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AMYLOID IN ACQUIRED APONEUROTIC PTOSIS

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ABSTRACT – PURPOSE: Histopathological and immunohistochemical evaluation of levator aponeurosis in patients with aponeurotic ptosis vs. control aponeurosis obtained from autopsies of cadavers without history of ptosis, especially in relation with presence of amyloid deposition.
MATERIAL AND METHODS: Seven levator specimens were taken from 6 patients, undergoing anterior levator repair due to aponeurotic ptosis. The control group consisted of 12 levator aponeurosis specimens harvested from both lids of 6 cadavers, during autopsy. Congo red was used to detect amyloid and Orcein stain for elastic fibers. Immunohistochemistry was applied as a final marker for amyloid.
RESULTS: In 2 out of 6 (33,3%) aponeurotic patients we demonstrated amyloid. Orbital and systemic involvement was excluded. The levator aponeurosis was attenuated, stretched and grayish in all patients, but still attached to the tarsus. Collagen bundles were thinned with fatty and elastotic degeneration of different grade in all samples. Control group was negative for amyloid.
CONCLUSION: We demonstrated amyloid in 33,3% of patients with aponeurotic ptosis, without systemic amyloidosis, which may open a new perspective in aponeurotic ptosis etiology explanation.

Key words: *aponeurotic ptosis, amyloidosis, aponeurosis histopathology*

Introduction

The normal levator aponeurosis is composed of compact collagen bundles and elastic fibres directly connected to it, forming a network, with the fusion of the posterior orbicularis fascia and the orbital septum at the lid crease area.[1] The levator aponeurosis differentiates independently before attaching to the striated muscle part[2] being the thickest at that attachment due to the vessels and the muscle fibres from striated muscle that grow down into the aponeurosis.[3] Multiple insertions of the aponeurosis are recognized.[2,4,5] Current belief in acquired aponeurotic ptosis emphasizes aponeurotic fibres fanning out through the pretarsal and preseptal orbicularis[6] plus attachments to the lower 7-8 mm of anterior surface of the tarsus and the firmest one, 3 mm above the lid margin.[5] Dehiscence and disinsertion of the aponeurosis have been described as possible causes of acquired ptosis.[2,4,7,8] However, Martin and Tenzel[3] consider the network of aponeurosis attachments so extensive that "complete disinsertion seems unlikely from involutional changes". The area of aponeurosis thinning and fat infiltration[9,10] is "area of structural weakness more susceptible to the damage from any form of trauma".[3] Their study found the reason for aponeurotic defect to be iatrogenic, most likely blunt dissection during surgery. Caroll[11] employed a technique using cautery to dissect tissue planes and found aponeurosis to be intact in 95% of cases. Wilkes and Adams[12] observed a fatty infiltration of levator muscle with attenuated aponeurosis, but no disinsertion or dehiscence.

Amyloidosis is a group of disorders of protein metabolism characterised by the extracellular deposition of abnormal insoluble protein.[13] Many organ systems may be affected. Clinical clasification recognise primary, secondary and familial subtypes of the disease.[14] Currently a new clasification, based on the biochemical composition of amyloid

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subunit protein, indentifies five subtypes. All forms of amyloid share common light microscopic properties and a fibrilar ultrastructure.[15] Amyloidosis may involve the eye and its adnexa as a localized disorder or as a part of systemic illness.[16] Progressive ptosis secondary to localized amyloidosis of the tarsal conjuctiva and tarsus has been described.[15] Amyloid of aging occurs as senile cardiac or cerebral amyloidosis (Alzheimer's disease).

The purpose of this paper is histopathological and immunohistochemical evaluation of levator aponeurosis specimens in patients with aponeurotic ptosis vs. control aponeurosis tissue obtained from autopsies, especially in relation to presence of amyloid.

