

Microscopic characteristics of the inferior tarsal muscle and its surroundings in Korean

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Abstract

• **AIM:** To investigate the detailed microscopic anatomic structures of the lower eyelid in Korean cadavers.

• **METHODS:** Eight lower eyelids of 4 formalin-fixed Asian cadavers (4 males; age range, 48–69 years; mean age, 60.2 years) were examined. Three perpendicular dissected sections with a 2mm thickness were obtained from each eyelid to investigate anatomic shapes, size and relationship with surrounding structures. One section was obtained from the midline and 2 the other sections were obtained from a 3mm apart from the lateral and medial tarsus margins.

• **RESULTS:** The inferior tarsal muscle fibers were not directly attached to the tarsus but were only linked to the tarsus with enclosed fibrous fascia. The inferior tarsal muscles connected loosely with the capsulopalpebral fascia anteriorly and the conjunctiva posteriorly. The inferior tarsal muscle runs horizontally to the tarsus according to the shape of muscle fibers. The capsulopalpebral fascia consisted of an anterior and posterior layer. The anterior layer reached the orbital septum and subcutaneous fat but the posterior layer forwarded into the tarsus. Lockwood's ligament was separated from the inferior tarsal muscle and capsulopalpebral fascia or fused into the capsulopalpebral fascia.

• **CONCLUSION:** This study suggests that the inferior tarsal muscle which runs horizontally and doesn't insert directly into the tarsus, plays an important role in the movement and localization of the lower eyelid.

• **KEYWORDS:** capsulopalpebral fascia; inferior tarsal muscle; lower eyelid

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INTRODUCTION

The inferior tarsal muscle of the lower eyelid is a homologous organ with the Müller muscle of the upper eyelid and it is innervated by sympathetic nerves like the Müller muscle [1]. Generally, the lower eyelid retractors include the capsulopalpebral fascia and the smooth muscle fibers surrounding the capsulopalpebral fascia. The inferior tarsal muscle is commonly described as a part of the capsulopalpebral fascia and the smooth muscle fibers under the fornix adjacent to the capsulopalpebral fascia [2,3]. Also, in most cases, it is irregular and immature and runs from the back of the capsulopalpebral fascia to the tarsal plate. Although the anatomy of the lower eyelid has been reported on cadavers, there are still obscure portions about the shape and function of the inferior tarsal muscle due to a lack of accurate explanation [4-6]. Knowledge of the anatomy of the inferior tarsal muscle may be important for determining the amount of surgical resection in ptosis, lower lid entropion and ectropion repair. However, the anatomic structure associated with the eyelid movement is complex and is still controversial. Furthermore, there are few studies on the anatomic structure of Asian eyelids [6-8]. Thus, we conducted this research focusing on the anatomical characteristics of the lower eyelid and the insertion of the inferior tarsal muscle to the tarsus to clarify their detailed anatomic relationship in Korean eyelid.

MATERIALS AND METHODS

Eight lower eyelids were taken from four Korean cadavers. The four cadavers were all male and the mean age was 60.2 years (range, 48-69). All eight lower eyelids of four cadavers had no other eyelid problems, such as eyelid malformation and eyelid surgery or trauma history.

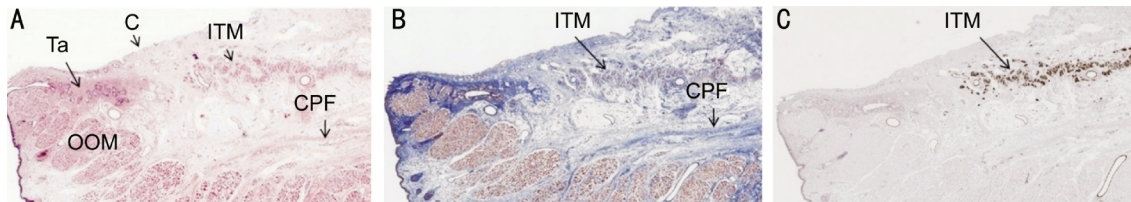


Figure 1 Histologic findings of the lower eyelid according to staining methods A: Hematoxylin-Eosin stain; B: Masson trichrome stain; C: Immunohistochemical study for smooth muscle actin antibody. B and C, The smooth muscle components of the inferior tarsal muscle are emphasized. (Ta: Tarsus; C: Conjunctiva; ITM: Inferior tarsal muscle; CPF: Capsulopalpebral fascia; OOM: Orbicularis oculi muscle; magnification $\times 10$).

