Cadaveric Study

Ultrasound-guided Injection of the Levator Scapulae Muscle in a Cadaver Model

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Background: Despite the advantages of ultrasound and previous anatomical data on neuromuscular junction locations, to the best of our knowledge, the feasibility and accuracy of precise ultrasound-guided injection techniques into the proposed injection site of botulinum neurotoxin for the levator scapulae muscle have not been assessed in any publication.

Objective: In the present cadaver-based study, the ultrasound-guided injection technique in the middle and distal portions of the levator scapulae muscle was evaluated to determine whether this method distributes injections properly to the target muscle in fresh cadavers.

Study Design: Cadaveric study.

Setting: A cadaver laboratory.

Methods: Twenty fresh cadavers were used. Real-time B-mode ultrasound scanning was performed interfaced with a linear array transducer. A mixture of 0.5 mL of dye and yellow filler was injected transverse in-plane with a 6 cm 21-G. needle. Each specimen was dissected to determine whether the dye was correctly targeted to the middle and distal portions of the levator scapulae muscle and to evaluate the accuracy of the injections and any complications.

Results: All 40 injections were successfully injected within the middle and distal portions of the levator scapulae muscle. When dissecting the cadavers, the dye spread was evenly distributed along the muscle fiber.

Limitation: Despite successful injection into the middle and distal portions of the levator scapulae muscle, the usefulness of this technique was not verified in clinical practice.

Conclusions: The ultrasound-guided injection technique presented in this study might facilitate precise visualization and localization of the levator scapulae muscle, thereby enhancing the effectiveness and safety of botulinum neurotoxin treatment in cervical dystonia.

Key words: Levator scapulae muscle, ultrasonography, injections, pain, cervical dystonia, cadaver

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Cervical dystonia (CD) is a neurological condition in which neck muscles involuntarily contract, twist, or repeatedly move, and may accompany tremors and pain. This disorder can limit activities of daily living and social functioning, negatively affecting patient quality of life (1). The most common treatment options to ameliorate most types of focal dystonia are botulinum neurotoxin

(BoNT) injection, oral medication, and physiotherapy/physical therapy (2).

A BoNT injection has been considered the first-line treatment for CD since the US Food and Drug Administration approved its use (3). The most frequently involved muscles for treating CD are the sternocleidomastoid, trapezius, levator scapulae, splenius capitis, semispinalis capitis muscles, and the deep neck muscle group (Fig. 1) (4). Comella and Bhatia (5) reported that approximately 62% of patients with CD experienced symptom improvement with BoNT treatment; however, 25% of patients reported no improvement and 13% reported worsening of CD symptoms. The main adverse reaction is unintended muscle weakness in neighboring muscles (6).

A possible reason for this adverse reaction could be an inaccurate injection into the target muscle. Although a BoNT injection is usually performed via palpation and under instrument guidance, such as electrical stimulation and electromyography (4,7), more safe and precise injection techniques to target the muscle should be performed because the posterior neck muscles are small, closely attached, and related to several essential blood vessels and nerves. To improve a BoNT injection's effectiveness, using an ultrasound (US) is recommended along with injecting into the neuromuscular junction- or motor endplate-targeted region of the intended muscle (8,9). Consequently, many anatomical studies have been performed to pinpoint the precise location of the neuromuscular junctions in various muscles (10,11). However, these previous studies only provided the location of the neuromuscular junction; the feasibility and accuracy of a precise injection into the neuromuscular junction area of the levator scapulae muscle has not been evaluated.

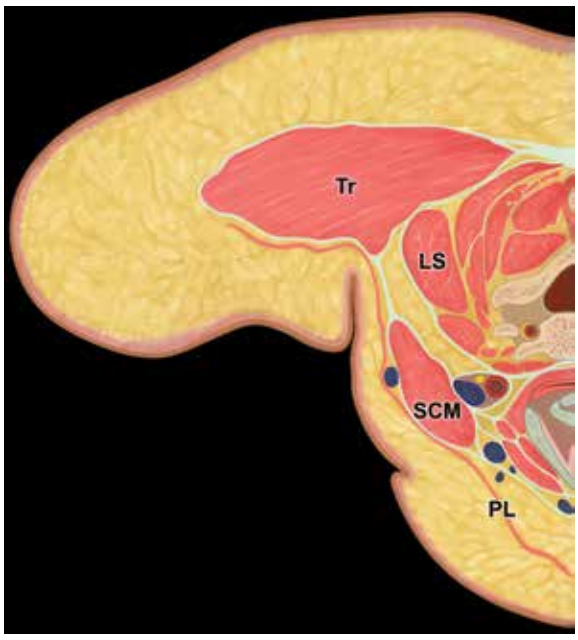

Fig. 1. *Cross-section image at the level of C6. The levator scapulae muscle lies deep within the trapezius muscle and prevertebral fascia.*
Tr, trapezius muscle; SCM, sternocleidomastoid muscle; LS, levator scapulae muscle; PL, platysma.

