Clinical Anatomy of the Temporalis Tendon and Anterior Belly of the Digastric Muscle Using Intraoral Ultrasonography and Sihler's Method

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Clinical Anatomy of the Temporalis Tendon and Anterior Belly of the Digastric Muscle Using Intraoral Ultrasonography and Sihler's Method

Directed by Professor Hee-Jin KIM, D.D.S, Ph.D

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Soo-Bin Kim

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This certifies that the Doctoral Dissertation of
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해부학이라는 학문을 배우기 위해 2021년 낯선 교실에 처음 입학했었던 지난 3년을 되돌아보니 정말 많은 일이 있었습니다. 난관에 부딪힐 때마다 저 자신에 대한 실망과 그로 인한 위기도 많이 겪었지만, 반대로 하나씩 성취를 하면서 얻은 만족과 기쁨의 순간도 있었습니다. 그럴 때마다 항상 저를 응원해 주시고 지지해 주신 많은 분들께 감사의 인사를 전하고 싶습니다. 가장 먼저, 숭고한 뜻으로 시신을 기증해 주신 분들과 유가족분들께 깊은 존경과 경의를 표합니다.

해부학뿐만 아니라 학자로서 가져야 할 마음가짐과 자세를 몸소 보여주시며, 아낌없는 조언과 무한한 믿음으로 지도해 주신 김희진 교수님께 먼저 감사와 존경의 마음을 전하고 싶습니다. 또한, 연구실 생활 동안 항상 따뜻하게 챙겨주시고 부족한 저에게 애정 어린 조언을 아끼지 않으시는 허경석 교수님께도 마음 깊이 감사를 드립니다. 두 분의 가르침이 부끄럽지 않도록 항상 노력하며 정진하는 제자가 되도록 하겠습니다.

너무도 부족함이 많은 논문을 끝까지 읽어주시고 논문 작성에 대한 조언을 아끼지 않으신 길영천 교수님, 묵묵히 교실의 어른으로서 대학원 생활에 부족함 없도록 저희를 돌보아 주시는 강민규 선생님, 선배 해부학자로써 후배들에게 귀감이 되어주시는 허미선 교수님께 고개 숙여 감사의 인사를 드립니다. 또한, 석사 시절뿐만 아니라 지금까지도 항상 많은 관심 가져주시고 따뜻한 눈으로 지켜봐 주시는 김지연 교수님께도 진심으로 감사드립니다.

학위기간 동안 가족들보다 더 오랜 시간을 함께한 해부학 교실 식구들에게도 감사드립니다. 먼저, 비록 지금은 같은 공간에서 함께하진 못하지만, 내용 하나하나 꼼꼼히 챙겨주시고 논문이 완성되기까지 깊은 가르침을 주셨던 최

유진 교수님, 언제나 자신감을 북돋아 주시며 학문적인 조언을 주시는 이형진 교수님, 실험적으로 부족한 부분을 항상 진심으로 도와주신 이지현 교수님, 밤낮없이 부족한 저를 도와주시고 관심 가져주시는 박현진 선생님, 제가 나태해질 때마다 강한 행동력으로 이끌어 주시는 이규호 선생님께 감사드립니다.
또한, 많이 부족한 내용이었지만 논문의 중심을 잘 잡을 수 있도록 곁에서 조언을 아끼지 않으신 이규림 교수님께도 진심으로 감사드립니다. 대학원 생활이 낯설었던 저에게 오아시스 같던 든든한 버팀목이 되어 준 저의 동기 안혜련 선생님, 연구와 논문에 대한 조언뿐만 아니라 박사과정 중의 연구실 생활에 대해 언제나 도움을 주시고 조언해 주신 배형규 선생님, 안효상 선생님께 감사드립니다. 그리고 언제나 교실 업무에 많은 도움을 주셨던 박형수 선생님, 김유영 선생님, 지현주 선생님께도 감사의 말씀을 드립니다. 항상 좋은 그림으로 같이 논문 작성을 도와주신 허혜원 선생님께 큰 감사의 말씀을 전하고 싶습니다.

마지막으로 박사학위를 받기까지 끝없는 응원과 한없는 희생으로 저를 키워주신 사랑하는 어머니, 아버지, 늘 기도로 보살펴 주시는 할머니에게 참 많이 감사하고 존경한다는 말 전하고 싶습니다. 끝으로, 철없는 동생이자 언니를 언제나 격려해주고 지지해주는 형제들 경태오빠, 새언니, 시윤, 제부에게도 미안하고 고맙다는 말을 담아, 이 논문을 바칩니다.

2023년 12월

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Temporomandibular disorders (TMD) encompass various clinical signs and symptoms associated with the masticatory system, including the masticatory muscles and the temporomandibular joint (TMJ). Typical symptoms include restricted mouth opening, asymmetrical lower jaw movements during opening or closing, ear pain, and facial pain such as headache. TMD can be caused by factors such as stress, trauma, and bruxism, with sleep bruxism (SB) being a primary contributing factor. While conventional treatments involve muscle

relaxation through physical therapy and splint devices, injectable treatments such as botulinum toxin (BoNT) are commonly suggested when other methods prove ineffective. An anatomical understanding of the treatment area is crucial for effective management.

In particular, the mandibular coronoid process that attaches to the temporalis tendon is surrounded by a complex anatomical structure and is deeply located in the zygomatic arch, making access challenging. Therefore, foundational data for diagnosis or treatment is lacking. Furthermore, advancements in understanding SB suggest a new approach. There is a growing emphasis on new approaches to SB, as theories propose that the activity of the suprahyoid muscle precedes that of the masseter and temporalis muscles. Therefore, unlike the existing method of injecting BoNT into the masseter (a mouth-closing muscle), it is necessary to investigate whether a method targeting the suprahyoid (a mouth-opening muscle) can more effectively alleviate bruxism. This study aimed to 1) identify the anatomical structures surrounding the coronoid process using intraoral ultrasonography (US) to provide guidelines for safe and efficient injection treatment of temporal tendinitis, 2) clarify the neural distribution patterns of the anterior belly of the digastric muscle (ABDM) using Sihler's staining for BoNT injection treatment in SB patients, and 3) based on this information, administer US-guided injections into the ABDM and/or the masseter and compare their therapeutic effects.

This study consisted of a US study (part I), an anatomical study (part II), and a clinical study (part III). In part I, we analyzed the normal anatomical structures around the coronoid process using 116 half-faces from 58 subjects. For the coronoid process, an US image was obtained below the occlusal aspect of the maxillary second molar using intraoral US. The horizontal distance from the

anterior border of the coronoid process observed at the midpoint of the US image, along with the depth of the coronoid process and temporalis tendon from the intraoral mucosa, were measured. In part II, Sihler's staining was used on 12 specimens of the ABDM extracted from 6 Korean cadavers. The ABDM was then analyzed by dividing it into three parts along the midline connecting the tip between the gnathion and the hyoid bone. In part III, 24 subjects were randomly divided into two groups to verify the effect of BoNT injection on the ABDM of patients with SB, based on Sihler's staining performed in part II. Group A received injections into the masseter muscle only, while Group B received injections into the masseter muscle and the ABDM. Bite force and muscle thickness were measured before injection, 1 month, and 2 months after injection.

In Part I, the anterior border of the coronoid process was observed in all US images. The observed structures, from superficial to deep, included the oral mucosa, buccinator muscle, buccal fat pad, temporalis muscle and some tendon, and the coronoid process. The temporalis tendon was located at an average depth of 3.12 ± 0.68 mm from the oral mucosa.

In Part II, we confirmed that in all specimens, a single branch of the mylohyoid nerve entered the ABDM, with concentration of nerve ending in the middle 1/3 (100%, 12/12).

In Part III, BoNT injection into the masseter muscle alone or the masseter muscle and the ABDM resulted in decreased bite force and masseter muscle thickness in all subjects before injection, at 1 month, and 2 months after injection. However, there was no significant difference ($p > 0.05$, repeated measures analysis of variance) between the two groups, and there was also no significant difference in ABDM thickness ($p > 0.05$, repeated measures analysis of variance).

According to the findings of this study, the landmarks used in the anatomical study act as guidelines for safe and effective injections when injecting BoNT into the ABDM. These landmarks are bony structures whose positions on the surface of the patient's head and neck can be easily anticipated. Additionally, the maxillary second molar can serve as an intraoral landmark for visualizing the anterior border of the coronoid process. These findings can be used as guidelines for distinguishing anatomical structures during US-guided intraoral injections. Lastly, when treating SB with BoNT, a single injection into the masseter muscle is sufficient. Additional polysomnography to verify the effectiveness of the injection on the mouth-opening muscle may yield more meaningful results.

Keywords: Temporomandibular disorder, Intraoral ultrasonography, Temporal tendinitis, Sleep bruxism, Coronoid process, Anterior belly of the digastric muscle, Masseter muscle

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Ⅰ. INTRODUCTION

Temporomandibular disorders (TMD) encompass a wide range of clinical signs and symptoms involving the structures of the masticatory system, including chewing muscles, nerves, related tendons, ligaments, bones, teeth, and the temporomandibular joint (TMJ) itself. Common symptoms include restricted mouth

opening, asymmetrical lower jaw movements during opening or closing, pain in the ear, and facial pain (LeResche, 1997; Wadhwa et al., 2008), with TMD ranking as one of the most prevalent causes of orofacial pain following odontogenic pain (Dworkin et al., 1994; Lipton et al., 1993). These disorders may be associated with various other pain types, including migraines, fibromyalgia, and widespread pain (Velly et al., 2010; Velly et al., 2011; Aggarwal et al., 2006). Factors contributing to these symptoms can range from stress, trauma, and occlusal conditions, with bruxism recognized as a significant contributing factor (Poveda Roda et al., 2007).

TMD treatment involves conservative approaches, such as physical therapy, massage, and splint use, as primary interventions for mild pain (Wieckiewicz et al., 2015). Management of severe acute or chronic pain involves minimally invasive procedures, such as administration of muscle relaxants, corticosteroids, botulinum toxin (BoNT), and similar substances directly into the painful area (Freesmeyer, et al., 2005; Cairns et al., 2010). The facial area, including the TMJ, constitutes a complex structure comprising blood vessels, nerves, facial muscles, salivary glands, and the oral cavity. Accurate knowledge of the anatomical complexity is essential for effective and safe minimally invasive procedures in this area. Detailed information on this subject is outlined as follows.

1.1. Temporal tendinitis

The temporalis muscle arises from the bony surface of the temporal fossa and the deep temporal fascia, extending deeply into the zygomatic arch. It splits into two lower layers, with medial and lateral attachments to the coronoid process, and extends towards the retromolar triangle behind the rearmost mandibular molars. Functionally, the temporalis muscle is a masticatory muscle that facilitates mouth closure by elevating and retracting the mandible (Iturriaga et al., 2016; Dupont et al., 2012).

Excessive physiological force exerted during jaw movement or clenching can lead to pain in various structures, including the TMJ, masticatory muscles, and upper and lower teeth. Among these symptoms, the degenerative and inflammatory processes affecting the temporalis tendon are categorized as masticatory muscle disorders and termed temporal tendinitis (Dupont et al., 2012; Shankland et al., 1997; Mohamed et al., 1997; Duffin et al., 2020; Bressler et al., 2020).