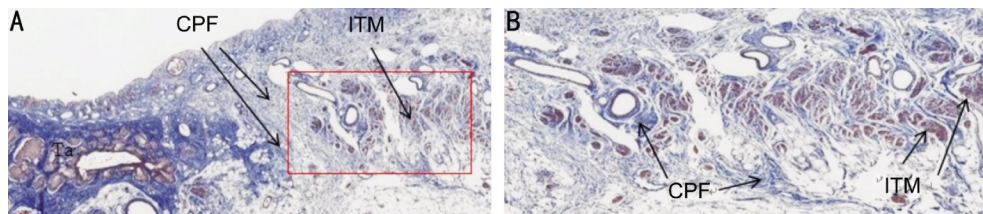


Figure 2 Microscopic findings of the inferior tarsal muscle (ITM) and tarsus (Ta) in Masson trichrome stain This photograph shows that the inferior tarsal muscle fibers are not directly attached to the tarsus and are only linked to the tarsus with enclosed fibrous fascia (capsulopalpebral fascia). (Ta: Tarsus; ITM: Inferior tarsal muscle; CPF: Capsulopalpebral fascia; A: Magnification $\times 10$; B: Magnification $\times 40$).

The sample eyelids were fixed in formalin solution for a minimum of 6 months. Full-thickness resection at three different sections was performed with a surgical blade. One section was obtained from midline and 2 the other sections were obtained at the lateral portion and medial portion. The vertical incision was joined by a horizontal incision 3cm above the eyelid margin. To examine relationship of the inferior tarsal muscle with surrounding structures, one specimen was obtained by dissecting the midline portion, and the other 2 specimens were obtained by dissecting the portion 3mm apart centrally from the lateral and medial tarsus margins, respectively. Each specimen was perpendicularly dissected with a 2mm full thickness and then was fixed in 10% formalin solution. The specimens were dehydrated over 24 hours in alcohol solution, and then processed for routine paraffin embedding over a period of 12 hours. The embedded specimens were microtomed to $4\mu\text{m}$ thickness sections. Sagittally sliced sections of all eyelids were stained with Hematoxylin-eosin, Masson trichrome, and anti-smooth muscle actin antibodies (DakoCytomation, Glostrup, Denmark) to observe nuclei, phosphoric acid, collagen fiber and smooth muscle fibers easily (Figure 1). Sections were examined with light microscopy (Olympus Inc. Japan) and photographs were taken using a digital camera (Carl Zeiss) affixed to the microscope. Dimensions of the structure and distance between them were measured by analyzing the digital photographs. To find out the relationship between the inferior tarsal muscle insertion and the tarsus, we measured the distance between the first identifiable smooth muscle bundle and the inferior tarsal margin in samples of the midline, lateral, and medial sections, respectively. In the same way, the distance from the capsulopalpebral fascia to the inferior tarsal border was measured.

RESULTS

Inferior Tarsal Muscle Did Not Insert Directly into the Tarsus

The inferior tarsal muscle of the lower eyelid consisted of bundle of smooth muscle fibers. The smooth muscle components of the inferior tarsal muscle were demonstrated by staining with Hematoxylin-eosin, Masson's trichrome, and anti-smooth muscle actin antibody. The findings from the samples according to 3 types of staining methods are illustrated in Figure 2. The inferior tarsal muscle did not insert directly into the tarsus, but its fibers enclosed some part of the capsulopalpebral fascia and tapered to the inferior margin toward the tarsus (Figure 1). The fibrous fascia surrounded the inferior tarsal muscle, became thinner and then was connected to the tarsal plate (Figure 2).

Distance Between the Superior Margin of The inferior Tarsal Muscle and the Inferior Margin of the Tarsus

The distance between the tarsal plate and inferior tarsal muscle varied from 1.4mm to 2.5mm. It measured as $(1.96 \pm 0.42)\text{mm}$ at the lateral side, $(1.98 \pm 0.34)\text{mm}$ at the midline and $(1.83 \pm 0.47)\text{mm}$ at the medial side respectively and therefore, any specific differences among them were not observed (Table 1).

Inferior Tarsal Muscle Connected Loosely with the Capsulopalpebral Fascia Anteriorly and the Conjunctiva Posteriorly

The anterior side of the inferior tarsal muscle is loosely adjacent to the capsulopalpebral fascia and its posterior side is loosely adjacent to the conjunctiva, respectively (Figure 3). The inferior tarsal muscle is surrounded by capsulopalpebral fascia, however, most of muscle fiber is independent and runs loosely adjacent to the capsulopalpebral fascia and its posterior side loosely adjacent to the conjunctiva.