US imaging equipment shows anatomical structures such as muscles, nerves, and blood vessels in real-time without radiation exposure and is fast and safe. US-guided BoNT injections have been used on the face and shoulders because the physician can monitor the syringe needle's position in real-time during the procedure (12,13). Despite the advantages of US and the previous anatomical data on neuromuscular junction location, to the best of our knowledge, the feasibility of US-guided injection techniques into the proposed injection site of BoNT for the levator scapulae muscle has not been assessed in any publication. Therefore, in the present cadaver-based study, a US-guided injection technique in the middle and distal portions of the levator scapulae muscle was evaluated and to determine whether this method distributes injections properly to the target muscle in fresh cadavers.

Methods

In the present study, 20 fresh cadavers were used (10 men and 10 women, average age 71.7 years, range, 65–79 years). There was no history of previous trauma or surgical procedures in the posterior neck and scapular region. This study was performed in accordance with the principles set forth in the Declaration of Helsinki. This cadaveric 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 Review Board of the College of Medicine, The Catholic University of Korea (Approval No MC22SISI0056). All donors or authorized representatives provided written informed consent for use of the cadavers and consent for use of the related materials in future research.

US Scanning Protocol

Real-time B-mode US scanning was performed using the HS50 (Samsung, Seoul, Republic of Korea) interfaced with a linear array transducer (LA3-14AD, 3–14

MHz). Two investigators (J.H.L and K.H.Y),each with more than 5 years of experience in musculoskeletal US, led the procedures.

The cadavers were placed in a lateral decubitus position. After localizing the sternocleidomastoid (SCM) and trapezius muscles using palpation, the posterior border of the SCM and the lateral border of the trapezius were marked on the cadaver's skin. The cricoid cartilage was palpated and confirmed on the anterior side of the neck. The C6 and C7 spinous processes were also confirmed on the posterior side of the neck. Then, a transverse line at the C6 level was drawn (Fig. 2).

The transducer was placed horizontally and scanned along the transverse section of the SCM, superior part of the trapezius, and levator scapulae muscle to ensure the targeted muscle was identified on the US image. The SCM was found from the medial side, and the levator scapulae muscle and trapezius were confirmed by moving the transducer laterally (Fig. 3). After finding the levator scapulae muscle, the transversely located transducer was moved downward along the muscle to examine the shape of the muscle that gradually becomes bigger. The levator scapulae muscle was confirmed by passively elevating and depressing the scapula that moves the levator scapulae muscle but not the surrounding muscles.

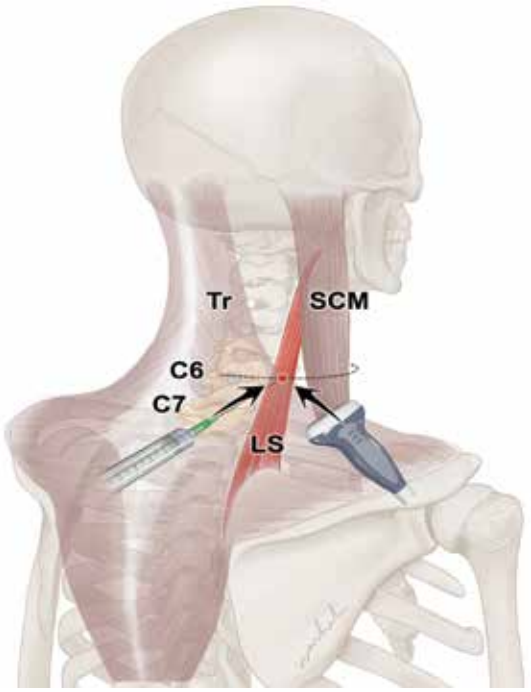

Fig. 2. *An illustration of the target area of the levator scapulae muscle. The injection point was established based on Sihler staining results on the location of the motor endplate. Black dotted line indicates the transverse reference line passing through the C6 spinous process.*

Injection Procedures and Assessment of Injected Dye

Injections were performed with a 6 cm 21-G needle after estimating target depth using US. Using a real-time technique, the probe was placed close to the puncture site, and the needle was advanced under direct guidance using US. The injection was performed transverse in-plane (Fig. 4). When the needle was positioned in the targeted muscle, 0.5 mL of dye mixed with yellow filler was injected. For each cadaver, the same technique was implemented on both sides.