The treatment of temporal tendinitis typically involves the administration of local anesthetics, BoNT, steroids, or autologous blood via intraoral injections into the temporalis tendon (Coombes et al., 2010; Mishra et al., 2009) with the mouth open after palpating the coronoid process that attaches to the medial section of the temporalis tendon (Brown et al., 1996; Ernest, 2006; Ernest, 2008).

However, it is important to note that the coronoid process is located deep within the oral cavity and has a complex anatomical structure. Blind injections carry the risk of undesired injection sites and potential harm to surrounding structures (Ernest et al., 1991). Therefore, it is crucial to consider the precise location and depth of the coronoid process in the oral cavity to ensure the

effective and safe delivery of these substances. Furthermore, the use of intraoral ultrasonography (US) to locate and analyze the surrounding structures of the coronoid process is an unexplored area and necessitates the establishment of an approach protocol.

1.2. Sleep bruxism

Bruxism is characterized by repetitive activity in the masticatory muscles, involving teeth grinding or clenching and lower jaw tension or thrust (American Academy of Sleep Medicine, 2014). It can be categorized as awake bruxism and sleep bruxism (SB). Awake bruxism involves repetitive or sustained muscle activity in the masticatory muscles, including tooth contact and lower jaw grinding, typically not classified as a movement disorder in healthy individuals. SB is characterized by rhythmic (phasic) or non-rhythmic (tonic) muscle activity in the masticatory muscles during sleep, usually not considered a movement or sleep disorder in healthy individuals (Lobbezoo et al., 2018). The prevalence of SB is approximately 8% in the population (Lavigne et al., 2001) and can result in tooth damage, damage to dental restorations, and orofacial pain. It is also considered a risk factor for TMJ disorders (Carra et al., 2012; Okeson, 2011). Therefore, it is crucial to alleviate excessive mechanical stress to maintain the morphological and physiological functions of teeth, periodontal tissues, masticatory muscles, and TMJ.

The application of BoNT for treating SB began in 1990 with the pioneering work of Van Zandijcke and Marchau. They reported a significant reduction in SB following BoNT injections into the masseter and temporalis muscles, and subsequent cases have provided evidence of BoNT effectiveness in SB management (Van Zandijcke et al., 1990; Tan et al., 2000; See et al., 2003; Ivanhoe et al., 1997). Consequently, BoNT has become a frequently used method, often in conjunction with splints, for bruxism treatment, with a primary focus on the mouth-closing muscles, including the masseter and the temporalis muscles.

Recent research has identified rhythmic masticatory muscle activity (RMMA) in

patients with sleep bruxism, characterized by repetitive contractions at a rate of 1 Hz (Lavigne et al., 1996; Lavigne et al., 2001). RMMA consists of repetitive activities involving phasic muscle contractions or sustained isolated biting, primarily observed in mouth-closing muscles such as the masseter and temporalis muscles. Polysomnographic studies have revealed a series of events leading to bruxism episodes. Four minutes before the onset of bruxism, there is an increase in sympathetic cardiac activity (Huynh et al., 2006), followed by a rise in brain wave frequency four seconds before, leading to an elevated heart rate and tachycardia one second before bruxism onset (Kato et al., 2001; Kato et al., 2003). Subsequently, there is an increase in suprahyoid muscle activity, an enlargement of respiratory volume, and electromyographic (EMG) evaluation recordings indicating mouth-closing muscle activity along with RMMA, highlighting the involvement of suprahyoid muscles in the early stages of RMMA occurrence (Lavigne et al., 2001; Khoury et al., 2008; Lavigne et al., 2008). Considering these findings, the impact of targeting the mouth-opening muscle when treating SB patients with BoNT injections needs evaluation.

The outcomes of BoNT injections depend on the absorption of the toxin by presynaptic membranes at the neuromuscular junction (Van Campenhout et al., 2013; Gracies et al., 2009). Therefore, injections should be directly administered to the neuromuscular junction area where it predominantly exists. For precise injection points of BoNT, an understanding of the intramuscular neural distribution is necessary. An excessive amount of BoNT may potentially spread to surrounding muscles, causing paralysis (Hsu et al., 2004). However, precise guidance on targeting the suprahyoid muscle is lacking (unlike masticatory muscles). Furthermore, there is no available literature on the nerve distribution of the anterior belly of the digastric muscle (ABDM) among the suprahyoid muscles.

Among the symptoms of TMD, temporal tendinitis can be confused with TMJ symptoms. In particular, the coronoid process that attaches to the temporalis tendon is surrounded by a complex anatomical structure and is located deep in the zygomatic arch, resulting in a severe lack of basic data for diagnosis or treatment. Additionally, there is growing emphasis on new approaches to SB, as theories suggest that the activity of the suprahyoid muscle precedes that of the masseter and temporalis muscles. Thus, effective treatment for SB requires research on the efficacy of a procedure targeting the mouth-opening muscle, compared to the conventional method of injecting BoNT into the mouth-closing muscle, especially the masseter muscle.

Therefore, our study aimed to 1) identify the anatomical structures surrounding the coronoid process using intraoral US to provide guidelines for safe and efficient injection treatment of temporal tendinitis, 2) clarify the neural distribution patterns of ABDM using Sihler's staining for BoNT injection treatment in SB patients, and 3) based on this information, administer US-guided injections into the ABDM and/or the masseter and compare their therapeutic effects.

Ⅱ. MATERIALS & METHODS

Part Ⅰ: Intraoral US anatomy of the temporalis tendon

Participants

The study enrolled 58 healthy Korean woman volunteers (average age of 43.3 ± 14.5 years). Exclusion criteria were as follows: (1) history of TMJ treatment; (2) diagnosis of TMJ abnormalities; (3) absence of maxillary second molars. Ethical approval for all study procedures was obtained from the Institutional Review Board of Yonsei University College of Dentistry (IRB identification code: No. 2-2017-0023). Informed consent was acquired from all participants, and they were allowed to withdraw from the study at any time.

Reference point

All volunteers were positioned semi-supine with their mouths opened to 4 cm using a mouth prop for the intraoral US examination. Real-time two-dimensional B-mode US images were obtained using a high-frequency (12 MHz) intraoral transducer (E-CUBE 15 Platinum, ALPINION Medical Systems, Seoul, Korea). Specific reference points for intraoral US scanning were established as follows (Figure 1): (1) the left cheek mucosa at the maxillary second molar on the occlusal plane, referred to as the LC point; (2) the right cheek mucosa at the maxillary second molar on the occlusal plane, referred to as the RC point.

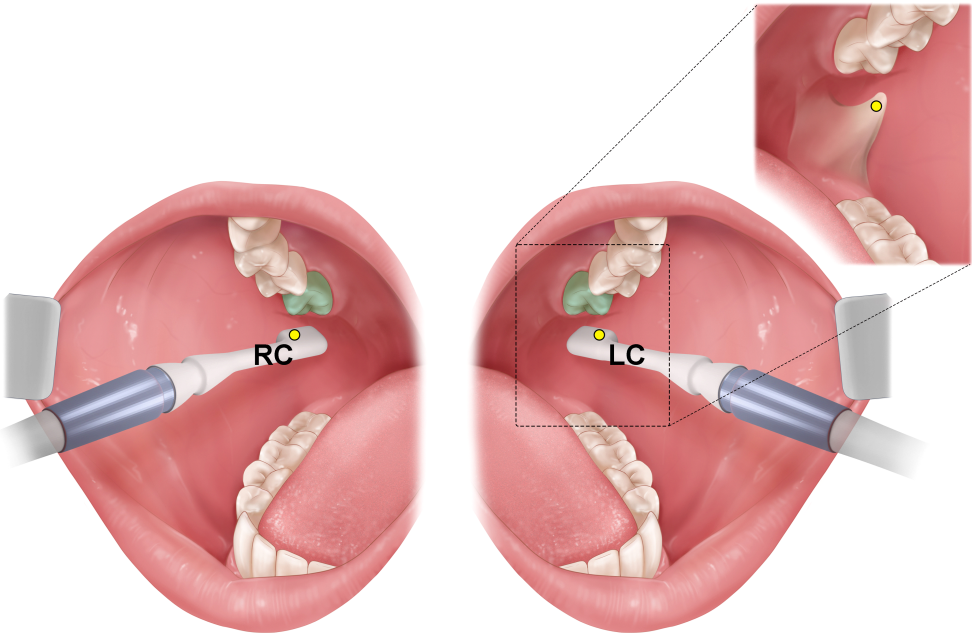

Figure 1. Reference point for ultrasonography scan and analysis. On the occlusal plane, the left cheek mucosa at the second molar (green) is designated as LC, while the right cheek mucosa at the second molar (green) is designated as RC.

Intraoral US analysis protocol

Bilateral intraoral US images were obtained horizontally at the LC and RC points (Figure 1), with the intraoral transducer positioned just beneath the occlusal aspect of the maxillary second molar. The intraoral transducer, wrapped with US gel, was carefully positioned to prevent any gel from entering the oral cavity. Out of the 116 acquired US images, four unclear images were excluded from subsequent analysis. Horizontal distances and depths were measured using an image analysis program (ImageJ; National Institutes of Health, Bethesda, MD, USA) according to the following criteria:

1) Determination of the horizontal distance and position of the anterior border of the coronoid process, both anteriorly and posteriorly, from the midpoint of the US image (referred to as the MP).

2) Assessment of the depth from the oral mucosa to the coronoid process and temporalis muscle based on the US images.

Horizontal distances were specifically measured at the deepest point of the anterior border of the coronoid process.

Part Ⅱ: Sihler's staining for ABDM

This study used 12 digastric muscle specimens derived from 6 embalmed Korean human cadavers (3 men, 3 women; mean age, 78.5 years; range, 69–88 years). None of the cadavers showed any gross pathology or surgical procedures in the examined area. This study was performed in accordance with the principles of the Declaration of Helsinki. All cadavers used in this study were donated to the Surgical Anatomy Education Center of Yonsei University College of Medicine. Appropriate consent and approval were obtained from the families of the cadaver subjects before dissection.

Dissection

The digastric muscle was exposed by carefully dissecting the skin and subcutaneous tissue covering the muscles. The specimens were dissected in detail, using extreme care to avoid damage to the underlying muscles and nerves. Meticulous dissection was performed to expose the mylohyoid nerve innervating the ABDM. The ABDM was removed from the digastric fossa of the mandible. After removing the ABDM laterally, the mylohyoid nerve was carefully dissected to identify the nerve entry point to the muscle. The number of nerve fibers entering the muscle and their precise entry locations were observed and recorded. Subsequently, we removed the tendon attached to the body of the hyoid bone after disarticulating the posterior belly from the mastoid notch of the temporal bone to extract the specimen.

Sihler's staining

The nerve distribution pattern within the muscle was visualized using Sihler's staining method as modified by Liem and Douwe van Willigen, and Lim et al. (Liem and Douwe van Willigen, 1988; Lim et al., 2004). The modified Sihler's staining comprised seven steps, as outlined below.

Fixation

The ABDM specimens were fixed in 10% unneutralized formalin for approximately one month. The fixation duration depends on the specimen size. The solution was changed when it became cloudy.

