



Intramuscular neural distribution of the teres minor muscle using Sihler's stain: application to botulinum neurotoxin injection

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Abstract: The aim of this study was to elucidate the intramuscular arborization of the teres minor muscle for effective botulinum neurotoxin injection. Twelve specimens from 6 adult Korean cadavers (3 males and 3 females, age ranging from 66 to 78 years) were used in the study. The reference line between the 2/3 point of the axillary border of the scapula (0/5), where the muscle originates and the insertion point of the greater tubercle of the humerus (5/5). The most intramuscular neural distribution was located on 1/5–3/5 of the muscle. The tendinous portion was observed in the 3/5–5/5. The result suggests the botulinum neurotoxin should be delivered in the 1/5–3/5 area of the teres minor muscle.

Key words: Teres minor muscle, Sihler's method, Botulinum neurotoxin, Trigger point injection, Intramuscular neural distribution

Received March 29, 2023; Accepted May 16, 2023



Introduction

Botulinum neurotoxin has become a popular treatment in recent years due to its proven safety and effectiveness in reducing muscle tone from aesthetics to rehabilitations in focal spasticity of the upper limb, leading to an improvement in active upper limb function [1-9]. Several clinical trials have included the shoulder muscles, and they have shown

positive results in reducing shoulder spasticity and improving shoulder function [10-16]. In fact, an observational study of chronic post-stroke spasticity has also demonstrated that botulinum toxin injections can reduce pain and improve shoulder function [17]. However, despite these findings, no specific injection points have been suggested for the teres minor muscle so far.

To achieve maximum effectiveness, administering botulinum neurotoxin injections requires precise dosage and location. Studies have shown that injections near the neural arborized region, which has a high density of neuromuscular junctions, are especially effective [18-24]. However, it can be challenging to locate small nerves accurately through visual inspection. To overcome this issue, researchers have used a technique called Sihler staining, which highlights nerves while making muscle fibers transparent [25-34]. The objective of the current study is to utilize Sihler staining to better understand the distribution of intramuscular nerves in the teres minor muscle.

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Materials and Methods

This study was conducted in compliance with the Act on Dissection and Preservation of Corpses of the Republic of Korea (Act number: 14885) and approved by the Institutional Cadaver Research Committee of the College of Medicine, the Catholic University of Korea (MC22SISI0056).

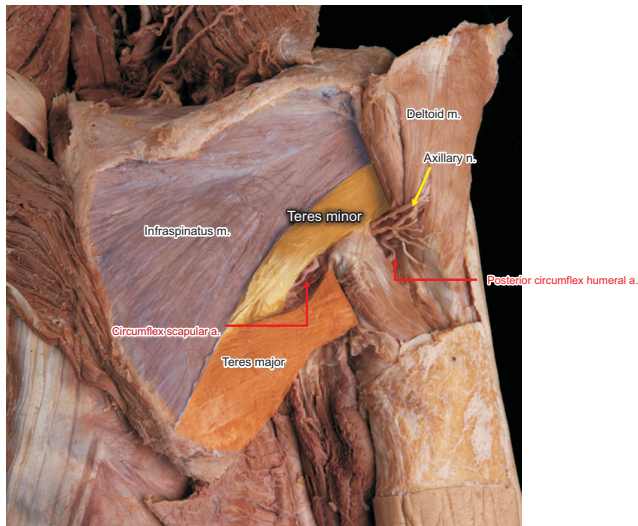


Fig. 1. The teres minor muscle has been revealed after removing posterior deltoid and dissected with surrounding muscles of infraspinatus, teres major, and long head of triceps brachii.

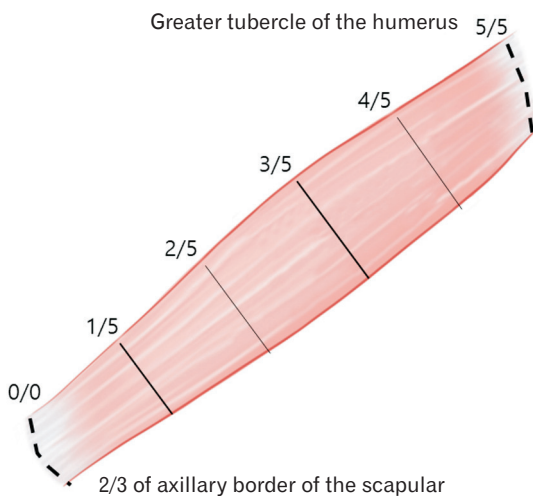


Fig. 2. The teres minor muscle was harvested from the 2/3 point of the axillary border of the scapula (0/5), where the muscle originates, and the insertion point of the greater tubercle of the humerus (5/5). The arborization patterns in the muscles were elucidated with respect to the horizontal length of the muscle with 5 divisions.

