

Analysis of mast cells, melanophages and keratin deposition in the primary localized cutaneous amyloidosis

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Objective: To analyse the presence of mast cells, melanophages and keratin deposition on dermis with cases of cutaneous amyloidosis and to compare with normal skin biopsy.

Study design and methods: Primary localized cutaneous amyloidosis (PLCA) classifies into three major types: macular, lichen and nodular. Lichen amyloidosis (LA) and macular amyloidosis (MA) are clinically and histologically distinct varieties. Chronic pruritis, epidermal degeneration and degenerated epithelial cells are thought to contribute to the etiopathogenesis. We have reviewed the clinical and histopathological findings and differential diagnosis of 19 cases of LA and 5 cases of MA. The patients were clinically evaluated and biopsy procedures were performed. Paraffin sections were stained with hematoxylin-eosin, crystal violet, Congo red, toluidin blue, Masson-Fontana, PAS and anti-pankeratin antibody. Dermal and epidermal findings were examined under light microscopy.

Results: Marked hyperkeratosis, keratinocyte degeneration, liquefaction degeneration of the basal layer, superficial perivascular infiltration of mononuclear cells were present in LA cases while these findings were slighter in MA cases. With PAS stain, degeneration of the basal membrane was observed and with crystal violet and Congo red stains amyloid deposits were demonstrated at the papillary dermis. Number of mast cells and melanophages accompanying these findings were also detected. Immunohistochemically, dermal keratin was demonstrated with using pankeratin antibody. Keratin deposits frequently found in papillary dermis.

Conclusions: As a result, clinically suspicious PLCA cases are discussed with morphological and histochemical methods and it is emphasized that sequential biopsies should be taken in selected cases in the differential diagnosis of LA and MA.

Keywords: Cutaneous, lichen, macular, amyloidosis

Introduction

Primary localized cutaneous amyloidosis (PLCA) is defined as cutaneous amyloid deposition in the absence of any other systemic or dermatological disease. It may be classified into three major types: macular, lichen and nodular. Nodular type is a rarely seen form that is characterized with the development of single or multiple nodules, most commonly involving the trunk or limbs. It results from a localized plasma cell dyscrasia¹.

Lichen amyloidosis (LA) and macular amyloidosis (MA) are clinically and histologically distinct varieties that are rarely encountered in clinical practice. In the lichenoid form, discrete, firm and hyperkeratotic papules are seen on the anterior aspect of the shins and the extensor surfaces of the forearms. Sometimes these hyperpigmented papules may coalesce into plaques. Intense pruritis is invariably present. MA is a pruritic eruption of small dusky brown or greyish pigmented macules distributed typically in a symmetric fashion on the upper aspect of

the back, limbs, and occasionally chest and buttocks, which may have a characteristic rippled or reticulated pattern and which tend to persist unchanged for many years. Sometimes hyperpigmented macules 2-3 mm in diameter may coalesce into larger confluent lesions. LA and MA have characteristic epidemiological profiles. MA is commoner in Central and South America, India and the Middle East, whereas LA is more common among the Chinese.^{1,2,3}

One proposed mechanism in the etiopathogenesis of LA and MA is that degenerating keratinocytes drop off into the papillary dermis where they are transformed into "amyloid bodies" in an unknown way. Degeneration induced by prolonged scratching. It is also proposed that amyloid fibril formation begin to form at the basal cells.^{4,5,6} Histopathologically, epidermal hyperplasia, hypergranulosis, irregular rete ridges, orthokeratosis, liquefaction degeneration of the basal layer, limited amyloid deposition in the dermal papilla, mild to moderate perivascular lymphocytic infiltration are seen.^{5,6,7,8,9}

Materials and methods

In our study, 19 LA and 5 MA cases that were examined in the Department of Dermatology at the University of Çukurova and got the clinical diagnosis of PLCA were evaluated in department of pathology according to their morphological and clinical characteristics. Systemic, hereditary, secondary amyloidosis and lymphoproliferative diseases were excluded with clinical and histopathologic findings.

Formalin-fixed, paraffin-embedded skin biopsy specimens were stained with hematoxylin-eosin, crystal violet, Congo red, toluidin blue, masson fontana, PAS and anti-pankeratin antibody and examined under light microscopy. The number of mast cells was determined in 10 high power field (x400 HPF). Ten normal skin biopsy (uninvolved skin part of breast carcinoma cases) were used for control group. Toluidin blue and Masson-Fontana stain were performed also on control group and number of mast cells and dispersion of melanophages in the skin were evaluated. Number of mast cells in the control and patient groups were compared statistically. For the dispersion of mast cell numbers were different, Mann-

Whitney-U test was used. P value less than 0.05 was considered as significant.

