

Histopathologic Evaluation and Tensile Strength of the Levator Aponeurosis-Tarsus Junction after Ptosis Surgery

Ptozis Cerrahisi Sonrası Levator Aponevroz-Tars Kavşağının Histopatolojik İncelenmesi ve Gerilme Kuvveti

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Summary

Purpose: The aim of this study was to investigate the recovery period histopathologically and the tensile strength of the levator aponeurosis (LA)-tarsus junction following LA surgery.

Material and Method: Twenty-one New Zealand rabbits were used in this prospective study. The left eye of each rabbit had LA surgery and formed the study group; the right eyes formed the control group. According to the postoperative follow-up time, the study group was divided into 5 as follows: group 1-5 days, group 2 - 10 days, group 3-2 weeks, group 4-4 weeks, and group 5-6 weeks. The control group had no ptosis surgery. After the follow-up period determined for each group, total eyelid excision was performed. The LA and the tarsus were evaluated histopathologically, and the stretching test was applied.

Results: The average separation force in the control group was detected to be 14.36 Newtons (N). It was 1.41 N in group 1, 2.74 N in group 2, 3.56 N in group 3, 4.4 N in group 4, and 6.03 N in group 5. The histopathological evaluation performed in group 1 revealed vascularisation and polymorphonuclear cell infiltration. In group 5, the histopathological appearance of the tissue was close to normal, except for intense eosinophilic infiltration.

Discussion: After ptosis surgery at the postoperative 6th week, the healing process was almost complete histopathologically, however, the tensile strength of the LA-tarsus junction did not return to the preoperative level. (*Turk J Ophthalmol* 2010; 40: 314-7)

Key Words: Ptosis, levator aponeurosis, surgery, tensile strength

Özet

Amaç: Levator aponevroz (LA) cerrahisinden sonra LA-tars kavşağının histopatolojik olarak iyileşme süreci ve gerilme kuvvetinin incelenmesi.

Gereç ve Yöntem: Prospektif olarak yapılan bu çalışmada 21 adet Yeni Zelenda tavşanı kullanıldı. Tavşanların sol gözlerine LA cerrahisi yapıldı. Sol gözler çalışma grubunu sağ gözler kontrol grubunu oluşturdu. İzlem süresine göre çalışma grubu aşağıdaki şekilde beşe ayrıldı; grup 1: 5 gün, grup 2: 10 gün, grup 3: 2 hafta, grup 4: 4 hafta, ve grup 5: 6 hafta. İzlem süresini takiben bütün gruplara total kapak eksizyonu yapıldı. Levator aponevrozu ve tars histopatolojik olarak incelendi ve bu yapıların gerilme testi uygulandı.

Sonuçlar: Kontrol grubunda ortalama ayrılma gücü 14,36 Newton (N) olarak tespit edildi. Ortalama ayrılma gücü grup 1'de 1,41 N, grup 2'de 2,74 N, grup 3'te 3,56 N, grup 4'te 4,4 N, grup 5'te 6,03 N olarak belirlendi. Histopatolojik incelemede grup 1'de vaskülarizasyon ve polimorfonükleer hücre infiltrasyonu gözlenirken grup 5'te histopatolojik görünüm olarak yoğun eozinofilik infiltrasyon dışında iyileşme hemen hemen tamamlanmıştı.

Tartışma: Ptozis cerrahisi sonrası 6. haftada histopatolojik olarak iyileşme tamamlanmasına karşın LA-tars kavşağındaki gerilme gücü ameliyat öncesi döneme göre oldukça düşüktür. (*Turk J Ophthalmol* 2010; 40: 314-7)

Anahtar Kelimeler: Ptozis, levator aponevroz, cerrahi, gerilme gücü

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Introduction

Ptosis is the downward displacement of the upper eyelid caused by the underaction of the eyelid retractors relative to the eyelid protractors. Levator palpebralis superior (LPS) muscle, which attaches to the upper tarsal plate by the lowest fibres of the levator aponeurosis (LA), is the major retractor of the upper eyelid. Ptosis is most frequently associated with decreased LPS muscle function or disinsertion of its aponeurosis (1).

Levator palpebralis superior muscle surgery is one of the employed techniques in the correction of ptosis in patients with greater than 5 mm of levator function. In this procedure, the LA is detached from its normal point of insertion and advanced by folding or excising the LA, and reattached to the anterior surface of the tarsus in order to allow a more complete retraction of the upper eyelid (2). This procedure can be carried out either using a skin incision or a transconjunctival approach (3, 4).

