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Two different types of botulinum toxins: Is there a difference in efficacy and longevity?

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Abstract

Background: OnabotulinumtoxinA and incobotulinumtoxinA are two botulinum toxin A (BoNT-A) formulations commonly used in esthetic medicine. They are distinguished by whether complexing proteins are included with the active neurotoxin. While OnabotulinumtoxinA has complexing proteins, incobotulinumtoxinA does not; yet, it is unclear whether these differences affect their efficacy, longevity, and immunogenicity, especially in practices with high ambient temperatures.

Objectives: To assess the efficacy and longevity of unreconstituted incobotulinumtoxinA with unreconstituted OnabotulinumtoxinA when stored and transported in a cold box to areas with high external ambient temperatures and to understand the implications of storing and transporting botulinum toxin to tropical areas with high ambient temperatures.

Methods: A prospective, randomized, and evaluator-blinded split-face trial was conducted in 30 patients with symmetrical, moderate-to-severe forehead lines. Following routine transportation and storage in thermocol cold boxes, OnabotulinumtoxinA or incobotulinumtoxinA was injected into corresponding sides of the frontalis to facilitate analysis within the same patient. Using a 4-point facial wrinkling grading scale and a clinical improvement scale, patients' outcomes were assessed over 24 weeks.

Results: Forehead lines reappeared in OnabotulinumtoxinA-treated patients after 8.3 weeks, compared to 10.1 weeks in incobotulinumtoxinA-treated patients. While side-vs-side improvements in forehead lines were observed for both toxins, after 8 weeks, improvements from were diminished relative to incobotulinumtoxinA, indicating that incobotulinumtoxinA was more effective at prolonged wrinkle relief.

Conclusions: These results suggest that incobotulinumtoxinA is more stable at higher ambient temperatures, thus contributing to its better efficacy and longevity. IncobotulinumtoxinA is therefore more appropriate for practices in tropical climates.

KEYWORDS

botulinum toxin, efficacy, incobotulinumtoxinA, OnabotulinumtoxinA, stability

1 | INTRODUCTION

Botulinum toxin (BoNT) is used to treat various neurologic disorders and provides esthetic enhancements. Seven different serotypes of BoNT exist: types A,B, C, D, E, F, and G.[1] Each has a unique molecular structure and function and each produced from a different strain of the *Clostridium Botulinum* bacteria. Currently, three formulations of botulinumtoxinA (BoNT-A) are commonly used: (Botox® or Vistabel®, Allergan), incobotulinumtoxinA (Xeomin® or Bocouture®, Merz Pharmaceuticals), and abobotulinumtoxinA

FIGURE 1 Toxin storage cold box (A) thermocol nonconducting cold box (B) thermometer near vials in box (C) interior temperature

(Dysport®, Medicis or Azzalure® Ipsen).[2] Each formulation is purported to have unique benefits; however, it is unclear whether their structural and functional differences are clinically significant. Factors that distinguish each formulation include dose potency or equivalency, onset of action, duration of action, local diffusion, side effect profile, and differences in immunogenicity.[3] A major difference between these different formulations is the presence or absence of complexing proteins. Manufacturers typically produce BoNT as a 150-900 kDa protein that comprises both the primary active component (the 150 kDa polypeptide chain) and the complexing proteins. The 150 kDa protein is the neurotoxin and has low toxin activity; however, once cleaved into its 50 kDa (light chain) and 100 kDa (heavy chain) constituents, the toxin activity increases.[4] The complexing proteins consist of hemagglutinin and smaller nonhemagglutinin proteins. Complexing proteins are sometimes referred to accessory proteins, protective proteins, or neurotoxin-associated proteins.[5] These are important in protecting the toxins in their natural environment (pH range of 5-7) but will dissociate at a physiologic pH of 6-8.

