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Incidence and characterization of age related amyloid deposits in the human anterior pituitary gland*

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Summary. To identify amyloid deposits in the anterior pituitary gland, we have immunohistochemical, histochemical and alkaline Congo red staining. The anti-human P component reacted positively with these amyloid deposits, while antisera against prealbumin, AA type amyloid fibril protein and various anterior pituitary hormones were negative. A combination of Congo red and anti-human P component staining was most sensitive and reliable for detection of amyloid in the anterior pituitary glands of 300 randomly autopsied patients. Amyloid deposits increased in parallel with the age of the patients, however, they appeared earlier and more frequently than heretofore reported. Deposition of amyloid was seen initially in the 3rd decade and the positivity rate of amyloid deposits was 73% in the 5th decade. The histochemical characteristics of these pituitary amyloid deposits differed from those of cerebral and systemic deposits, particularly those found in the amyloid of senile systemic amyloidosis.

Key words: Pituitary Gland – Amyloid – Amyloid P Component – Immunohistochemistry – Aging

Introduction

Amyloid accumulates in endocrine tissues secreting peptide hormones (Wright et al. 1969; Pearse et al. 1972; Westermark 1977). In the anterior lobe of the pituitary gland, amyloid deposits are sometimes detected in aged persons (Ravid et al. 1967; Saeger et al. 1983; Störkel et al. 1983). Recent studies using the Congo red stain disclosed that the positivity rate of amyloid is 27.6% to 49% in people in the 7th decade (Saeger et al. 1983; Störkel et al. 1983) and thus amyloid deposition in the anterior pituitary gland is thought to be a senile form. We surveyed the relationship between aging and amyloid deposits of anterior pituitary gland, as well as the type of amyloid deposit, using immunohistochemical and histochemical methods.

Materials and methods

The pituitary glands from 300 autopsies were examined (Table 2). All cases with systemic amyloidosis were excluded. The tissues were fixed in 10% formalin for over 1 week, embedded in paraffin, and cut into 5 μm sections.

All sections were stained with haematoxylin-eosin, and alkaline Congo red (Puchtler et al. 1962). In some cases, sections were also stained with thioflavin T, and periodic acid Schiff (PAS). Sections stained with Congo red were examined by polarized microscopy and those stained with thioflavin T were analyzed by fluorescent microscopy.

All sections were stained for demonstration of the amyloid P component (1:1000), using the immunohistochemical method. The sections were neighboring serial sections to the Congo red stained sections. Anti-human P component was purchased from DAKO (Denmark).

To characterize the type of amyloid, eight cases with many amyloid deposits (Grade II) were stained with the following antisera.

(a) Antiserum against prealbumin (1:1000) (Kitamoto et al. 1986)

(b) Antiserum against AA type amyloid (1:2000) (Kitamoto et al. 1987)

(c) Antibodies against pituitary hormones (LH, FSH, GH, ACTH, prolactin, TSH) were purchased from DAKO (Denmark). Avidin-Biotin system reagents were purchased from Vector Laboratories (Vectastain, Burlingame, C.A USA), and PAP system reagents from DAKO. Using these antisera and reagents, the ABC method (for antisera against human P component, prealbumin and AA type amyloid fibril protein) of Hsu et al. (1981) and the PAP method (for antibodies against pituitary hormones) of Sternberger et al. (1970) were used.

The potassium permanganate method (Wright et al. 1977). Autoclave, and Alkaline-Guanidine methods (Kitamoto et al.

