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# Diffusion characteristics and efficacy of letibotulinum toxin a in forehead wrinkle treatment

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**ABSTRACT**

**Background:** Facial wrinkles, caused by aging and repetitive muscle contractions, are commonly treated with botulinum neurotoxin type A (BoNT-A). However, excessive toxin diffusion can cause side-effects like muscle weakness.

**Objectives:** This study aimed to compare the diffusion, efficacy, and safety of letibotulinum toxin A with two other BoNT-A products for treating forehead wrinkles.

**Methods:** In a double-blind, randomized, split-face controlled trial, 20 participants with moderate-to-severe horizontal forehead wrinkles received letibotulinum toxin A on one side and prabotulinum or onabotulinum toxin A on the other. The primary outcome was diffusion profile assessed via anhidrosis area (iodine-starch test at 2 weeks); secondary outcomes included wrinkle reduction, assessed by photographic analysis, and safety.

**Results:** Results show that letibotulinum toxin A exhibited diffusion patterns and wrinkle-reduction efficacy comparable to the control products. No statistically significant differences were observed be-tween the groups for the primary or secondary outcomes.

**Conclusion:** Letibotulinum toxin A is a safe, effective alternative for wrinkle treatment, potentially minimizing excessive diffusion risks and related side effects, making it a valuable addition to available treatment options.

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**KEYWORDS**
Diffusion; botulinum toxin; forehead wrinkle; split-face; controlled trial

## Introduction

Facial wrinkles are a common esthetic concern in dermatology and are often associated with intrinsic aging and repetitive muscle activity. Dynamic wrinkles such as glabellar lines and horizontal forehead lines are particularly prominent. Glabellar lines are caused by contraction of the corrugator and procerus muscles during frowning, whereas horizontal forehead lines arise from the activity of the frontalis muscle, which elevates the eyebrows (1). Repetitive facial muscle contractions accelerate the degradation of skin elasticity and reduce tissue compliance and resilience. Over time, this leads to the progression of dynamic wrinkles into static wrinkles, necessitating early and effective intervention (2). Botulinum neurotoxin injections have become the cornerstone of nonsurgical wrinkle treatment due to their ability to temporarily reduce muscle activity, thereby preventing and alleviating dynamic wrinkles (3,4).

Despite its widespread use, these treatments require careful consideration of toxin diffusion to optimize therapeutic outcomes and minimize adverse effects. Diffusion refers to the passive spread of botulinum neurotoxin type A (BoNT-A) beyond the injection site, which significantly affects its clinical performance (5,6). The common method for evaluating diffusion of botulinum toxin is *via* the measurement of anhidrosis area, typically using the Minor's iodine-starch test (7). Excessive diffusion can result in unintended paralysis of non-target muscles, leading to complications such as ptosis, facial asymmetry, or diplopia (8,9). Conversely, inadequate diffusion may compromise the efficacy of treatment by failing to target the desired muscle sufficiently. Factors such as dose, dilution, and injection technique play critical roles in controlling diffusion and ensuring predictable results (6).

Among the currently available BoNT-A products, onabotulinum toxin A and prabotulinum toxin A are Food and Drug Administration-approved agents with well-established clinical profiles. Letibotulinum toxin A received regulatory approval in 2024, offering dermatologists a new option with potential advantages in terms of diffusion control and efficacy. Understanding the unique characteristics of each BoNT-A product is essential for selecting the most suitable agent to meet the diverse patient needs. One study found that Letibotulinum toxin A could potentially outperform Onabotulinum toxin A in targeted, low-volume esthetic applications, owing to tighter diffusion, stronger receptor engagement, and faster immune clearance. However, these results have not been validated in clinical settings (10). Therefore, the current study aims to evaluate the diffusion characteristics and clinical utility of letibotulinum toxin A for treating horizontal forehead lines, one of the most frequently requested areas in esthetic practice. By

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comparing letibotulinum toxin A with two other commercially available BoNT-A products, we provide clinicians with valuable insights to guide product selection and optimize patient outcomes.

## Materials and methods

### Study design

This randomized, double-blind, split-face, controlled prospective study was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine (Institutional Review Board number 4-2021-1097). This study was also registered on clinicaltrials.gov (Identifier: NCT07072806, registration date: 18 July 2025).

The sample size was calculated based on the primary endpoint, the area of anhidrosis. According to prior literature, the expected mean ± standard deviation values were 300 ± 60 $mm^2$ for the intervention and 200 ± 40 $mm^2$ for the control group, with an assumed correlation of 0.3 between paired groups (11–13). Using a two-sided test with a type I error (α) of 0.05 and a statistical power of 95% (β = 0.05), the required number of participants was estimated as 8 per group for a matched-pair design. To account for an anticipated 20% dropout rate, the sample size was increased to 10 per group. Given the split-face design, in which each participant received both the test and control treatments, the final total sample size was set at 20 participants. Based on this, a total of 20 adult participants with moderate to severe horizontal forehead wrinkles (Facial Wrinkle Scale score ≥ 2, Table 1) were enrolled.