After the US-guided injection, expert anatomists (K.W.L and H.J.L) dissected each specimen to determine whether the dye was correctly targeted to the middle and distal portions of the levator scapulae muscle where most of the motor nerve endings were located. They also evaluated the accuracy of the injections and any complications (defined as

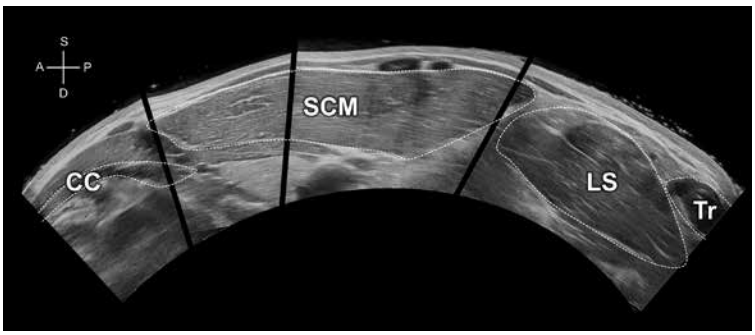

Fig. 3. *Ultrasonography (US) scan and the corresponding US panoramic image were used to visualize the muscle arrangement at the C6 level for guiding the injection into the levator scapulae muscle (LS). The probe was positioned transversely and scanned from the anteroposterior direction, revealing the trapezius (Tr), LS, sternocleidomastoid muscles (SCM), and cricoid cartilage (CC). The muscles appeared hypoechoic, while the fascial structure, including the prevertebral fascia, appeared hyperechoic.*
A, anterior; P, posterior; S, superficial; D, deep

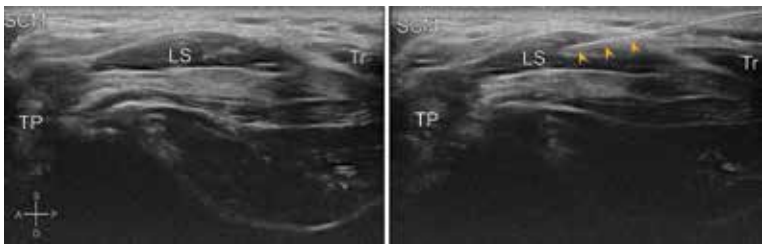

Fig. 4. *Image of ultrasound-guided injection into the levator scapulae muscle along the line passing through the cricoid cartilage level, or C6. The probe was positioned close to the injection site, and the 6-cm 21G needle was advanced under ultrasound guidance using the in-plane approach. Orange arrowhead indicates the needle targeting the levator scapulae muscle. TP = transverse process of the cervical vertebrae; S = superficial; D = deep; A = anterior; P = posterior.*

needle passage through unintended structures, such as significant neurovascular structures). First, the skin and subcutaneous tissue were removed from the posterior to anterior direction, and the trapezius and SCM were sequentially exposed. In addition, cutaneous nerves that emerged posteriorly to the SCM, accessory nerves, and fat tissue were removed, and the levator scapulae, splenius capitis, and posterior and middle scalene muscles were exposed.

Results

The levator scapulae muscle was observed between the trapezius and SCM at the posterolateral side of the neck. Notably, in cadavers with less fat tissue, the levator scapulae muscle between the trapezius and SCM was very superficially observed. Conversely, in cadavers with moderate fat tissue (fat tissue thickness between those of the SCM and the trapezius), the levator scapulae muscle was located deeper than in cadavers with less fat. Because the levator scapulae muscle lies deep within the prevertebral fascia, a strong sound is heard when the needle penetrates this fascia to reach the levator scapulae muscle.