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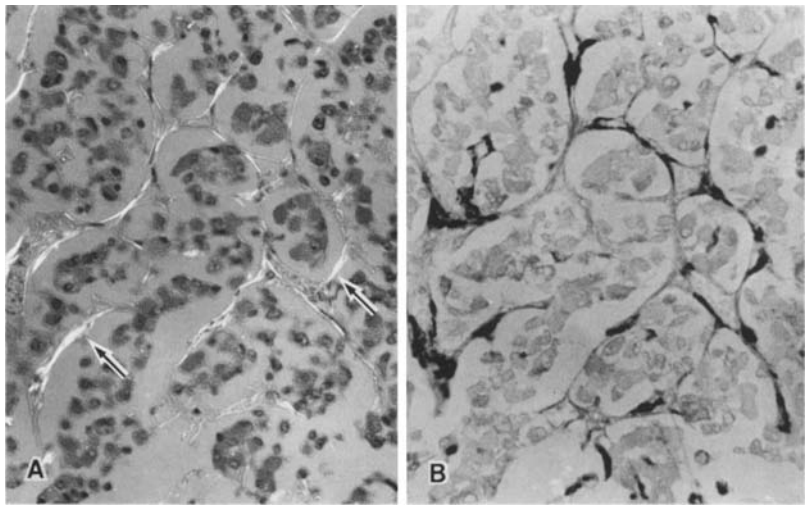

Fig. 1. Amyloid deposits in anterior pituitary glands. **a** Bright white foci represent green birefringence (*arrows*). Wispy birefringent material represents collagen. (alkaline Congo red, polarized light × 207). **b** Anti-human P component antiserum reacted positively with amyloid deposits in serial sections of the pituitary gland. (× 207)

1985; Tashima et al. 1986) were used. Briefly, after deparaffinization, tissue sections were heated in an autoclave or incubated with guanidine hydrochloride, for various periods of time. After cooling or washing with tap water, the sections were stained with Congo red and examined by polarized microscopy.

The presence of amyloid deposits was confirmed by green birefringence after Congo red stain and immunohistochemical positivity for anti-human P component using neighboring serial sections. A semi quantitative evaluation of the amyloid deposits into three grades which Störkel et al. used was applied. *Grade 0*: No amyloid deposition was recognized in the serial sections stained with Congo red or anti-human P component. *Grade I*: Slight amyloid deposition (a single or a few isolated amyloid deposits stained with both histochemical and immunohistochemical methods). *Grade II:* Pronounced amyloid deposition (reticular deposition surrounding the glandular cells).

Table 1. Staining characteristics of amyloid deposits in the human pituitary gland

Immunohistochemical methods		Result
Anti-P component		Positive
Anti prealbumin		Negative
Anti AA		Negative
Anti anterior pituitary hormones		Negative
Histochemical approaches		
Congo red-birefringence		Positive
Thioflavine T		Positive
Potassium permanganate method		Resistant
Autoclaving (130° C, 30 min)		Resistant
(130° C, 120 min)		Sensitive
Alkaline Guanidine method	(1 min)	Resistant
	(120 min)	Sensitive

Results

Amyloid deposits were found in various portions in the vascular walls and connective tissue stroma between the cell groups of the anterior lobe of the pituitary gland. These deposits were slightly eosinophilic and slightly positive with PAS and Congo red stain, by light microscopy. They fluoresced brilliant yellow after staining with thioflavine T and showed a green birefringence after staining with Congo red (Fig. 1a). The amyloid deposits which showed a green birefringence were usually stained positively after immunohistochemical staining for the anti-human P component (Fig. 1b). Antisera against prealbumin, AA type amyloid fibril protein and pituitary hormones of the anterior lobe did not stain the pituitary amyloid deposits (Table 1).

Amyloid deposits in the pituitary gland were resistant to potassium permanganate treatment. In the autoclave treatment, the amyloid deposits showed a green birefringence after heating at 130° C for 30 min. When the duration of the treatment was prolonged to 120 min, the amyloid deposits lost the green birefringence. In alkaline guanidine treatment for one minute, the amyloid deposits showed a green birefringence. When the duration of the treatment was prolonged to 120 min, the amyloid deposits lost green birefringence (Table 1).