OnabotulinumtoxinA contains complexing proteins, whereas incobotulinumtoxinA does not.[6] The amount of neurotoxin product, along with complexing proteins and residual proteins, defines the foreign protein load.[7] The human immune system may recognize any part of this protein load as a foreign substance and trigger an immune reaction, especially after injection. Several studies, mostly in clinical literature, have suggested that a higher total protein content might increase the risk of antibody formation.[8] As a result, BoNT-A products have evolved correspondingly with a reduction in the total protein content. The current formulation of onabotulinumtoxinA contains only 5 ng of foreign bacterial protein per 100 units (U).[9] [Correction added on August 29, 2019, after first online publication: The sentence has been changed from "The current formulation of incobotulinumtoxinA contains only 5 ng of complexing protein per 100 units (U)." to "The current formulation of onabotulinumtoxinA contains only 5 ng of foreign bacterial protein per 100 units (U)."] Clinically, however, it is unclear whether these molecular differences have a significant impact on antigenicity and efficacy.[10]

Due to the large number of nonrandomized, nonblinded, industry-sponsored trials, clinicians have difficulties in determining whether a specific toxin product is more advantageous than another in terms of efficacy and safety.[11] The shelf life of nonreconstituted incobotulinumtoxinA is much longer at room temperature (3-4 years) than that of nonreconstituted onabotulinumtoxinA* (2-3 years at 2-8°C or in a freezer at −20°C).[12] IncobotulinumtoxinA maintains efficacy at higher ambient temperatures, as found in earlier studies and as described in the manufacturer's prescribing information[13] than onabotulinumtoxinA* . We therefore evaluated and compared the efficacy and longevity of unreconstituted incobotulinumtoxinA with unreconstituted onabotulinumtoxinA* when stored and transported in a thermocol (expanded polystyrene) cold box, to areas with high external ambient temperatures. We also sought to understand the ramifications of storing and transporting botulinum toxin to multiple clinical centers located in tropical areas with high ambient temperatures.

*[Correction added on August 29, 2019, after first online publication: The term "onabotulinumtoxinA" was included to make it a complete sentence.]

1.1 | Study design

A prospective, randomized evaluator-blinded split-face clinical trial was conducted. The study protocol was approved by The Esthetic Clinics institutional review board.

1.2 | Participants

Thirty follow-up patients, with symmetrical moderate-to-severe forehead lines at maximal frown, were enrolled. The two groups were age- and gender-matched to avoid any confounding variables. Carruthers' Forehead Lines Grading Scale was used to evaluate the lines.[14] Informed consent was obtained from each participant.

1.3 | Study duration

Eight months, between May 2017 and January 2018.

1.4 | Inclusion criteria

Patients with symmetrical moderate-to-severe forehead lines during frowning and follow-up patients, who had previously received injections for forehead lines, were included.

1.5 | Exclusion criteria

Patients with substantial forehead line asymmetry, baseline frontalis muscle atrophy, ptosis, any sign of underlying/latent ptosis, those who were pregnant or lactating, or had concomitant conditions such as myasthenia gravis or muscular dystrophy were excluded.

1.6 | Methodology

Unreconstituted vials of onabotulinumtoxinA* and incobotulinumtoxinA were kept underneath ice packs which had been frozen for 12 hours and in a cold box composed of thermocol nonconducting material. Using a platinum-based digital thermometer, temperatures were checked and recorded at the start and at 2-hour intervals. The cold box was maintained below 8°C. After 24 hours of storage in the cold box, onabotulinumtoxinA* and incobotulinumtoxinA were reconstituted in 2 ml of normal saline to yield 5U/100 µL of reconstituted solution (Figure 1).

Patients were randomized by a blinded observer using a random number enumerator. Each patient was injected with either 25U of onabotulinumtoxinA* or 25U of incobotulinumtoxinA into corresponding parts of the frontalis muscle in only half of the forehead (Figure 2).