Table 1 Distance between the smooth muscle fiber of inferior tarsal muscle and the inferior tarsal border of the tarsus

Case	1		2		3		4		Mean(mm)	¹ P
	OD	OS	OD	OS	OD	OS	OD	OS		
Lateral	2.5	2.4	1.8	2.0	1.7	1.6	1.5	1.4	1.96±0.42	
Center	2.4	2.2	2.3	1.5	1.9	1.7	1.6	1.4	1.98±0.34	0.919
Medial	2.1	2.2	1.6	1.8	1.7	2.0	1.8	1.8	1.83±0.47	

Distance between tarsus and inferior tarsal muscle. ¹Kruskal-waills test.

Table 2 Distance between the capsulopalpebral fascia and the smooth muscle of the inferior tarsal muscle

Case	1		2		3		4		Mean(mm)	¹ P
	OD	OS	OD	OS	OD	OS	OD	OS		
Lateral	0.5	0.9	1.0	0.9	0.6	0.6	1.1	1.1	0.84±0.24	
Center	0.5	1.4	0.7	0.6	0.9	1.0	0.8	0.8	0.84±0.28	0.970
Medial	1.0	0.7	0.9	0.7	1.1	0.5	1.3	0.6	0.85±0.27	

Distance between capsulopalpebral fascia and smooth muscle fibers of inferior tarsal muscle. ¹ Kruskal-waills test.

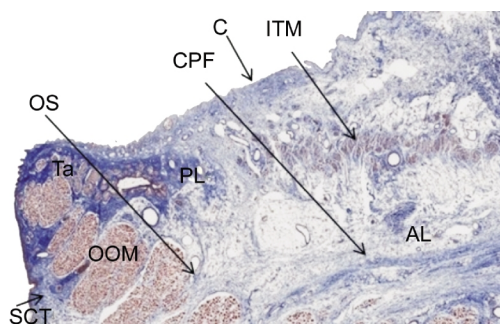


Figure 3 Microscopic finding of the inferior tarsal musclein Masson trichrome stain The inferior tarsal muscles connected loosely to the capsulopalpebral fascia anteriorly and the conjunctiva posteriorly. The capsulopalpebral fascia consists of an anterior and posterior layer. The anterior layer reached the orbital septum and subcutaneous fat but the posterior layer forwarded into the tarsus. (Ta, tarsus; C, conjunctiva; ITM, inferior tarsal muscle; CPF, capsulopalpebral fascia; AL, anterior layer of capsulopalpebral fascia; PL, posterior layer of capsulopalpebral fascia; OOM, orbicularis oculi muscle; OS, orbital septum; SCT, subcutaneous tissue; magnification ×10).

Distance Between the Inferior Tarsal Muscle and Capsulopalpebral Fascia The distance ranges from 0.5mm-1.1mm at the lateral side, from 0.5mm to 1.4mm at the midline and from 0.5mm-1.3mm at the medial side and averages (0.84±0.24)mm, (0.84±0.28)mm, (0.85±0.27)mm at the lateral side, the midline and the medial side respectively. This result demonstrates no difference in the distance according to a region (Table 2).

Inferior Tarsal Muscle Runs Horizontally When observing the perpendicular cutting plane of the inferior tarsal muscle through an light microscope, we found that the muscle fiber of the inferior tarsal muscle was cut perpendicular to the section and did not run toward the tarsal plate, instead, it ran toward the lateral and medial canthus (Figure 4A).

Relationship of the Capsulopalpebral Fascia and Surrounding Structures

The capsulopalpebral fascia could be separated into the anterior layer and posterior layer at the time of observing the running pattern of the capsulopalpebral fascia and the relationship between the capsulopalpebral fascia and surrounding structures. After reaching the orbital septum, the anterior layer met the orbicularis oculi muscle fiber bundle and then arrived at the subcutaneous layer by surrounding each muscle fiber bundle. Reaching the inferior tarsal muscle, similar to the state of the anterior layer, the posterior layer encircled each muscle fiber bundle and became thinner where it connected to the lower lid tarsal plate (Figure 3).

Lockwood's ligament was short on the section and observed as similar in size on the lateral side, the midline and the medial side. Lockwood's ligament was separated by the adipose tissue that is located below the inferior tarsal muscle and then it was located between the inferior tarsal muscle and the capsulopalpebral fascia (Figure 4B) or fused near the capsulopalpebral fascia (Figure 4C).