Randomization was performed by an independent statistician using Microsoft Excel to generate a random sequence of 0s and 1s to allocate the treatment. Each random assignment was sealed individually in a nontransparent envelope. For each participant, the forehead was divided along the midline, with one side randomly assigned to receive the letibotulinum toxin A (Botulax®, Hugel Inc., Gangwon, Republic of Korea; intervention group) and the contralateral side assigned to receive one of two comparator botulinum toxin A formulations, prabotulinum toxin A (Nabota®, Daewoong Pharmaceutical, Seoul, Republic of Korea) or onabotulinum toxin A (Botox®, Abbvie, Chicago, United States) (control groups 1 and 2, respectively). Ten participants were assigned to receive letibotulinum toxin A versus prabotulinum toxin A, and the remaining ten received letibotulinum toxin A versus onabotulinum toxin A. The allocation of treatment to the left or right side of the forehead was determined according to the randomization process and was concealed from both the investigators administering the treatment and the evaluators conducting outcome assessments, thereby maintaining a double-blind protocol throughout the study period.

### Inclusion and exclusion criteria

Participants were eligible if they were aged ≥20 years with visible horizontal forehead wrinkles scored as moderate to severe on the Facial Wrinkle Scale using a photonumeric guide. Exclusion criteria included a history of BoNT-A injection or cosmetic procedures near the forehead/orbital area within the past year; signs of infection or inflammation at the injection site; compensatory frontal hyperactivity; neuromuscular disorders including myasthenia gravis or amyotrophic lateral sclerosis; use of anticoagulants, aminoglycosides, curare-like agents, or neuromuscular inhibitors (stable doses of muscle relaxants or benzodiazepines are permitted); and hypersensitivity to the investigational product or iodine.

**Table 1.** Facial wrinkle scale at maximum frown (14).

| Score | Severity | Explanation |
|---|---|---|
| 3 | Severe | Wrinkles are clearly visible. The depth of wrinkles cannot be assessed from the surface. |
| 2 | Moderate | Wrinkles are clearly visible. The depth of wrinkles can be assessed from the surface. |
| 1 | Mild | Wrinkles are visible. |
| 0 | None | Wrinkles are not visible. |

### Treatment protocol

Vials containing letibotulinum toxin A and the two control BoNT-A products were diluted with saline to a standardized concentration of 20 U/mL. Using a 31-gauge, 0.3-ml syringe, 2 U (0.1 ml) of each BoNT-A product was injected at four designated points per participant's forehead (Figure 1). The injection sites were spaced 3 cm apart, and subcutaneous injections were performed at a 30°–40° angle to the dermis. Light pressure was applied using a gauze to minimize bleeding without rubbing the area.

### Evaluations

The primary endpoint was the area ($cm^2$) of anhidrosis, assessed using the Minor iodine-starch test two weeks after injection. After drying the forehead of each participant, 2% iodine in ethanol is applied, followed by starch powder. Each participant then walks inside the room, maintained at ~32 °C/90 °F and constant humidity, until the sweating areas and the surrounding anhidrotic regions near the injection sites become clearly visible. To record the results, photographs are immediately taken. All photographs are captured with the same camera settings, lighting, stabilization headset, and a centimeter scale ruler, ensuring identical positioning of each participant. Computer-assisted image processing is then used to calculate the surface area, vertical, and horizontal dimensions of the anhidrotic regions.

Secondary endpoints included the assessment of wrinkle relaxation two weeks after injection. We took photographs at rest and during forced upward gaze to assess the area of wrinkle reduction, using a digital camera. The photographs were then analyzed *via* 3D imaging systems. Namely, we used the Antera 3D (Miravex, Dublin, Ireland) to assess the indentation index and maximal wrinkle relaxation length at baseline and two weeks post-treatment. Additionally, we utilized the Morpheus 3D (Yongin-si, Republic of Korea) system that allowed three-dimensional (3D) visualization of overall muscle contraction patterns to evaluate maximal wrinkle depth two weeks after treatment. All measurements were conducted on the lateral and medial sections of the forehead. To ensure the reproducibility and validity of these 3D measurements,

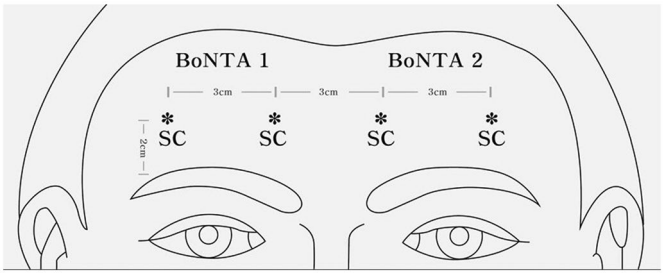


**Figure 1.** Botulinum neurotoxin type A (BoNTA) injections sites on the forehead. BoNTA: botulinum toxin type A; SC: subcutaneous.

we implemented a standardized operating procedure. For each imaging session, participants were seated in a fixed position with their heads stabilized by a chin and forehead rest to maintain consistent orientation and distance from the camera. Ambient room lighting was kept constant for all acquisitions. Prior to each use, both the Antera 3D and Morpheus systems were calibrated according to the manufacturers' specifications. To ensure intra-rater reliability, all quantitative analyses of the images were performed by a single trained evaluator who was blinded to the treatment allocation. The regions of interest on the forehead were manually defined using consistent anatomical landmarks across all images before automated software analysis of wrinkle depth and indentation. The Antera 3D system, specifically, has been previously validated and shown to have high reliability and accuracy for measuring skin surface topography (15). The Morpheus 3D also showed high degrees of accuracy and decent conformity with traditional anthropometry (16).

