Observational Study

Novel Ultrasound-guided Injection Method for Thoracic Outlet Syndrome Based on Anatomical Features: A Cadaveric Study

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Background: Despite the positive effect of botulinum neurotoxin (BoNT) injections in thoracic outlet syndrome (TOS) treatment, there is insufficient anatomical evidence of its use in the anterior scalene (AS) and middle scalene (MS) muscles.

Objectives: This study aimed to provide safer and more effective guidelines for the injection of botulinum neurotoxin into scalene muscles for the treatment of thoracic outlet syndrome.

Study Design: The study was based on an anatomical study and ultrasound studies.

Setting: This study was conducted at the Division in Anatomy and Developmental Biology, Department of Oral Biology, Human Identification Research Institute, BK21 FOUR Project, Yonsei University College of Dentistry (Seoul, Republic of Korea)

Methods: Ten living volunteers underwent ultrasonography, and the depths of the anterior scalene (AS) and middle scalene (MS) muscles were calculated from the skin surface. In cadaveric specimens, fifteen AS and 13 MS muscles were stained using the Sihler staining procedure; the neural arborization pattern was identified, and localized dense portions were investigated.

Results: The mean depth of the AS was 9.19 ± 1.56 mm, and that of the MS was 11.64 ± 2.73 mm at 1.5 cm above the clavicle. At 3 cm above the clavicle, the AS and MS were clearly located 8.12 ± 1.90 mm and 10.99 ± 2.52 mm deep, respectively. The nerve ending points were highest in the lower three-quarters of the AS (11/15 cases) and MS muscles (8/13 cases), followed by the lower quarter (AS muscle, 4/15 cases; MS muscle, 3/13 cases).

Limitations: There are many difficulties for clinics to directly perform ultrasound-guided injections in clinical practice. However, results of this study can be used as basic data.

Conclusion: According to anatomical features, the appropriate location for botulinum neurotoxin injection in the AS and MS muscles for the treatment of TOS is the lower portion of the scalene muscles. Therefore, it is recommended to inject at a depth of approximately 8 mm for AS and 11 mm for MS at a point 3 cm above the clavicle.

Key words: Thoracic outlet syndrome, ultrasound-guided injection, nerve arborization pattern, botulinum neurotoxin, noninvasive treatment, scalene muscles, anterior scalene, middle scalene

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Thoracic outlet syndrome (TOS) is a condition that causes various symptoms related to nerve or vascular structures in the upper limb (1-3). TOS results in pain, fatigue, and paresthesia in the arm and is caused by neurovascular compression applied to the neck, just above the first rib (1-3).

Although there is no standard treatment for TOS, recent studies have reported the highly effective use of botulinum neurotoxin (BoNT) in improving musculoskeletal disorders, including TOS. Previous studies have suggested that the application of BoNT therapy can help relieve symptoms in patients who do not obtain positive results from physical therapy (2). BoNT therapy is one of the most effective and safe treatments for muscle tone relief and as a solution for stiffness in local areas (2,4). Further, BoNT injection therapy in the anterior scalene (AS) and middle scalene (MS) muscles can be applied for several pain syndrome treatments. The scalene muscles are located behind the sternocleidomastoid (SCM) muscle and the deep layer of the neck. The AS muscle originates from the anterior tubercles of the transverse processes of the third to the sixth cervical vertebra and inserts into the first rib, while the MS muscle originates from the posterior tubercles of the transverse processes of the second to the seventh vertebra and inserts into the first rib. Since various anatomical structures are located around the AS and MS muscles, such as the brachial plexus and transverse cervical artery, it is difficult to specify an optimal injection point.

