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ORIGINAL ARTICLE

Ultrasonographic analyses of Crow's feet and novel guideline for botulinum toxin injection

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Abstract

Background: Crow's feet are bilateral orbital wrinkles formed by the orbital portion of the orbicularis oculi muscle, which is the target muscle for botulinum neurotoxin (BoNT) injection.

Objectives: This study's aim was to demonstrate a novel BoNT injection guideline by assessing muscle width, thickness, and dynamic features using ultrasonography.

Methods: Twenty healthy Korean volunteers (10 men, 10 women; mean age, 25.6) participated. The width, thickness, and dynamic movement of the orbicularis oculi muscle were measured using ultrasonography. Two volunteers were selected to receive BoNT injections. Injections were administered using a novel method with two curved reference lines passing a point 15 mm lateral to the lateral canthus (conventional injection) and a point 5 mm lateral to the lateral margin of the frontal process of zygomatic bone (additional injection).

Result: At the lateral canthus level, the distance between the lateral margin of the frontal process and the most lateral margin of the orbicularis oculi muscle was 12.5 ± 1.3 mm. The thickness of the orbicularis oculi muscle at the midpoint of the frontal process, the lateral marginal of the frontal process, and 5 mm lateral to the lateral marginal of the frontal process was 0.7 ± 0.3 mm, 1.1 ± 0.3 mm, and 1.2 ± 0.3 mm, respectively. The crow's feet of the two volunteers began to disappear from day 3 and completely disappeared on day 7 after the injection.

Conclusion: The novel injection technique based on the ultrasonographic anatomy resulted in improvements in the appearance of crow's feet.

KEYWORDS
botulinum neurotoxin injection, crow's feet, orbicularis oculi muscle, ultrasound-guided injection

Jiong-Zhen Piao and Wook Oh should be considered joint first author.

1 | INTRODUCTION

The term 'crow's feet' refers to the appearance of noticeable bilateral orbital wrinkles caused by the orbicularis oculi muscle. When the orbicularis oculi muscle is active, the skin of the lateral orbit is drawn toward the medial side, and skin wrinkles are formed due to the contraction of the orbicularis oculi muscle. Noticeable crow's feet are among the most common signs of facial aging.[1]

The injection landmark for the crow's feet is recommended at a point 1.5–2 cm lateral to the lateral canthus. In general, two or three 2 U doses of BoNT are injected above and below this point along the orbital rim. Multipoint or multilevel injection techniques can be used to achieve a full zone of treatment, along with different injection methods according to the position of the eyebrows.[1–3]

Although extensive injection guidelines on BoNT treatment have been reported, most treatments are administered by blind injection.[1–3] Furthermore, few studies have investigated the dynamic analyses of the formation of crow's feet, including detailed anatomical investigation of the orbital portion of the orbicularis oculi muscle via ultrasonography, to date. Several attempts have been made to utilize ultrasonography in guided injections in the musculoskeletal areas.[4,5] A previous study comparing conventional blind injection and ultrasonography-guided BoNT injection found that ultrasonography-guided injection successfully increased the safety and reliability during the procedure. When using ultrasonography, clinicians can precisely confirm the target muscle and needle position to avoid complications.[6] Muscle movement and the detailed surrounding anatomy can also be monitored.

The aim of this study was to demonstrate a more effective novel BoNT injection guideline for improving the appearance of crow's feet by assessing the anatomical information and dynamic features of the orbicularis oculi using ultrasonography.

2 | MATERIALS AND METHODS

Forty images of the orbicularis oculi muscle obtained from 20 healthy Korean volunteers (10 men and 10 women; mean age, 25.6) using ultrasonography were included in this study.