Immunohistochemical studies by anti-pankeratin were performed on 24 cases. Four-µm thick sections were prepared and anti-pankeratin (Neomarker; 1/100 dilution) were performed with strept-avidin-biotin complex method for immunohistochemical stains. The distribution of keratin bodies was examined in the lesions of LA and MA. We have scored as + to +++ for the presence and intensity of keratin deposits in the papillary or reticular dermis.

Results

Ten of 19 LA cases were men, nine were women. One of the MA cases was man and four were women. Their mean age was 42.3 (with an age range of 25-76 years) in LA cases and 37.8 (with an age range of 22-48 years) in MA cases. In the LA lesions the most common location was the extremities (78.9% cases) and in the MA lesions, however, it was neck (%100 cases) (Table 1).

The main clinical presentation was pruritis (79.1% cases) (Table 1) and hyperpigmented macular or papular-like lesions. It was reported that pruritis was generally elevated in summer. In the physical inspection and laboratory tests of the patients no other sign of a systemic disease was detected.

On histopathologic examination of the biopsies; hyperkeratosis, hypergranulosis, irregular acanthosis, liquefaction degeneration of the basal layer, deposits of homogeneous eosinophilic globules in the papillary dermis, superficial perivascular lymphocytic infiltration were observed (Figure 1). Histochemical staining of the homogeneous eosinophilic globules in the papillary dermis was positive for crystal violet and Congo red stains (Figure 2). Apple-green birefringence on Congo red stain of amyloid was demonstrated under polarized light. With PAS stain, in 14 of the LA cases (73.6%) and 4 of the MA cases (80%) mild-moderate disruption of continuity and vacuolar degeneration of the basal layer was observed. When they were compared with the 10 normal skin biopsies (3-4 melanophages x40 HPF) marked increase of the melanophages (16-20 melanophages x40 HPF) was obtained with the masson-fontana stain (Figure 3). The 24 patient and 10 normal cases were inspected

Table 1: Clinical features of PLCA cases and distribution of keratin deposits on dermis.

Patient #	Patient protocol #	PLCA type	Age	Sex**	Localization of the lesion	Pruritis	Distribution of keratin*
1	3537/97	LA	40	M	Lower legs	+	PD+
2	6492/97	LA	40	F	Anterior aspects of the shins	+	PD++
3	9526/97	MA	48	F	Back	+	PD+
4	9887/97	LA	33	M	Groin, back, anterior aspects of the shins	–	PD+
5	15426/97	MA	38	M	Back, limbs, arms	+	PD+
6	15683/98	LA	32	M	Lomber region, arms, back, shoulder	+	–
7	17053/98	LA	32	F	All body	–	PD++
8	5131/99	LA	43	F	Anterior aspects of the shins	+	RD+
9	5741/99	LA	34	M	All body, extremities	+	RD+
10	15861/99	LA	25	M	Anterior aspects of the shins, lateral parts of the forearms	+	PD++
11	11165/00	LA	33	F	All body	+	PD+++ RD+
12	17929/00	MA	46	F	Back of the neck, forearms	–	PD+ RD+
13	577/01	MA	35	F	Back	–	PD+
14	4750/01	LA	40	M	Lateral part of the right tibia	+	–
15	9272/01	LA	41	M	Extremities	+	PD++ RD++
16	14619/01	LA	46	M	Anterior aspects of the shins	+	PD+
17	809/02	LA	76	F	Back	+	RD+
18	4937/03	LA	43	M	Back, hairy skin	+	PD+
19	5806/03	LA	48	F	Back, extremities	+	PD++
20	12281/03	LA	74	F	Back	+	PD+ RD+
21	12293/03	LA	35	M	Back	–	PD+++
22	12975/03	MA	22	F	All body	+	PD+++ RD+
23	13540/03	LA	44	F	Anterior aspects of the shins	+	PD+ RD+
24	17163/03	LA	45	F	Trunk, extremities	+	PD+

*Presence and intensity were scored as + to +++ in the papillary (PD) or reticular dermis (RD)

** M: Male; F: Female

according to their mast cell numbers with toluidin blue stain (10 x 40 HPF). The median mast cell number was 9.25 in patient group while it was 5 in the control group. In the Mann Whitney U test p value was 0.018. The increase in mast cell numbers was statistically meaningful.