1.7 | Assessment

Standard global photographs of the forehead were taken at baseline (preinjection) and at 2, 4, 6, and 8 weeks postinjection, and weekly thereafter. Subjective and objective assessments were performed. Investigators' assessments were performed by four surgeons and dermatologists blinded to the treatment group and timing of the photographs. These individuals used the 4-point facial winkling grading (FWG) and clinical improvement scale (CIS). The 4-point FWG scales were as follows: 0 = no wrinkling, 1 = mild wrinkling, 2 = moderate wrinkling, and 3 = severe wrinkling at full contraction of the frontalis, with averaged FWG values used for statistical analyses by paired *t* test (Table 1). A CIS for each patient was calculated by subtracting the FWG score at each follow-up visit from that at baseline (Table 2). Subjective evaluations were completed by each patient through a self-assessment questionnaire to gauge satisfaction at each follow-up visit; scores ranged from 0 (not satisfied at all) to 3 (very satisfied; Table 3). The CIS, FWG, and subject satisfaction scores were compared at each visit

2 | RESULTS

Out of the 30 patients enrolled for this study, 19 were male and 11 were female. The patients ranged in age from 21 to 61 years (38.6 ± 10.0 years). No patients withdrew due to adverse events. All patients were followed up for an average of 24 weeks, with long-term patients being evaluated from 12 weeks onwards.

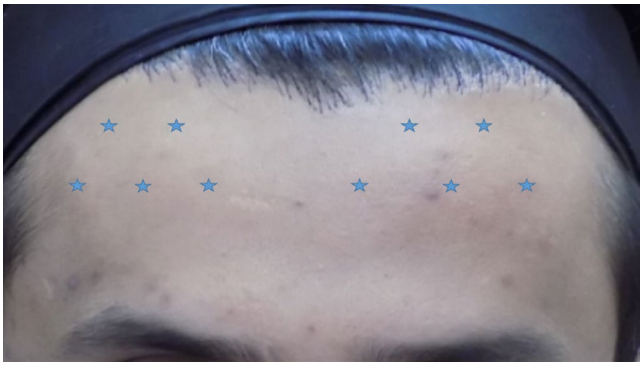

FIGURE 2 Toxin administration. Random points (marked by star symbols) were injected by a blinded physician

TABLE 1 Average facial wrinkling grading of vs incobotulinumtoxinA over time

Score	Facial Wrinkling Grade (FWG)
0	No wrinkles with expression
1	Mild facial wrinkling with expression
2	Moderate facial wrinkling with expression
3	Severe facial wrinkling with expression

The mean time of reappearance of forehead lines in sides injected with OnabotulinumtoxinA was 8.3 weeks (range 6-10 weeks) vs 10.1 weeks (range 8-12 weeks) with incobotulinumtoxinA (Table 4). The mean baseline FWG in patients was 2.6. This side-vs-side comparison of FWG and CIS was performed at every follow-up visit. Forehead lines improved from 2.6 at baseline to 0.9, 0.5, and 0.5 for both toxins at weeks 2, 4, and 6, respectively, and continued in all patients for at least 12 weeks (Figures 3 and 4). Notably, after the 8th week, sides treated with OnabotulinumtoxinA were less improved than sides treated with incobotulinumtoxinA and the former also had a higher average FWG. Paired *t* testing supported this observation and suggested that OnabotulinumtoxinA was less efficacious than IncobotulinumtoxinA for prolonged wrinkle relief, with more significant ($P < 0.0.05$) appearance of forehead lines following OnabotulinumtoxinA treatment. Sides of the face injected with IncobotulinumtoxinA showed a more consistent mean improvement in CIS than sides injected with OnabotulinumtoxinA, where the CIS scores decreased more drastically from week 8 onwards (Figure 5). The mean FWG also indicated that significantly more forehead lines reappeared on the sides injected with OnabotulinumtoxinA from 8 weeks onwards ($P < 0.05$; Figures 4 and 5). Conversely, paired *t* testing showed a significantly higher improvement in CIS in areas injected with IncobotulinumtoxinA than in areas injected with OnabotulinumtoxinA. Taken together, these results indicate a superior longevity of IncobotulinumtoxinA. Also, all patient assessment scores paralleled that of the investigators' (Table 5). However, two patients developed mild upper eyelid drooping in the sides injected with IncobotulinumtoxinA (Figure 6).