The volunteers were placed in a semi-supine position for ultrasonography. The width, thickness, and dynamic movement of the orbicularis oculi muscle were measured and monitored using a real-time two-dimensional B-mode ultrasound device with a high-frequency (13 MHz) linear transducer (LOGIQ e, GE Healthcare). Volunteers were asked to relax their face, then smile while squinting their eyes hard. This caused the orbicularis oculi muscle to lift the labial commissure to maximize the visualization of the crow's feet to determine the movement of the orbicularis oculi muscle during the formation of the crow's feet and allowed the researchers to measure the width and thickness of the orbicularis oculi muscle. As shown in Figure 1, a horizontal line was drawn laterally from the lateral canthus, and landmarks were marked where this line intersected with the orbital rim and lateral margin of the frontal process of zygomatic bone (point B). Point A refers to the midpoint of the orbital rim (OR) and point B, and point C is 5 mm lateral to point B.

Ultrasonographic examination was performed at this horizontal line by placing the transducer on the outer orbit at the lateral canthus level. The depth of the orbicularis oculi muscle from the skin surface (combining thickness of the skin and subcutaneous tissue) and the thickness of the orbicularis oculi muscle were measured using the ImageJ program (National Institutes of Health) (Figure 2). Descriptive statistics were used to describe the distribution of orbicularis oculi muscle. Independent *t*-tests were performed to assess the differences between male and female patients and between the left-and right-side measurements. The level of significance was set at $p < 0.05$.

All procedures performed in this study were approved by the Institutional Review Board (approval no. 2-2017-0023; date of approval: June 22, 2017). All subjects received a sufficient explanation of the study purpose and protocols and provided written informed consents. Written consent was provided, by which the patients agreed to the use and analysis of their data.

3 | RESULTS

3.1 | Location and thickness of the lateral portion of the orbicularis oculi muscle under ultrasonographic images

At the lateral canthus level, the mean length between the lateral margin of the frontal process of zygomatic bone (point B) and the most lateral margin of the orbicularis oculi muscle was 12.5 ± 1.3 mm. These measurements did not differ significantly between the sexes and sides ($p > 0.05$) (Figure 2).

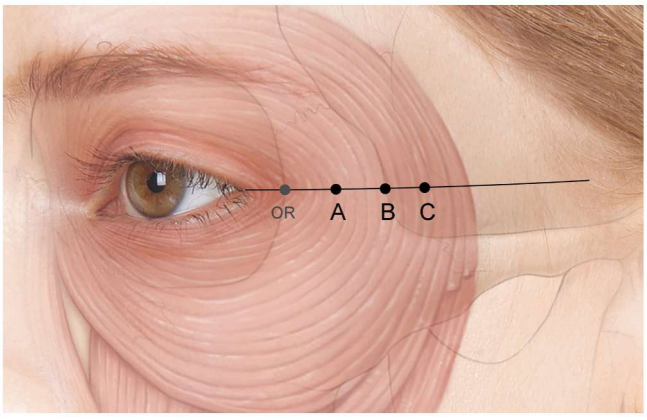

FIGURE 1 Surface landmarks used in the present study. A horizontal line was drawn laterally from the lateral canthus and landmarks were designated where this line intersected with the frontal process of the zygomatic bone (OR, a point at the orbital rim; (A) midpoint of the frontal process; (B) a point at the lateral margin of the frontal process; (C) a point 5 mm lateral to point B at the lateral canthus level, respectively)

The depth of the orbicularis oculi muscle from the skin surface at points A, B, and C was 2.0 ± 0.4 mm, 2.8 ± 0.4 mm and 3.1 ± 0.5 mm, respectively. The depth of the orbicularis oculi muscle differed between the sexes, and it was significantly greater in females compared to that of males ($p < 0.05$) (Figure 3 and Table 1).

The muscle thicknesses of the orbicularis oculi muscle at points A, B, and C were 0.7 ± 0.3 mm, 1.1 ± 0.3 mm, and 1.2 ± 0.3 mm, respectively. No significant differences in muscle thickness measurements were found between the sexes and sides at most points except gender differences in muscle thickness at point C. The lateral portion of the orbicularis oculi muscle located lateral to point B was clearly demonstrated to be the thickest (Figure 3 and Table 1).