Gaining knowledge about the durations of the recovery period of the LA-tarsus junction and the wound healing following ptosis surgery is important for both follow up and instituting various precautions to the patients postoperatively. In the current study, we aimed to evaluate the changes in the tensile strength of the LA- tarsus junction and the duration of the wound healing after ptosis surgery performed in rabbits. We planned to observe the subsequent histopathological changes in the LPS muscle and the tensile strength of the LA- tarsus junction after LA surgery. To our knowledge this is the first study demonstrating the healing period of the LA-tarsus junction after ptosis surgery.

Materials and Methods

This prospective study was conducted at Ankara Research and Training Hospital Animal Laboratory, in compliance with the approval of the Ethics Commission on Animal Studies of Ankara Research and Training Hospital and Ankara Atatürk Research and Training Hospital. All rabbits were housed and handled in accordance with Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Humane use of Animals in Vision Research.

Twenty-one healthy New Zealand white male rabbits weighting 2500-3500gr were used in this study. Left eyes of each rabbit had LA surgery, and formed the study group. According to the postoperative follow up time, the study group was divided into 5 as follows; group 1 : 5 days, group 2: 10 days, group 3 : 2 weeks,

group 4: 4 weeks, and group 5: 6 weeks. Right eyes formed the control group. No ptosis surgery was performed to the control group.

Surgery

After intramuscular injection of Xylazine HCl (50mg) and Ketamine HCl (50mg), the left eyelid was shaved. An horizontal incision 3mm. from the edge of the upper eyelid was made. As seen in Figure 1, the LA was dissected and separated from the edge of the tarsus. The LA was then sutured onto the upper 1/3 part of the anterior tarsus by 3 interrupted 6.0 polyglactine sutures. The skin was sutured with the same suture material. Postoperatively topical antibiotic ointment was applied for 5 days.

After the follow up period determined for each group, total eyelid excision was performed to all eyes. Eyelid material consisting the LPS muscle and tarsus was divided vertically into two parts. First part was fixed in 10% neutral solution of formaline (pH 7.4) solution for hystopathological evaluation. The second part was performed the stretching test.

Histopathological Evaluation

The biopsy specimens were fixed in 10% neutral solution of formaline (pH 7.4) for 2 days before embedded in paraffin for histopathological examination. The 4-6 µm serial cross-sections were obtained with the Leitz-1512 type microtome. After staining with Hematoxyline-Eosin (HE) dye, the specimens were evaluated with a photomicroscope.

Stretching Test

Full-thickness suturing of the two ends of the LPS muscle and tarsus with 4.0 silk was performed. In between the two ends of the LPS muscle continuous suturing was performed using 4.0 silk again (Figure 2). The two sutures at either end were then secured to the hooks of the Tensile Test Machine (Lloyd Inst., UK) (Figure 3a). To determine the separation point, a pro-



Figure 1. The dissection of the levator aponeurosis (LA)

gressively increasing force (in Newton units=N) was applied to the sections (Figure 3b). The test speed was set at 10mm/min.

Results

Twenty-one eyes of 21 New Zealand white male rabbits formed the study group. The numbers of eyes per groups are as follows: 3 in group 1, 3 in group 2, 5 in group 3, 5 in group 4, and 5 in group 5.

The average separating force in the control group was detected as 14.36 Newtons (N) during the stretching test. In group 1 this was decreased to 1.41 N. The gross evaluation of the LPS muscle before applying the stretching test in this group showed some separated muscle fibers. The average separating force was 2.74 N in group 2, 3.56 N in group 3, 4.4 N in group 4, and 6.03 N in group 5.

The percentage of the tensile strength of LA-tarsus junction was 9.82% in group 1, 19.08% in group 2, 24.79 % in group 3, 30.64% in group 4, and 41.99% in

group 5 when compared with the controls. According to these results it was seen that even at the 6th postoperative week, the tensile strength-endurance level was below normal.

The histopathological evaluation performed in group 1 revealed, vascularisation and polymorphonuclear cell (PMN) infiltration. An increase in the number of mononuclear cells and fibroblasts were detected in group 2. In group 3, and group 4, marked elevation in the number of fibroblasts, mononuclear cells and eosinophils along with the narrowing of the incisional space were observed (Figure 4). At the early stages after surgery, demonstrating an increase in the number of active fibroblasts and an elevation of the concentrations of collagen fibers around the incisional site were proofs of the repairing process of the tissue. In group 5, the histopathological appearance of the tissue looked close to normal except intense eosinophilic infiltration, which indicated that the tissue reached the end stage of healing.

Discussion

Previous knowledge has stated that after 1 week from the ptosis surgery, the developing cutaneous scar tissue had only 10% strength of resistance. This was reported to be increased to 30-50% by the postoperative 4th week and finally reached up to 80% (5). As we detached the eyelid skin and applied stretching force only to the LA-tarsus junction in our study, the lower values of the strength of resistance were demonstrated in contrast with the previous findings. The percentage of the tensile strengths compared to the preoperative values were 9.82% on the 5th, 19.08% on the 10th, 24.79% on the 15th, and 41.99% on the 42nd postoperative day.