Maceration and depigmentation

The fixed specimen was washed in running water overnight and then placed in maceration solution (3% aqueous KOH and 0.2 ml of 3% hydrogen peroxide per 100 ml) for 4 weeks.

Decalcification

The macerated ABDM specimens were transferred into Sihler's solution (1:1:6 = glacial acetic acid: glycerin: 1% aqueous chloral hydrate). The solution was replaced with a new solution every week for 4 weeks.

Staining

Decalcified ABDM specimens were stained with Sihler's solution II (1:1:6 = Ehrlich's hematoxylin: glycerin: 1% aqueous chloral hydrate). All tissues, including nerves and muscle fibers, were stained.

Destaining

The stained ABDM specimens were placed in Sihler's solution I for 2–8 hours. At this stage, muscle fibers were destained, excluding the stained nerves. Researchers frequently checked the specimen in an observation box that transmitted light to observe the condition of the specimen.

Neutralization

The acidic ABDM specimen was neutralized in running water for approximately 1 hour and placed in 0.05% lithium carbonate for 30 min.

Clearing

The ABDM specimens were cleared by increasing the concentration of glycerin (40%, 60%, 80%, and 100%) for 1 day until the excessive stain washed out.

After Sihler's staining, as described in the modified Sihler's sections, we visualized the distribution pattern of nerves within the muscles. Following the staining process, samples were observed using a medical film viewer (Meditech, Ansan City, Korea) that provided sample illumination to elucidate the detailed course of the nerves. The reference points for staining were established by dividing the midline connecting the gnathion and the hyoid bone into three equal parts, distinguishing the anterior, middle, and posterior thirds. Subsequently, we analyzed the entry points and nerve terminal density of the mylohyoid nerve in each region.

Part Ⅲ: Clinical study of the effect of additional injections on ABDM during SB

Participants

Twenty-four subjects (6 males, 18 females; average age: 34.3±6.8 years) were recruited from the Orofacial Pain Department outpatient clinic at Yonsei University Dental Hospital. Participants were randomly assigned to two groups: group A consisted of 12 participants who received bilateral BoNT injections into the masseter muscles, and group B consisted of 12 participants who received bilateral BoNT injections into the masseter and ABDM.

Each participant received a consultation with a clinician, with documentation of age, sex, medical history, and medication history. Participants exhibited specific clinical signs and symptoms associated with SB, including (1) a history of clenching during sleep at least three times a week; (2) morning jaw stiffness; (3) clinical evidence of tooth wear. Exclusion criteria were as follows: (1) prior treatments, such as splint therapy or BoNT injections, for SB; (2) a history of TMJ disorders; (3) ongoing orthodontic treatment; (4) severe malocclusion, including anterior open bite; (5) two or more missing molars (excluding third molars); (6) pregnancy or breastfeeding; (7) allergies to BoNT; (8) use of medications that could impact muscle relaxation. Participants were instructed to refrain from other treatments or medications affecting muscle function during the study.

The research protocol received approval from the Institutional Review Board (IRB) of Yonsei University Dental Hospital (IRB No. 2-2021-0122; approval date: February 23, 2022) and was registered with the Clinical Research Information

Service (CRIS) of the Ministry of Health and Welfare of the Republic of Korea (CRIS number: KCT 0008721). Before participation, all individuals were thoroughly briefed on the study objectives and procedures, and they retained the option to withdraw from the study or treatment at any point.

Reference line

Reference lines were established for both BoNT injections and US measurements. For the masseter muscle, the transducer was horizontally positioned along an imaginary line connecting the cheilion and otobasion inferius (Figure 2A). The ABDM was located by palpation towards the gnathion and the hyoid bone, and the imaginary line was divided into thirds to pinpoint the ABDM in the middle third region (Figure 2B). Before injections and measurements, all reference lines were marked on the skin using waterproof eyeliner.

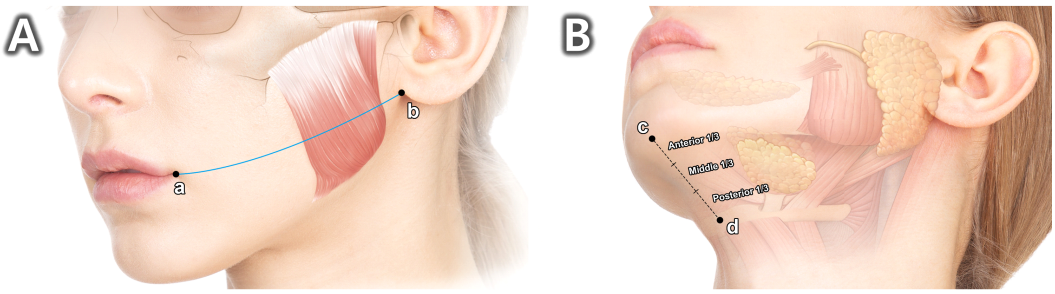

Figure 2. Reference lines for injections and measurements. (A) masseter muscle, the line passing through the cheilion (a) to otobasion inferius (b), (B) anterior belly of the digastric muscle, the line passing through the gnathion (c) to the hyoid bone (d).

US-guided injection method

Both groups underwent BoNT injections (Nabota, Daewoong Pharmaceutical, Seoul, Korea) using US-guided techniques. The BoNT, provided as a freeze-dried powder of 100 U, was reconstituted to a concentration of 4 U/0.1 ml by diluting it in 2.5 ml of 0.9% normal saline. A 1-ml syringe with a 27-gauge, 0.5-inch needle was used to administer 25 U into each masseter and 10 U into each ABDM. A US-guided injection was performed using sufficient gel to cover the entire masseter and ABDM, without applying pressure to the transducer to avoid distorting the depth information (Figure 3A, B).

For masseter muscle injections, the US device was used to visualize the internal structure of the masseter muscle, including the deep inferior tendon (DIT) and surrounding structures. US-guided injection followed an out-of-plane technique, with the transducer positioned along the reference line to ensure dual plane injection, a deeper and more superficial BoNT injection method than DIT (Figure 3C). Regarding ABDM injections, the internal and surrounding structures of the ABDM were identified using US imaging. Doppler mode was activated to verify the presence of major blood vessels along the injection path, and subsequent US-guided injections were performed (Figure 3D). The thickness of the masseter and ABDM was measured at the reference line before, at 4 weeks, and 8 weeks after BoNT injection using US.

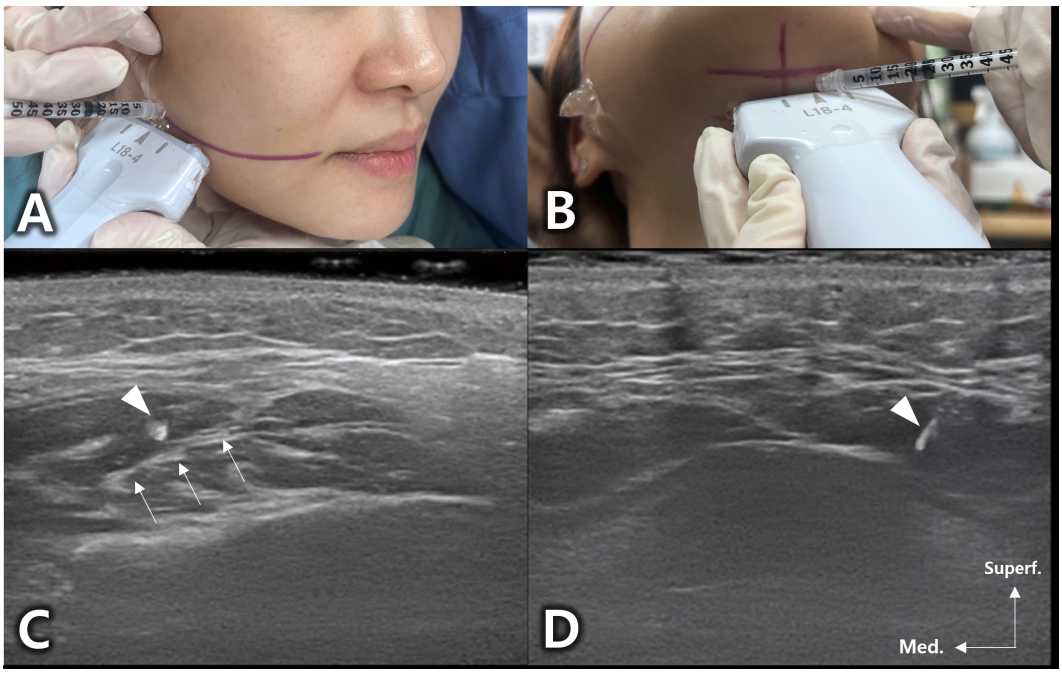

Figure 3. Ultrasonography (US)-guided injection and US images. (A, C) masseter muscle, and (B, D) anterior belly of the digastric muscle. Arrows, deep inferior tendon; Arrowhead, needle tip

Bite force measurements

All participants underwent examination using a pressure-sensitive sheet (Dental Prescale, 50 H, type R, 97 μm thick, GC, Tokyo, Japan) at baseline, 4 weeks, and 8 weeks following BoNT injection. The pressure-sensitive sheet comprises a film bite foil and an analyzer (Occluser 709, GC). When bitten, the microcapsules within the foil burst upon impact, identifying the locations of occlusal contacts. Bite forces (Newton) were measured using the Occluser 709 software.

Participants were seated upright during the assessment to ensure consistent measurements because the mandibular position could influence occlusion location. Participants were instructed to bite as forcefully as possible in their habitual bite position for 3 seconds. The initial two recordings served to acquaint participants with the procedure, with the third and final recording used for subsequent data analysis (Figure 4).

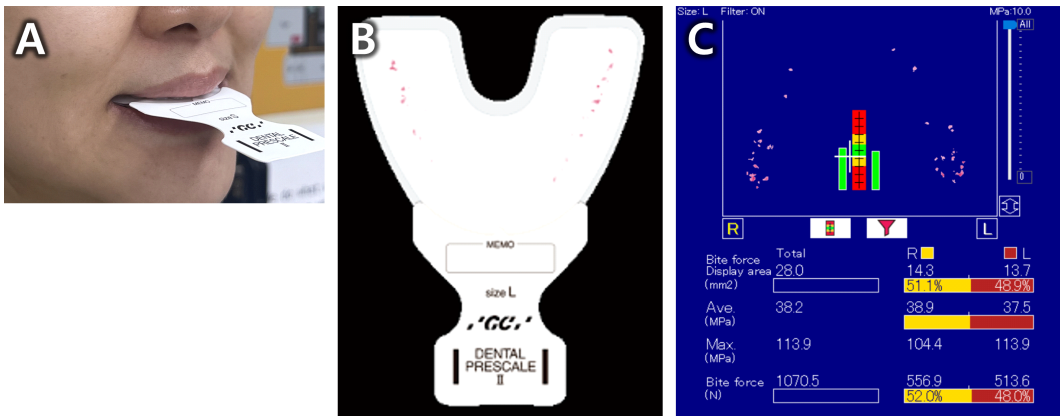

Figure 4. Bire force measurement process. (A) Placement of pressure-sensitive sheets (dental prescale) on the dental arch, followed by biting down for 3 seconds, (B) Application of pressure during biting results in the collapse of microcapsules, releasing the encapsulated red color former, and (C) Presentation of a typical display upon placing pressure-sensitive sheets in the analyzer (Occluser 709).

