertebral arteries, many elderly patients showed amyloid deposits in the perivascular tissue. These proved to be senile systemic amyloidosis of the transthyretin-type by immunohistochemistry. Amyloid deposits were also found in the arterial wall. These intramural amyloid deposits showed significant affinity to elastic material of the arterial wall. The intramural amyloid deposits did not react with any of the known or available antibodies to amyloid subtypes. Only a polyclonal antibody to human elastin could mark this type of amyloid. It may therefore be assumed that the precursor protein of this amyloid is derived from elastin molecules. By electron microscopy, the light microscopic amyloid deposits were of fibrillary structure, typical for amyloid with a direct contact to elastic material.

Key words: Amyloid, transthyretin-type, elastin, immunohistochemistry, fibrillary structure

INTRODUCTION

Research on amyloid dates back to the beginning of early pathologic and patho-biochemical medical research. This protein complex with a rigid, non-ramified microfibril structure cannot be found in a healthy mammalian organism (Teng *et al*., 2001). Thus, under pathological conditions, it is deposited locally or generally, in the extracellular tissue (Shin *et al*., 2007). To date, many different types of amyloid have been identified. They have been categorised according to their distribution pattern in many different organs, to the associated basic disease or, more recently, according to their different proteins. All these deposits have in common a specific secondary biophysical structure (β-pleated sheet-structure), which gives amyloid its proteinase resistance and consequently its pathogenicity as well as its diagnostic recognition.

Amyloid deposits increase with age, though these deposits are not limited to a single type of amyloid but simultaneously form progressive multiple types of amyloid (Del Mercato *et al*., 2008). The vertebral artery is one of the sites of the human body so little examined under this aspect. In comparison to other arteries, the vertebral artery plays a special role owing to its anatomical and physiological characteristics. Due to its hidden location in the lateral processes of the cervical spinal column, its complicated course with reserve loops and its difficult accessibility render routine examination in the course of pathological or forensic autopsies extremely cumbersome. Its presentation demands considerably intensified preparatory efforts, which might be a reason why this artery is only rarely examined. When searching for mural cerebral β-A4-amyloid in intracranial vertebral arteries amyloid has often been found in the arterial wall. The deposition of β-A4-amyloid in intracranial vertebral arteries causes the major variety of cerebral amyloid angiopathy (Shin *et al*., 2007; Zekry *et al*., 2003). But immunohistological examination showed that this is not of the senile cerebral amyloid type. Subsequent systematic examinations revealed that this amyloid is an amyloidosis of the extra-and intracranial parts of the vertebral artery, frequently found with increasing age and of a different protein type.

MATERIALS AND METHODS

In our study, 61 autopsy cases (30 female, 31 male) between 1983 and 1999 have been examined to prove the presence of amyloid in vertebral arteries. The age range was between 50 and 97 years. Vertebral arteries, from pars transversalis to the craniospinal transition were dissected

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out of cervical vertebral columns, isolated and fixed in formalin. These arteries were embedded in paraffin. To demonstrate the existence of amyloid, the Puchtler technique of Congo-red staining was used Khurana *et al*. (2001). Furthermore, an EvG-preparation was produced parallel to the Congo-red one to determine the precise location of the deposited intramural amyloid.

To assess sensibility, several Congo-red preparations were first treated with potassium permanganate. The preparations were analysed with a light-and polarization-microscope. The amyloid was immunohistologically classified with the APAAP-method by using mono-or polyclonal antibodies to amyloid A, β-A4-protein and transthyretin-type amyloid as well as amyloid of β-2-microglobuline origin (Table 1). Additionally, two more antibodies to human elastin and actin were used. For immunohistological subtyping of the amyloid, a serial Congo-red section was employed in all cases to identify

Table 1: Antibodies used in this study

Antibody	Clone	Isotype	Host	Dilution	Source/Product N.
Anti-AA	mc1	IgG2a	Mouse	1:1000	Dako/M0759
Anti-AB	P	IgG	Rabbit	1:2000	Dako/A072
Anti-ATTR	P	IgG	Rabbit	1:2000	Dako/A0002
Anti-β-A4	6F/3D	IgG1	Mouse	1:400	Dako/M872
Anti-HEL	P	IgG	Rabbit	1:300	Biogenesis/4060-1054
Anti-HAC	1A4	IgG2a	Mouse	1:200	Dako/M0851

Incubation time: over night, incubation temperature: room temperature P = Polyclonal, AA = Amyloid A, AB = Amyloid of β-2-microglobuline origin, HAC = Human actin, β-A4 = β-A4-protein ATTR = Transthyretintype amyloid, HEL = Human elastin

clearly amyloid as the reaction product. Conventional methods were used to provide electron-micrographs of amyloid in a vertebral artery fixed in glutaraldehyde and embedded in Epon, of an 80-year-old patient, to demonstrate the fibrillar structure of the mural amyloid and its location with regard to the elastic fibres.