All 40 injections were successfully injected within the middle and distal portions of the levator scapulae muscle (Fig. 5). Because a transverse in-plane injection was performed, the inserted needle exhibited high echogenicity. When dissecting the cadavers, the dye spread was evenly distributed along the muscle fiber. The other muscles, such as the trapezius, splenius capitis, and posterior scalene muscles, were not unintentionally injected. These muscles were significantly remote from the injection site. Accidental penetrations did not occur at adjacent neurovascular structures.

Discussion

In the present study, the accuracy of US-guided injection into the levator scapulae muscle was evaluated in a cadaver model for the treatment of CD and other pain syndromes. All levator scapulae muscles were successfully injected without neurovascular damage or inappropriate injection into other muscles.

A BoNT injection is the first-line treatment for CD., and A BoNT injection with US guidance provides several advantages (14). First, application of US prevents injection errors, including unintended muscle weakness due to diffusion of BoNT into adjacent muscles, consequently reducing the incidence of adverse reactions (14). This is especially important in CD, because neck muscles might be small or thin and lie in close proximity to each other. Dysphagia or unexpected weakness of the neck extensor muscles can be prevented when performing a US-guided injection (14). Second, US guidance offers the potential for dose reduction and a decrease in the total number of injections/injected muscles, factors that potentially reduce the risk of neutralizing antibodies, ensuring long-term efficacy of BoNT treatment in CD (14). Third, US guidance ensures a highly standardized injection procedure, resulting in a stable effect, better comparability between injection sessions, and consistency among treating physicians, which is especially important for long-term treatment (14). Despite the aforementioned advantages, a US-guided injection technique and protocol that ensures injection effectiveness to levator scapulae muscle in CD has not been previously reported.

The levator scapulae muscle is a commonly injected muscle in patients with CD (14-16). Localization of the levator scapulae muscle is reportedly quite difficult, with an injection accuracy of 50% using only anatomical landmarks (17). In addition, access for localizing and targeting the levator scapulae muscle is somewhat difficult (14). Ko et al (17) reported the accuracy of US-guided and non-US-guided injections as 91.7% and 50%, respectively. Injection errors into the levator scapulae muscle can lead to injecting into the adjoining muscles, such as the posterior scalene, lower part of the splenius capitis, and the trapezius (14). Despite the access difficulty and potential injection error, a US-guided injection technique as well as a protocol to ensure in-

jection effectiveness into the levator scapulae muscle in patients with CD have not been demonstrated.

In the present study, surface landmarks such as the C6 and C7 spinous processes were used to localize the levator scapulae muscle. Kaymak et al (16) also recommended using C6 and C7 and injecting at a specific point at the level of these 2 landmarks. However, the position of the transducer on the skin differed compared with the present study. Kaymak et al (16) positioned the transducer in the sagittal (oblique) plane at the midpoint between the C7 spinous process and the acromion of the scapula. The US image obtained in their study showed that the trapezius is located superficial to the levator scapulae muscle. In the present study, the transducer was positioned at the horizontal line passing through the C6 spinous process level; the levator scapulae muscle was visualized by moving the transducer between the SCM and trapezius muscle. A US image of the levator scapulae muscle was obtained showing a very small portion of the trapezius muscle covering the levator scapulae muscle at the posterior aspect. Although the location of the transducer and the obtained images are slightly different between the studies, the C6 and C7 spinous processes can be considered useful surface landmarks to localize the levator scapulae muscle.

In the present study, the feasibility of a US-guided injection into the middle and distal portions of the levator scapulae muscle was investigated by identifying whether the colored dye was precisely injected into the target regions where most neuromuscular junctions or motor endplates are located in fresh adult cadavers. Our results support the previous hypothesis that US guidance can effectively target the intended muscle for a precise and safe injection. All injections delivered dye to the primary target portions in the levator scapulae muscle. Any adjoining neurovascular structures were not affected, and the injected dye did not spread to neighboring muscles or other structures of clinical importance.

To the best of our knowledge, this is the first study in which the accuracy of a US-guided injection into the middle and distal portions of the levator scapulae muscle has been validated in a cadaver model. To optimize the therapeutic effect of a BoNT injection, needling the proper pathologic muscle and eliciting pain sites are very important. The BoNT impedes acetylcholine release at the neuromuscular junction. The location of the neuromuscular junction or motor endplate can be a potential site for an effective BoNT injection.