Statistical analysis

The normality of data distribution was assessed through the Kolmogorov-Smirnov test. The variations in bite force and masseter muscle thickness between the two groups were examined at baseline, 4 weeks, and 8 weeks post-BoNT injection, using repeated measures ANOVA. Before BoNT injection, the Student t-test and Mann-Whitney U test were used to determine group differences in each variable, while paired t-tests and Wilcoxon signed-rank tests were used to assess differences within groups over time. Age and sex differences were evaluated using the Mann-Whitney U test and Fisher's exact test, respectively. The threshold for statistical significance was set at $P < 0.05$. Statistical analyses were conducted using SPSS software (version 23.0 for Windows, SPSS, Chicago, IL, USA).

Ⅲ. RESULTS

Part Ⅰ: Intraoral US anatomy of the temporalis tendon

Normal intraoral US anatomy at reference points

LC is the left cheek mucosa at the maxillary second molar on the occlusal plane, and RC is the right cheek mucosa at the maxillary second molar on the occlusal plane. The oral mucosa and buccinator muscle are observed most superficially. The coronoid process appears as a strong hyperechoic structure, and a hypoechoic temporalis muscle is observed directly above it. Inside the muscle, some hyperechoic temporalis tendons are observed. Above the temporalis muscle, the buccal fat pad is irregularly hyperechoic (Figure 5).

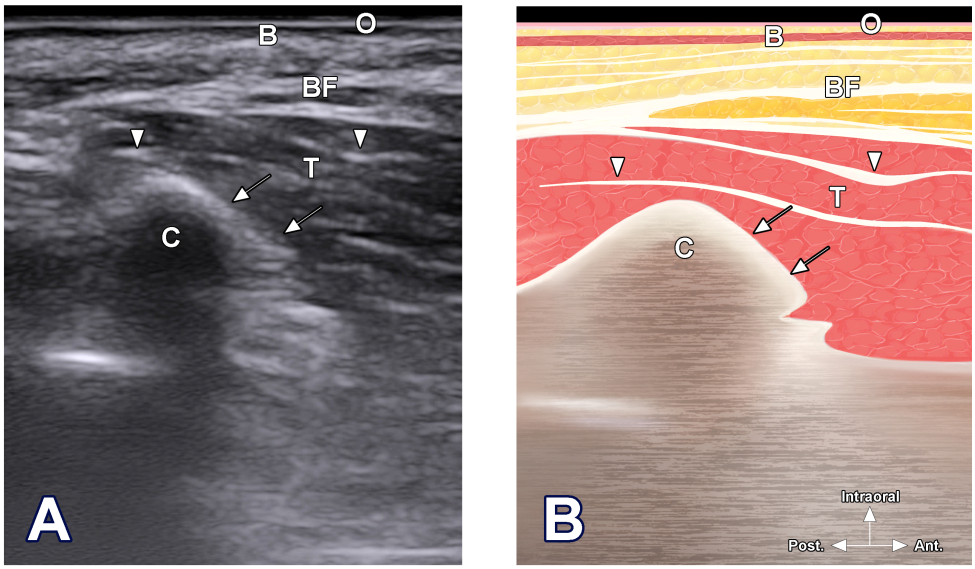

Figure 5. (A) Ultrasonography images acquired from designated reference points. (B) Diagrammatic representation. Arrows, anterior border of coronoid process; Arrowheads, temporalis tendon; B, buccinator muscle; BF, buccal fat pad; C, coronoid process; O, oral mucosa; T, temporalis muscle

Coronoid process observation in US images

The coronoid processes were identifiable in all US images at the LC and RC points. These observed patterns of the coronoid processes could be categorized into three types: type A, where the anterior border of the coronoid process was positioned anterior to the MP (56.2% [63 out of 112 cases]); type B, with the anterior border of the coronoid process coinciding with the MP (16.1% [18 out of 112 cases]); type C, where the anterior border of the coronoid process lay posterior to the MP (27.7% [31 out of 112 cases]) (Figure 6). In types A and C, the horizontal distances from the MP to the anterior border of the coronoid process measured 1.3±1.3 mm (ranging from 5.4 mm to 0.3 mm) and 1.8±1.4 mm (ranging from 5.1 mm to 0.4 mm), respectively.

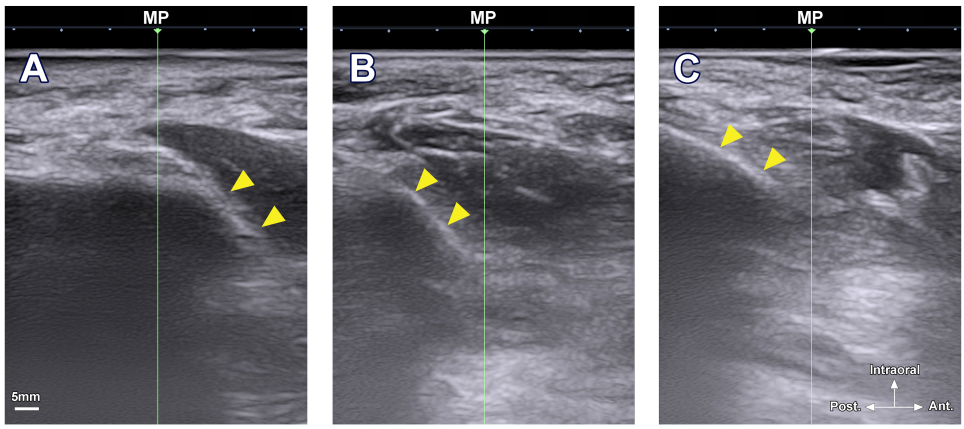

Figure 6. Three types of the coronoid processes identified in the ultrasonography images. (A) Type A, coronoid process seen anteriorly from the MP; (B) Type B, coronoid process seen at the MP; (C) Type C, coronoid process seen posteriorly from the MP. MP, midpoint of the US image; yellow arrowhead, front edge of the coronoid process

Depth of the temporalis muscle and coronoid process

The vertical measurement from the oral mucosa to the coronoid process was 5.8±1.2 in type A, 5.2±1.3 in type B, and 5.5±1.0 in type C. Additionally, the distance from the oral mucosa to the temporalis muscle was 3.2±0.7 in type A, 3.1±0.8 in type B, and 3.0±0.6 in type C. The mean depths of the coronoid process and temporalis muscle from the oral mucosa were 5.6±1.2 and 3.1±0.7 mm, respectively. There were no significant differences among the three types (Table 1).

Table 1. Measurement of the distance from the oral mucosa to the temporalis muscle and the coronoid process based on the types of the coronoid process

Depth	Type A	Type B	Type C	All types
Coronoid process	5.8±1.2	5.2±1.3	5.5±1.0	5.6±1.2
Temporalis muscle	3.2±0.7	3.1±0.8	3.0±0.6	3.1±0.7

Data are expressed as mean±SD values (mm); type A, coronoid process seen anteriorly from MP; type B, coronoid process seen at MP; type C, coronoid process seen posteriorly from MP; MP, midpoint of the US image

Part Ⅱ: Sihler's staining for ABDM

Locations of nerve entry points of the ABDM

The digastric muscle is the most superficial muscle beneath the submandibular, consisting of two muscle bellies connected by a tendon attached to the hyoid bone. The anterior and posterior bellies are innervated by the mylohyoid and the facial nerve, respectively. Specimen extraction revealed that a single branch of the mylohyoid nerve entered the ABDM in all 12 specimens, with no instances of more than two branches. Furthermore, the entry point of the mylohyoid nerve existed only in the middle third region of the ABDM (Figure 7).

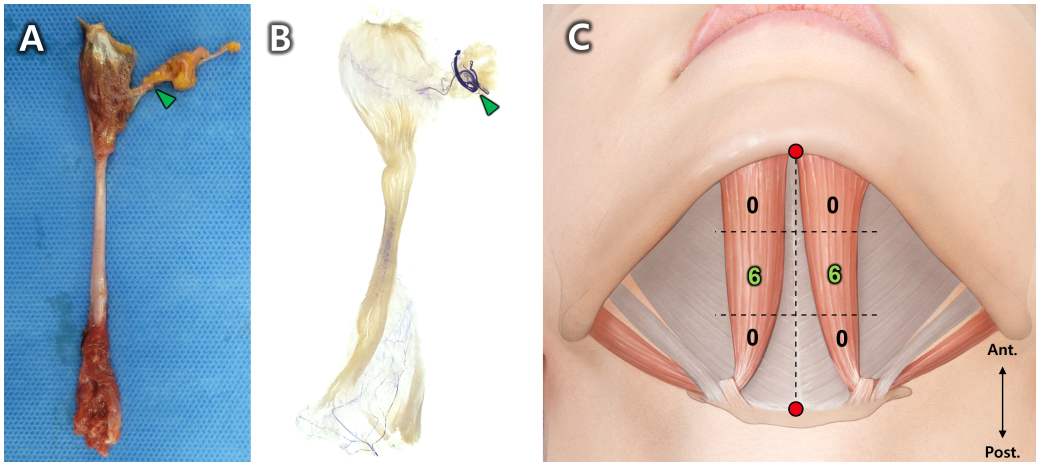

Figure 7. Nerve entry points in the anterior belly of the digastric muscle (ABDM). The ABDM is innervated by a single branch of the mylohyoid nerve (green arrowheads), and the nerve entry point is located in the middle third, dividing the gnathion and hyoid bone into three equal parts. (A) Harvested digastric muscle, (B) Sihler's stained digastric muscle, (C) illustration of the general ABDM. Green number 6 represents the count of branches through which the mylohyoid nerve enters the ABDM

Concentration of nerve endings in the ABDM

We tracked the nerve course and termination of the mylohyoid nerve for each sample. The nerve endings were most frequently observed near the middle of the ABDM (Figure 8). The concentration of the intramuscular nerve ending was highest in meddle third of the ABDM (100%, 12/12 cases), followed by the anterior third (58.3%, 7/12 cases), with no observations in the posterior third (Figure 9).

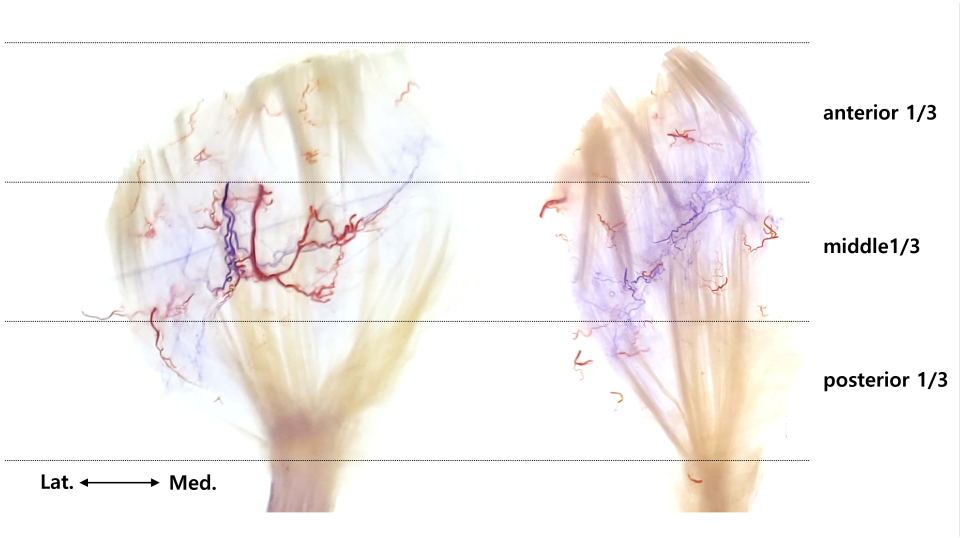

Figure 8. Results obtained by applying Sihler's staining to the anterior belly of the digastric muscle with mylohyoid nerve and set to the same ratio. The stained neural structures, highlighted in purple, are primarily observed in the middle third, followed by observation in the anterior third.