With immunohistochemical method; amyloid deposition on dermis was revealed positive reaction to anti-pankeratin except two cases (Figure 4). Results were shown in Table 1. The intensity and diffuseness of the +++ positivity was accepted as similar to that of the epidermal basal layer.

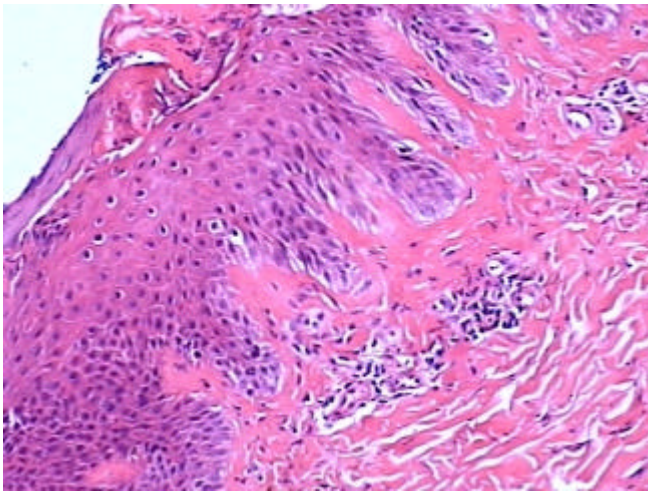


Figure 1. Hyperkeratosis, hypergranulosis, irregular acanthosis, liquefaction degeneration of the basal layer, and superficial perivascular lymphocytic infiltration were observed in biopsies from cutaneous amyloidosis cases (Hematoxylin Eosin x 400).

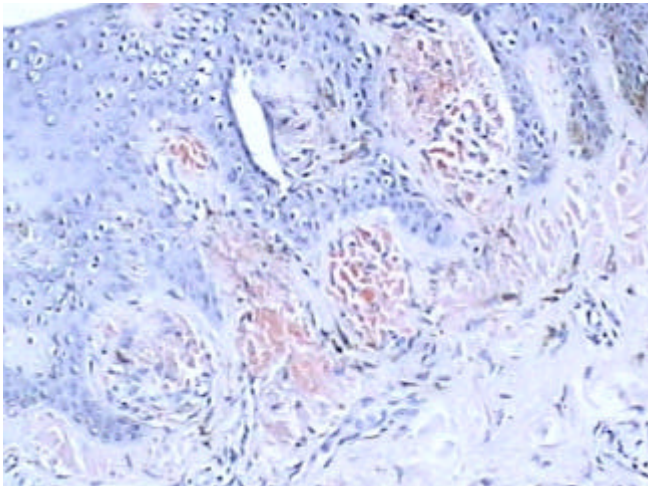


Figure 2. Amyloid deposition on the dermis is determined as orange-red colour with congo-red stain (Congo red x 100).

Discussion

Amyloid deposits may occur not only in the skin like PLCA but in several other organs in systemic amyloidosis (SA).¹ In SA, amyloid deposits may be found deep dermis, in small blood vessels, nerves and around adnexal structures, as well as surrounding individual fat cells in the subcutis. However, in LA and MA the deposits are usually confined to the papillary dermis and do not involve blood vessels or adnexal structures. The deposits in the papillary dermis are slightly larger in LA than MA.^{5,7} Crystal violet and Congo red stains showed positive reaction in the

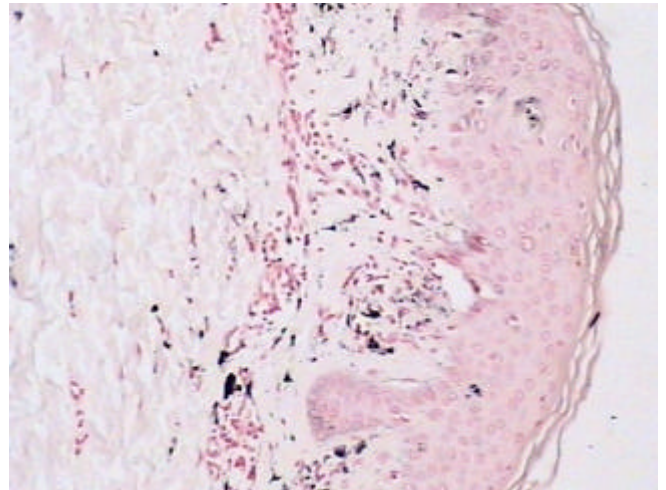


Figure 3 Marked increase of the melanophages was showed with the Masson-Fontana stain (Masson-Fontana x 100).