### *Safety assessments*

Adverse events were monitored through patient reports. Prior to treatment, the investigator educated all participants regarding potential adverse events associated with the investigational product and instructed them to report any post-treatment events at each study visit. Patient-reported adverse events were categorized as depicted in Table 2.

**Table 2.** Classification of patient-reported adverse events by severity.

| Grade | Severity | Description | Impact on daily activities | Treatment / study participation |
|---|---|---|---|---|
| 1 | Mild | Minimal symptoms | Does not interfere | Generally not required; study participation continues |
| 2 | Moderate | Symptoms causing discomfort | Interferes | May require treatment; study participation can continue |
| 3 | Severe | Severe symptoms | Makes daily activities impossible | Requires treatment or hospitalization; study participation not possible |

### *Statistical analyses*

Data were presented as mean ± standard deviation. The comparison of anhidrosis data and maximal wrinkle width between the intervention and control groups was conducted using independent t-tests or Mann-Whitney tests, depending on the normality assumptions. Subgroup analyses comparing the anhidrosis area between the medial and lateral forehead were also performed in each group. For the indentation index and maximal wrinkle depth, we compared the change from baseline of each metric between the intervention and control groups, using ANCOVA adjusted for baseline values. Analyses were performed on both intent-to-treat and per-protocol sets. Statistical significance was set at $p < .05$.

## Results

### *Participant characteristics*

A total of 20 participants (18 women and 2 men; mean age, 51.4 ± 8.04 years old) were recruited from Severance Hospital, Yonsei University College of Medicine, between June and August 2022. All participants completed the study without dropping out. The baseline wrinkle indices are depicted in Table 3. Figure 2 summarizes the process of recruitment, allocation, and follow-up of participants throughout the trial.

**Table 3.** Baseline wrinkle indices of participants.

| | *Intervention vs. Control 1* | | *Intervention vs. Control 2* | |
|---|---|---|---|---|
| Measurements | Intervention ($n = 10$) | Control 1 ($n = 10$) | Intervention ($n = 10$) | Control 2 ($n = 10$) |
| Indentation (AU, mean ± *SD*) | 35.92 ± 10.54 | 34.69 ± 9.59 | 31.45 ± 8.39 | 32.25 ± 10.24 |
| Maximal wrinkle depth (mm, mean ± *SD*) | 0.39 ± 0.12 | 0.37 ± 0.11 | 0.34 ± 0.11 | 0.36 ± 0.13 |

*Notes: SD*: standard deviation; AU: artificial unit. Intervention, Letibotulinum toxin A; Control 1, Prabotulinum toxin A; Control 2, Onabotulinum toxin A.

