

The Anatomic Features of Müller's Muscle: A Histology Study in Chinese

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Background: The purpose of this article is to clarify the character of Müller's muscle in Chinese specimens.

Methods: Ten upper eyelids of 10 formalin-fixed Chinese cadavers (9 elderly people, from 68 to 86 years of age; 1 male child, 10 years old) were examined. Full-thickness sagittal sections of the central part of the upper eyelids were microscopically examined using hematoxylin-eosin, Masson trichrome, and anti-smooth muscle actin antibodies staining.

Result: In 9 elderly specimens, Müller's muscle inserted onto tarsus via fibers, and the smooth muscle components decreased gradually and even faded away from the originated inferior branch of levator palpebrae superioris muscle to the upper margin of tarsus, although, in 1 child specimen, Müller's muscle inserted directly onto tarsus rather than connective fibrous structure, and smooth muscle components did not decrease obviously. Both in elderly and young specimens, the aponeurotic structure that originated from Müller's muscle extended to the tarsal plate.

Conclusion: This study suggests that Müller's muscle inserts onto the tarsus via fibrous tissue or smooth muscle, and also indicates that the smooth components of Müller's muscle may decrease gradually in elderly people. (*Plast Reconstr Surg Glob Open* 2021;9:e3437; doi: 10.1097/GOX.0000000000003437; Published online 18 February 2021.)

INTRODUCTION

Müller's muscle is a sympathetically innervated muscle, which originates from the inferior branch of the levator palpebrae superioris muscle.¹⁻⁵ It takes part in the movement of upper eyelid together with levator palpebrae superioris muscle and levator aponeurosis. The anatomic structures between Müller's muscle and the tarsal plate are controversial and blurry, and previous studies indicated that the superior and anterior surface of the tarsus is covered by Müller's muscle tendon.^{3,4,6} Another research found that Müller's muscle inserted onto the tarsal plate

by fibrous connective tissue strands rather than inserted directly onto the tarsus.^{7,8}

In different studies, the results of the extension and attachment of Müller's muscle on the tarsus were conflicting and incompatible.³⁻⁹ In Asians, it has been reported that Müller's muscle tendon extends further downward to cover the upper two-third of tarsus.^{3,4,6} However, Hee et al found that Müller muscle fibers were tapered to the superior margin of the tarsal plate in 8 Asian specimens.⁸ An additional study performed by Kakizaki et al⁹ in 4 whites indicated that Müller's muscle extended beyond the superior margin of the tarsus and did not attach further than the upper third of the tarsus, but in the remaining 7 specimens showed that Müller's muscle attached to the superior aspect of the tarsus.

Although many studies have reported the relationship between Müller's muscle and the tarsal plate, the results were different and debatable.³⁻⁹ Therefore, it is important to explore the anatomic features of Müller's muscle and its relationship with the tarsus by histological studies. This study focuses on the characters and extensions of Müller's muscle on the tarsus to clarify the structures between Müller's muscle and tarsus in Chinese.

METHOD

Ten upper eyelids of 10 formalin-fixed Chinese cadavers aged at time of death from 10 to 86 years (8 elderly

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men, aging from 68 to 86 years, mean age 77 ± 5 years; 1 elderly woman, 82 years old; 1 boy, 10 years old) were microscopically examined. All cadavers were registered at Nanjing Medical University, and proper consent and approval were obtained before use. The methods for securing human tissue were humane and complied with the tenets of the Declaration of Helsinki.

After fixing in 10% formalin, the paraffin embedding was performed routinely. Each eyelid was dissected perpendicularly with 4-mm thickness. The embedded specimens were microtomed in 3- μ m-thick sections. To examine the fine anatomic relationship between Müller's muscle and the tarsus, the authors stained these specimens with hematoxylin-eosin, Masson trichrome, and anti-smooth muscle actin antibodies (α -SMA, Dakocytomation, Glostrup, Denmark). The authors also measured the distance between the first identifiable smooth muscle bundle in the distal end of Müller's muscle and the superior border of the tarsus, and measured the distance of fibers and smooth muscle components on the surface of the tarsus. All specimens were examined under an optical microscope and scanned by Panoramic Digital Slide Scanners (3DHISTECH, Budapest, Hungary).

RESULT

Nine elderly Asian specimens in the histological study showed that Müller's muscle did not insert directly onto the tarsus, but its fibrous tissue strand was attached to the tarsus (Figs. 1A, B and 2A). To observe the continuity and extension of the fibers from Müller's muscle, the fibers divided into 2 parts. One of them extended along the surface of the tarsal plate and then attached to the marginal artery. The extended along the conjunctiva and then

attached approximately to one-third part of the posterior surface of the tarsus (Fig. 1A, B).