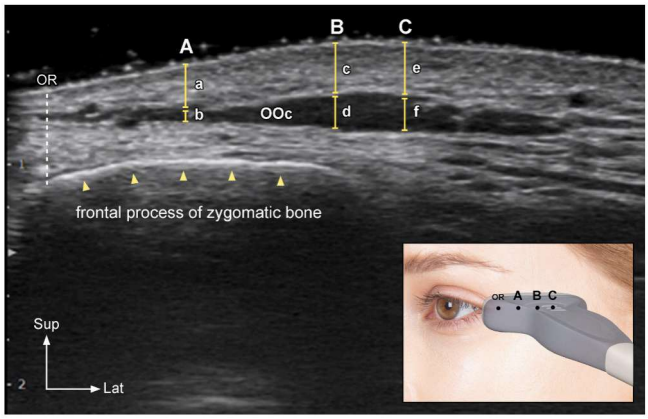

FIGURE 2 Ultrasonographic analysis of the orbicularis oculi muscle (B mode, transverse view, 16-MHz linear transducer). Each parameter was measured at three landmarks. (A) the depth of the orbicularis oculi muscle from the skin surface at point A; (B) the thickness of the orbicularis oculi muscle at point A; (C) the depth of the orbicularis oculi muscle at point B; (D) the thickness of the orbicularis oculi msucle at point B; (E) the depth of the orbicularis oculi muscle at point C; (F) the thickness of the orbicularis oculi muscle at point C

3.2 | Ultrasonography of crow's feet formation

The orbicularis oculi muscle was shown as a hypoechoic image beneath the skin with irregular hyperechoic subcutaneous fat above the muscle during ultrasonography. Lateral movement of the transducer revealed that the orbicularis oculi muscle became thicker, and the lateral margin of the orbicularis oculi muscle was found at the superficial layer of the anterior temple (Figure 4A).

During the formation of dynamic crow's feet, the contraction of the orbital portion of the orbicularis oculi muscle moved the skin of the lateral orbital rim toward the lateral canthus, causing fine wrinkles in the thin skin (Figure 4B). Under ultrasonography in the horizontal view, it was also observed that the lateral portion of the orbicularis oculi muscle contracted during medial movement as the

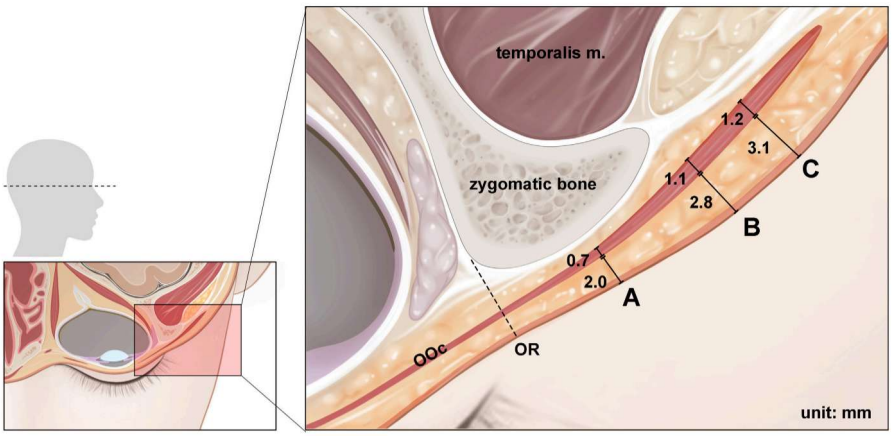

FIGURE 3 Mean depth and thickness of the lateral portion of the orbicularis oculi muscle (OR, a point at the orbital rim; (A) midpoint of the frontal process; (B) a point on the lateral margin of the frontal process; (C) a point 5 mm lateral to B at tha lateral canthus level)

TABLE 1 Mean depth and thickness of the orbicularis oculi muscle

Landmarks		Thickness	*p*-Value	Depth	*p*-Value
A	Male	0.7 ± 0.2	0.616	1.8 ± 0.4	0.001[a]
	Female	0.7 ± 0.3		2.2 ± 0.3	
B	Male	1.1 ± 0.3	0.311	2.6 ± 0.5	0.034[a]
	Female	1.0 ± 0.3		2.9 ± 0.3	
C	Male	1.4 ± 0.3	0.001[a]	2.9 ± 0.5	0.003[a]
	Female	1.1 ± 0.3		3.4 ± 0.5	

Note: Data are presented as mean ± SD values. Values are expressed in mm.