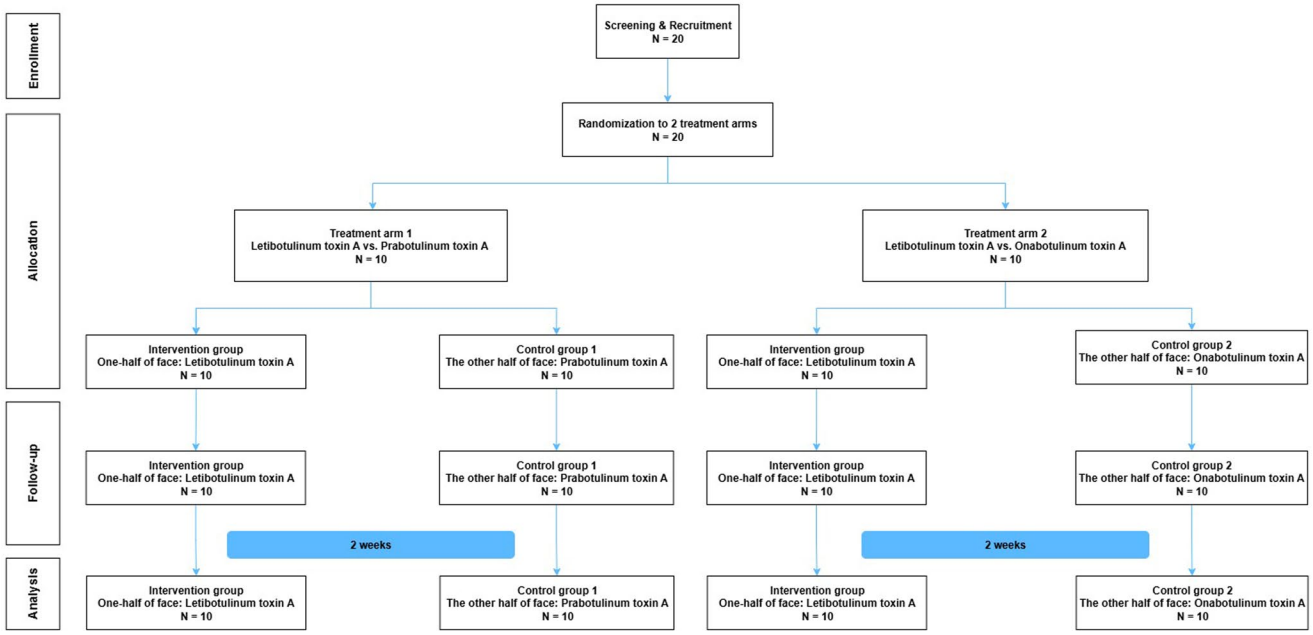


**Figure 2.** Flowchart of the recruitment, allocation, and follow-up processes of the study.

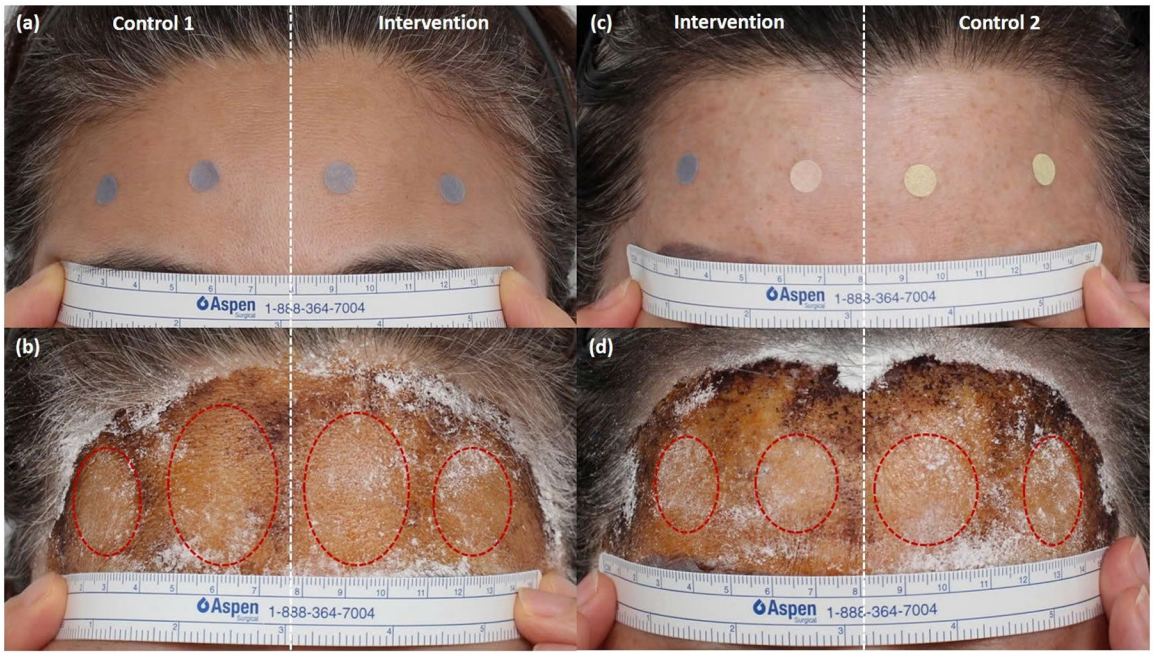


**Figure 3.** Measurement of the anhidrosis area. (a,b) Clinical photograph and anhidrosis area after administration of prabotulinum toxin A (left) and letibotulinum toxin A (right). (c, d) clinical photographs and anhidrosis area after administration of onabotulinum toxin A (right) and letibotulinum toxin A (left). The red circles represent the anhidrosis areas.

**Table 4.** Comparison of post-treatment anhidrosis area between the intervention and control groups.

| Treatment group | Anhidrosis area ($cm^2$, mean ± *SD*) | 95% CI | Effect size | *p* Value |
|---|---|---|---|---|
| Intervention group vs. Control 1 group | | | | |
| Intervention group ($n=10$) | 11.69 ± 5.48 | −3.231–8.449 | 0.049 | .36 |
| Control 1 group ($n=10$) | 14.3 ± 6.84 | | | |
| Intervention group vs. Control 2 group | | | | |
| Intervention group ($n=10$) | 10.99 ± 4.74 | −3.205–5.675 | 0.018 | .57 |
| Control 2 group ($n=10$) | 12.23 ± 4.71 | | | |

*Notes: SD*: standard deviation; CI: confidence interval. Intervention, Letibotulinum toxin A; Control 1, Prabotulinum toxin A; Control 2, Onabotulinum toxin A.

### *Assessment of anhidrosis area using minor's iodine starch test*

All participants showed anhidrosis after BoNT-A treatment. The mean horizontal and vertical axis lengths of the anhidrosis areas of all participants were 1.74 ± 0.52 and 2.19 ± 0.61 cm, respectively, with the vertical axis being significantly longer ($p < 0.001$, Figure 3).