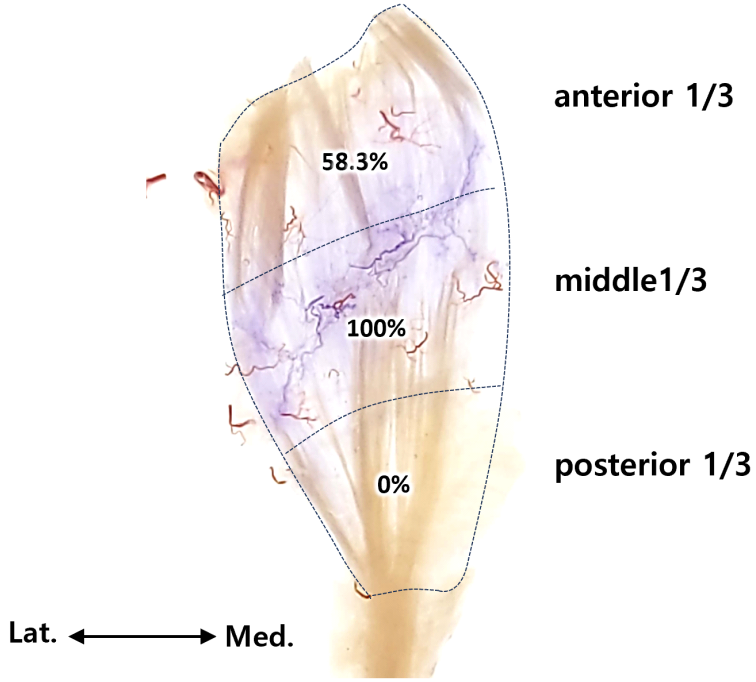

Figure 9. Proportion of intramuscular distribution of the mylohyoid nerve to the anterior belly of the digastric muscle.

Part Ⅲ: Clinical study of the effect of additional injections on ABDM during SB

Table 2 outlines the basic characteristics of the subjects, with no significant differences observed between the groups. The administration of injections was well-tolerated, with no reports of serious side effects or injection-related complaints.

Table 2. The characteristics of the subjects

Variable	Group A	Group B	Total	P value
Age[a]	32.2 ± 6.3	36.5 ± 7.1	34.3 ± 6.8	0.2
Sex[b]	M (3) F (9)	M (3) F (9)	M (6) F (18)	1.0

[a]Data are analyzed by Mann-Whitney test. [b]Data are analyzed by Fisher's exact test

Normal US anatomy at reference lines

Masseter muscle

Masseter muscle was observed along a line passing through cheilion and otobasion inferius. This location typically included the epidermis, dermis, and subcutaneous layer. The surface of the inferior mandibular ramus appeared as a strong hyperechoic line at the deepest position. The masseter muscle appeared as a hypoechoic structure, with some hyperechoic DIT observed between the muscles. Anterior to the masseter muscle, the extension of the buccal fat pad of the hyperechoic line and posteriorly, the parotid gland was observed uniformly. The platysma muscle of the hypoechoic line was identified on the surface of the masseter muscle and the parotid gland (Figure 10).

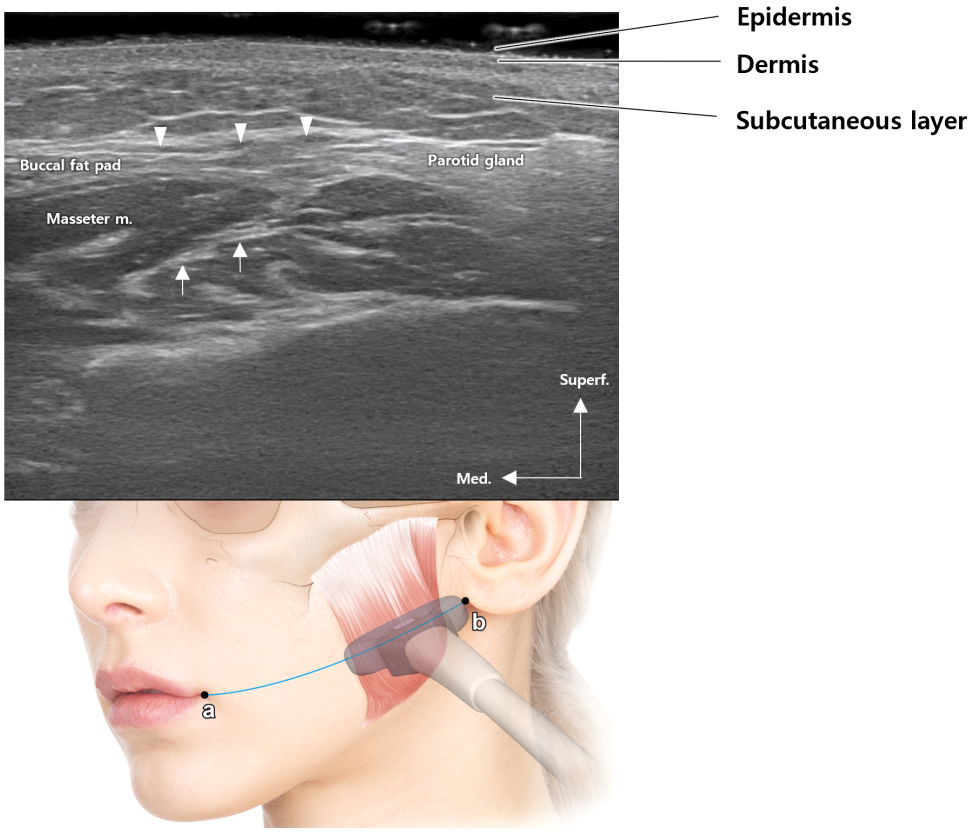

Figure 10. Ultrasonography image of the masseter muscle on the transverse line passing the cheilion (a) and the otobasion inferius (b). Arrows, deep inferior tendon; arrowheads, platysma muscle

ABDM

The ABDM was visualized along a line passing through the middle third of the gnathion and hyoid bone. In this location, the epidermis, dermis, and subcutaneous layers are typically present. At its deepest point, the hypoechoic mylohyoid muscle as an arch across the image. The ABDMs appeared round-shaped and clearly hypoechoic on the images. Additionally, the platysma muscle observed as a hypoechoic line on the surface of the ABDM (Figure 11).

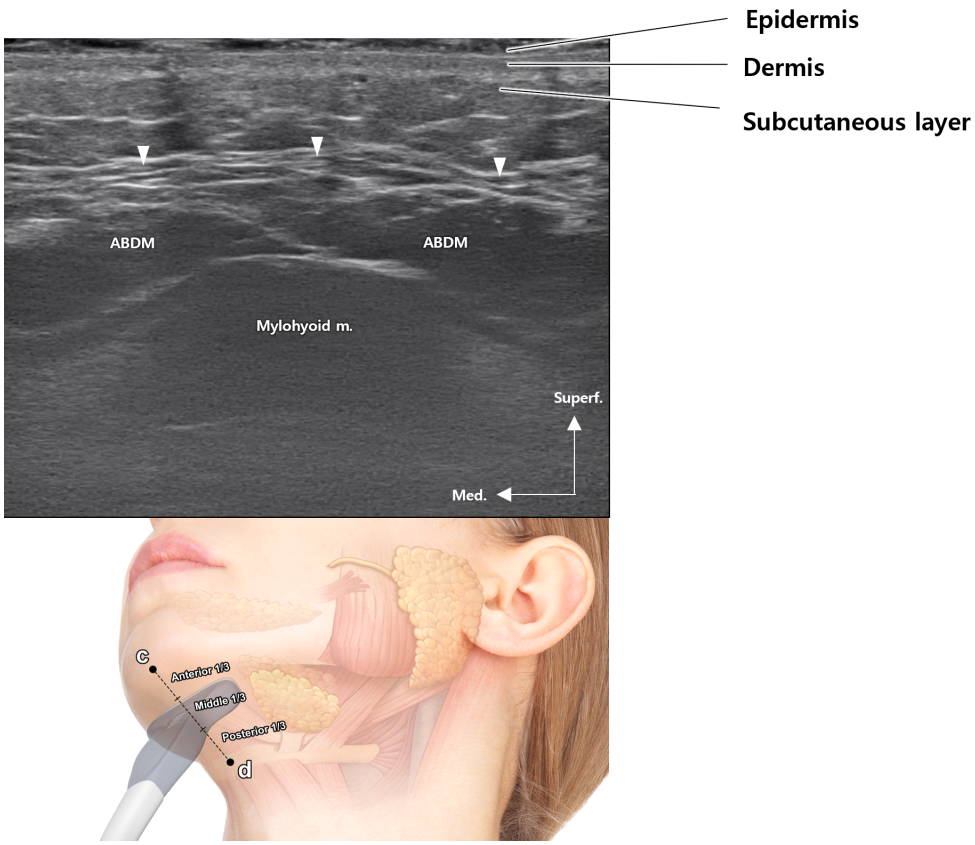

Figure 11. Ultrasonography image of the anterior belly of the digastric muscle in the middle third the longitudinal line passing through the gnathion (c) and the hyoid bone (d). Arrowheads, platysma muscle

Table 3 presents data for all variables, indicating no significant differences between the groups before BoNT injection ($p > 0.05$). Bite force significantly reduced in both groups, with a 36% decrease in group A and a 22% decrease in group B at 4 weeks post-injection compared to pre-injection levels. Similarly, at 8 weeks post-injection, group A exhibited a 6% reduction, and group B showed an 8% reduction (Figure 12A). Masseter muscle thickness also demonstrated a significant 10% decrease at 4 weeks post-injection for both groups compared to pre-injection levels. Compared to before injection, group A showed an 8% decrease at week 8, while group B showed a 12% decrease (Figure 12B). Notably, in group B, no significant reduction in ABDM thickness was observed at any time point after injection into the ABDM (Figure 12C). No significant time and group interactions were observed for all variables ($p > 0.05$).

Table 4 presents data on clinically subjective symptoms recorded during follow-up. All participants reported a reduction in teeth grinding at the 4-week follow-up. In group B, two participants initially experienced problems with mouth closure after BoNT administration. However, it was reported that mouth closure improved by the 8-week mark. No adverse effects were reported in group A.

