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Histological Changes in Levator Aponeurosis According to Blepharoptosis and Aging

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Background Many studies about the levator aponeurosis complex of the blepharoptosis have already been presented. However, the studies about the changes of the levator aponeurosis are relatively insufficient. So, this study was performed to observe histological changes of levator aponeurosis that arise depending on the severity of blepharoptosis and the age.

Methods Twenty patients who have undergone surgical treatment for blepharoptosis from 2013 to 2014 were analyzed in this study. Patients were categorized mild or severe group according to the severity of blepharoptosis, and the age. Through the blepharoplasty incision, we harvested the specimens of the levator aponeurosis on the upper border of tarsal plate. After staining the specimens with the Verhoeff-van Gieson technique, the changes of elastin was analyzed in a histopathological manner.

Results Light microscopy of the levator aponeurosis stained positively for elastic fibers using the Verhoeff-van Gieson technique. Elastic fibers appear to have direct connections with the collagen fiber of the levator aponeurosis. The amount of the elastin was decreased in the old age group. And the amount of elastin was decreased markedly in severe blepharoptosis group.

Conclusions The elastin of the levator aponeurosis was decreased in old age and elastin tended to decreased markedly in severe levator function group. The levator aponeurosis plays a greater role in the eyelid ptosis. Therefore, knowledge about the histologic changes of the levator aponeurosis may give more help us to understand the high recurrence rate of the blepharoptosis in old age. Also, considering this information, will be helpful to the blepharoptosis surgery.

Keywords Aging, Blepharoptosis, Eyelids

INTRODUCTION

Blepharoptosis is a medical condition in which the upper eyelid margin is located at a lower position than the normal eyelid margin when looking straight ahead at eye level. The normal position of the upper eyelid margin is at 12 o'clock, 2 mm below the superior corneal limbus; an upper eyelid margin located more than 2 mm below the superior corneal limbus is considered blepharoptosis. Myogenic blepharoptosis accounts for 50% of all blepharoptosis cases; aponeurotic blepharoptosis occurs when the force of the levator muscle is not sufficiently transferred to the tarsal plate. Although this occurs frequently in elderly people, it can also occur in young people [1]. Many research studies have described the histopathology of the levator muscle of blepharoptosis patients. In acquired blepharoptosis, the striated muscle isobserved to be in a normal form, but in congenital blepharoptosis, the muscle fibers of the levator muscle are observed to be few in number or atrophied [2-4].

Despite many reports on the histological changes of the levator muscle in both congenital and acquired blepharoptosis patients, few reports on the levator aponeurosis exist. Since blepharoptosis

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can be caused not only by the levator muscle, but also by changes in the levator aponeurosis, this study intended to examine the histological changes in the levator aponeurosis.

METHODS

This study was conducted on 20 patients (7 male and 13 female) who visited the hospital for bilateral blepharoptosis from May 2013 to August 2014. This study received the Institutional Review Board (IRB) approval from the Medical Ethical Committee of Inje University. Written informed consent was obtained from all participants.

The patients were divided into two groups, depending on age and severity of blepharoptosis. The patients ranged in age between 15 and 71 years. Patients under the age of 25 years, whose faces had not yet begun to show aging changes, were classified as Group A; patients over the age of 50 years, who had serious aging changes to the areas around the eyes, were classified as Group B [5].

To compare the apparent differences based on age, patients between the ages of 25 and 50 years were excluded. Evaluation of levator muscle function was performed using Berke's method. The severity of blepharoptosis was categorized by margin reflex distance 1 (MRD1), by measuring in units of 0.5 mm the distance from the central pupil reflection point to the upper eyelid margin.

If the function of the levator muscle was between 5 mm to 7 mm and MRD1 was between 1.5 to +3.0, the patient was classified into the mild group. If the function of the levator muscle was less than 4 mm and the MRD1 was below 0.5, the patient was classified into the severe group [6]. Cases where the function of the levator muscle was greater than 8 mm and moderate cases where the MRD was from 0.0 to +1.0 were excluded from the study. None of the patients experienced trauma to the eye or eye surgery, such as double eyelid surgery, vision correction surgery such as LASIK, eximer laser, or photorefractive keratectomy. Patients who wore contact lenses were excluded from the study. Additionally, patients who did not have congenital blepharoptosis were excluded after a general examination.