Abbreviation: SD, standard deviation.

[a]Statistically different between sexes ($p < 0.05$).

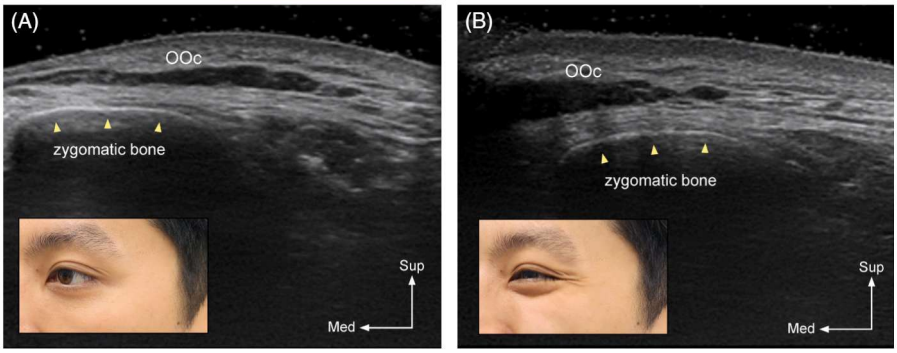

FIGURE 4 Dynamic ultrasonography performed during crow's feet formation. (A and B, ultrasound images taken while the face was relaxed and when crow's feet formed, respectively)

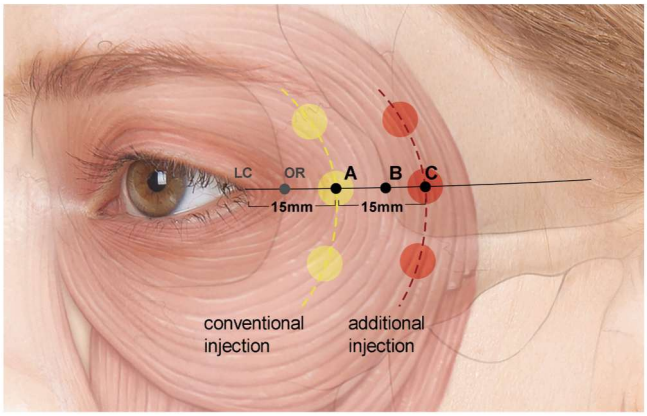

FIGURE 5 An illustration showing the injeciton points for crow's feet using the two-line injection technique (LC, lateral canthus; yellow circle, conventional injection into the dermis; red circle, additional inejction into the muscle)

crow's feet formed. At the crow's feet formation stage, it was shown that the contracted orbital part of the orbicularis oculi muscle was thicker than that in the relaxed state (Figure 4A,B).

3.3 | Determination of BoNT injection points for crow's feet and clinical applications

The movement of the orbicularis oculi muscle can be visualized and monitored using dynamic images of ultrasonography. The orbicularis oculi muscle contracted toward the lateral canthus and appeared to thicken during the formation of the crow's feet. Closer inspection of the dynamic ultrasound images showed that the thicker lateral portion of the orbicularis oculi muscle lateral to point B played a major role in the formation of crow's feet.

Due to the anatomical location of the most lateral margin of the orbicularis oculi muscle 12.5 ± 1.3 mm lateral to B at the lateral canthus level, additional botulinum neurotoxin injections should be administered at a point 5–10 mm lateral to point B. When considering botulinum neurotoxin diffusion, injections administered at a point 5 mm lateral to point B may be clinically reasonable.

Therefore, based on our results, we propose a more effective novel botulinum neurotoxin injection guideline for improving the crow's feet. Compared to conventional injection guidelines, the novel botulinum neurotoxin injection included additional injections at points 5 mm lateral to point B. This two-line novel injection technique comprised injections along two curved reference lines passing points 15 mm lateral to the lateral canthus (conventional injection) and 5 mm lateral to point B (approximately 30 mm lateral to the lateral canthus, additional injection). For the conventional injections, a 2 U intradermal injection was administered into a point at the lateral canthus level, and two more were injected intradermally 1 cm above and below that point. For the novel injections, an additional three intramuscular injections were administered at the relevant points along the curved line in the same way (two-line injection) (Figures 5 and 6).