Table 3. Changes in Bite force and masseter muscle thickness before and after injection

Variable		Before	After		P^{+}	Time P^{*}	Interaction $P^{\dagger}$
			4weeks	8weeks			
Bite force (N)[a]	Group A	674±414	433±248	406±286	.319	.000	.852
	Group B	869±559	682±466	612±335			
Masseter muscle thickness (mm)[b]	Group A	14.24±1.81	12.75±1.50	13.04±1.71	.053	.000	.349
	Group B	15.38±2.17	13.88±2.28	13.56±2.02			

Data are presented as mean ± standard deviation values. $^{+}$Comparison of differences between groups for each variable before BoNT injection ([a] Mann-Whitney U test and [b] student t-test). *intragroup and † intergroup p-values were obtained from repeated measures ANOVA ($p < 0.05$)

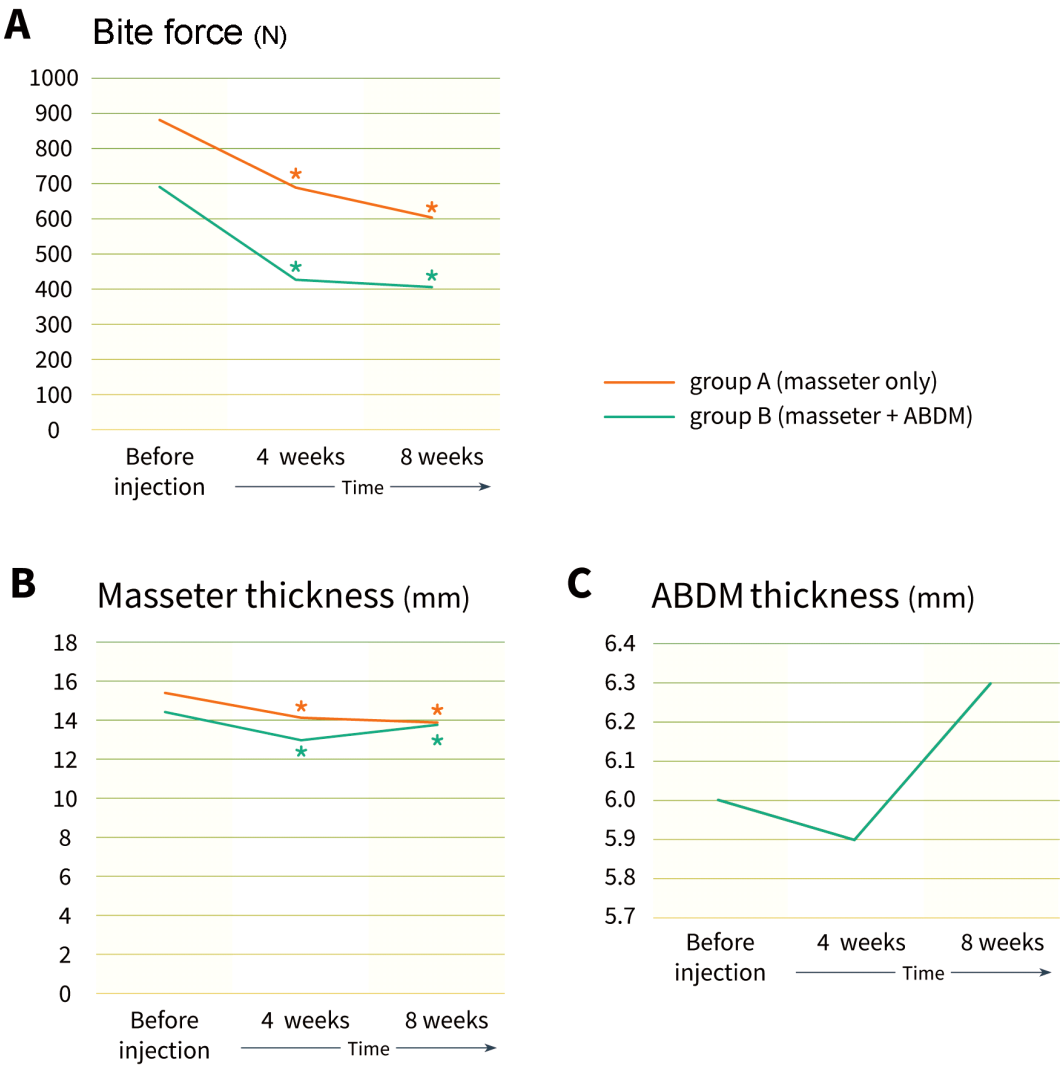

Figure 12. The changes in (A) bite force, (B) masseter muscle, and (C) anterior belly of the digastric muscle (ABDM) thickness before and at 4 and 8 weeks after injection. *p-value was obtained from paired t-test ($p < 0.05$)

Table 4. Subjective clinical symptoms

Symptoms	Group A *(n=12)*	Group B *(n=12)*
Decrease of SB event	100 %	100 %
Side effect		16.7 %

Ⅳ. DISCUSSION

Part Ⅰ: Intraoral US anatomy of the temporalis tendon

Temporal tendinitis, a form of orofacial pain, may be mistaken for TMJ and often goes undiagnosed due to the intricate nature of its anatomy. Furthermore, the limited availability of scholarly literature on temporal tendinitis diagnosis and management is exacerbated by the predominant research focus on the TMJ (Bressler et al., 2020).

The coronoid process, situated deep within the zygomatic arch, presents challenges to access through extraoral palpation when the mouth is closed (Lumley, 2008). Accurate palpation of the temporalis tendon is also difficult and requires the ability to differentiate between various anatomical structures, such as the lateral and medial pterygoid muscles, oral mucosa, and buccinator muscle (Schiffman et al., 2014; Conti et al., 2008; Johnstone et al., 1980; Türp et al., 2001). In this context, intraoral US transducers prove invaluable for identifying the temporalis tendon, coronoid processes, and adjacent anatomical structures (Geers et al., 2005). Few studies have used intraoral US to investigate structures influencing TMJ movements.

Ultrasonography offers advantages such as the absence of ionizing radiation, shorter image acquisition time compared to conventional radiography, real-time assessment of dynamic anatomical structures, and the ability to detect major blood vessels using Doppler mode (Bressler et al., 2017; Shah et al., 2014; Oeppen et al., 2010). Despite these benefits, the practical application of US as an imaging tool in clinical dentistry remains uncommon mainly due to the lack of standardized

criteria for analyzing US images, variations in image quality based on operator skill and experience, and the need for clinical knowledge and expertise for accurate interpretation. The challenge of gaining access to the oral cavity using US transducers is compounded by anatomical complexities (hard palate, soft tissues, and a narrow palate). Therefore, consistent patient position, operator placement, and adherence to the correct image acquisition protocol are critical for ensuring image consistency (Lee et al., 2022).

Volunteers were initially positioned semi-supine, and a mouth prop was used to achieve a 4 cm mouth opening, ensuring consistent anatomical US images of the coronoid process and surrounding structures. Precise positioning implied aligning the mandibular border parallel to the ground, instructing participants to maintain a fixed gaze to minimize head movement, and stabilizing the examiner's arm at the participant's shoulder position to minimize transducer movement. The intraoral transducer was then positioned horizontally on the occlusal plane of the maxillary second molar to visualize the coronoid process, with an average mouth opening of 4 cm selected for image acquisition (Gallaghe et al., 2004; Li et al., 2017).

Identification of surrounding structures was achieved by monitoring muscle movements associated with mandibular movement. As a result, the anterior border of the coronoid processes consistently appeared hyperechoic, positioned just below the occlusal plane of the maxillary second molar in all US images (Figure 5). Numerous studies (Yu et al., 2021; Harn et al., 1982; Parker et al., 2012; Dunn et al., 1996) have highlighted considerable variability in the attachment of tendons to the coronoid process of the temporalis muscle, and the anatomical complexities at the myotendinous junction pose challenges in differentiating between the muscle and tendon. Nevertheless, we successfully distinguished the temporalis muscle surrounding the coronoid process as hypoechoic in the intraoral US images

obtained in this study. Additionally, certain tendons within the muscle exhibited echogenicity similar to that of the coronoid process. Validation of each structure position was facilitated by comparing it with cross-sectional images of the head (Figure 13).

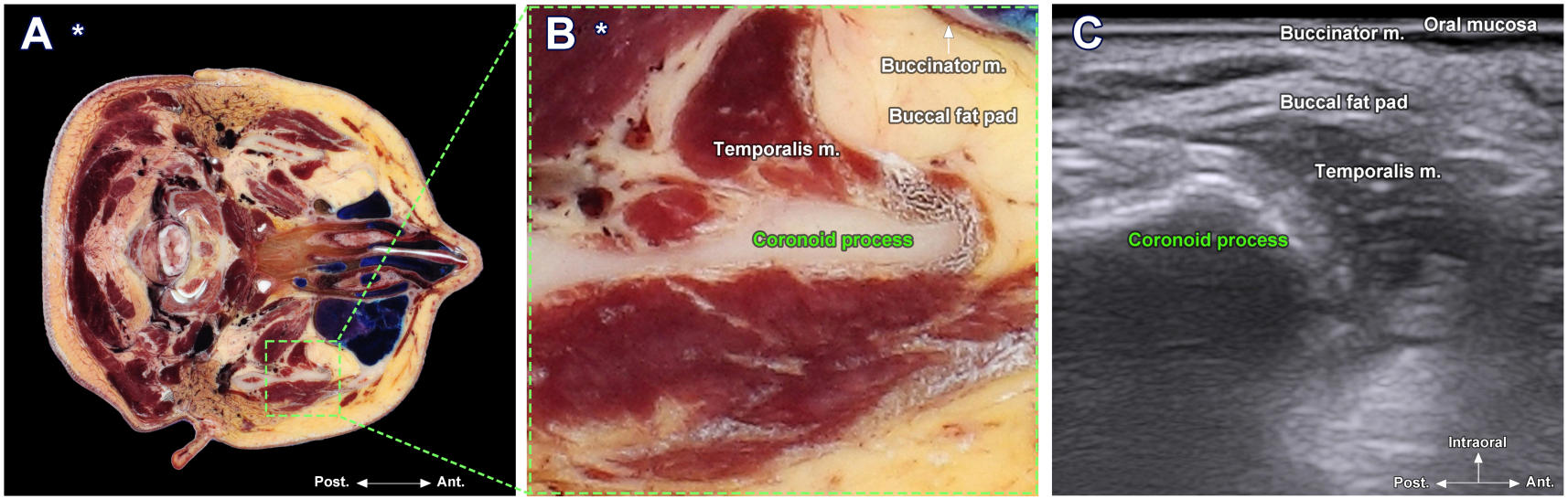

Figure 13. Comparison of cross-sectional head and ultrasonography (US) images. Cross-sectional head image of the position of the second molar occlusal plane with the mouth open (A). Magnification of the head sectional image (B). US image obtained from the second molar occlusal plane (C). *cross-sectional head images from (Park et al., 2005)

The coronoid processes observed at the MP using US were categorized into three types, with the majority being type A (56.2%), where the anterior border of the coronoid process was located in front of the MP (Figure 6). Based on measured horizontal distances at the MP, the anterior border of the coronoid process was within a maximum of 5.4 mm and a minimum of 0.3 mm anteriorly from the MP. Studies report that medial portions of the temporalis tendon are typically located at an average width of 1.4 cm and a height of 5.2 cm from the lingula of the mandible to the anterior border of the ramus (Geers et al., 2005; Parker et al., 2012; Dunn et al., 1996). Hence, in type A cases, injections targeting the temporalis tendon could often be accurately administered at the location of the maxillary second molar, even when considering the observed maximum and minimum values of the horizontal distance for the coronoid process. The average distance from the oral mucosa to the temporalis muscle was 3.1±0.7 mm, indicating that an injection depth of approximately 3 mm was necessary for precise injection.