Material and methods

Seven specimens of levator aponeurosis were taken from 6 consecutive patients with acquired aponeurotic ptosis operated an our Department in period from September 1999 to January 2000. A detailed history of the disease was taken and a standard protocol of ptosis evaluation[17,18,19] was used. Since levator function was better than 10 mm in all patients, anterior levator repair[20,21,22,23] has been performed under the local anesthesia. Sharp spring scissors dissection was used. Aponeurosis was detached from superior tarsal border and lower 6-7 mm of Müller's muscle prior to suturing of healthy tissue to the tarsus. After healthy part of the aponeurosis had been fixated to the tarsus, attenuated aponeurotic inferior end has been cut off along the upper lid. This strip of the cut aponeurosis, 2-4 mm wide, was put in a container filled with 10% formalin and sent to the Department of Pathology and Citology, University Clinic "Merkur", Zagreb, Croatia, within maximum of 6 hours postoperatively. During that time the specimens were kept on 4ºC. Control group consisted of 12 levator aponeurosis specimens harvested from both lids of 6 cadavers during autopsy at the Department of Patology, General Hospital "Sveti Duh", Zagreb, Croatia. The autopsy was performed 12-24 hours post-mortem. Documentation of known disease and cause of death was made for each specimen. History of preexisting blapharoptosis, chronic lid edema, ocular trauma or surgery and known connective tissue disease has been excluded based on the patients charts. The 4x4 mm aponeurosis specimens were kept in containers filled with 10% formalin on 4ºC, no longer than 12 hours prior to analysis.

The paraffin-embedded 6-8μm sections were stained with hematoxylin-eosin, Mallory trichrome, Congo red and

Table 1. Data of the patients. Standard protocol of ptosis evaluation is used

No	1	2	3	4	5	6
Age, years	54	78	64	37	75	40
Sex	M	F	F	F	F	F
R/L/bil	R	R	Bil	L	L	L
Etiology, duration	postop. cataract, 7 years	involutive 1 year	involutive 10 years	edema 2 years	postop. cataract, 1 year	long-standing buphthalmos
R/PA/L	6 / 10	6 / 10	8 / 9	10 / 8	7 / 4	9 / 8
R/MRD/L	-3 / 1 / 0	0 / 5 6 / 5	3 / 3 5 / 6	4 / 2 6 / 6	2 /-2 5 /	4 / 3 5 / 5
R/LF/L	11 / 15	18 / 18	18 / 18	18 / 18	15 / 15	14 / 14
R/sc/L	12 / 11	11 / 8	14 / 13	8 / 10	11 / 14	8 / 12
Close/ retract test	positive	positive	positive	inconclu-sive	positive	negative
Bell's	+	+	+	+	+	+
Down gaze ptosis	+++	++	R +++ L ++	+	++	++
Schirmer test, R/L (mm)	13 / 12	6 / 8	10 / 13	17 / 20	7 / 9	15 / 15

M- male, F- female, R- right, L- left, bil-bilateral, PA-palpebral aperture, MRD-margin-to-reflex distance, LF-levator function, s/c-skin crease

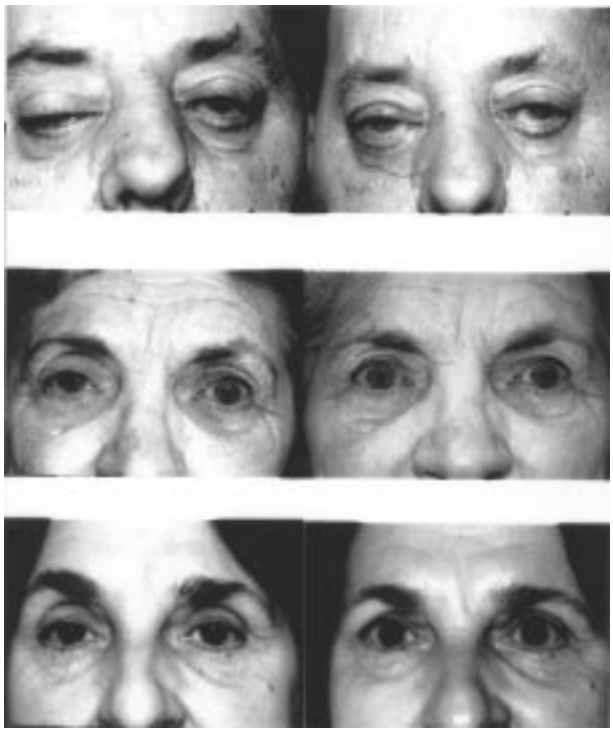

Figure 1. Patients No 1-3, top to bottom of the figure. Photos are taken preoperatively (left side) and 3 months postoperatively (right side). In patient No 1 hypocorrection of the right upper lid was aimed to match the contralateral lid height.