Among the 20 volunteers, two were selected to receive the botulinum neurotoxin injection (Liztox, Humedix Inc.) to improve the appearance of their crow's feet. After diluting botulinum neurotoxin in 0.9% NaCl solution to a concentration of 100 units/2.5 ml, 4 units/0.1 ml were injected into each site using a 30G needle. Both a 35- and 36-year-old man complained of a negative crow's feet appearance on smiling. The two male volunteers had normal skin elasticity with no fine static wrinkles or photoaged skin. Hence, the clinician diagnosed that their crow's feet were caused by a hyperactive orbicularis oculi muscle. Botulinum neurotoxin was injected into each volunteer using a novel injection technique. The crow's feet disappeared from day 3 and completely improved on day 7 after the injection, with no side effects such as infection or edema (Figure 7).

4 | DISCUSSION

Two major etiologic factors of wrinkle formation have been proposed in the literature, namely the skin and facial expression muscles, and an effective treatment is paralysis of the facial muscles.[7] To reduce the obvious appearance of wrinkles, various esthetic treatments such as laser, high-intensity focused ultrasound, filler, and botulinum neurotoxin treatments have been developed for patients to choose from.[8] Among them, botulinum neurotoxin is one of the representative treatments targeting muscles and has been used extensively to treat crow's feet.[9] Botulinum neurotoxin for facial wrinkles is the most frequently performed and popular cosmetic procedure approved by the Food and Drug Administration, with few side effect, significant effect, and good patient satisfaction.[10–12]

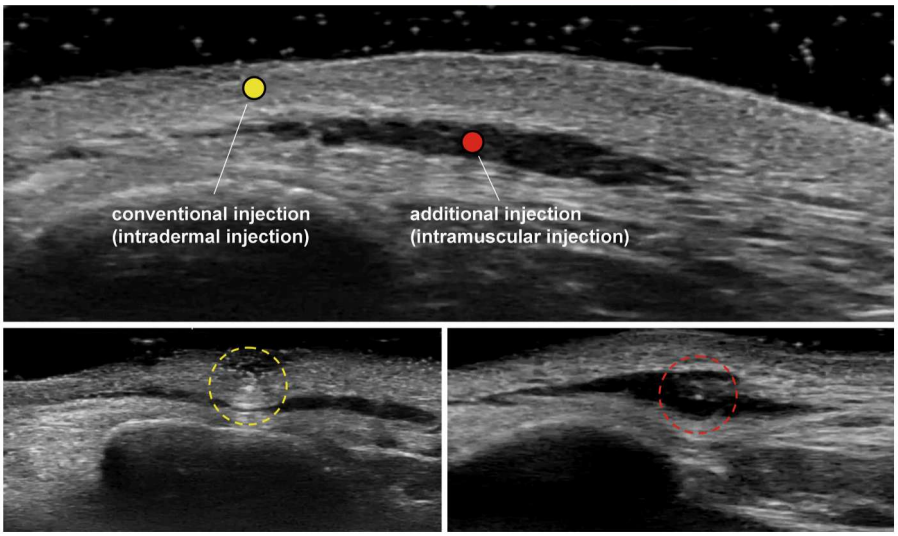

FIGURE 6 Ultrasonography demonstrating the two-line injection technique (yellow circle, intradermal injection; red circle, intramuscular injection; red and yellow dotted circles, needle tip visualized during ultrasound-guided injeciton)