BoNT injections have high directivity towards motor endplates. To ensure a therapeutic effect, it is important to accurately position the needle in the target muscle (4,5). In addition, if the physician can target the exact area, the expected effect can be realized with a smaller dose. For this purpose, various studies have been conducted, but they have limitations in clinical application owing to factors such as cost, the required time, and stability. A procedure that relies only on the clinician's experience to properly place the needle results in unintended side effects, such as inadvertent somatic and sympathetic blocks. Therefore, continual research is required in various fields based on anatomical considerations of the target muscle. However, more detailed information on the proper location based on surface landmarks, depth, and innervation analysis of the scalene muscles also requires elucidation.

This study aimed to 1) reveal the anatomical features of the scalene muscles and 2) present anatomy-based guidelines for safer and more effective BoNT injections in the AS and MS muscles for the treatment of TOS.

Methods

Ethics Statements

In this study, we simultaneously conducted research using both cadaveric specimens and living participants. The cadaver specimens were legally donated to the Yonsei University College of Medicine. The use of cadavers was carried out with the informed consent of the donors before their death, and the consent of the next of kin was also obtained posthumously, allowing the research to proceed. Before conducting the ultrasound (ULSD) examination, all volunteers (living participants) were provided with a detailed description of the study's purpose, methods, and risks. They were informed that they could withdraw from the experiment at any time if they desired. Subsequently, the participants signed an informed consent form. All study procedures were approved by the Ethics Committee of the Yonsei University College of Dentistry (IRB No. 2-2021-0122). This study was conducted in accordance with the principles of the Declaration of Helsinki.

Harvesting Specimens

Fifteen AS (right side, 7; left side, 8) and 13 MS muscles (right side, 7; left side, 6) were harvested from 8 cadavers. There was no medical history of the cadavers' anatomical structure. Sihler staining of the scalene muscles was performed to identify their neural arborization pattern. This method involves multiple stages to obtain a visual image of intramuscular neural arborization. We applied this method with modifications as described below. The process was conducted very carefully to avoid damaging the nerve entry point. After Sihler staining, the stained specimens were divided into quarter sections according to a set standard, and then the innervation pattern and localized dense portions of the stained specimens were investigated.

Modified Sihler Staining

The staining method was as follows:

1) Fixation: The procured scalene muscles were placed in 10% unneutralized formaldehyde for one month.
2) Maceration and depigmentation: Fixed samples were washed in clean water for one hour. Later, the samples were depigmented for 2–3 weeks in 3% aqueous potassium hydroxide in combination with hydrogen peroxide.
3) Decalcification: Depigmented samples were placed in a Sihler I solution, a mixture of glacial acetic acid and glycerin in distilled water, for 3 days.

4) Staining: Once the samples were decalcified, they were placed in a Sihler II solution, a mixture of Ehrlich hematoxylin and glycerin in distilled water, for 24 hours.
5) Destaining: The stained samples were destained with Sihler I solution for 3–5 hours.
6) Neutralization and bluing: The destained samples were neutralized in running tap water for 30 minutes. Then, the samples were blued in 0.05% lithium carbonate for 30 minutes to block nerve fibers.
7) Clearing: The neutralized samples were cleaned in increasing concentrations of formamide from 70% to 100%.

Ultrasound Examination

Ten living volunteers underwent a USLD examination and ultrasonograms were acquired from both sides of the scalene muscles.

The AS and MS muscles exist at the level of the C3 and C2 vertebrae and tC2, respectively. Therefore, based on the anatomical characteristics of the scalene muscles and Sihler staining results, we determined that the injection point of the scalene muscles should be set lower than the C7.

The AS and MS muscles originate from C2 to C7 they are inserted into the first rib, which is behind the clavicle. Usually, the vertebral level of the clavicle is the height of T3. Therefore, based on the anatomical characteristics of the scalene muscles and Sihler staining results, we determined that the injection point of the scalene muscles should be set in the T1 or T2, which are the lower parts of the scalene muscles. According to the results of previous studies, the average height of the upper thoracic vertebra is 1.5 cm, so about 1.5 cm and 3 cm above were set as target heights for AS and MS, respectively. In the mid-height region of the neck, the scalene muscles are covered by the SCM muscle, but at the clavicle level, they are located lateral to the SCM muscle. In addition, the SCM is a large, prominent muscle that can be easily palpated, making it a landmark. In addition, to empirically target AS and MS, they were set to 1.5 and 3 cm laterally, respectively.