Orcein. Collagen changes due to fatty degeneration, and elastotic degeneration were graded semiquantitatively on a scale ranging from:

+ = roughly 1/3 of the collagen on the slide was replaced with fat/ thick bundles of elastic fibers are still seen

to +++ =collagen was found in traces, replaced with fat/ elastic fibers are reduced to short thorn wavy lines.

Inflammatory cells are marked as +/- . If present, they were described. Congo red was applied to demonstrate amyloid deposits, which should stain bright orange and show apple-green birefrigence.[24] On immunohistochemistry monoclonal antibody against AA (amyloid associated) and polyclonal antibody against P component (polyclonal) were applied as a final marker for amyloid. Orcein stain was used to detect elastic fibres in aponeurosis.

Results

Patients subjected to this study aged from 37 to 78 years, male/female 1/5. They all suffered from acquired aponeurotic ptosis, which lasted from 1 to 10 years, with causes listed in Table 1. It was symptomatic unilateral ptosis in five cases (83,4%) and bilateral in one case. Levator function was good, 11 to 18 mm. All patients had raised skin crease (8 to 14 mm), deep upper lid sulcus and a thinned lid. The levator aponeurosis was attenuated, stretched and grayish in all patients, almost transparent in patient No 2, but still attached to the tarsus. No dehiscence or disinsertion was found. More graduate than definite transition from healthy white aponeurosis to stretched, grayish, attenuated aponeurosis was seen. Postoperative outcome was good in 3 patients (Figure 1 and 2). In others we consider it satisfactory.

Collagen bundles were thinned, showing different grade of fatty degeneration (Table 2) (Figure 3). Elastotic degeneration is also detected in all patients (Table 2). In patient No 2 and 4 (Figure 4) amyloid was positive. Further clinical and laboratory investigations gave no evidence to suggest orbital or systemic involvement. Lymphocytes and plasma-cells are only found in one of the patients with amyloid (Table 2). Mononuclears are found in two patients (Table 2).

The age of cadavers ranged from 67 to 80 years. There were 2 males and 4 females. Levator aponeurosis was attached to the tarsus in all cadavers. It was grey-yellow and

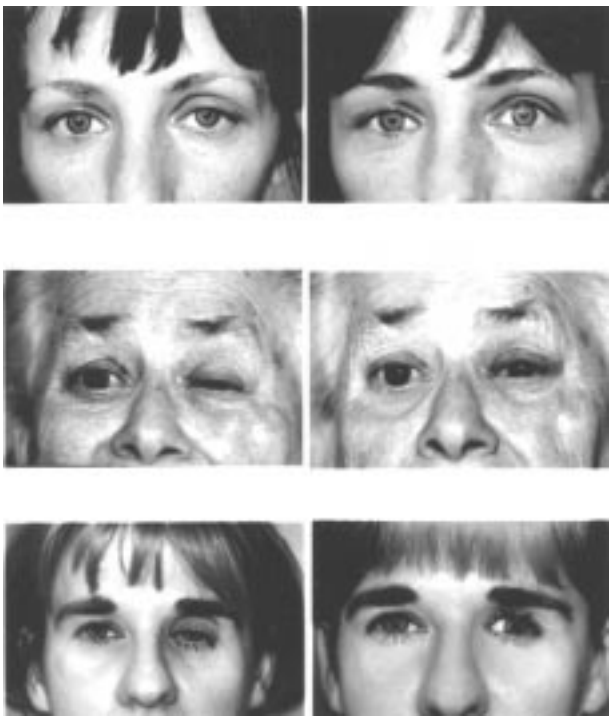

Figure 2. Patients No 4-6, top to bottom of the figure. Photos are taken preoperatively (left side) and 3 months postoperatively (right side).

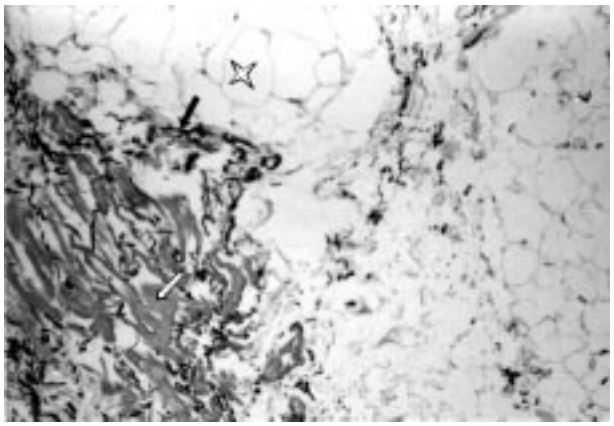

Figure 3. Patient No 5. Residual thinned collagen fibres are seen in the lower right part of the figure (white block arrow) with abundance of the fat tissue (star). Elastic fibres are seen as diffusely, scattered short wavy dark lines (black block arrow) (Orcein stain, x 400).