Additionally, in 9 elderly specimens, the smooth muscle in Müller's muscle decreased gradually and even faded away from the originated inferior branch of levator palpebrae superioris muscle to the upper margin of the tarsal plate, but the fibrous tissue strand increased gradually (Fig. 2A). The smooth muscle component was not found on the anterior or posterior surface of the tarsus.

One child specimen in the histological study showed that Müller's muscle attached to tarsus via smooth muscle components, and then it transformed into aponeurotic structure which extended to the tarsal plate. The smooth muscle components were divided into 2 parts: the anterior part extended along the anterior surface of tarsus, and the posterior part extended along the conjunctiva (Figs. 1C, D and 2B). On the anterior and posterior surface of the tarsus, it could be found the smooth muscle component in this child specimen (Fig. 3). By observing the continuity and extension of the fibers from Müller's muscle, it can be found that the fibers from the anterior part were attached to the marginal artery and those from the posterior part approximately to the one-third part of the posterior surface of the tarsus (Fig. 1C, D).

Additionally, in these child specimens, the smooth muscle components in Müller's muscle did not decrease obviously from an originated inferior branch of levator palpebrae superioris muscle to upper margin of the tarsal plate, and the fibrous tissue strand did not increase conspicuously (Fig. 2B).

DISCUSSION

The relationship between Müller's muscle and the tarsal plate is controversial and inconsistent, with there being

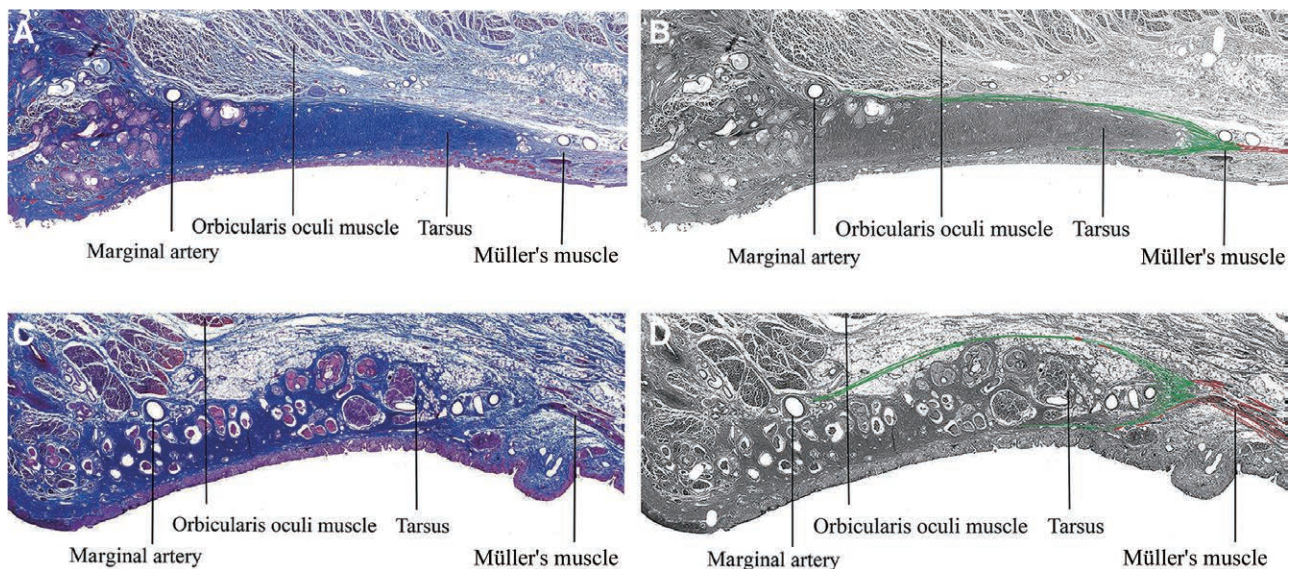


Fig. 1. Diagrams showing the insertion and extension of Müller's muscle on tarsus. A and B, An elderly specimen shows that Müller's muscle attaches to tarsus via fibrous tissue. C and D, A child specimen shows that Müller's muscle inserted directly onto tarsus, and then it transformed into aponeurotic structure which extended to the tarsal plate. A–D, Both pictures indicate that the fibers originated from Müller's muscle on the anterior surface of tarsus attached to the marginal artery, and the fibers on the posterior surface of tarsus attached approximately to one-thirds part of the posterior surface of the tarsus. B and D, the green color shows the extension of Müller's muscle and the red color indicates Müller's muscle. Bar = 0.5 mm.