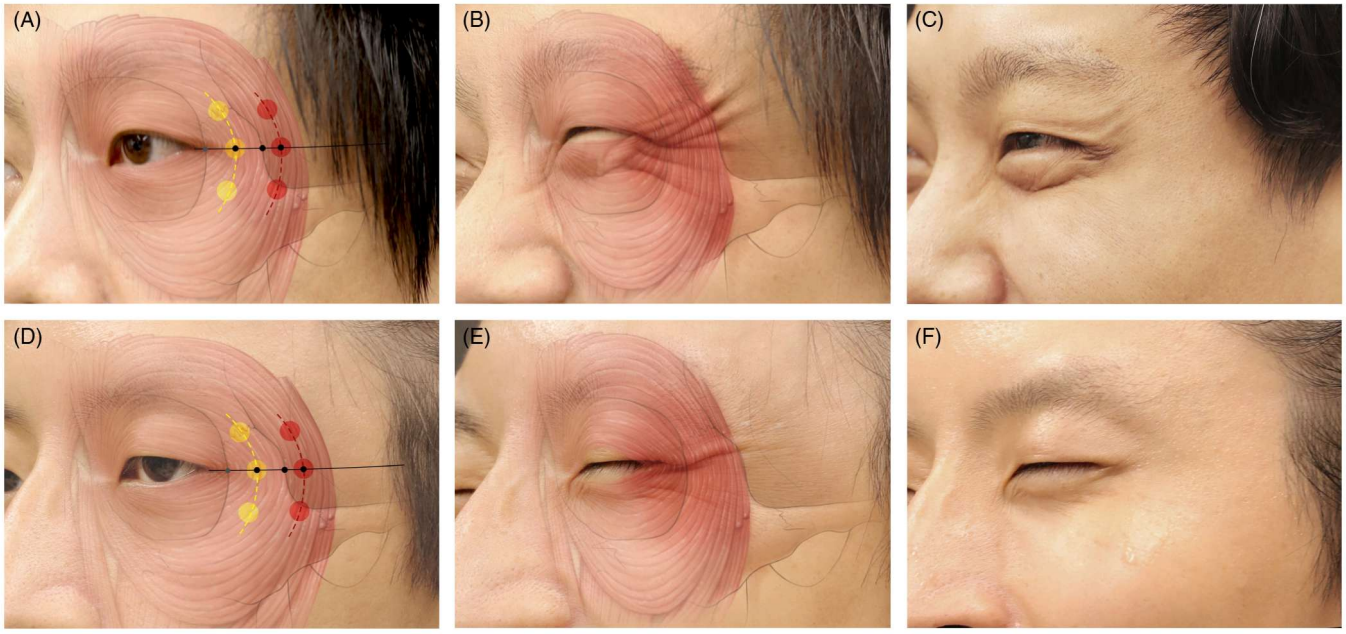

FIGURE 7 Clinical photos showing the crow's feet of the volunteers. (A and D) Relaxed orbicularis oculi muscle before injection. (B and E) Contracted orbicularis oculi muscle before injection. (C and F) Contracted orbicularis oculi muscle 7 days after injection

Repeated facial muscle contraction with the expression of smiling and squinting, especially of the orbicularis oculi muscle, leads to the formation of crow's feet, which are classified as dynamic wrinkles. Botulinum neurotoxin has been injected into the orbital part of the orbicularis oculi muscle using multipoint, intradermal, and intramuscular injection methods.[1–3]

The average length from the lateral canthus to the lateral margin of orbicularis oculi muscle is reportedly 3.1 cm in Asians.[13] Hence, injections more than 3 cm lateral to the lateral canthus into the surrounding muscle component, such as the lateral muscular band, are not recommended.[1] Meanwhile, to prevent adverse effects such as diplopia caused by botulinum neurotoxin diffusion toward the eyeball, the injection point should not be too close to the lateral canthus, and the needle should be pointed outward.[14] Although extensive injection guidelines on crow's feet have been provided, no studies on the anatomical analyses of the orbicularis oculi muscle exist to our knowledge. The aim of the present study was to provide a new injection method by visualizing and assessing the orbicularis oculi muscle during the formation of crow's feet under ultrasonography.

In the present study, we proposed a novel two-line injection technique in addition to the conventional injection points (Figure 5) and established two curved reference lines passing 15 and 30 mm lateral to the lateral canthus, respectively. The lateral additional reference line was included to allow the novel injection technique to target the thicker lateral portion of the orbicularis oculi muscle. The portion of the orbicularis oculi muscle lateral to the frontal process tended to be thicker in the relaxed state, and we observed that the lateral part of the orbicularis oculi muscle strongly contracted and moved medially during dynamic crow's feet formation during ultrasonography. From the clinical applications of the two volunteers, this

two-line botulinum neurotoxin injection technique clearly demonstrated that the dynamic crow's feet began disappearing from day 3 and completely disappeared on day 7 after injection.