Therefore, using the meeting point between the lateral border of the clavicle portion of the SCM muscle and the superior border of the clavicle designated as a marker, reference points were marked at 4 points, each 1.5 cm and 3 cm on the superolateral sides, respectively. Then, ultrasonograms were obtained at 1.5 cm and 3 cm above the clavicle on the 3 cm lateral line of the lateral border of the SCM muscle (Fig.1).

The lateral borders of the SCM, AS, and MS muscles were identified from the acquired ultrasonograms, and the depths of the AS and MS muscles were measured. All ULSD examinations to measure depth were performed using a real-time 2-dimensional B-mode ULSD scanner (SONIMAGE HS1, Konica Minolta) with a B-mode high-frequency linear array transducer (18 MHz, Konica Minolta).

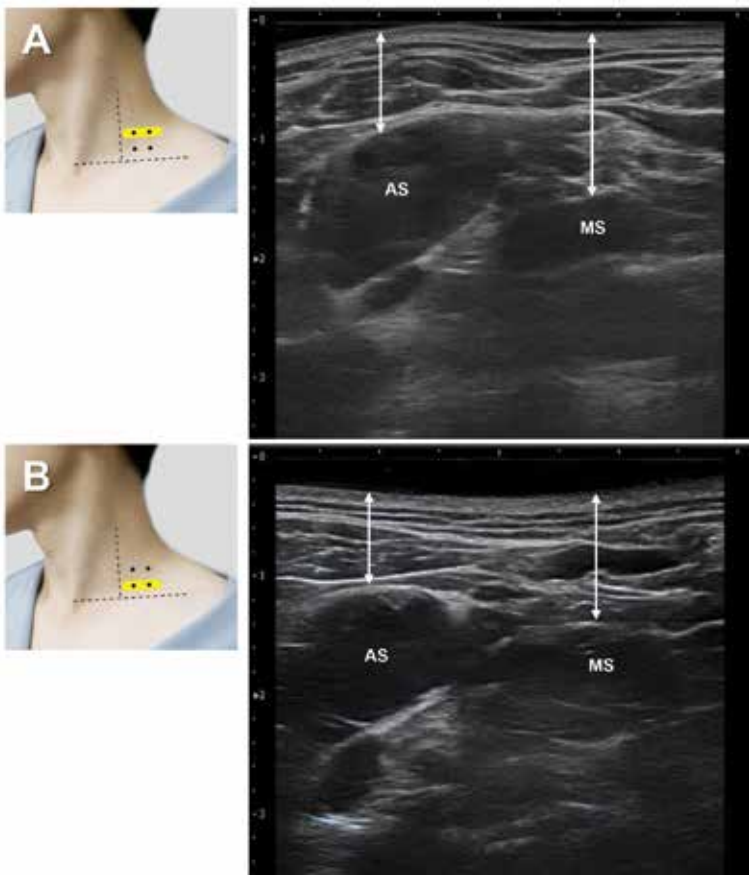

Fig. 1. *Depth measurement point and ultrasonogram of the scalene muscles at 3 cm (A) and 1.5 cm (B) points above the clavicle for injection guidelines.*

Results

Modified Sihler Staining

The nerve ending points were highest in the lower three-quarters of the AS (11/15 cases) and MS muscles (8/13 cases), followed by the lower quarter (AS muscle, 4/15 cases; MS muscle, 3/13 cases). By analyzing the quadrant sections of the AS and MS muscles, we found that most nerve endings were located at the lower three-quarters and lower quarter points in both muscles. Figure 2 shows the region where the nerve endings were densely distributed.