After incising 7 mm, on average, above the upper eyelid margin, the orbicular oculi muscle was dissected and the pretarsal fat was partially removed. The tarsal plate was then partially exposed, and the levator aponeurosis, which was removed, was harvested during levator advancement and balanced tucking of the Müller muscle for blepharoptosis (Fig. 1A and B). The specimen was first harvested slightly above the tarsal plate in a parallel direction (Fig. 1C); then, making a cross-section by fixation in 10% formalin, it was

Table 1. Patients summary

Age group	Mean age	Gender (Male/Female)	Ptosis degree	
			Mild	Severe
Group A (≤ 25 years)	21	3/5	3	5
Group B (≥ 50 years)	65	4/8	5	7

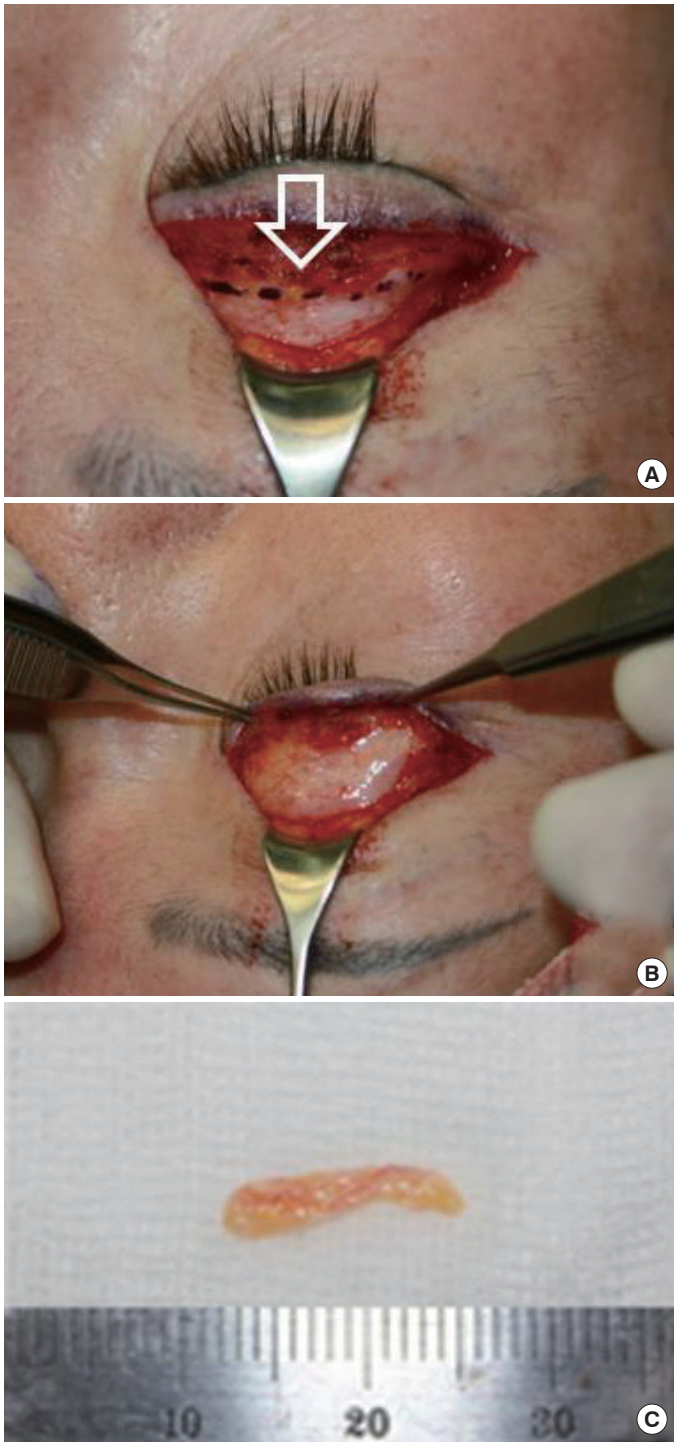

Fig. 1. (A, B, C) The levator aponeurosis of right eye was resected and the specimens of the levator aponeurosis on the upper border (A, white arrow) of tarsal plate was harvested (B). The average of the specimen's size was 12 mm (C) (Photographs view : upper part - caudal position, lower part - cranial position).

stained by hematoxylin-eosin (H&E) and Verhoeff-van Gieson stain for the examination of the elastic fiber distribution in the levator aponeurosis under 40× magnification (Carl Zeiss, Oberkochen, Germany) [7].

Based on the elastin distribution of Group A and the mild group, the distribution level was compared with the other groups. Tissues of both eyes were collected from two people in each group and differences between the eyes were assessed.

RESULTS

The results of examining the state of the outer ocular muscles, myasthenia testing, and the existence of the Bell phenomenon were unremarkable. Using the patients' medical history and general examinations of the decreased function of the levator muscle, innate blepharoptosis was diagnosed. Out of 20 patients, 8 patients in Group A were 21 years of age on average (3 male and 5 female). In Group B, there were 12 patients whose average age was 65 years (4 male and 8 female). Sixty percent of patients were categorized with severe blepharoptosis, and the remaining 40% of patients were in the mild group (Table 1).