The intervention group had smaller mean anhidrotic areas compared to both control groups; however, these differences were not statistically significant. Specifically, for letibotulinum toxin A versus prabotulinum toxin A (control 1), the areas were 11.69 ± 5.48 and 14.3 ± 6.84 $cm^2$, respectively ($p = .36$; Table 4). Meanwhile, for letibotulinum toxin A versus onabotulinum toxin A (control 2), the areas were 10.99 ± 4.74 and 12.23 ± 4.71 $cm^2$, respectively ($p = .57$; Table 4). Furthermore, subgroup analyses showed no considerable differences in anhidrosis area between the lateral and medial aspects of the forehead in all products ($p > .05$, Table 5).

### *Forehead wrinkle relaxation*

Wrinkle relaxation was observed in all participants regardless of the BoNT-A product (Figure 4). The maximal wrinkle relaxation widths measured two weeks post-injection showed no significant differences between the intervention and control 1 groups (17.84 ± 2.47 vs. 18.62 ± 2.35 mm, $p = .48$; Table 6) or the intervention and control 2 groups (16.78 ± 3.16 vs. 18.09 ± 3.78 mm, $p = .41$; Table 6).

Both groups showed marked reductions in wrinkle metrics. In the first treatment arm, the mean indentation indices decreased

**Table 5.** Comparison of post-treatment anhidrosis area between lateral and medial foreheads in each group.

| Treatment group | Anhidrosis area ($cm^2$, mean ± *SD*) | | | | |
|---|---|---|---|---|---|
| | Lateral forehead | Medial forehead | 95% CI | Effect size | *p* Value |
| Intervention group vs. Control 1 group | | | | | |
| Intervention group ($n=10$) | 9.77 ± 4.68 | 13.62 ± 7.01 | −2.790–9.950 | 0.26 | .35 |
| Control 1 group ($n=10$) | 12.85 ± 6.56 | 15.76 ± 8.51 | −10.08–4.264 | 0.042 | .4 |
| Intervention group vs. Control 2 group | | | | | |
| Intervention group ($n=10$) | 9.71 ± 3.02 | 12.28 ± 7.41 | −2.953–8.087 | 0.079 | .33 |
| Control 2 group ($n=10$) | 11.69 ± 4.49 | 12.77 ± 6.38 | −6.308–4.142 | 0.012 | .67 |

*Notes: SD*: standard deviation; CI: confidence interval. Intervention, Letibotulinum toxin A; Control 1, Prabotulinum toxin A; Control 2, Onabotulinum toxin A.

**Table 6.** Comparison of post-treatment maximal wrinkle width between intervention and control groups.

| Treatment group | Mean maximal wrinkle width (mm, mean ± *SD*) | 95% CI | Effect size | *p* Value |
|---|---|---|---|---|
| Intervention group vs. Control 1 group | | | | |
| Intervention group ($n=10$) | 17.84 ± 2.47 | −1.481–3.051 | 0.029 | .48 |
| Control 1 group ($n=10$) | 18.62 ± 2.35 | | | |
| Intervention group vs. Control 2 group | | | | |
| Intervention group ($n=10$) | 16.78 ± 3.16 | −1.962–4.592 | 0.039 | .41 |
| Control 2 group ($n=10$) | 18.09 ± 3.78 | | | |

*Notes: SD*: standard deviation; CI: confidence interval. Intervention, Letibotulinum toxin A; Control 1, Prabotulinum toxin A; Control 2, Onabotulinum toxin A.

