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 part2 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 Current belief in acquired aponeurotic ptosis emphasizes aponeurotic fibres fanning out through the pretarsal and preseptal orbicularis5 plus attachments to the lower 7-8 mm of anterior surface of the tarsus and the firmest one, 3 mm above the lid margin.2 Dehiscence and disinsertion of the aponeurosis have been described as possible causes of acquired ptosis.2,4,7,8 However, Martin and Tenzel3 consider the network of aponeurosis attachments so extensive that “complete disinsertion seems unlikely from involutional changes”. The area of aponeurosis thinning and fat infiltration9,10 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. Carroll11 employed a technique using cautery to dissect tissue planes and found aponeurosis to be intact in 95% of cases. Wilkes and Adams12 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 classification recognise primary, secondary and familial subtypes of the disease.14 Currently a new classification, based on the biochemical composition of amyloid...
Kuzmanović B. Amyloid in ptosis 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 conjunctiva 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 on in 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 was used. Since levator function was better than 10 mm in all patients, anterior levator repair 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 Cytology, University Clinic “Merkur”, Zagreb, Croatia, within maximum of 6 hours postoperatively. During that time the specimens were kept on 4°C. Control group consisted of 12 levator aponeurosis specimens harvested from both lids of 6 cadavers during autopsy at the Department of Pathology, 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 blepharoptosis, 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 4°C, no longer than 12 hours prior to analysis.

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

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<td>6 / 8</td>
<td>10 / 13</td>
<td>17 / 20</td>
<td>7 / 9</td>
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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
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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. 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 (83.3%) 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 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.

Figure 2. Patients No 4-6, top to bottom of the figure. Photos are taken preoperatively (left side) and 3 months postoperatively (right side).
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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 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. Others found marked attenuation and fatty degeneration. Shore suggested primary myopathic process in some cases. Martin and Tenzel 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. 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. 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%. Multiple factors have been involved in its development: preoperative ptosis, volume and myotoxic effect of local anesthet-

![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).](image1)

![Figure 4. Patient No 4. An apple-green birefringence is seen by polarization microscopy (black block arrow) (Congo stain, polarization, x 600).](image2)

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m-monomonuclear phagocytes, l-leucocytes, ly-lymphocytes, p-plasma-cells, PMN’S-polimorphonuclears, ma-mastocytes
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.

References

AMILOID KOD APONEUROTSKE PTOZE

B. Kuzmanović

CILJ: Patohistološka i imunohistokemijska evalvacija aponeuroze m. levator palpebrae superior, posebice istraživanje prisustva amiloidnih depozita, u pacijenata sa 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 vježe kadavera tijekom obdukcije. Congo red bojenje je korišteno za dokaz amiloida, a Orcein za dokazivanje elastičnih vlakana. Imunohistokemijom 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 sa aponeurotskom ptozom, bez sistemne bolesti, što otvara novu perspektivu u rješavanju etiologije aponeurotske ptoze.

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

Sažetak

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