During the blepharoptosis surgery, the tissue collected from the tarsal plate of the top eyelid in a parallel direction was between 10 mm to 15 mm, with an average length of 12 mm. After collecting the tissue through advancement and balanced tucking of the Müller muscle, blepharoptosis was corrected. Changes in the distribution of elastin were examined and analyzed using an optical microscope [7]. Elastin becomes a navy color when stained with Ver-

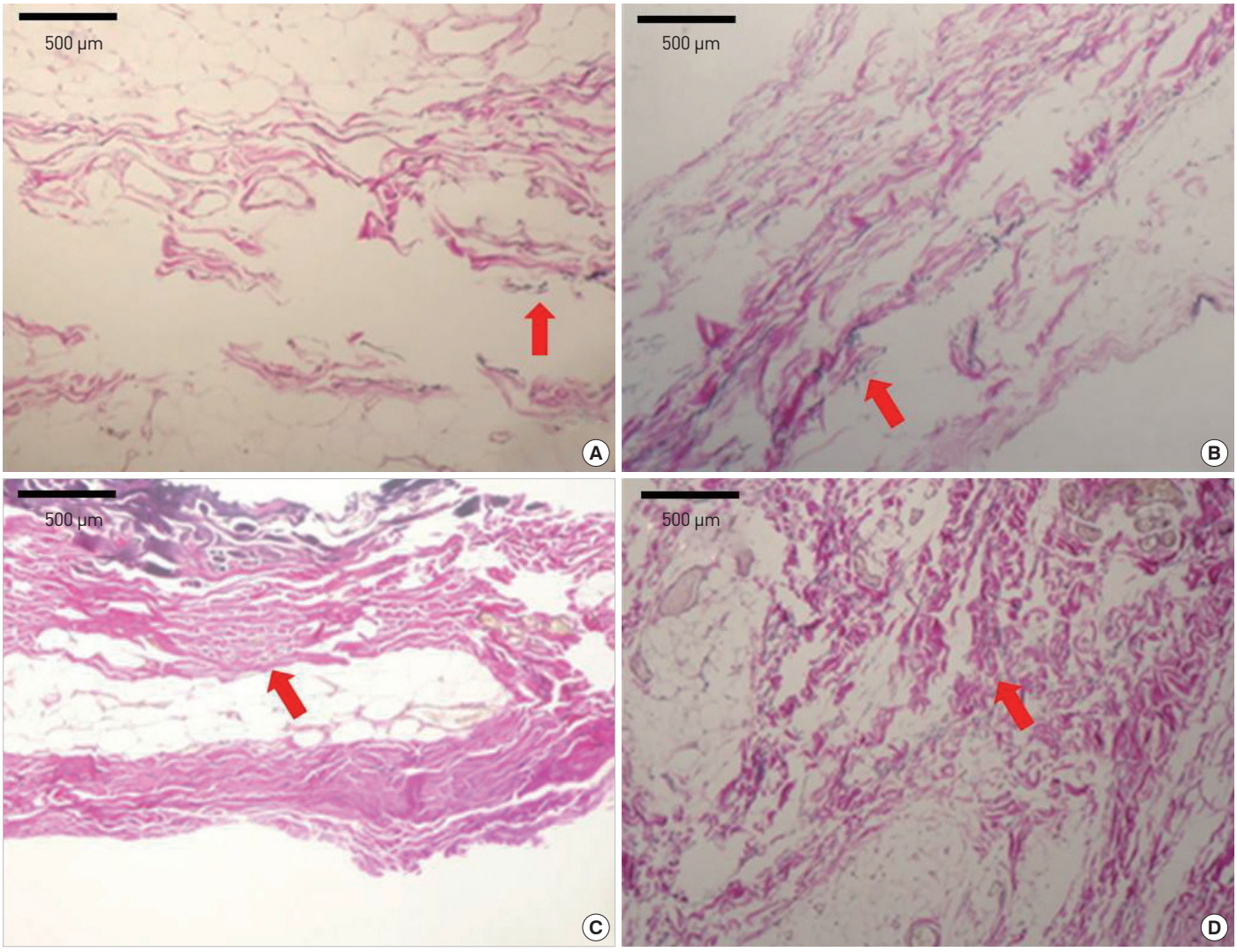

Fig. 2. Histopathologic photograph showing old age and severe blepharoptosis group (A), Old age and mild blepharoptosis group (B), young age and severe blepharoptosis group (C), young age and mild blepharoptosis group (D) (hematoxylin-eosin [H&E] stain, Verhoeff's/van Gieson's stain × 40, Red arrows: stained elastic fibers).