Before beginning the dissection, consent and approval were obtained from the families of the cadaver subjects. The study utilized a modified version of Sihler’s method to reveal the intramuscular nerve patterns in the teres minor muscle, using 6 cadavers. The cadavers were of Korean origin, 3 males and 3 females, age ranging from 66 to 78 years were used in the study. The twelve teres minor muscles were dissected and aligned according to anatomical structure (as depicted in Fig. 1) and stained to detect intramuscular neural distribution. The teres minor muscle was extracted from the 2/3 point of the axillary border of the scapula (0/5), where the muscle originates and the insertion point of the greater tubercle of the humerus (5/5) (as illustrated in Fig. 2). The arborization patterns in the muscles were analyzed in relation to the vertical length of the muscle, which was divided into 5 parts. This division was based on previous studies that found botulinum neurotoxin to spread a few centimeters from the injection site [17-19].

Sihler’s staining

The intramuscular neural arborization pattern of the teres minor muscles was studied using a modified Sihler’s staining technique, as shown in Figs. 3, 4. The muscle samples underwent multiple steps, which included fixation in non-neutral formalin for a month, treatment with potassium hydroxide and hydrogen peroxide to enhance transparency for a month, decalcification in a mixture of glycerin, acetic acid, and chloral hydrate for four weeks, staining with hematoxylin, glycerin, and chloral hydrate for five weeks, re-soaking in the decalcification solution to highlight the nerves, and finally display through immersion in glycerin solutions of increasing concentration (40%, 60%, 80%, and 100%). For a

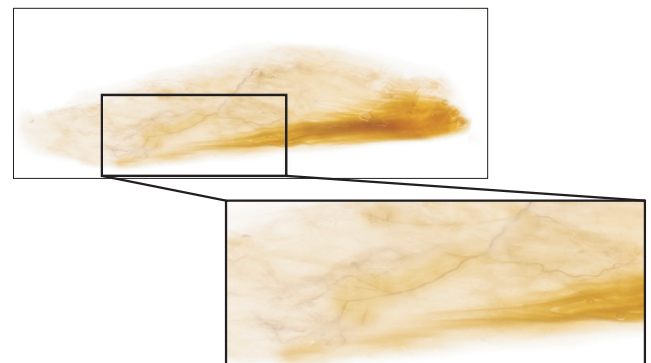


Fig. 3. A specimen of Sihler’s stained teres minor muscle with enlarged panels of intramuscular arborizations. The intramuscular pattern and nerve entry points are revealed by dissection and staining.

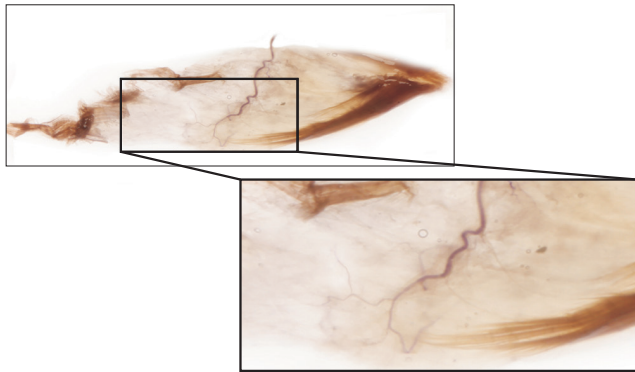


Fig. 4. A different example of Sihler's stained teres minor muscle is demonstrated, showing larger sections of internal branching. By dissecting and staining, the nerve entry points and internal pattern of the muscle can be observed.

more comprehensive overview of the procedures, refer to Yi et al. [34].

Results

Locations of nerve entry points

Ten of the 12 specimens had a nerve entry point between the 2/3 point of the axillary border of the scapula (0/5), and greater tubercle of the humerus (5/5), while the other two specimen had a nerve entry point between 1/5 to 2/5.

Intramuscular arborization patterns

Nine out of the 12 teres minor muscles had the largest arborization patterns, in the located on 1/5–3/5. Two teres minor muscle had the largest arborization patterns in the 2/5–3/5. One teres minor muscle had the largest arborization patterns in the 1/5–2/5.