RESULTS

Older patients showed amyloid deposits more often in the perivascular tissue and in the arterial wall (Fig. 1). Furthermore, the cranial sections of this artery, almost

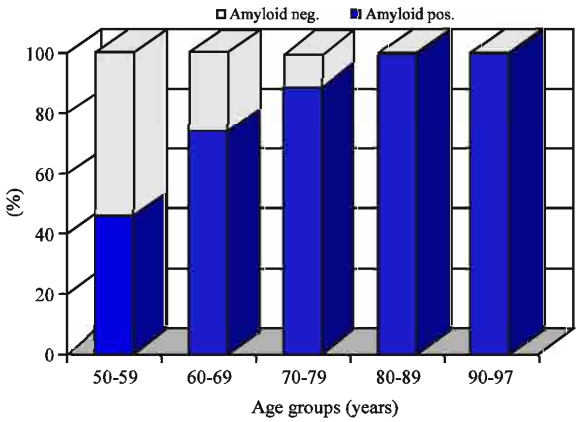

Fig. 1: Age dependency of amyloid deposits in the vertebral artery (n = 61)

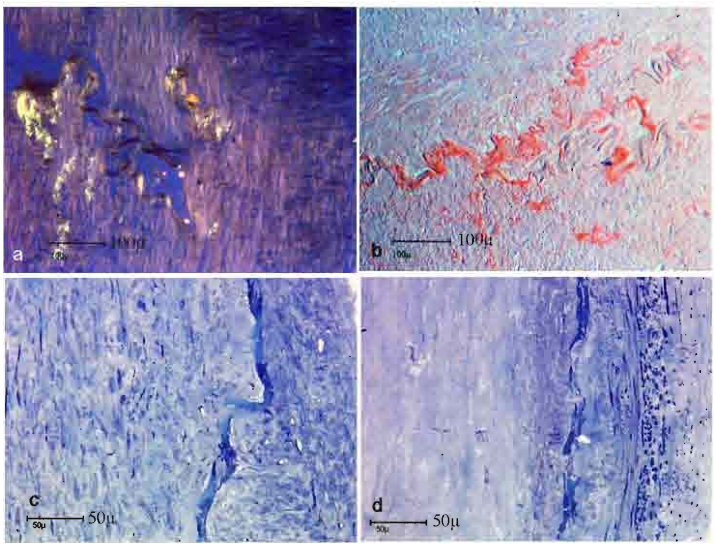

Fig. 2: Elastin-associated amyloid deposits in vertebral arteries, (a) Typical birefringence of amyloid deposits in polarised light after Congo-red staining, (b) Positive immunohistochemical reaction of amyloid deposits with an antibody to human elastin, (c and d) Close affinity of amyloid deposits to internal elastic membrane of the vertebral artery. Epon embedding, semithin section, staining according to Richardson

consisting of the muscular type and being exposed to a higher mechanical stress, had also accumulated amyloid deposits more frequently. Related to the different basic diseases of the patients, only cardiovascular diseases were significantly related to the amyloid deposits. In 55.7% of the amyloid-positive cases, cardio-vascular diseases were known from past history or the patho-anatomic diagnosis, respectively. Men and women were equally affected by amyloid deposits.

In this study, amyloid was located at two different sites: perivascular and/or intramural. Apart from one case with generalised AL-amyloidosis, all other cases examined showed that the perivascular amyloid was of the transthyretin-type. According to Wright, this type of amyloid was potassium permanganate-resistant.