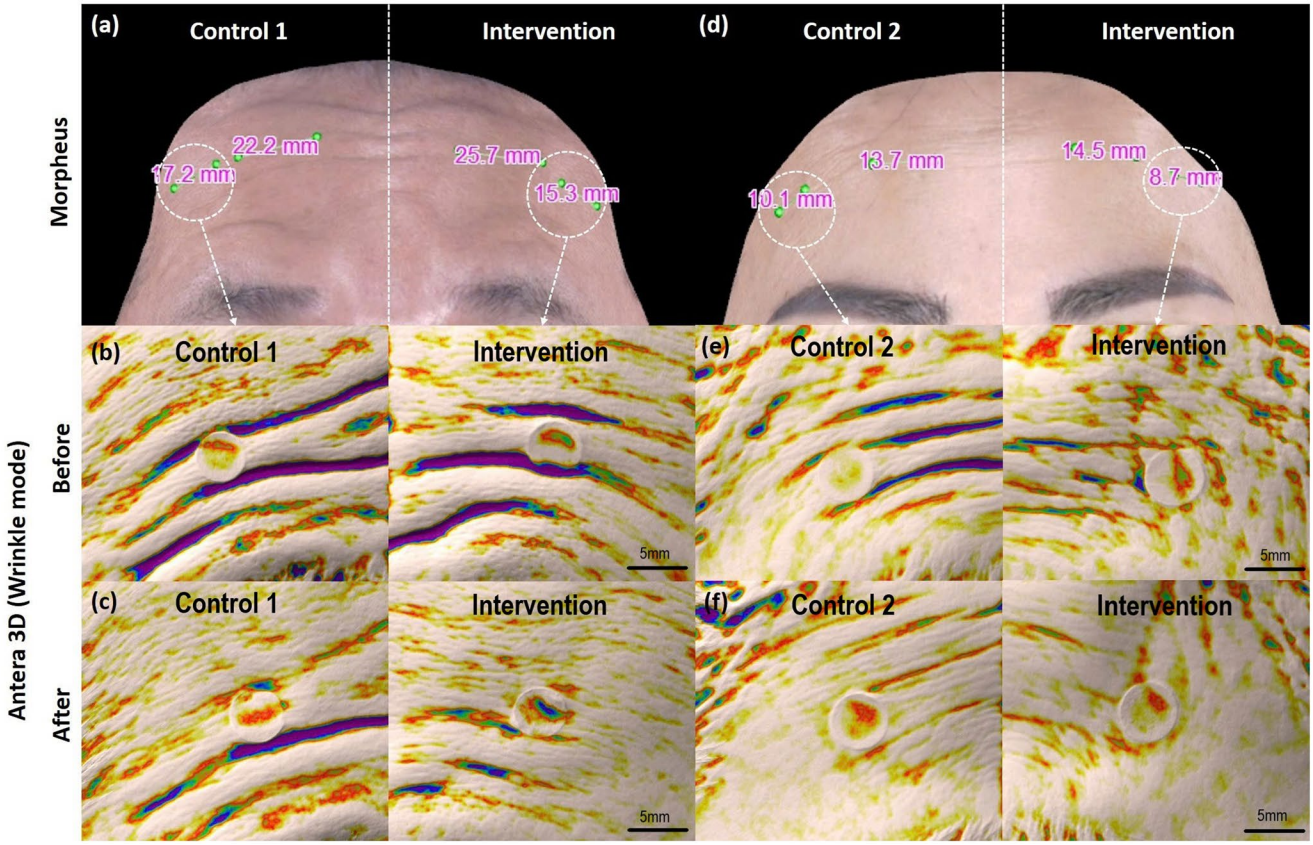


**Figure 4.** Assessment of wrinkle relaxation indices. Clinical photographs show maximal wrinkle width measurement sites at the medial and lateral forehead using Morpheus 3D for control 1 and intervention (a) and for control 2 and intervention (d). Representative Antera 3D wrinkle-mode images illustrate indentation and maximal wrinkle depth at the lateral forehead for control 1 and intervention before (b) and after (c) treatment, and for control 2 and intervention before (e) and after (f) treatment. White circles indicate the measurement regions at the lateral forehead. Scale bars, 5 mm. Intervention, letibotulinum toxin A; control 1, prabotulinum toxin A; control 2, onabotulinum toxin.

**Table 7.** Comparison of the change from baseline in the indentation index between intervention and control groups.

| | Indentation (AU, mean ± *SD*) | | | | |
|---|---|---|---|---|---|
| Treatment group | Baseline | 2 Weeks | Mean change from baseline (%) | 95% CI | *p* Value |
| Intervention group vs. Control 1 group | | | | | |
| Intervention group ($n=10$) | 35.92 ± 10.54 | 17.84 ± 2.47 | 18.36 ± 9.54 (51.1%) | −4.94–1.36 | .25 |
| Control 1 group ($n=10$) | 34.69 ± 9.59 | 18.62 ± 2.35 | 15.95 ± 6.63 (46.0%) | | |
| Intervention group vs. Control 2 group | | | | | |
| Intervention group ($n=10$) | 31.45 ± 8.39 | 16.78 ± 3.16 | 12.74 ± 5.47 (40.5%) | −1–2.6 | .36 |
| Control 2 group ($n=10$) | 32.25 ± 10.24 | 18.09 ± 3.78 | 14.05 ± 8.06 (43.6%) | | |

*Notes:* AU: arbitrary unit; *SD*: standard deviation; CI: confidence interval. Intervention, Letibotulinum toxin A; Control 1, Prabotulinum toxin A; Control 2, Onabotulinum toxin A.

**Table 8.** Comparison of the change from baseline in the maximal wrinkle depth between intervention and control groups.

| | Maximal wrinkle depth (mm, mean ± *SD*) | | | | |
|---|---|---|---|---|---|
| Treatment group | Baseline | 2Weeks | Mean change from baseline (%) | 95% CI | *p* Value |
| Intervention group vs. Control 1 group | | | | | |
| Intervention group ($n=10$) | 0.39 ± 0.12 | 0.18 ± 0.06 | 0.21 ± 0.1 (53.9%) | −0.08–0.005 | .08 |
| Control 1 group ($n=10$) | 0.37 ± 0.11 | 0.21 ± 0.08 | 0.16 ± 0.08 (43.2%) | | |
| Intervention group vs. Control 2 group | | | | | |
| Intervention group ($n=10$) | 0.34 ± 0.11 | 0.19 ± 0.10 | 0.15 ± 0.10 (44.1%) | −0.02–0.05 | .4 |
| Control 2 group ($n=10$) | 0.36 ± 0.13 | 0.20 ± 0.14 | 0.18 ± 0.11 (50%) | | |