A previous study reported cases of intraoperative injection of botulinum neurotoxin into the orbicularis oculi muscle as a treatment for crow's feet. Intraoperative injection permits exact injection of the toxin due to direct vision and results in improvement in the crow's feet. The same effect can be obtained through precise targeted injection with noninvasive ultrasound-guided injections.[7]

The establishment of various injection guidelines has resulted in intramuscular injection no longer being the only injection method for botulinum neurotoxin administration. The level of injection determines the potency of botulinum neurotoxin into the muscle; intramuscular injection has a strong effect, whereas intradermal injection has a soft effect.[2,15] Consequently, we administered the injection intradermally into the relatively thin orbicularis oculi muscle portion (0.7 mm) with a relatively thinner subcutaneous layer (2.0 mm) that was not easy to target at the points 1.5 cm lateral to the lateral canthus under ultrasonographic guidance. The fine wrinkles near the lateral canthus can be eliminated by these intradermal injections. Simultaneously, we administered the ultrasonography-guided intramuscular injection into the portion of the thicker orbicularis oculi muscle (1.2 mm) with an abundant subcutaneous layer (3.1 mm) at the additional points 3.0 cm from the lateral canthus (Table 1 and Figures 5 and 6). These intramuscular injections could help in improving the deep furrows at the outer orbital rim during the dynamic contraction of the orbicularis oculi muscle.

Botulinum neurotoxin treatment is recommended for dynamic crow's feet responsive to botulinum neurotoxin, whereas it is not recommended for static crow's feet due to photodamage and loss of skin elasticity. A note of caution is warranted here, as this study is unable to encompass the entire wrinkle pattern and anatomical relationship due to the high customization of the detailed facial muscle structure.[16–19] Individualized injection techniques may be applied based on the patterns of the crow's feet. A note of caution is not to give deep injections and large does into the lateral and lower part with the zygomaticus major muscle underneath, it may cause the mouth corner cannot be elevated due to the weakness of zygomaticus major muscle.[14] In addition, we should consider anatomical variations, such as a type C lateral muscular band of orbicularis oculi muscle (8% of the specimens) that originate from the lateral orbicularis oculi muscle and insert into the mouth angle. When botulinum neurotoxin is injected at the most lateral portion of the orbicularis oculi muscle in cases of type C pattern, it may result in an asymmetric or unnatural smile due to the weakness of the lateral muscular band of orbicularis oculi muscle.[13] To reduce these side effects, the facial expressions of various patients must be evaluated before the procedure.

5 | CONCLUSIONS

The additional injection points based on the ultrasonographic anatomy in this study offer effective guidelines for targeting the orbicularis oculi muscle. The insights gained from this study may be of assistance to clinicians to design the injection point.

AUTHOR CONTRIBUTIONS

Hee-Jin Kim: Overall organization and direction of the research (supervision), providing anatomical and clinical opinion, and final revision and drafting of manuscript. Jiong-Zhen Piao: Overall planning the research, writing – original draft preparation, data acquisition. Oh Wook: Data acquisition, performing clinical procedure, providing anatomical opinion. You-Jin Choi: Analysis and interpretation, photographic works and providing anatomical opinion. Ji-Hyun Lee: Data acquisition, interpretation, and photographic works of harvesting data. Hyungkyu Bae: Data acquisition, photographic works. Kyung-Seok Hu: Organization and direction of the research (supervision), providing anatomical and clinical opinion. Hyoung-Moon Kim: Providing anatomical opinion. Hee-Jin Kim: Overall organization and direction of the research (supervision), providing anatomical and clinical opinion, and final revision and drafting of manuscript.

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CONFLICT OF INTERST

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. All procedures performed in this study were approved by the Institutional Review Board of the Yonsei University College of Dentistry (approval no. 2-2017-0023; date of approval: June 22, 2017).

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