The intramural amyloid was always located close to the lamina elastica interna of the muscular type or in the tunica media of the elastic type of the vertebral artery (Fig. 2). This amyloid could not be classified with available antibodies to amyloid-subtypes. Since both parts of the vertebral artery wall contain a large quantity of elastic material this type of amyloid showed high affinity to elastic fibres suggesting derivation from components of elastin. To verify this assumption, several selected cases were treated with a polyclonal antibody to human elastin. Surprisingly, this antibody, appropriately diluted, only reacted with elastin close to amyloid deposits (Fig. 2b). A monoclonal antibody to human actin excluded origin of this amyloid from smooth muscle cells of the tunica media. This suggests that this type of amyloid is derived rather from the elastic material. By electron-microscopy, the fibrillary structure of this intramural amyloid as well as its contact to elastic fibres could be demonstrated (Fig. 3). The elastin-associated amyloid also showed to be resistant to potassium permanganate according to Wright.

DISCUSSION

The antibody to transthyretin-type amyloid used in our study normally reacts with transthyretin of sporadic senile cardiovascular amyloidosis (ATTR) as well as of autosomal-dominantly inherited amyloidosis (AF) with polyneuropathy. Ruling out familial amyloid polyneuropathy among our cases it can be suspected that the perivascular amyloid deposits are indeed the cardiovascular amyloid of the transthyretin-type found at old age. This systemic senile amyloidosis is not related to any underlying disease. It is mainly found in heart, lungs, aorta, joint capsules and rectum (Ueda *et al*., 2006; Hobbs *et al*., 2004; Cornwell *et al*., 1995) Cardiovascular amyloid of the transthyretin-type was ubiquitously distributed as smaller deposits in the perivascular tissue of vertebral arteries.

Clinical significance of the mural elastin-type amyloid is unknown (Jacob *et al*., 2007; Meng *et al*., 2006). Narrowing of the vascular lumen in the examined sections was not encountered. These age-dependent elastin-associated intramural amyloid deposits however could have led to an increased vulnerability and fragility (e.g., tears, hemorrhage, defects) of the vessel wall. Due to their location, i.e., at the lamina elastica interna, they could cause the damage of the intima as well as of the media.

Whether the elastin-associated amyloid can only be found in the wall of the vertebral arteries in old age or whether other arteries of the body also show similar local intramural amyloid deposits can not yet be answered and needs further systematic examination (Bochicchio *et al*., 2007; Zekry *et al*., 2003) But since this type of amyloid was increasingly found in the cranial parts of the vertebral artery, especially in the cranio-spinal transitional region where the artery is exposed to higher mechanical stress,

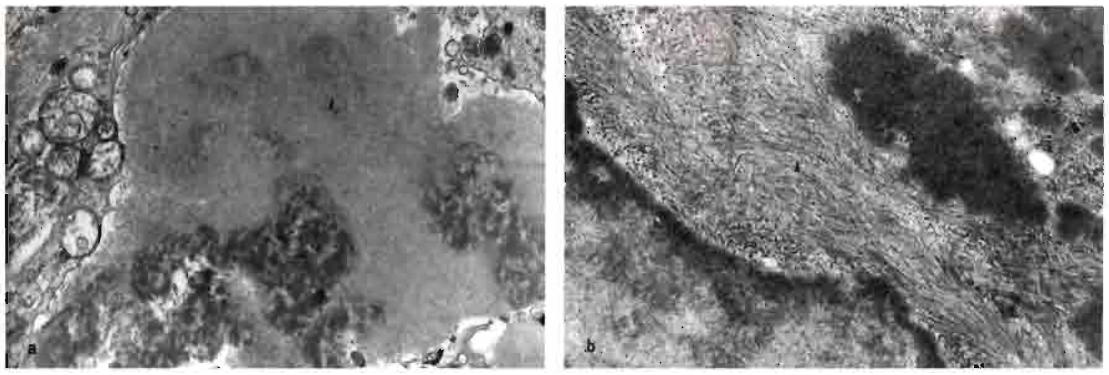

Fig. 3: Electron-micrographs of amyloid in a vertebral artery. Typical haphazardly arranged fibrils (arrow head) with a direct contact to elastic material (arrow), Magnification a: 28.000x. b: 70.000x

mechanical stress may play a role in the pathogenesis and the anatomical transitional zone between the two types of vertebral arteries may be a locus for predilection.

To which extent these intramural elastin-associated or elastin-dependent amyloid deposits, which are due to still unknown (Ostuni *et al*., 2007; Shah *et al*., 2002). Local pathomechanisms, induce a mediolytic arteriopathy or aneurysma formation of the vessel wall, is still subject to research (Peng *et al*., 2007).

Whether other tissues, i.e., elastic ligaments or elastic cartilage tissue, may also be affected by deposition of local elastin-associated amyloid of old age remains unclear and needs further systematic examination.

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