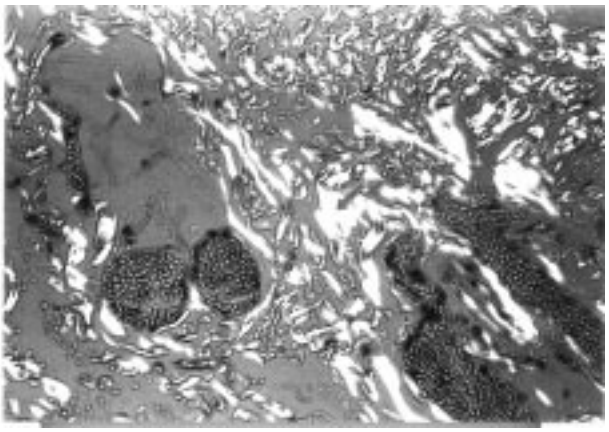

Figure 4. Patient No 4. An apple-green birefrigence is seen by polarization microscopy (black block arrow)(Congo stain, polarization, x 600).

sinewy in specimens of 4 cadavers. Samples taken from female cadavers age 79 and 80 years revealed very thin, tender, light yellow aponeurosis, almost see-through, without any resistance during cutting. It could be easily thorn and had to be handled with very carefully. Collagen changes due to fatty degeneration and elastotic degeneration grading are given in Table 2. Mononuclears, plasma-cells and surprisingly, mastocytes are found. Amyloid was negative in all control specimens.

Discussion

The acquired aponeurotic ptosis has characteristic clinical signs[17,25] recorded in all our patients. Its proposed anatomical etiology is manifold. Dehiscence or disinsertion of levator aponeurosis has been demonstrated by histopathology and ultrasound biomicroscope.[26,27] Others found marked attenuation[11] and fatty degeneration.[12] Shore[10] suggested primary myopathic process in some cases. Martin and Tenzel[3] observed intact aponeurosis firmly attached to the tarsus requiring dissection off the tarsus in their series of 98 cases of acquired ptosis. There is a general agreement that the underlying Müller's muscle appears to be intact.[5,10,28] Macroscopic appearance of Müller's muscle in our patients was unchanged as well. There was no difference in macroscopic appearance of levator aponeurosis in aponeurotic ptosis of different etiology. In all our patients the levator aponeurosis was attenuated, stretched and grayish, but still attached to the tarsus.

Postoperative ptosis is defined either as a 2 mm or greater drop in the position of the operated lid without correction for the fellow eye or a relative change between the operated and unoperated eye.[29] The incidence of postoperative ptosis after cataract extraction at the first postoperative day is high 48%. However, most of the cases resolve in the first 6 months with residua in only 7,3%.[30] Multiple factors have been involved in its development: preoperative ptosis, volume and myotoxic effect of local anesthet-

Table 2. PHD results of the patients / postmortem control specimens

No	1	2	3	4	5	6
Fatty deg.	++/+	+++/+++	++/++	++/+	++/++	++/+
Elastotic deg.	++/+	++/+++	++/++	++/+	+++/++	+/+
Inflam. cells	- / -	m / -	-/ m,ma	ly,p,		
PMN'S/p	m / m,ma	-/ ma				
Amyloid	neg/neg	**pos**/neg	neg/neg	**pos**/neg	neg/neg	neg/neg

m-mononuclear phagocytes, l-leucocytes, ly-lymphocytes, p-plasma-cells, PMN'S- polimorphonuclears, ma-mastocytes

ics, ocular massage, eyelid speculum, superior rectus traction suture and prolonged postoperative patching.[29,30,31,32] In our two cases, postcataract ptosis was the result of extracapsular cataract extraction with all the mentioned provoking factors present, except prolonged patching.