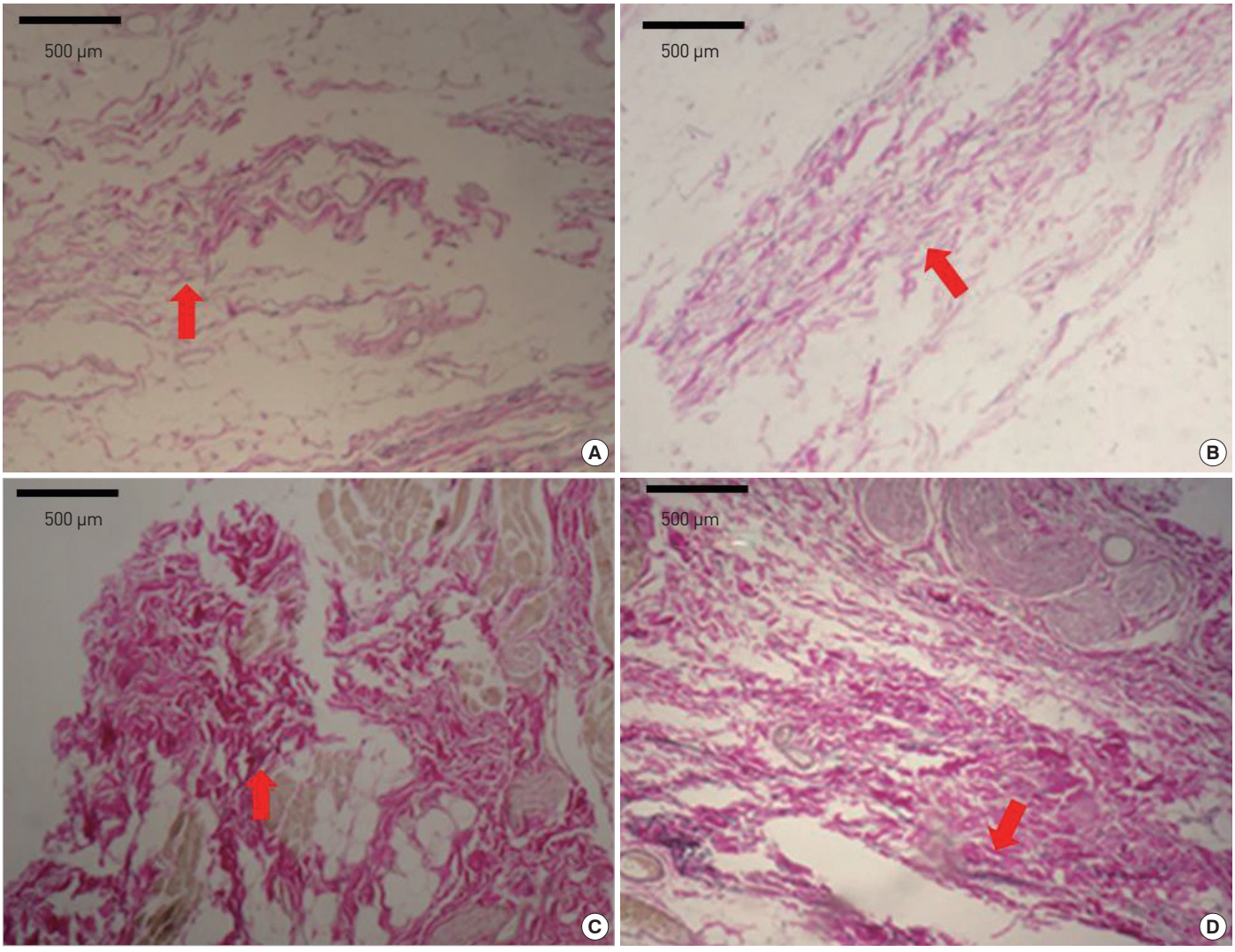

Fig. 3. Histopathologic photograph in the other side of the same patient, showing old age and severe blepharoptosis group (A), Old age and mild blepharoptosis group (B), young age and severe blepharoptosis group (C), young age and mild blepharoptosis group (D) (hematoxylin-eosin [H&E] stain, Verhoeff's/ van Gieson's stain × 40, Red arrows: stained elastic fibers).

hoeff-van Gieson stain and was compared to the age and severity of blepharoptosis. Elastin was found sporadically over a wide region in both Groups A and B, with more elastin in Group A than in Group B; Group B showed a tendency for the distribution of elastin to decrease. A sporadic distribution of elastin was also found in the mild and severe groups. However, the elastin distribution was greater in the mild group than in the severe group. Compared with the Group A-severe patients, the Group B-severe patients showed a decrease in elastin distribution more clearly (Fig. 2).