The presence of amyloid deposits was confirmed by green birefringence after Congo red stain and immunohistochemical positivity for anti human P component. The combination of immunohistochemical methods using the anti human P component and alkaline Congo red stain confirmed the presence of amyloid in 155 of 300 cases. As seen in Fig. 2 and the Table 2 the number of cases increased in parallel with age of the patient. The deposition of amyloid appeared first in the 3rd decade of life. Statistical significane was evaluated using the chi square (χ^2) test compairing with

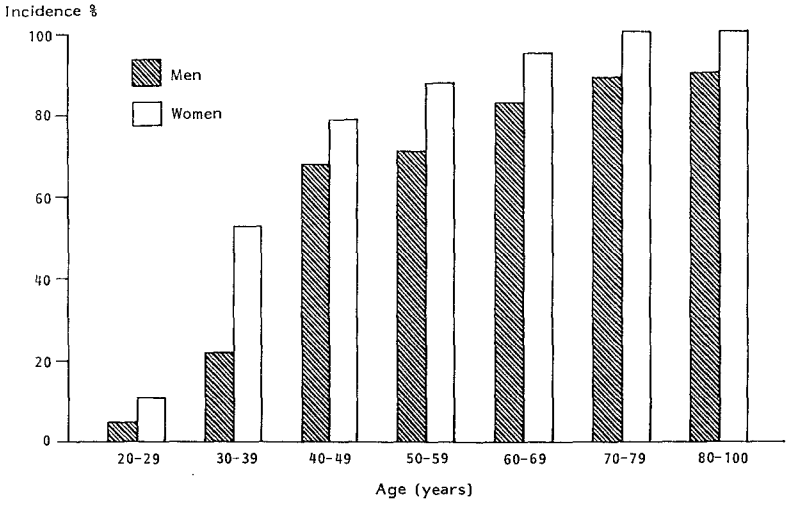

Fig. 2. Incidence of the amyloid deposits in relation to the age. Deposition of amyloid in the pituitary gland was detected initially in 3rd decade, and the deposits increased in parallel with aging. The incidence was higher in women than in men, in all decades

Table 2. Incidence of amyloid deposits in the human pituitary gland in relation to age groups and sex

Age (year)	Men				Women				Total			
	Total	Amyloid deposits			Total	Amyloid deposits			Total	Amyloid deposits		
	n	GII	GI	Total (%)	*n*	GII	GI	Total (%)	*n*	GII	GI	Total (%)
0– 19	34	0	0	0 (0)	22	0	0	0 (0)	56	0	0	0 (0)
20– 29	20	0	1	1 (5)	19	0	2	2 (11)	39	0	3	3 (8)
30– 39	23	1	4	5 (22)	19	0	10	10 (53)	42	1	14	15 (36)
40– 49	19	3	10	13 (68)	14	5	6	11 (79)	33	8	16	24 (73)
50– 59	28	8	12	20 (71)	8	3	4	7 (88)	36	11	16	27 (75)
60– 69	24	12	8	20 (83)	19	14	4	18 (95)	43	26	12	38 (88)
70– 79	18	15	1	16 (89)	11	11	0	11 (100)	29	26	1	27 (93)
80–100	10	7	2	9 (90)	12	12	0	12 (100)	22	19	2	21 (96)
Total	176	46	38	84 (48)	124	45	26	61 (57)	300	91	64	155 (52)

GI: Grade I; GII: Grade II

Table 3. Statistical analysis of the incidence of amyloid deposits in the human pituitary gland

	Age				
	0–19	20–39	40–59	60–79	80–100
n	56	81	69	72	22
G I	0 (0%)	17 (21%)	32 (46%)*	13 (18%)	2 (9%)
G II	0 (0%)	1 (1%)	19 (28%)**	52 (72%)*	19 (86%)*
total	0 (0%)	18 (22%)	51 (74%)*	65 (90%)**	21 (95%)***

* $p<0.001$ significant
** $p<0.001$ not significant, but $p<0.005$ significant
*** $p<0.005$ not significant

There was significant increased proportion of cases compairing with a younger subject group

a younger subject group (Table 3). There was a significant increase ($p<0.001$) of the total incidence of amyloid deposits in middle age (40–60), but a low or non-significant change over 60. However Grade II deposition increased in the over 60's ($p<0.001$). The incidence was higher in women than in men, but this difference was not significant statistically.