Tendinous portion

The tendinous portion was observed in the 3/5–5/5 of the teres minor muscle in 6 specimens. And other 6 specimen had tendinous portion of 2/5–5/5.

Discussion

The teres minor muscle originates from the back surface of the axillary border of the scapula, covering about two-thirds of its length. Additionally, it originates from two aponeurotic laminae that separate it from the infraspinatus and teres major muscles. The main function of the teres minor muscle is lateral rotation, and it also contributes to shoulder

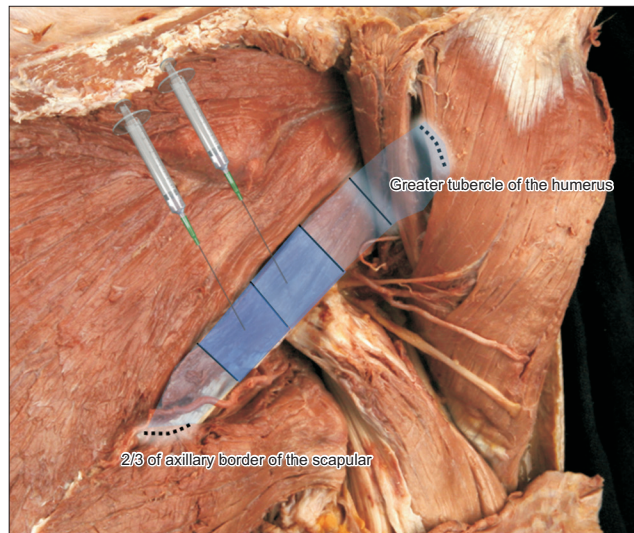


Fig. 5. The teres minor muscles had the largest arborization patterns (shaded in blue), in the located on 1/5–3/5. The tendinous portion was observed in the 3/5–5/5. The needles in the photograph show ideal injection areas along the teres minor muscle where the largest arborization was observed.

adduction in conjunction with the infraspinatus muscle. However, the two muscles are distinguished by their nerve supply, with the teres minor muscle being supplied by the axillary nerve and the infraspinatus muscle by the supra-scapular nerve. If either muscle becomes stiff, it can restrict movement of the shoulder joint, leading to limitations in movement [35].

Chemical neurolysis with phenol, alcohol, and botulinum neurotoxin injections is a potential treatment for muscle stiffness [36]. Botulinum neurotoxin works by blocking the release of acetylcholine from nerve endings at the neuromuscular junction, resulting in selective effects on motor nerves without impacting sensory nerves [27, 37-39]. Compared to phenol and alcohol, botulinum neurotoxin has lower side effects, making it a popular option for managing spasticity. To achieve the maximum effect, it is recommended that these injections be administered close to areas where neuromuscular junctions are most densely distributed.

The muscles located at the back of the shoulder include the posterior deltoid, infraspinatus, and teres minor muscles, which can cause limited internal rotation due to muscle stiffness [40]. The findings of this study on the injection point may help to improve shoulder movement.

The significance of using neuromuscular junction-targeted botulinum neurotoxin injections has been confirmed in clinical studies in the biceps brachii muscle and iliopsoas

muscle. Neuromuscular junction-oriented injections result in much greater volume reduction than control injections [18, 19].

In this study, the origin and insertion point of the teres minor muscle were selected as the reference point for injections, as they are easily identifiable, especially in patients with limited upper extremity movement that may make it challenging to locate other landmarks.

There are limited studies that have investigated the anatomy of the teres minor muscle. Our study identified the location of the intramuscular neural dense area of the nerve branch that connects to the teres minor muscle. This area was found to be located between 1/5 and 3/5 from the reference point and these areas should be considered in clinical fields (Fig. 5). This finding could serve as a helpful guideline for injections targeting the teres minor muscle.

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Conceptualization: KHY, SBK. Data acquisition: KHY, SBK, KL, HH. Data analysis or interpretation: KHY, SBK, KL, HH. Drafting of the manuscript: KHY, SBK. Critical revision of the manuscript: JHL, HJL. Approval of the final version of the manuscript: all authors.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

Funding

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (NRF-2020R1A2B5B0100223811).

Acknowledgements

The authors sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge that can then improve patient care. Therefore, these donors and their families deserve our highest gratitude.

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