*Notes: SD*: standard deviation; CI: confidence Interval. Intervention, Letibotulinum toxin A; Control 1, Prabotulinum toxin A; Control 2, Onabotulinum toxin A.

by 51.1% in the intervention group and 46.0% in control 1 (Table 7), while the maximal wrinkle depths decreased by 53.9% and 43.2%, respectively (Table 8). Similarly, reductions were observed in the intervention and control 2 groups, with indentation index reductions of 40.5% and 43.6% (Table 7), and maximal wrinkle depth reductions of 44.1% and 50%, respectively (Table 8). None

of these differences were statistically significant ($p > .05$; Table 7 and Table 8), showing a comparable wrinkle-reducing efficiency of the intervention group compared to control products.

### *Safety outcomes*

No SAEs, protocol violations, or injection-site reactions were reported. No adverse events, including erythema, swelling, hypersensitivity, or secondary infections, were observed. In addition, no cases of excessive muscle weakness, ptosis, or other complications interfering with daily activities were observed.

## Discussion

This study represents the first double-blind, randomized, split-face controlled evaluation of letibotulinum toxin A's diffusion characteristics in the forehead, assessed *via* areas of anhidrosis and wrinkle reduction. Letibotulinum toxin A demonstrated diffusion patterns comparable to those of the two control BoNT-A products, with consistently smaller anhidrosis areas in the intervention group, albeit without statistical significance.

The frontalis muscle, a fan-shaped structure located beneath the superficial fascia, makes the forehead particularly susceptible to diffusion of BoNT-A (17). This unique anatomical feature makes the forehead an ideal site for studying diffusion patterns, as demonstrated in multiple studies that have provided valuable insights for optimizing clinical applications (7,11,13). BoNT-A typically spreads uniformly within a radius of 2.5–3 cm from the injection site; however, adjustments to the injection site may be required depending on individual forehead dimensions, wrinkle distribution, and muscle depth. For instance, Kwon et al. reported that a 2-U BoNT-A injection (0.05 ml per point) results in a diffusion radius of approximately 1.5 cm or a diameter of 3 cm (18).

BoNT-A has become a cornerstone treatment for esthetic concerns caused by muscle contractions, such as glabellar lines, lateral canthal lines, and forehead wrinkles (2). However, the small size and proximity of facial muscles pose challenges, as excessive diffusion can lead to unintended paralysis of adjacent muscles (6,19). For example, diffusion during glabellar line treatment may affect the levator palpebrae superioris, resulting in ptosis, while diffusion from lateral canthal line treatments could weaken the extraocular or lateral rectus muscles, causing diplopia (6,20). These risks highlight the importance of selecting a BoNT-A product with a controlled and predictable diffusion profile to ensure precise localization and minimize complications.

The results of this study suggest that letibotulinum toxin A offers a favorable diffusion profile with a relatively low risk of excessive spread, making it suitable for precise facial esthetic procedures. Additionally, no cases of excessive muscle weakness, upper eyelid ptosis, or other adverse events that disrupt daily life were reported, further supporting its safety profile. Proper reconstitution protocols are crucial for maintaining BoNT-A efficacy and safety (6). Per manufacturer guidelines, letibotulinum toxin A should be reconstituted with preservative-free 0.9% sodium chloride solution under aseptic conditions to preserve stability and prevent denaturation, ensuring optimal therapeutic outcomes (21).

Our findings align with the current understanding of BoNT-A pharmacodynamics, which is increasingly informed by both molecular research and computational modeling. While it was once theorized that the molecular weight of the toxin-complex dictates diffusion, the current literature has clarified that the core 150 kDa neurotoxin rapidly dissociates from its accessory proteins at physiological pH (22). This suggests that the toxin's field of effect is primarily determined by injection volume and dose, not the size of the original complex (22). Another factor influencing the toxin's diffusion is its receptor affinity. This was complemented by in silico research, such as the model by Rahman et al. which predicted that letibotulinum toxin A would exhibit tighter, more localized diffusion due to stronger receptor engagement (10). These aforementioned factors affecting the diffusion profile are consistent with our clinical observations, where letibotulinum toxin A produced a numerically smaller area of anhidrosis. Together, the theoretical and clinical evidence suggest a mechanism that could be highly advantageous for esthetic applications requiring precision.