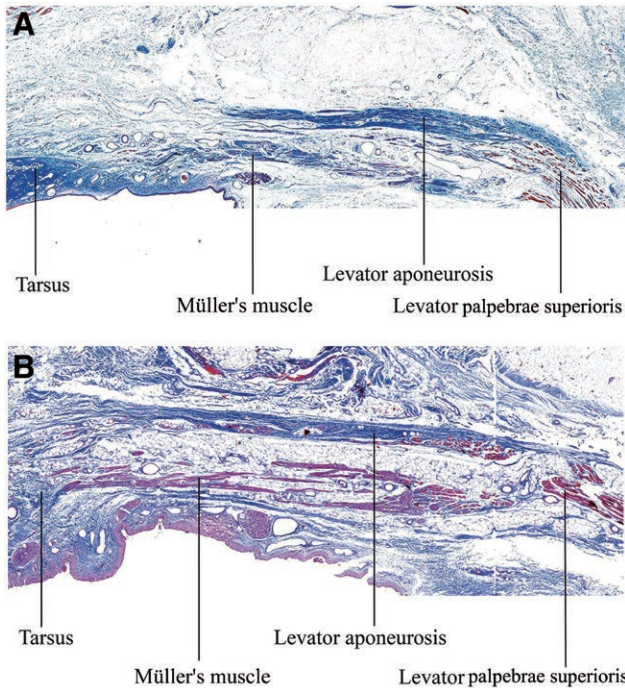


Fig. 2. Diagrams showing the transformation of Müller's muscle on tarsus. A, The elderly specimen shows that the smooth muscle in Müller muscle decreased gradually, even faded away from the originated inferior branch of levator palpebrae superioris muscle to upper margin of the tarsal plate, but the fibrous tissue strand increased gradually. B, From the origin of Müller's muscle to the upper border of the tarsus, the child specimen shows that the Müller's muscle does not decrease conspicuously, and the fibrous tissue strand does not increase obviously. Bar = 0.5 mm.

at least 3 different theories. The first is that Müller's muscle attaches directly to the superior border of the tarsus.¹⁰ The second is that Müller's muscle does not insert directly onto the tarsus, but its fibers attach to the superior margin of the tarsal plate.^{7,8} The third is that Müller's muscle transforms gradually into a tendon before inserting onto the superior tarsal plate border, and Müller's muscle tendon covers the anterior aspect of the tarsus.^{1-4,6}

The area which is attached or inserted by Müller's muscle is another dispute, and it includes a superior margin of the tarsal plate,^{7,11} a superior aspect of the tarsus,⁴ and covering by tapered fibers on superior tarsal plate border.⁸ In a previous study performed by Kakizaki et al, it reported that the extension of Müller's muscle surpassed the superior margin of the tarsal plate.⁷ Haramoto et al⁷ indicated that the fibrous tissue covering the anterior aspect of the tarsus is an extension of Müller's muscle rather than levator aponeurosis.⁴ Additional research considered that Müller's muscle might contribute to the main traction for the posterior lamella of the upper eyelid, because the superior and anterior surface of the tarsal plate was covered by Müller's muscle tendon.¹⁻⁴ All the above studies were concluded based on the observation of the histological section without direct evidence.

The present study found that, in 9 elderly specimens, Müller's muscle inserted onto tarsus via fibers rather

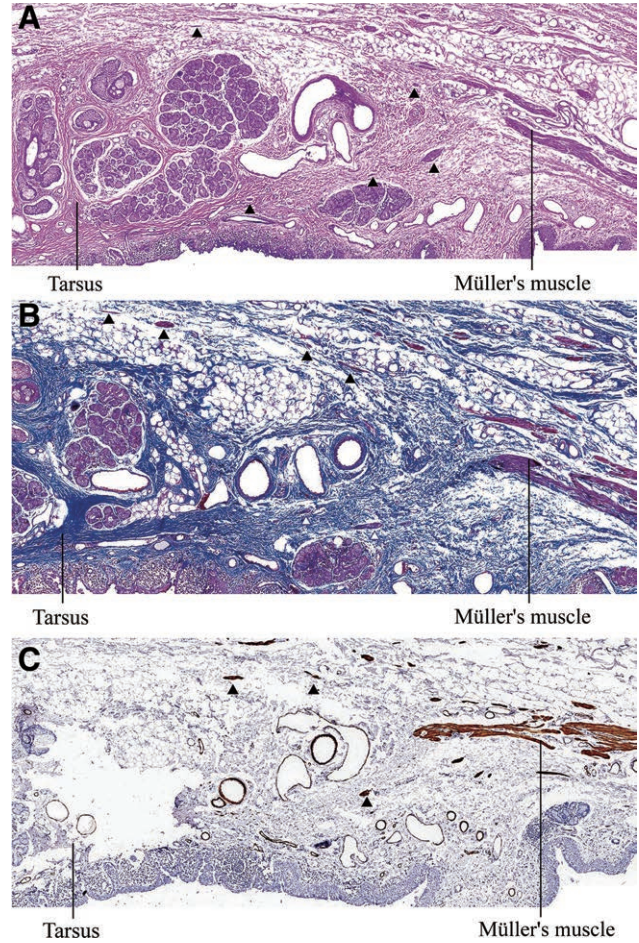


Fig. 3. Histologic findings of the smooth muscle components on the surface of the tarsal plate according to staining methods. A-C, (hematoxylin-eosin stain, Masson trichrome stain, and immunohistochemical study for smooth muscle actin antibody), the smooth muscle components (black and white triangle) can be found on the surface of the tarsus. Bar = 0.2 mm.