TABLE 2 Average clinical improvement of vs incobotulinumtoxinA over time

Clinical Index Severity	Difference of FWG
3 (Excellent, 50% Improvement)	Baseline FWG- post-treatment FWG > 1.5
2 (Good, 25%-50% Improvement)	Baseline FWG- post-treatment FWG < 1.5
1 (Fair, < 25% Improvement)	Baseline FWG- post-treatment FWG < 0.75
0 (Poor, no improvement)	Baseline FWG- post-treatment FWG < 0

Questions	Possible responses
Since the start of the study, I can see my forehead lines improving	Strongly agree/ Strongly disagree
Since the start of the study, how would you describe the improvement of your forehead lines?	Greatly increased/ No significant change
Since the start of the study, do you think the duration of reappearance of forehead lines has relatively increased from the previous BoNT injections	Yes/ No
Are the Lines lesser on one side of the forehead compared to the other, currently? If Yes, please specify which side.	Yes/ No

TABLE 3 Sample patient satisfaction self-assessment questionnaire

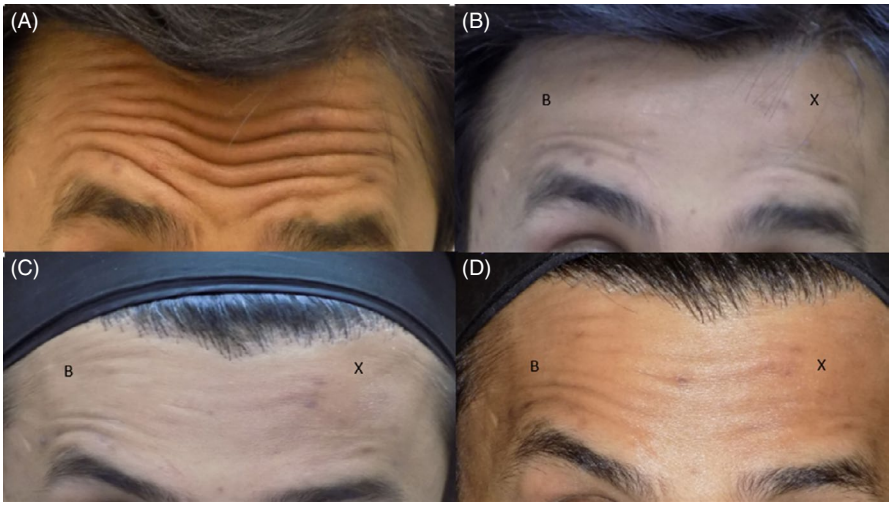

FIGURE 3 Representative patient results. Baseline (A), after 6 wk (B), 8 wk (C), and 10 wk (D) (X: IncobotulinumtoxinA; B: OnabotulinumtoxinA)

At long-term follow-up, IncobotulinumtoxinA-mediated improvements in the patient's left forehead had diminished and wrinkles were visible again after 16 weeks (Figure 7). [Correction added on August 29, 2019, after first online publication: The phrase "after 16 weeks" has been added at the end of previous sentence.] However overall, wrinkling was significantly improved, as shown in a patient's IncobotulinumtoxinA-treated right forehead compared to her OnabotulinumtoxinA-treated left forehead after 14 weeks (Figure 8). [Correction added on August 29, 2019, after first online publication: The phrase "after 14 weeks" has been added at the end of previous sentence.]

3 | DISCUSSION

This is the first clinical study reporting a relevant clinical comparison of efficacy and longevity of unreconstituted, unrefrigerated vials of OnabotulinumtoxinA and IncobotulinumtoxinA, stored in a cold box.