The differences in the elastin distribution were analyzed using optical microscopy, by collecting tissues from the opposite eye of the same patients. Similar results were obtained for both eyes (Fig. 3).

DISCUSSION

The clinical aspects of blepharoptosis are determined mainly by the severity of blepharoptosis and the function of the levator muscle, based on which there could be a risk of complications, such as abnormal head posture and amblyopia [8]. Stasior et al. [9] described the mechanism of raising the eyelid, such that in the area of the levator aponeurosis near the double eyelid, the posterior orbicularis oculi muscle and orbital septum merge to form a conjoined fascia; the eyelid would then be raised by sending the elastic fibers from this conjoined fascia to the tarsal plates and allowing the functioning of the anterior orbicular muscle together with the Müller muscle. Therefore, for the correction of blepharoptosis, it is essential to understand the levator aponeurosis. However, there are several re-

ports on the histological changes of the levator muscle in both innate and acquired blepharoptosis patients. Heuck [2] and Ahlström [10] reported that a histological examination of the levator muscle in congenital blepharoptosis showed cases in which levator muscles were extremely atrophied or even where finding any muscle tissue was difficult. Furthermore, in innate and acquired blepharoptosis with decreased function of the levator muscle, it was observed that the striated muscles of the levator muscle were both decreased and fiberized [11-13]. In the levator muscles of patients with acquired blepharoptosis, Berke and Wadsworth [11] observed striated muscle in a normal form, but in congenital blepharoptosis, they found reduced striated muscle and fibrosis in the levator muscle. These authors claimed that the severity of blepharoptosis was related to the state of the striated muscle, and in the case of congenital blepharoptosis, blepharoptosis would occur due to a defect in the levator muscle.

Many studies on both the levator muscle of patients with blepharoptosis and on the histological changes of the levator muscle have been reported. However, there are few findings on the levator aponeurosis. Some reports claim that there is no change in a blepharoptosis patient's levator aponeurosis. Landolt [14] reported that he performed an examination by H&E staining part of the levator aponeurosis that was cut from the tarsal plate during a blepharoptosis surgery, but he found no histological changes. However, considering that the levator aponeurosis plays an important role in raising the eyelid, and that blepharoptosis can be corrected by advancing the levator aponeurosis, it is necessary to check for any change in the levator aponeurosisin blepharoptosis patients. Morris et al. [7] reported that an elastin stain identified the elastic fiber network of the levator aponeurosis. However, they only evaluated the role of the levator aponeurosis in the Müllerectomy procedure for blepharoptosis correction. Moreover, they described no statistically significant difference between the amount of resection and the presence of the Müller or levator muscle in specimens from cadavers ($P > 0.05$) or patients ($P > 0.05$), but they did not examine the levator aponeurosis with regard to the amount of resection. Our study was performed to observe the histological changes in the levator aponeurosis that arise depending on the severity of blepharoptosis and age.

The epidermis consists of 1% elastin fibers, which maintain the elasticity of skin and other organs [15,16]. Elastin changes with age; when skin becomes old, it sags, which is thought to be due to the changes in the elastin itself [17]. With aging, the amount and diameter of elastin fibers is reduced; elastin also becomes shorter, as if it had been split into pieces [18].

Histologically, it is known that elastin exists in the levator aponeurosis [9]. As eyelids lose their elasticity, and sag with aging, the elastin in the levator aponeurosis is also thought to change with age. By monitoring for changes in the levator aponeurosis, as is done for the skin, it was determined that the older the elastin, the lower its distribution. As blepharoptosis becomes severe, the elastin distribution further decreases. Therefore, elderly blepharoptosis patients could manifest changes in the levator aponeurosis due to aging. In many cases of extreme blepharoptosis, symptoms reoccur after surgery [19]. In such cases, it is assumed that a reduction of elastin in the levator aponeurosis is the main cause.

The limitation of this study was that the size of the test subject pool was small (20 test subjects), patients between the ages of 25 to 50 were excluded, and the amount of elastin was not quantitatively analyzed (only the elastin distribution for each group was analyzed). However, considering the results of this study, it is recommended that when performing surgery in a patient who is too old or has severe blepharoptosis, and among patients who have undergone blepharoptosis surgery previously, better results can be achieved by deciding on the amount of correction.

PATIENT CONSENT

Patients provided written consent for the use of their images.

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