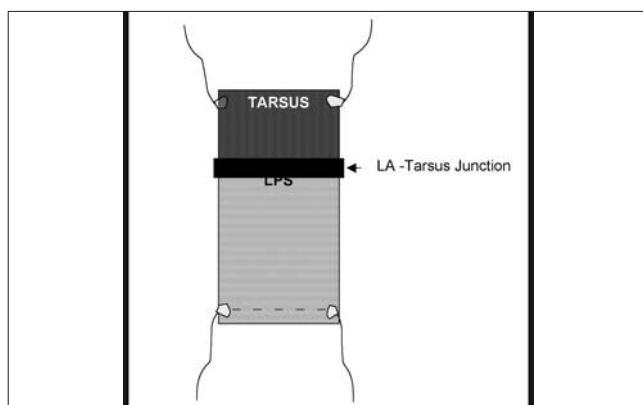


Figure 2. The suturing of the LPS muscle and tarsus



Figure 3. a. The tensile strength test machine (Lloyd Inst, UK), b: A progressively increasing force (in Newton units=N) was applied to the two ends of the section's including the LPS muscle and the tarsus which were tied to the hooks with 6.0 polyglactine

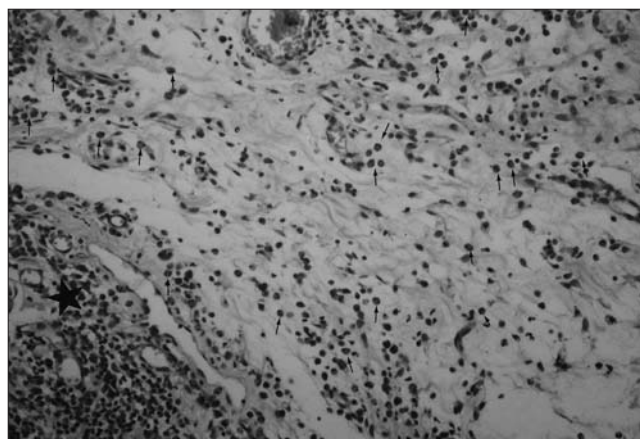


Figure 4. Mononuclear cells (star) and eosinophilic infiltration (arrows) were still existing on the postoperative 15th day (H.E x 50)

Clinically, it was stated that stabilization of the wound after ptosis surgery was achieved at the 6th postoperative week (6). In our study the tensile strength detected during the same period was 41.99 %. As we investigated only the LA-tarsus junction, it was clearly demonstrated that the healing process takes much more time than the cutaneous repairing process.

The surgical wound healing is a complex process, and has been demonstrated as follows (7, 8): After the incision, a blood clot rapidly fills the produced tissue defect. Polymorphonuclear cell infiltration is seen within the first 24 hours. During the next 24 hours fibroblasts migrate to the site while the fibrin filaments develop a bridge drawing the edges of the incision together. On the 3rd day, macrophages begin to replace PMNs. On the 5th day, the supporting tissue is composed of neovascularisation plus fibroblasts and inflammatory cells which then form the granulation tissue. Later, fibroblasts continue to synthesize collagen which appears in the wound site in about 72 hours and its synthesis increases progressively during the 4 to 6 weeks after the surgery. In this period, the proteoglycans, which strengthen the collagen bonds and effect the diameter and direction of the fibres, are synthesised by the fibroblasts. At the 6th week the total amount of collagen reaches to the maximum level while the tensile strength remains still low. Within months type II collagen replaces with type I and intramolecular collagen fibrils increase to achieve an anatomic integrity. As inflammatory material begins to recede, more avascular and noncellular tissue is left.

In our study we observed a heightened, and prolonged inflammatory response, and cellular infiltration during the healing process. An intense eosinophilic infiltration, continued during the postoperative 6th week was remarkable in our study group. This may be related to the suture material used in the surgery for the LA and the tarsus which may lead to an allergic reaction or it can be due to a different healing period seen in the rabbits used in the study. The longer period of the

cellular infiltration detected histopathologically should be assessed by more detailed studies dealing specifically with this issue.

The absorption period for the the polyglactine sutures used in our study is about 3 months. Bearing in mind the rate of returning of the tensile strength of the LA reinsertion, it was considered that the absorbable polyglactine sutures can be used safely in levator surgery.

In conclusion, our study demonstrated that, after LA surgery, the reinsertion site of the LA was considerably weak on the postoperative 5th day. Although the strength of the LA-tarsus junction was demonstrated to increase over time, it did not return to the preoperative level even after 6 weeks postoperatively. Further studies should be carried out to show the long term status of the LA-tarsus junction after ptosis surgery.

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