Notably, this research involved participants lacking TMJ movement disorders. The range of motion during maximum mouth opening encompasses the condylar process of the lower jaw rotating within the mandibular fossa while simultaneously translating forward and backward on the articular eminence (Acri et al., 2019). The degree of disc rotation varies depending on the inclination of the articular eminence surface and the size and shape of the condylar process (Çağlayan et al., 2014; Pandis et al., 1991). Consequently, in types B and C, the anterior border of the coronoid process could be observed posteriorly. In type C, this anterior border was positioned within a range of 5.1 mm to 0.4 mm posteriorly from the MP. Types A and C may exhibit a difference of approximately 10 mm from the MP. Considering the well-established fact that the mesiodistal width of maxillary second molars typically measures around 11 mm,

even a minor variance, as minute as the size of the maxillary second molar, can hold significance in the intraoral region. Thus, the real-time visualization of the coronoid process and temporalis tendon through US is imperative to address these individual variations and ensure the precision and stability of injections.

Part Ⅱ: Sihler's staining for ABDM

The digastric muscle is the most superficial muscle below the submandibular and comprises two muscle bellies attached to the hyoid bone by tendons. The ABDM and the posterior belly are innervated by the mylohyoid and the facial nerves, respectively. This study aimed to define the distribution pattern of the mylohyoid nerve in the ABDM and provide an effective injection site for BoNT in the treatment of SB.

BoNT has been applied to masseter muscle and/or temporalis muscles for decades in treating bruxism patients, with reported reductions in pain and jaw stiffness following injections (Van Zandijcke et al., 1990; Ivanhoe et al., 1997; Nash et al., 2004). Recent research indicates that the mechanism underlying the onset of SB involves initial activity in the suprahyoid muscles, followed by the activation of the masseter muscles, leading to the development of RMMA. Thus, studies conducted so far suggest that BoNT injections administered in SB patients might impact only the concluding phase of RMMA episodes without affecting its initiation (De la Torre Canales et al., 2017). Therefore, we needed to examine the effects of BoNT injections into the suprahyoid muscles in SB patients.

The suprahyoid muscles comprise four muscles (e.g., stylohyoid, geniohyoid, mylohyoid, and digastric muscles) located in the anterior part of the neck, positioned between the hyoid bone and the mandible. During swallowing, they physically pull the hyoid bone and larynx through muscle contraction (Park and Hwang, 2021; Matsuo and Palmer, 2008), resulting in the downward rotation of the epiglottis and the opening of the upper esophageal sphincter, contributing to normal swallowing (Lang, 2009; Ertekin and Aydogdu, 2003). In this study, the mylohyoid and geniohyoid muscles were excluded among the four suprahyoid

muscles. This decision was based on evidence demonstrating their significant roles in moving the hyoid bone superiorly and anteriorly, respectively (Doty and Bosma, 1956; Burnett et al., 2005). Additionally, EMG measurement methods included needle EMG and surface EMG. Due to the challenges of conducting measurements during sleep in SB patients, we were limited to using the surface EMG approach. This limitation could explain why other studies have also conducted EMG assessments on the masseter and temporalis muscles, while excluding the pterygoid muscle. (Lee et al., 2010; Shim et al., 2014; Shim et al., 2020). Consequently, we chose to focus on the most superficial and isolatable ABDM among the four suprahyoid muscles, believing that this approach minimizes potential complications related to dysphagia after BoNT injection.

BoNT inhibits acetylcholine release at the neuromuscular junction, leading to muscle relaxation. To optimize the effects of BoNT, precise injection at the motor nerve terminals is essential (Childers et al., 2004). When injecting BoNT into the masseter muscles, Considering the nerve distribution of the masseteric nerve, BoNT injections are commonly administered at 3-4 points in the lower 1/3 of the muscle. (Kim et al., 2010). Moreover, DIT located within the masseter muscle can impede uniform diffusion of BoNT; therefore, a dual-plane injection technique is usually performed to inject deeper than this tendon (Lee et al., 2016).

Contrastingly, BoNT injections in the ABDM have been used in oral and maxillofacial surgery, including the treatment of open bite due to bilateral mandibular fractures or prevention of surgical relapse in patients with deep bite and open bite malocclusions treated by orthognathic surgery (Seok et al., 2013; Mücke et al., 2016; Kang et al., 2019). The administration of such injections is performed without sufficient anatomical evidence and protocols.

This study used a modified Sihler's staining method to preserve neuromuscular

junctions and illustrate the nerve pattern within the ABDM (Figure 8). Most nerves entered the muscle at the middle third, and nerve ending points were observed in the middle third (100%, 12/12 cases) and anterior third (58.3%, 7/12 cases) regions (Figure 9). Therefore, the rich distribution of neuromuscular junctions in the ABDM, as revealed in this study, may assist in predicting potential injection points for BoNT in clinical settings. BoNT injections should be performed near motor endplates for optimal effects (Childers, 2004). Injecting BoNT 5 mm away from the neuromuscular junction can result in a 50% reduction in efficacy (Parratte et al., 2008), and insufficient dose of BoNT may decrease therapeutic effects. Conversely, excessive injections may lead to unintended paralysis of adjacent muscles. Hence, accurate anatomical knowledge is crucial for effective treatment.

The surface landmarks proposed in this study can facilitate localization of the ABDM, enabling safe and effective injection practices. As demonstrated in this research, Sihler's staining offers an efficient method for observing neuromuscular distribution within skeletal muscles (Bae et al., 2018), benefiting anatomists, who traditionally dissect and trace nerve endings with the naked eye, and clinicians seeking effective injection points. Gnathion and the hyoid bone, used as surface landmarks, simplify the establishment of trisected points and aid in the precise localization of the ABDM on the skin surface. This clinician-friendly surface landmark ensures the safe and effective administration of BoNT treatment.

Part Ⅲ: Clinical study of the effect of additional injections on ABDM during SB

BoNT injections have been applied to the mouth-closing muscles for decades to treat bruxism (Van Zandijcke et al., 1990; Ivanhoe et al., 1997; Nash et al., 2004). Most patients self-reported reductions in pain and jaw stiffness caused by bruxism after BoNT injection (Lee et al., 2010; Shim et al., 2014; Guarda-Nardini et al., 2008; Bolayir et al., 2005). However, studies using EMG, an objective tool for evaluating SB, have indicated that bruxism activity decreases after masseter muscle injections but continues in the temporalis muscle (Lee et al., 2010). Furthermore, polysomnography showed that BoNT injection into the masseter and/or temporalis muscles attenuated muscle contraction and alleviated SB but did not reduce the occurrence of RMMA episodes (Shim et al., 2020).

The insignificant reduction in RMMA episodes by BoNT injections can be explained via the physiological sequence of SB events. Recent studies on the mechanism of SB suggest the activation of the suprahyoid muscles before the onset of SB, followed by the initiation of mouth-closing muscle activity and the movement of the RMMA, in which the movements of both muscles contract simultaneously (Lavigne et al., 2001; Khoury et al., 2008; Lavigne et al., 2008). Therefore, we needed to investigate the effects of BoNT injections, including the activation of the suprahyoid muscles, which is the mouth-opening muscle, during the treatment of SB patients.

In this study, we targeted the lower 1/3 region, the conventional injection site for the masseter muscle (Kim et al., 2016), and the middle 1/3 region of the ABDM identified through our previous Sihler's staining study (Part Ⅱ) as the area densely innervated by mylohyoid nerve.

Unlike many studies on the masseter muscle, ABDM lacks a clear protocol and injection dosage. Previous research has emphasized the safety and effectiveness of using EMG and US examinations as a secure method for targeting ABDM during injection therapy (Kim et al., 2022; Fernández-Pajarín et al., 2021). Moreover, the use of US is highlighted as a method to screen for ABDM variations in advance, preventing unwanted side effects (Zdilla et al., 2015). Therefore, this study implemented a US-guided injection method to visualize the structure of the injection site for a safer and more effective procedure.

We measured bite force and muscle thickness as variables to compare the therapeutic effects of SB treatment. As a result, bite force and masseter muscle thickness decreased by 8 weeks after BoNT injection. The most significant reduction was observed at 4 weeks (Figure 12A, B), and all patients self-reported a reduction in SB. Lee et al. reported that the frequency of SB markedly reduced 4 weeks after injection of BoNT into the masseter muscle by EMG evaluation and was maintained for 12 weeks (Lee et al., 2010). Similarly, many studies reported a significant decrease in bite force 4 weeks after BoNT injection into the masseter muscle (Kim et al., 2007; Kim et al., 2009; Ahn et al., 2007). BoNT injection reduced muscle contractions, leading to a 20-40% decrease in maximum bite force and reduced SB frequency (Kwon et al., 2019). These findings indicate that BoNT injected into SB patients offers effective therapeutic benefits for SB by decreasing muscle activity rather than primarily affecting the central nervous system (Lee et al., 2010). However, no significant difference was found between the two groups (Table 3, $P > 0.05$). In particular, in group B, the thickness of ABDM was not affected by BoNT, confirming that ABDM injection had no direct effect on bite force and masseter muscle thickness (Figure 12C).

However, for our study, we could propose an effective injection depth by

measuring the pre-injection thickness of ABDM using US. The distance from the skin to ABDM was 6.0±1.3 mm, and the ABDM thickness was 4.7±1.1 mm. Based on these results, even considering the maximum and minimum values, an injection depth of 7.3–8.3 mm would precisely target ABDM (Figure 14). US provides a unique opportunity to collect data directly from living individuals, allowing for the analysis of muscle depth from the skin and overcomes the limitations of traditional dissection analysis, making it a groundbreaking tool for various clinical anatomical applications in the head and neck region (Kim et al., 2022).