DISCUSSION

The inferior tarsal muscle which composes part of the lower eyelid retractor performs a function to retract the lower eyelid and innervates sympathetic nerves like the Müller muscle, an organ homologous with the inferior tarsal muscle. Much has been written about the anatomic structures of the lower eyelid, particularly their relation to the cause and treatment of epiblepharon or racial trichiasis [9-11]. But, the role and the anatomy of the inferior tarsal muscle are controversial so far. There are so many controversial theories about the relationship between the capsulopalpebral fascia and the orbital septum. Hawes and Dorzbach [12] reported that the inferior tarsal muscle consisted of scattered smooth-muscle fibers and did not insert into the tarsus. The orbital septum fused with the capsulopalpebral fascia 5mm beneath the lower tarsal border to form a single, complex fascial layer.

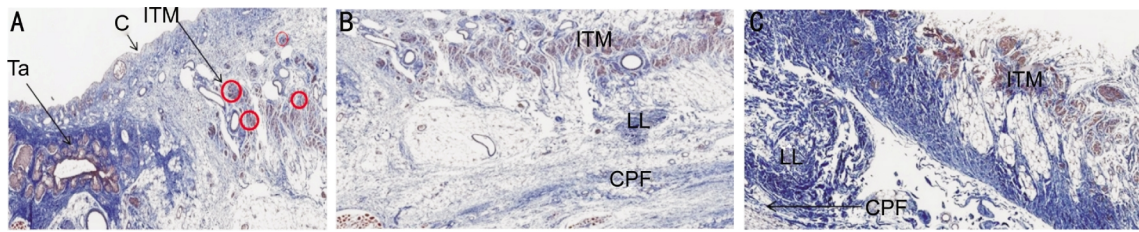


Figure 4 The cutting plane of the inferior tarsal muscle in Masson trichrome stain A: The inferior tarsal muscle runs horizontally to the tarsus according to the shape of the muscle fiber. A cutting plane of the muscle fiber is wrapped by circles of the fascia of many different sizes and run wide and horizontally. Lockwood ligament is separated from the inferior tarsal muscle and capsulopalpebral fascia (B) or fused into the capsulopalpebral fascia (C) in Masson trichrome stain. (Red circle: the cross section of inferior tarsal muscle; Ta: Tarsus; C: Conjunctiva; ITM: Inferior tarsal muscle; CPF: Capsulopalpebral fascia; LL: Lockwood ligament: magnification $\times 10$).

Hornblass^[13] described the capsulopalpebral fascia as proceeding anteriorly and superiorly to fuse with the orbital septum approximately 5mm below the lower tarsal edge, and above that point, he stated that a single fascial sheath passes upward to attach to the lower border of the tarsus. Bosniak^[14] said that the lower lid retractors insert at or slightly below the inferior tarsal border after fusing with the orbital septum. More inferiorly, the orbital septum is separated from the lower lid retractors by the preaponeurotic fat pad.

Also, the lower eyelid retractors has been considered a single layer since the detailed study by Hawes and Dorzbach^[12] was reported. Otherwise, Kakizaki *et al*^[15-17] reported that the lower eyelid retractors definitely consists of two layers and that a posterior thick, smooth muscle fibers bundle reaches the inferior tarsal plate to retract the lower eyelid. According to Kakizaki's opinion, the posterior layer is not a muscle itself but a fascial layer that includes scattered smooth muscles^[12], which have been referred to as the inferior tarsal muscle^[13], but these are not specific structures of the lower eyelid^[18]. Additionally, some authors have stated that there is no distinct fusion between the capsulopalpebral fascia and orbital septum. Cho *et al*^[7] reported that capsulopalpebral fascia was rarely fused with the orbital septum in sample of Korean lower eyelids and there was a tendency for the lower retractor to loosen from the tarsus and for increased fatty infiltration in the lower eyelids from elderly individuals. Kim *et al*^[8] described that the tarsal plate thickness in Korean lower eyelid was a difference in comparison to Chinese lower eyelids and there was no consistent fusion between the capsulopalpebral fascia and the orbital septum. Ellis and Zide^[19] contended that the orbital septum of the lower eyelid inserted into the inferior margin of the lower tarsus. Lim *et al*^[4] reported that no fusion of the orbital septum with capsulopalpebral fascia existed at the lower border of the tarsal plate in 70% of the specimens of Asian eyelids and very limited fusion in 30%.