than inserted directly onto tarsus. However, in the child specimen, Müller's muscle was attached to the tarsus by smooth muscle components. By observing the extension of the fibers in this study, it can be identified that the anterior fibers were attached to the marginal artery, and the posterior ones approximately to the one-third part of the posterior surface of the tarsus. In all elderly specimens, the α -SMA positive smooth muscle components are not observed on the anterior or posterior surface of tarsus, but it could be found in the child specimen. To the authors' knowledge, this is the first study to describe in detail that the smooth muscle component has been found on the surface of the tarsal plate. Furthermore, this is direct evidence that the anterior and posterior surfaces of the tarsus are covered by Müller's muscle rather levator aponeurosis.

In 9 elderly specimens, from the levator palpebrae superioris muscle to the upper border of the tarsus, the smooth muscle components in Müller's muscle decreased gradually, and the fiber components increased obviously. The reason might be that the smooth muscle transforms

into a fibrous aponeurosis at the upper border of the tarsus. However, the result is contrary to the child specimen. The smooth muscle components in Müller's muscle did not decrease obviously, and the fibrous tissue strand did not increase conspicuously. Müller's muscle inserted directly onto the superior border of the tarsal plate rather than onto the tarsus via fibers.

It is hard to clarify the extension of levator aponeurosis and Müller's muscle at the pretarsal plate. The previous study presented that the levator aponeurosis walked along the surface of the tarsal plate and inserted into the anterior surface at the lower half or lower third of the tarsus.^{6,11,12} On the contrary, the study performed by Haramoto et al indicated that there was no direct connection between the levator aponeurosis and the tarsal plate and regarded the pretarsal fibrous layer as the extensions of Müller's muscle.⁴

However, the present study demonstrates that the aponeurosis which covers the surface of the tarsus originates from Müller's muscle rather than the levator aponeurosis. In a precious child specimen, the smooth muscle components can be found on the anterior and posterior surface of the tarsal plate with immunohistochemical staining. To the authors' knowledge, this is the first study to find the smooth muscle component on the surface of tarsus. Compared with elderly specimens in this study, the authors speculate that the smooth muscle components gradually decrease, the fibrous tissue strands increase, and the adipose tissue atrophies with the increase of age. In some specimens with less pretarsal adipose tissue, slides show that pretarsal levator aponeurosis connect with Müller's aponeurosis so closely that we cannot distinguish the border of both. In clinical cases with thin pretarsal tissue, pretarsal orbicularis oculi connect with superficial tissue of tarsus. However, slides with much posterior fatty tissue of pretarsal orbicularis oculi show clearly that most posterior of levator aponeurosis extends over the upper edge of tarsus and insert into the orbicularis oculi muscle, and little levator aponeurosis extends into eyelid margin along the anterior of tarsus. In addition, we can observe some aponeurosis-like structures derived from Müller's aponeurosis on the surface of tarsus.

Compared with child's specimen, smooth muscle fibers of Müller's muscle decrease and fibroses of Müller's muscle increase in elderly specimens. In our consideration, alleviating fibrosis of Müller's muscle may improve ptosis, which needs further verification in clinic. In addition, changes in Müller's muscle may play a more important role, which suggested that we should reserve Müller's muscle as far as possible by folding Müller's muscle properly when we perform correction of ptosis, instead of excising it excessively.

The limitation of this study is that the numbers and types of specimens. First of all, most of the specimens (8/10) were from Asian males, and there was only 1

elderly female specimen and one male child specimen, no young or middle-aged specimens, resulting in a lack of universality and diversity. Second, most of these specimens were men. Perhaps, there will be different results among more children, women, young and middle-aged people, and whites. The second limitation is similar to the first one (only one female and one child specimen). The authors hope that future research can obtain specimens of different ages, genders, and races, to verify our conclusion and guess.

In conclusion, the present study indicates that Müller's muscle inserts onto tarsus via the fibrous tissue or smooth muscle component. Based on the evidence of smooth muscle component and extension of the fibers from Müller's muscle, it can be deduced that the fibers covering the tarsal plate originated from Müller's muscle.

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