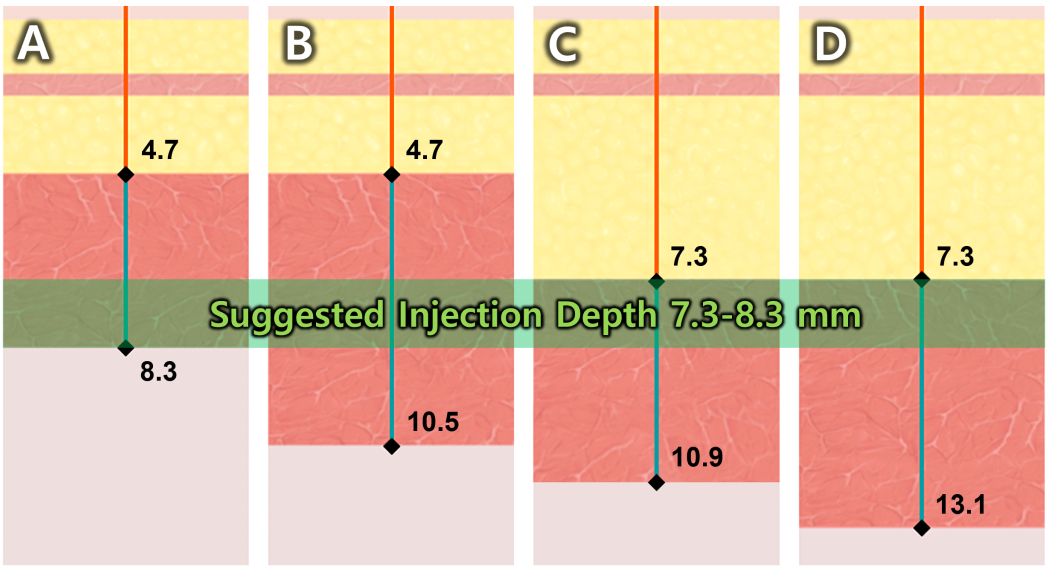

Figure 14. Schematic representation of the anterior belly of the digastric muscle (ABDM) injection site from the skin. (A) Minimum distance and thickness from the skin to ABDM, (B) Minimum distance from the skin to ABDM and the maximum thickness of ABDM, (C) Maximum distance from the skin to ABDM and the minimum thickness of ABDM, (D) Maximum distance and thickness from the skin to ABDM. Orange and green shaded bars indicate where the skin to ABDM and ABDM thickness

In addition, two participants in group B self-reported that they could not close their mouths well after injection, but these symptoms disappeared at the 8-week follow-up visit (Table 4). This finding suggests that BoNT may have diffused and affected the surrounding mylohyoid muscle. The clinical efficacy of BoNT is closely related to dosage and correlates with muscle mass (Campanati et al., 2017; Blitzer et al., 2001). Therefore, even if patients do not experience SB, additional research on effective dosages should be considered to prevent the diffusion of BoNT injected into ABDM.

However, there are several limitations to this study. First, the sample was small in size (n=24). Second, objective questionnaires to evaluate participants' subjective responses are necessary to assess the effects of BoNT injection for SB. Third, long-term effects were not examined because the study terminated after 8 weeks. Generally, the effects of BoNT injections last 2 to 3 months and then gradually decrease (Klein et al., 2004). As BoNT was injected into ABDM for the first time in patients with SB, long-term evaluation is necessary to confirm stability in this muscle. Lastly, the movement of the suprahyoid muscles is influenced by the complex and varied interactions of surrounding muscles. In an effort to minimize dysphagia, we specifically chose the ABDM among the suprahyoid muscles. However, the impact on the SB mechanism following BoNT injection into ABDM may be limited. Additionally, we evaluated the reduction in SB effects based solely on bite force and muscle thickness measurements after BoNT injection. Therefore, for a comprehensive understanding of the effects on RMMA after suprahyoid injection should be confirmed using polysomnography to record electroencephalography, EMG, and breathing-related variables.

V. CONCLUSION

This study provides detailed information on temporal tendinitis and SB, suggesting treatment guidelines based on anatomical evidence through 1) identifying the anatomical structures surrounding the coronoid process using intraoral US, which offers guidelines for the safe and efficient injection treatment of temporal tendinitis, 2) clarifying the neural distribution patterns of ABDM using Sihler's staining for BoNT injection treatment in SB patients, and 3) based on this information, administering US-guided injections into the ABDM and/or the masseter and comparing their therapeutic effects.

The conclusions of this study are as follows:

1) This study introduces novel insights into using intraoral US for managing temporal tendinitis. The maxillary second molar serves as a useful intraoral landmark for visualizing the coronoid process, providing clinicians with a valuable reference point. To enhance injection precision, US-guided intraoral injections present a precise option for treating temporal tendinitis.

2) This pioneering Sihler's staining study suggests a novel ABDM injection point, addressing the absence of standardized guidelines for BoNT injections. Sihler's staining enables a precise understanding of mylohyoid nerve distribution. Administering BoNT in the middle third region between the gnathion and hyoid bone prevents undesirable paralysis, ensuring optimal therapeutic outcomes with minimal dosage.

3) This study is the first to evaluate the short-term effects of BoNT injected into ABDM for SB control. In addition, we propose a 7.3-8.3 mm ABDM injection depth through US assessment. The findings confirm that BoNT injected into

ABDM does not influence the reduction of SB during sleep. However, further studies are needed to investigate whether BoNT can effectively control intense contractions of suprahyoid muscle during sleep and its potential impact on the occurrence of RMMA.

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Abstract (in Korean)

입안초음파와 쉴러염색을 이용한 관자근힘줄과 턱두힘살근 앞힘살의 임상 해부학적 분석

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김수빈

턱관절장애는 씹기근육과 턱관절을 포함한 씹기계통 구조의 많은 임상 징후와 증상을 총칭하는 용어로, 대표적 증상으로는 입벌림 제한 및 입을 벌리거나 닫을 때 비대칭적인 아래턱운동, 귀의 통증, 두통 등 얼굴 부위 통증을 나타낸다. 또한, 턱관절장애는 스트레스, 외상 등 여러 요인에 의해 발생 되나, 이갈이가 주된 요인 중 하나로 알려져 있다. 턱관절장애의 치료는 기본적으로 씹기근육을 이완시키기는 것을 권장하고 있으며, 주로 물리치료와 스플린트장치 착용 등이 시행되고 있지만 해결되지 않는 경우 통증 부위에 보툴리눔독소와 같은 주사치료를 일반적으로 시행하고 있다. 이를 효과적으로 치료하기 위해서는 치료 부위에 대한 해부학적인 이해가 필수적이다.

턱관절장애의 증상 중 관자근 힘줄염은 턱관절 증상과 혼동될 수 있다. 특히, 관자근 힘줄이 부착하는 아래턱뼈 근육돌기는 복잡한 해부학적 구조에 둘러싸여 있으며 광대활 깊숙이 위치하고 있어, 이를 진단하거나 치료하기 위한 기초자료가 매우 부족한 실정이다.

더불어. 수면이갈이의 경우 기존의 이론을 넘어, 아래턱 내림근인 목뿔위근육의 활동이 먼저 나타난 후 아래턱 올림근인 깨물근과 관자근이 작용한다는 이론이 대두됨에 따라 새로운 접근법에 대한 필요성이 강조되고 있다. 따라서, 아래턱 올림근에 보툴리눔독소를 주사하는 기존의 방식과는 달리, 아래턱 내림근을 목표로 하여 시술을 시행하는 방식이 이갈이를 보다 효과적으로 완화시키는가에 대하여 조사할 필요가 있다.

따라서, 본 연구에서는 1) 입안초음파를 활용하여 근육돌기 주변의 해부학적 구조물을 구명하고, 관자근 힘줄염의 주사치료 시 안전하고 효율적으로 치료하기 위한 가이드라인을 제시하고자 하였으며, 2) 수면이갈이환자의 보툴리눔독소 주사치료를 위해 턱두힘살근 앞힘살의 신경분포양상을 확인한 후, 이를 바탕으로 초음파 유도 주사를 시행하여 깨물근 또는 깨물근과 턱두힘살근 앞힘살 모두에 주사한 방법 간의 치료효과를 비교하여 턱관절장애의 치료를 위한 안전한 가이드라인을 제시하고자 시행되었다.

본 연구는 초음파 연구(Part Ⅰ)와 해부학적 연구(Part Ⅱ), 그리고 임상연구(Part Ⅲ)로 구성되어 있다. Part Ⅰ에서는 총 58명의 피험자의 116쪽의 양쪽 얼굴을 대상으로 근육돌기 주변 부위의 정상 해부학적 구조물에 대한 분석을 진행하였다. 근육돌기는 입안초음파를 이용하여 제2대구치 교합면 아래에서 초음파영상을 촬영하고, 초음파영상 중간지점(MP)에서 관찰되는 근육돌기 앞쪽경계의 수평거리와 입안점막으로부터 근육돌기와 관자근 힘줄의 깊이를 측정하였다. Part Ⅱ의 경우 한국인 시신 6구에서 추출한 총 12개의 턱두힘살근 앞힘살을 사용하여 쉴러염색을 진행하였으며, 턱두힘살근 앞힘살을 턱끝점과 목뿔뼈를 잇는 정중선을 3등분 하여 분석하였다. Part Ⅲ에서는 Part Ⅱ에서 수행한 쉴러 염색을 바탕으로 수면이갈이 환자의 턱두힘살근 앞힘살에 보툴리눔독소의 주사 효과를 검증하기 위해 24명의 대상자를 무작위로 12명씩 2그룹으로 나누었다. 그룹A는 깨물근에, 그룹B는 깨물근과 턱두힘살근 앞힘살에 보툴리눔독소를 주사한 후 주사 전, 주사 1개월, 2개월 후 씹는힘과 근육두께를 측정하였다.

Part Ⅰ연구결과에서는 근육돌기 주위 구조들을 초음파를 통해 분석하였다. 입을 4 cm 벌린 상태에서 근육돌기의 앞쪽경계가 모든 초음파에서 관찰되었으며, 입안점막, 볼근, 볼지방체, 관자근 힘줄, 근육돌기가 얕은 곳에서 깊은 곳 순서로 관찰되었다. 관자근 힘줄은 입안점막으로부터 평균 3.1±0.7 mm 깊이에 위치하였다. Part Ⅱ연구결과에서, 모든 표본에서 턱목뿔근신경의 단일 가지가 턱두힘살근 앞힘살에 들어가는 것을 확인하였으며, 중간 1/3 지점 (100%, 12/12)에 신경이 밀집되어 있는 것을 관찰할 수 있었다. Part Ⅲ 연구결과에서는 깨물근 또는 깨물근과 턱두힘살근 앞힘살에 보툴리눔독소를 주사한 결과, 주사 전, 주사 1개월과 2개월 후 모든 피험자의 씹는힘과 깨물근의 두께는 감소하였지만 두 그룹 사이에 유의한 차이는 없었으며 ($p>0.05$, 반복

측정분산분석), 턱두힘살근 앞힘살은 근육 두께 차이에 유의한 차이가 없었다 ($p>0.05$, 반복측정분산분석).

본 연구에서 도출한 결과에 따르면, 해부학적 연구에 사용된 표지점은 환자의 머리와 목 표면에서 쉽게 위치를 예측할 수 있는 구조물로, 턱두힘살근 앞힘살에 보툴리눔독소를 주사할 경우 안전하고 효과적인 주사를 위한 가이드라인으로 활용될 수 있을 것이다. 또한, 제2대구치는 근육돌기의 전방경계를 시각화하기 위한 입안 랜드마크로 활용될 수 있을 것이며, 이러한 초음파 연구의 결과를 바탕으로 입안, 얼굴부위의 초음파 유도 주사 시술 시 해부학적 구조물을 구분하기 위한 지침으로 활용될 수 있을 것이다. 마지막으로, 수면이갈이의 완화를 위한 보툴리눔독소 치료 시 아래턱 올림근에 단일 주사를 해도 충분한 효과를 기대할 수 있을 것이며, 아래턱 내림근에서의 주사 여부를 검증하기 위해서 수면다원검사를 추가적으로 실시할 경우, 보다 의미 있는 결과를 도출할 수 있을 것으로 기대된다.

핵심되는 말 : 턱관절장애, 입안초음파, 관자근 힘줄염, 수면이갈이, 근육돌기, 턱두힘살근 앞힘살, 깨물근