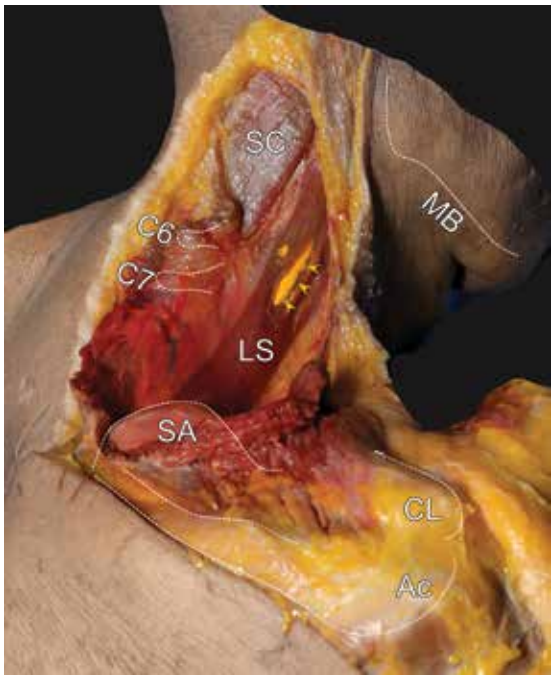

Fig. 5. *Cadaveric dissection image after an ultrasound-guided injection of 0.5 mL of dye solution into the levator scapulae. The trapezius muscle that lies superficial to the levator scapulae was removed. The spread of the injectate from the injection site was observed along the muscle fiber direction. The injectate was well located within the muscle fiber, neither spreading to the surrounding muscles nor neurovascular structures. Orange arrowheads indicate the location of the injected dye within the levator scapulae.*
SC, splenius capitis muscle; MB, mandibular border; SA, superior angle of the scapula; CL, clavicle; Ac, acromion of the scapula.

In a previous study, the location of the neuromuscular junction reportedly covers 30%–70% of the levator scapulae muscle, which corresponds to the middle and distal portions of the muscle (18). To identify the neuromuscular junction or motor end plate location, palpation and electromyography with an electrical stimulation method using equipment were mainly applied (4,7). However, palpation is time-consuming, accurately locating the motor endplate is difficult, and the other 2 methods are only used for detecting the neuromuscular junction of the superficial muscles rather than the deep muscle group (18). Therefore, accurately targeting and then injecting into the intended muscle are difficult. Accordingly, administration of BoNT should be limited to the muscle portion where the neuromuscular junction or motor endplate is located. In addition, a US-guided intramuscular BoNT

injection can increase the therapeutic effect compared with a blind injection, especially when injected into deep muscle tissue (19,20). In the present study, the levator scapulae muscles were located differently between cadavers with lower and moderate fat tissue. In addition, based on the morphology and size of the SCM and trapezius muscles, the location of the levator scapulae muscle differed. US guidance in this area can help physicians understand the anatomical relationship within the target area, resulting in a lower dose injection and improved treatment effectiveness.

A strength of this study was assessing the injection's feasibility using dissection following the injection. The injection was based on previous Sihler staining results that provide the most potentially effective injection area based on the location of the motor endplate zone. Dissection following injection has the advantage of directly evaluating and visualizing the precise location of the injectate. The injection's efficacy, based on functional outcomes such as dysphagia, was confirmed in previous studies (21). This can be attributed to several factors, such as injection volume, muscle volume, and the evaluation's timing. Therefore, the US-guided injection technique proposed in the present study could help manage CD.

Limitations

Our study has several limitations. First, despite successful injection into the middle and distal portions of the levator scapulae muscle, the usefulness of this technique has not been verified in clinical practice. Further clinical studies are needed to evaluate the therapeutic efficacy of an injection into the levator scapulae muscle using this US-guided approach in managing muscle-related levator scapulae pain. Second, the injection accuracy might have been affected by the small sample size. Therefore, a clinical trial with a larger sample size is warranted to confirm the treatment efficacy based on the US-guided injection technique presented.

Conclusion

In conclusion, this novel US-guided injection approach results in accurate needle insertion into the middle and distal portions of the levator scapulae muscle in adult fresh cadavers. The surface landmarks used in the present study, the C6 and C7 spinous processes, can be considered valuable structures for better visualizing the levator scapulae muscle in the posterolateral portion of the neck. Because the use of US with BoNT has gradually increased for the treatment of CD, the US-guided injection technique presented in this study might facilitate precise visualization and localization of the levator scapulae muscle leading to enhanced effectiveness and safety of using BoNT treatment for CD.

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