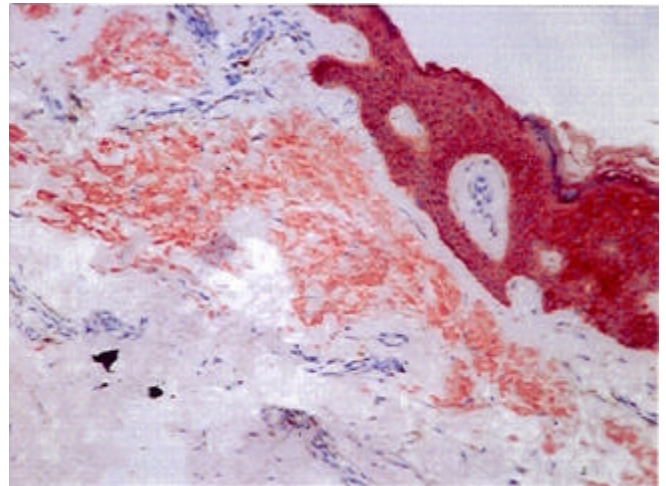


Figure 4. (+++) positivity of keratin deposits in the papillary dermis (IHC x 200).

amyloid deposits of all our cases. A variety of different cell types such as keratinocytes, fibroblasts, mast cells and melanocytes have been considered as possible synthesizers of amyloid fibril protein in the deposits of LA and MA.¹¹ It is now known that cutaneous amyloidosis is not derived from immunoglobulins or serum proteins, as it is in SA, but from keratin peptides of necrotic keratinocytes.^{2,4,5,6,11,12} Amyloid in LA and MA contains keratin epitopes and Huilgol et al.¹³ suggest that this is derived from fibrillar component of the keratin intermediate filaments. Immunohistochemical staining with anti-keratin antibodies appeared to be a useful method in making differential diagnosis of PLCA.¹⁴ Recently, Apaydin et al. have

been indicated that CK5 antibody is useful for diagnosis of LA and MA.¹⁵

Pruritis is not a presenting symptom in SA like PLCA. In PLCA, the cause of the formation of amyloid and the intense pruritis associated with it was said to be unknown. In 1984, Jambrosic, and co-workers¹⁶ demonstrated that the basic event in the conversion of filaments of necrotic keratinocytes into amyloid is the degeneration of keratinocytes. Ultrastructurally, tonofilaments in degenerating epidermal cells sequentially change their features ("filamentous degeneration") and become morphologically identical to amyloid filaments.⁶ Necrosis of keratinocytes may be induced by prolonged scratching. The important fact here is that the deposition of amyloid seems to be not the cause but the result of itching and scratching. 79.1% of our cases complained of pruritis as the main presenting feature. LA is often associated with pruritic diseases such as venous insufficiency, atopic dermatitis, cholestasis, keratosis lichenoides chronica, and Sipple syndrome (MEN type 2A).⁵ In our patients no other associated disease was observed. It was reported that histamine release from the mast cells had a triggering effect in the beginning and continuity of pruritis. Statistically meaningful increase in the mast cell numbers of our cases according to the control group also supported the "pruritis theory". Systemic treatment with sedating antihistamines and intense local treatment with corticosteroids were found to be effective in previous studies.^{3,4,5} Hyperkeratosis, papillomatosis, epidermal hyperplasia and acanthosis are found in variable degrees in LA.^{2,9,10} Epidermis is normal in MA. Dermal macrophages containing melanin pigment can be seen in both of them. This is a helpful finding and amyloid deposits should be searched when they are observed.¹⁰ In our study, an increase in dermal melanophages and melanocytes according to the control group was also detected. In PLCA cases, one way to exclude the skin involvement of systemic amyloidosis is fine needle biopsy of subcutaneous fat of clinically normal abdominal skin. In this way, amyloid reaction has been reported to be positive in up to 95% of primary SA, 66% of secondary SA and negative in LA ve MA cases.⁷ Sometimes skin biopsies of clinical PLCA cases show

negative pathologic results. In such cases, sequential skin biopsies are needed to confirm the diagnosis.³ Histochemical stains must be used for the differential diagnosis and systemic amyloidosis must be excluded. In conclusion, PLCA is a rare chronic disease with a prevalence of 0.15% among the most seen Middle East countries³ that should be kept in mind for the histopathologic evaluation of the pruritic and hyperpigmented skin lesions.

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