Discussion

In this study, amyloid deposits of the anterior pituitary glands were strongly positive for the amyloid P component. The amyloid P component is present in almost all forms of amyloid deposits, regardless of the chemical nature of the fibrils and the clinical type (Holck et al. 1979; Husby et al. 1981). Westermark et al. (1981) reported that it is present in amyloid deposits not only of all types of systemic amyloidosis but also in different types of localized amyloidosis. Our present report seems to be the first to show the presence of the amyloid P component in the anterior pituitary gland. The quantity of amyloid deposits in the pituitary gland was rarely sufficient to permit detection by routine light microscopic study. A combination of the immunoperoxidase method using anti P component and alkaline Congo red stain with neighbouring serial sections is a most reliable and sensitive tool for identifying amyloid deposits. The feature of this series showed a surprisingly earlier deposition and higher incidence of amyloid depositions than was noted in previous reports. Amyloid deposits appeared in the older population (Störkel et al. 1983; Saeger et al. 1983). We noted the deposition after the 3rd decade of life and a high frequency was found in those over middle age. The incidence of amyloid deposits in the pituitary gland reached a plateau about 6th decade, but the amount of the amyloid deposits increased with age.

In pituitary adenomas, an unusual accumulation of amyloid was sometimes evident (Bilbao et al. 1975; Mori et al. 1985; Kubota et al. 1986). In particular, the amyloid deposits in prolactinoma or growth hormone (GH) producing adenoma showed variable reactions to antibodies for prolactin or GH (Mori et al. 1985; Kubota et al. 1986). Immunohistochemical reaction to antibody for the amyloid P component was not done. The morphology of the amyloid deposits in the normal pituitary gland differs from that of amyloid deposits in these adenomas.

As to cerebral amyloid deposition, senile plaques and Congophilic angiopathy are found in aged persons, and in large amounts in patients with Alzheimer's disease. These amyloids do not stain with the amyloid P component (Westermark et al. 1982), and maintain Congophilia after heating at 130° C for 120 min (Kitamoto et al. 1985) and dipping in alkaline guanidine for 120 min (Tashima et al. 1986). Therefore, amyloid deposits in the anterior pituitary gland differ from those in cerebral tissues.

As the antisera against AA type and prealbumin type amyloid fibril proteins showed a negative reaction, deposits in the pituitary gland differed from AA type amyloidosis and familial amyloid polyneuropathy (FAP) and senile systemic amyloidosis (SSA). In visceral organs of aged persons, amyloid deposits of SSA were occasionally evident (Pitkänen et al. 1984). Amyloid fibril protein of SSA (Protein/ASc1) is closely related to prealbumin, and reacted positively with antiprealbumin antiserum (Sletten et al. 1980; Cornwell III et al. 1981) but did not lose Congophilia after autoclaving (Kitamoto et al. 1986). In the present study, the amyloid deposits did not react positively with antiprealbumin antiserum, and lost the Congophilia after autoclave treatment. Moreover the amyloid began to deposit after the 3rd decade of life. Therefore, there are clear differences between age related pituitary amyloid and SSA or senile amyloidosis. In case of autoclave treatment and alkaline guanidine treatment, amyloid deposits in the pituitary gland were similar to AL type amyloid fibril protein. The AL type amyloidosis belongs to the primary systemic form or is associated with myeloma and amyloid deposits throughout the body (Cohen et al. 1983). Patients with systemic amyloidosis were excluded from this study. The physico-chemical properties of the amyloid fibril protein in the anterior pituitary gland differ from those of the above mentioned systemic and cerebral amyloidosis.

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