Ultrasound Measurement

The mean depth of the AS muscle was 9.19 ± 1.56 mm, and that of the MS muscle was 11.64 ± 2.73 mm at the 1.5 cm point. The deepest AS muscle was 12.44 mm deep, and the shallowest value was 6.32 mm. The maximum and minimum values of the MS muscle depth were 17.07 mm and 6.99 mm, respectively.

At the 3 cm point, the AS and MS muscles were located 8.12 ± 1.90 mm and 10.99 ± 2.52 mm deep, respectively. At the same position, the maximum and minimum values of the AS muscle were 14.18 mm and 4.66 mm, and those of the MS muscle were 15.12 mm and 7.37 mm, respectively. These values are presented in Table 1.

Discussion

TOS can be classified as arterial, venous, or neurogenic; however, the majority of cases (approximately 95%) are neurogenic TOS (6,7). Neurogenic TOS is caused by damage to the trunks or cords of the brachial plexus formed by the C5–T1 spinal nerves, resulting in pain, paresthesia, and numbness in the neck, shoulder, arm, and hand regions (8). Symptoms are more severe when the arm is lifted upward, and pain may occur in the trapezius, neck, and occipital region muscles; in some cases, anterior chest wall pain can occur (1,6,9). Injury to the scalene muscles is the most common etiology of TOS, which causes tenderness in the AS, MS, and subcoracoid space (2). When an injury occurs, the patient feels pain within a few days and can develop bleeding and swelling. Within weeks, cramps in the arms and hands can occur (6,8,10). When spasms occur in the AS and MS muscles, pressure is applied to the brachial plexus located between those muscles, causing pain and paresthesia in the upper limbs (11).

TOS treatment can be broadly divided into conservative and surgical treatment options. Conservative treatment focuses on physical therapy, including neck and shoulder stretching, and medication, such as trigger point injections or anti-inflammatory drugs. Recently, various techniques have been implemented to relax muscles and reduce pressure by directly injecting drugs such as anesthetic agents, steroids, and BoNT. In particular, there is increasing evidence that BoNT is effective in pain management for TOS (2,3,9,12). Previous studies have reported that the effects of an anesthetic agent injection were short-term and only adjuvant, but BoNT injection facilitated more continuous symptomatic improvement (3,7,13,14). In addition, an advantage of BoNT injection is that it is able to predict improvement after surgical decompression (14,15).

BoNT injection treatment has proven to be effective in various cases, such as chronic neuropathic pain, myofascial pain syndromes, chronic neck pain, and low back pain (3,16-19). This method is considered the most efficient and reliable alternative to relieve muscle contraction (20,21), and previous studies have suggested it as the

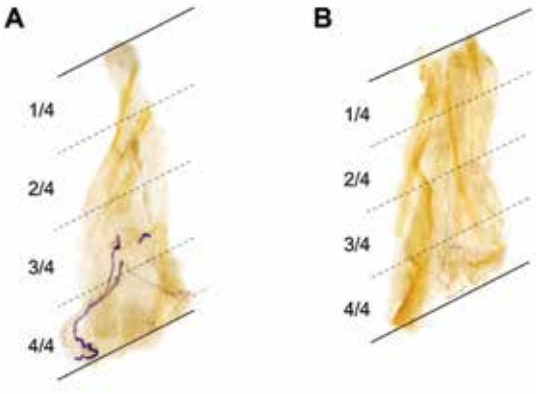

Fig. 2. *Separation for analysis of proportions of intramuscular innervation distribution of the anterior scalene (A) and middle scalene (B) muscles. In the stained specimen, the most locally dense part was analyzed and marked in number.*

Table 1. *Ultrasound measurement of the depths of the anterior scalene and middle scalene muscles from the skin surface.*

Unit: mm	1.5 cm Point		3 cm Point	
	Anterior Scalene	Middle Scalene	Anterior Scalene	Middle Scalene
Mean ± SD	9.19 ± 1.56	11.64 ± 2.73	8.12 ± 1.90	10.99 ± 2.52
Maximum	12.44	17.07	14.18	15.12
Minimum	6.32	6.99	4.66	7.37

most effective minimally invasive treatment method for TOS (7,22,23). BoNT injection in the AS and MS muscles can also be used to treat cervical dystonia (24,25).