Primary amyloidosis is a protein dyscrazia associated with a monoclonal population of plasma-cells.[24] Only two out of five amyloid protein subtypes are pertinent to the subject of orbital amyloidosis. The AA (amyloid associated) is the major component of the amyloid, deposited secondary to chronic inflammatory disorders. The amyloid light chain (AL) (consists of parts of either kappa or lambda immunoglobulin light chain) is common to cases of primary amyloidosis (with or without myeloma) (24). Localized amyloidosis of conjuctiva and tarsus that manifests as a painless nodular or diffuse thickening of the eyelid and progressive ptosis has been described.[15,33,34,35] To the best of our knowledge, this is the first study of aponeurosis specimens of patients with acquired aponeurotic blepharoptosis without lid thickening in relation with amyloid deposition. Fat has been described as replacement tissue between degenerated collagen bundles in patients with aponeurotic ptosis.[36] We presumed amyloid might play the similar role, being acellular aggregate that may be found in adipose tissue, walls of small blood vessels, extraocular muscles and lacrimal gland.[24] Histologically it looks pink with hematoxylin-eosin stain and can not be differentiated from collagen bundles without special stain and immunohistochemistry. "Some amyloid deposits incite very little reactive response in the host tissue", while others may show granulomatous foreign body response.[24] These are the reasons why amyloid can be missed if not explicitly searched for. Amyloid is in general not classified as a degeneration, but may be due to defective proteolytic degeneration. The findings of amyloid in aponeurosis should probably be classified as reactive amyloidosis, composed of AA protein, secondary to chronic inflammatory condition (supposed mechanism:chronic inflammation-macrophage activation-IL 1 and 6-liver cells-SAA protein-AA protein).

Stasior[1] demonstrated the elastic fibres in the levator aponeurosis, the pretarsal orbicularis muscle, Müller's muscle tendon, the conjoined fascia and the area of the upper lid crease. Elastic fibers, detected in all our specimens, showed a different grade of degeneration. Elastotic degeneration is a common phenomenon in aging and can be influenced by several factors, including solar degeneration. Our controls are not age-matched, and the groups are very small:it is impossible to draw any conclusions from these results.

Inflammatory cells and mastocytes have been described in ptosis. Mastocytes are connective tissue cells found close to small blood vessels. It is unknown why they are present only in three postmortem control tissues and missing in all the patients. Mononuclear phagocytes, in connective tissue called histiocytes, are critical components of inflammatory and immune reaction. They are found in roughly half of the specimens in both groups.

Conclusion

Positive amyloid in levator aponeurosis of 33,3% of patients with aponeurotic ptosis, without systemic amyloidosis, opens a new perspective in aponeurotic ptosis etiology explanation.

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Sažetak

AMILOID KOD APONEUROTSKE PTOZE

B. Kuzmanović

CILJ: Patohistološka i imunohistokemijska evaluacija aponeuroze m. levator palpebrae superior, posebice istraživanje prisustva amiloidnih depozita, u pacijenata s aponeurotskom ptozom, u usporedbi s kadaverima bez ptoze u anamnezi.
SUBJEKTI I METODE: Sedam uzoraka aponeuroze m.levator palpebrae superior su uzeti tijekom operacije ptoze. Kontrolnu skupinu čini 12 uzoraka aponeuroze dobivenih iz obje vjeđe kadavera tijekom obdukcije. Congo red bojenje je korišteno za dokaz amiloida, a Orcein za dokazivanje elastičnih vlakana. Imunohistokemija je poslužila kao krajnji dokaz za amiloid.
REZULTATI: Amiloid je dokazan u 2 od 6 (33,3%) pacijenata s aponeurotskom ptozom. Sistemna amiloidoza je isključena. Aponeuroza m.levator palpebrae superior je stanjena, istegnuta i sivkasta u svih pacijenata, ali još uvijek pričvršćena za tarzus. Degeneracija kolagenih vlakana, te masna i elastotična degeneracija različitog stupnja je dokazana u svim uzorcima. Amiloid je negativan u svim aponeurozama kadavera.
ZAKLJUČAK: Dokazali smo amiloid u čak 33,3% uzoraka aponeuroze m.levator palpebrae superior pacijenata s aponeurotskom ptozom, bez sistemne bolesti, što otvara novu perspektivu u rješavanju etiologije aponeurotske ptoze.

Ključne riječi: *aponeurotska ptoza, amiloidoza, histopatologija i imunohistokemija aponeuroze*