The findings of this study also align with previous research demonstrating that diffusion characteristics of BoNT-A are not uniform across formulations and can substantially influence clinical outcomes. Studies report varying diffusion halos between products like onabotulinum toxin A and abobotulinum toxin A or other derivatives, with the final field of effect often depending on the dose and injection techniques used in the comparison (11–13). More recently, a randomized clinical trial found that letibotulinum toxin A exhibited a smaller anhidrotic area—indicating more localized diffusion—than both onabotulinum toxin A and abobotulinum toxin A (23). Regarding prabotulinum toxin A, it demonstrated a similarly constrained diffusion profile to onabotulinum toxin A in one in silico study (24). Taken together, these studies reinforce that diffusion is influenced not only by the inherent properties of each BoNT-A formulation but also by dose, dilution, and injection technique. Our trial demonstrated that letibotulinum toxin A achieves a balance of efficacy and controlled diffusion, with results comparable to prabotulinum toxin A and onabotulinum toxin A, and with evidence of proper spread on forehead wrinkles. This supports its role as a clinically effective and safe alternative for forehead wrinkle treatment while potentially minimizing diffusion-related complications.

Despite these promising findings, this study had certain limitations. One key weakness lies in the statistical outcomes, as many of the analyses showed insignificance, suggesting no considerable differences between letibotulinum toxin A and the comparator agents. Additionally, the relatively small sample size and homogeneous demographic profile may restrict the generalizability of the findings. The study's design is also limited by its evaluation at a single two-week timepoint. This was chosen to assess peak toxin efficacy and diffusion, but it does not allow for an analysis of the duration of effect or potential late-onset adverse events; therefore, future studies should incorporate longer-term follow-up. Methodologically, while we used objective 3D imaging systems to assess wrinkle reduction, the study lacked direct neuromuscular function testing, such as electromyography, to quantify the physiological degree of muscle paralysis. Furthermore, our primary method for assessing diffusion, the Minor's iodine-starch test, has recognized constraints. While it is a practical and widely used tool for evaluating botulinum toxin diffusion, its reliability is highly sensitive to external variables such as ambient temperature and humidity, as well as inter-individual physiological differences in sweating. To mitigate these potential confounders, all of our assessments were performed within a strictly controlled environmental chamber, a crucial step for standardizing sudomotor function tests. However, incorporating more advanced techniques—such as the quantitative sudomotor axon reflex test, silicone impressions, or sympathetic skin response measurements, would provide a more comprehensive evaluation of diffusion dynamics (25,26). Finally, although potential evaluator bias was minimized by blinding the single analyst to treatment allocation, this design did not allow for an assessment of inter-rater reliability. Nonetheless, a notable strength of the study is the integration of diverse and

objective assessment methods. The use of both the iodine-starch test and advanced imaging tools like Antera 3D and Morpheus 3D to quantify wrinkle depth, width, and indentation significantly enhanced the analytical rigor and validity of the results. This multi-modal evaluation offers a robust framework for assessing BoNT-A efficacy and diffusion, supporting the clinical relevance of the findings. Future studies involving larger and more diverse populations are necessary to confirm these findings and further optimize clinical protocols in facial esthetics.

In conclusion, the findings from this study suggest that letibotulinum toxin A exhibits a diffusion profile and safety record comparable to established BoNT-A products for treating facial wrinkles. While the primary endpoint did not reach statistical significance, consistently more localized diffusion was observed for letibotulinum toxin A, alongside similar wrinkle-reduction efficacy when compared to prabotulinum toxin A and onabotulinum toxin A. These preliminary results indicate that letibotulinum toxin A may be a viable alternative in esthetic applications where precise toxin delivery is desired, potentially minimizing risks associated with excessive diffusion. However, given the small sample size, short follow-up duration, and lack of statistically significant findings, these conclusions should be considered exploratory. To validate these outcomes and better understand the clinical utility of letibotulinum toxin A, further research is essential. Future investigations should include large-scale, multicenter, randomized controlled trials involving more diverse patient populations. Additionally, incorporating longer-term follow-up assessments and more robust outcome metrics beyond the iodine-starch test would provide a more comprehensive evaluation of its efficacy and safety in broader clinical practice.

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## Ethical approval

This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine (Institutional Review Board number 4-2021-1097)

## Author contributions

Conceptualization, Y.I.L, N.H.C., and Jemin.K.; methodology, Y.I.L, N.H.C., Jemin.K.; software, Y.I.L, N.H.C., Jemin.K., Y.B., N.H.N., and Jihee.K.; validation, X.X., Y.Y. and Z.Z.Y.I.L, N.H.C., Jemin.K., Y.B., N.H.N., and Jihee.K.; formal analysis, Y.I.L, N.H.C., Jemin.K., Y.B., N.H.N., and Jihee.K.; investigation, Y.I.L, N.H.C., Jemin.K., Y.B., and N.H.N.; resources, Jemin.K. and Jihee.K.; data curation, Jemin.K. and Jihee.K.; writing—original draft preparation, Y.I.L, N.H.C., Jemin.K., Y.B., and N.H.N.; writing—review and editing, Y.I.L, N.H.C., Jemin.K., Y.B., N.H.N., Jihee.K., and J.H.L.; visualization, Y.I.L, N.H.C., Jemin.K., Y.B., and N.H.N.; supervision, J.H.L.; project administration, J.H.L. All authors have read and agreed to the published version of the manuscript.

## Consent form

Informed consent was obtained from all subjects involved in the study.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

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