BoNT injection reduces muscle contraction through local chemical innervation. Proper treatment can reduce pressure on neurovascular structures, relieve pain, and lead to decreased sensation and improved function (3,16). The most important aspect of BoNT injection is its administration to the correct motor end-plate area (26-28). Accidental spread of the toxin in the neck area can cause muscle weakness, aspiration, dysphonia, or dysphagia; technology that locates the target muscle more accurately with a smaller amount of injectate is important for effective and safe treatment (7,8). Therefore, this study focused on identifying intramuscular arborized zones and providing guidance on injection points for effective and safe BoNT treatment.

In a study of unguided injections based on anatomical landmarks, injection at an angle of 30° downward from the lateral corner of the SCM muscle toward the inner third of the clavicle was suggested (23). A more detailed reference point is required since an inaccurate injection can lead to unexpected results, such as dysphagia or excessive muscle weakness (29).

To accurately target the AS and MS muscles, which are small and have complex surrounding structures, guided injection methods such as fluoroscopic, ULSD, and computed tomography guidance have been suggested (7,8,12,14,22,24,30). In most cases, the injection point is determined through clinical examination, and even when the anatomical structure is considered, only superficial structures tend to be referenced. In previous studies, the space formed by the AS and MS muscles and the first rib was suggested by the scalene triangle as a reference point; however, these structures are not suitable for use as a surface reference point (15). Other studies have investigated the size and location of the muscles, but the relationship between surface structure and innervation patterns were not considered (25). In addition, no research on the AS and MS muscles has determined the nerve entry point or intramuscular neural arborization. It is difficult to trace the microscopic distribution of nerves in the muscle with the naked eye, and analysis of innervation through anatomy is difficult due to the possibility of nerve damage. Therefore, in this study, the anatomical positional relationships of both muscles were considered, and analysis was performed using Sihler staining, which efficiently revealed the distribution of nerves in the muscles without damage to the nerves.

Between the AS and MS muscles, the brachial plexus, a major anatomical structure, is located. The transverse cervical artery has also been observed in this location on ULSD imaging in many cases (31). Therefore, USLD-guided injection is essential to ensure a safe injection.

At the 1.5 cm point above the clavicle, to distinguish the AS and MS muscles on the ultrasonogram, we had to adjust and track the transducer based on the anatomical structure. In comparison, in the case of the 3 cm point above the clavicle, the shape and structure of the AS and MS muscles could be clearly identified without operator manipulation. Hence, it was determined that targeting the 3 cm point was an appropriate injection method (Fig. 3). This is consistent with the results of neural terminal distribution analysis.

We performed USLD-guided injections on frozen-thawed cadaveric specimens using colored fillers. The AS muscle was injected at a depth of approximately 8 mm, while the MS muscle was injected at a depth of 11 mm, 3 cm above the clavicle. We then carefully dissected the muscle layers to verify the accuracy of the injections. Based on the measurements obtained