The microscopic findings in the lower eyelids reported so far show some similarities and differences from the results of our study. First, the inferior tarsal muscle of the lower eyelid is not a single muscle but a grouping of several smooth muscle

fibers like Hawes's and Kakizaki's study. The inferior tarsal muscle did not insert directly into the tarsus, but its fibers were enclosed with some part of the capsulopalpebral fascia and tapered to the inferior margin of the tarsus. These reports do not differ from existing theories. Second, the anterior side of the inferior tarsal muscle is loosely adjacent to the capsulopalpebral fascia and its posterior side is loosely adjacent to the conjunctiva. The inferior tarsal muscle is surrounded by some capsulopalpebral fascia, however, most muscle fibers is independent and runs loosely adjacent to the capsulopalpebral fascia and its posterior side loosely adjacent to the conjunctiva. Third, the muscle fibers were wrapped by circles of the fascia of many different sizes and ran wide and horizontally. This aspect differs from a cutting section of upper eyelid of which Müller muscle fiber was cut vertically and generally traveled toward the tarsal plate. As previously stated, Hornblass^[13] concluded that the mean maximum total excursion of the superior tarsal muscle as 3.0mm, with 1.5mm of upward displacement and 1.5mm of downward displacement from its tonic position. The inferior tarsal muscle had a mean maximum total excursion of 0.3mm, with 0.1mm downward displacement and 0.2mm upward displacement from its tonic position, but the amount was variable, depending on the topical drugs used. The lifting effect of the Müller muscle was demonstrated clinically by the improvement of some ptotic eyelids after stimulation with phenylephrine eyedrops^[12]. Because of this anatomical structure that the inferior tarsal muscle runs horizontally, we could know clinically that there was no differences in the position of the lower eyelid compared with the upper eyelid after phenylephrine test. Fourth, the capsulopalpebral fascia consists of an anterior and posterior layer. The anterior layer reached the orbital septum and subcutaneous fat but the posterior layer forwarded into the tarsus. Unlike some authors had stated that there is no distinct fusion between the capsulopalpebral fascia and orbital septum, the result of our study showed fusion between the capsulopalpebral fascia and orbital septum.

This study suggests that the inferior tarsal muscle is wrapped by fibrous capsulopalpebral fascia and inserts into the inferior

tarsal plate indirectly. However, these smooth muscle fibers loosely form a group and their attachment to the lower eyelid retractor anteriorly, and palpebral conjunctiva posteriorly is not firm. So, it is not clear whether these smooth muscle fibers are involved in retracting the lower eyelid or not. A light microscope showed that the inferior tarsal muscle fibers have horizontal arrangement in cross-section view. Thereby, it suggests that the attachment to palpebral conjunctiva is not strong. According to Iwanami's study [20], the smooth muscle was not connected to the tarsus in all the sagittal sections and the smooth muscle fiber was punctuate in the midsection, indicating the cross-sections of the muscle as our study. But Iwanami and Tsurukiri [20] reported that increased muscle fiber was observed at the lateral edge of the tarsus. From these findings, they speculated that the smooth muscle runs from the temporal side of the rectus inferior muscle mainly toward the medial side in a diagonal direction unlike our study. A distance between the tarsal plate and inferior tarsal muscle was measured to be about 1.96mm, 1.98mm, and 1.83mm respectively on average in the lateral, median, and medial region (Table 2). These values were similar to each other. Through an experiment that used 15 male cadavers, Hwang *et al* [18] reported that the closest distance between the inferior tarsal border and the first identifiable smooth muscle nuclei of the inferior tarsal muscle was 2.1-2.7mm, and differently, (2.7±0.7)mm on the medial limbus line, (2.7±0.7)mm on the midpupillary line and (2.1±0.9)mm on the lateral limbus line. They suggested that there was no correlation between the distance and a region. Before Hwang *et al*'s study, Hawes and Dorzbach [12] showed that the average of 2.55mm was measured in the median region in the lower eyelids of twenty-two normal specimens that were studied microscopically to examine the normal anatomy of the lower eyelid retractors in 1982.

The Lockwood's ligament was isolated by a layer of fat or was united to the capsulopalpebral fascia between the inferior tarsal muscle and the capsulopalpebral fascia. It is known that Lockwood's ligament runs toward both the medial and the lateral canthus. Some think that further research on the relation and function between Lockwood's ligament and the inferior tarsal muscle would be needed in the future.

There are some limitations to our study. The specimens in this study were taken from elderly Korean, and it is possible that different findings might be seen in Westerners or in younger patients. Specifically, it is possible that the findings of the inferior tarsal muscle may actually be attributable to dehiscence caused by involuntional changes in the eyelid. It is also possible that findings may be different in fresh, rather than formalin-fixed, cadaver specimens. And the most critical drawback of the present study is the small specimen number. This study is about the anatomical findings of the lower

eyelid of Korean using a light microscope, with a focus on the unknown functions and localization of the inferior tarsal muscle and the relationship with surrounding structures. The results from this study can play an important role in lower blepharoplasty, as a baseline research. Also, a precise understanding of the lower eyelid might lead to improved treatments for various related diseases.

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