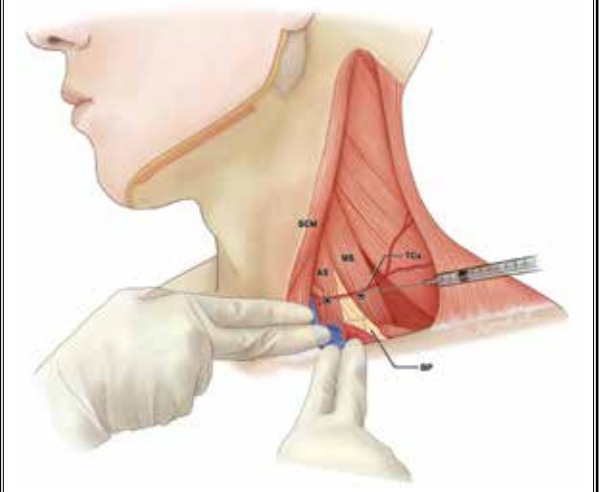

Fig. 3. *Injection guidelines based on anatomical landmarks. Aim for a 3 cm point based on the meeting point between the LCS and the upper boundary of the clavicle, designated as the reference point. Since 2 adult fingers reach approximately 3 cm deep, the LCS and clavicle can be identified smoothly by manual palpation. LCS, lateral border of the clavicle part of the sternocleidomastoid muscle; SCM, sternocleidomastoid muscle; AS, anterior scalene; MS, middle scalene; TCA, transverse cervical artery; BP, brachial plexus.*

during the cadaveric injections, we confirmed that the injections were accurately placed within the AS and MS muscles, as depicted in Fig. 4.

The advantages of the ULSD-guided injection technique have been reported in several studies (12,15,32-34). Therefore, we aimed to determine a more effective and safe treatment method by providing in-depth information on the target muscles to ensure accurate treatment and analyze the distribution of nerves for BoNT treatment. Several methods can be used to ensure that the needle is correctly placed in the target muscle; however, the safest and most effective method is ULSD-guided injection. Following the appropriate anatomy-based BoNT treatment guidelines presented in this study, we are confident that clinicians can easily and effectively target the scalene muscles for BoNT treatment.

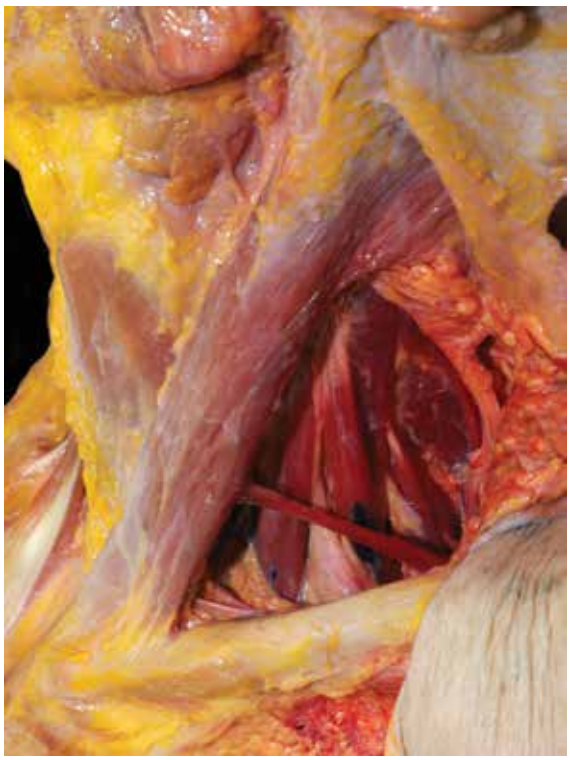

Fig. 4. *Results of ultrasound-guided injection in a frozen-thawed cadaver based on the suggested injection points.*

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Author Contributions

All authors were informed and understand the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects - and confirmed that the present study fulfilled the declaration. None of the authors have financial or private relationships with commercial, academic, or political organizations or people that may have improperly influenced this research. Author contributions were: overall planning of the research, data acquisition, analysis and interpretation, and major drafting and revision of manuscript submission, K.-L. L.,; data acquisition, analysis and interpretation, and major drafting and revision of manuscript submission, J.-H. L.,; figure creation of key results, analysis, and interpretation, drafting and revision of the manuscript, H.-W. H.,; provided the anatomical and clinical opinion for conception, overall organization, and direct supervision of the research, H.-J. K.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Yonsei University College of Dentistry (protocol code 2-2021-0122; date of approval, February 23, 2022).

Informed Consent Statement: Informed consent was obtained from all patients involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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