TABLE 4 Average time of forehead line reappearance

RANGE (reappearance of forehead lines)	MEAN (reappearance of forehead lines)
BOTOX: 6-10 wk	8.3 wk
XEOMIN: 8-12 wk	10.1 wk

The comparatively better longevity and efficacy of IncobotulinumtoxinA could be attributed to it being more stable than OnabotulinumtoxinA during storage at higher ambient temperatures.

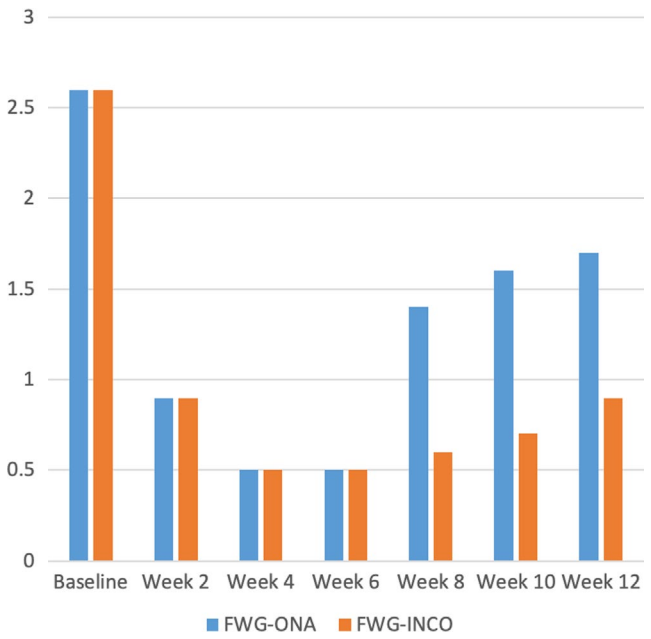

FIGURE 4 Average facial wrinkling grading (FWG) Following OnabotulinumtoxinA or IncobotulinumtoxinA treatment over 12 Wk (*=P < 0.005)

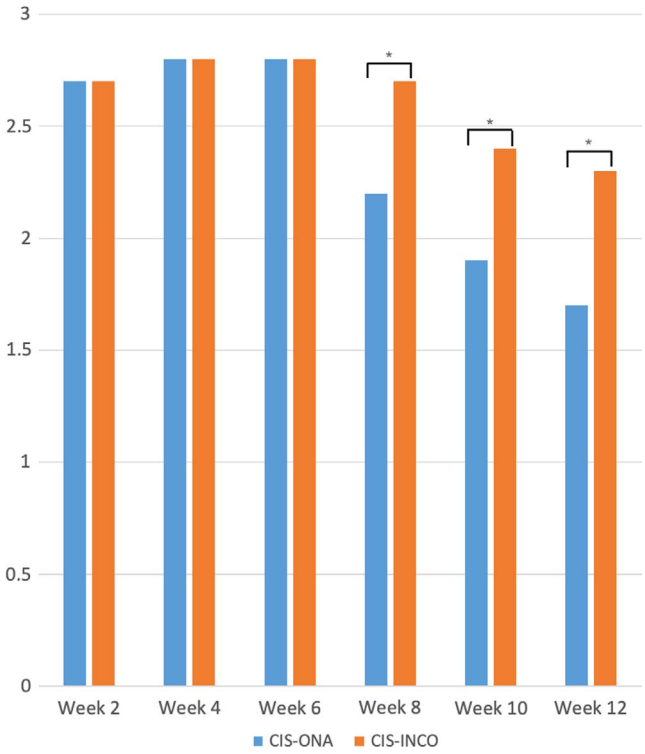

FIGURE 5 Average clinical improvement scaling (CIS) Following OnabotulinumtoxinA or IncobotulinumtoxinA treatment over 12 Wk (*=$P < 0.05$)

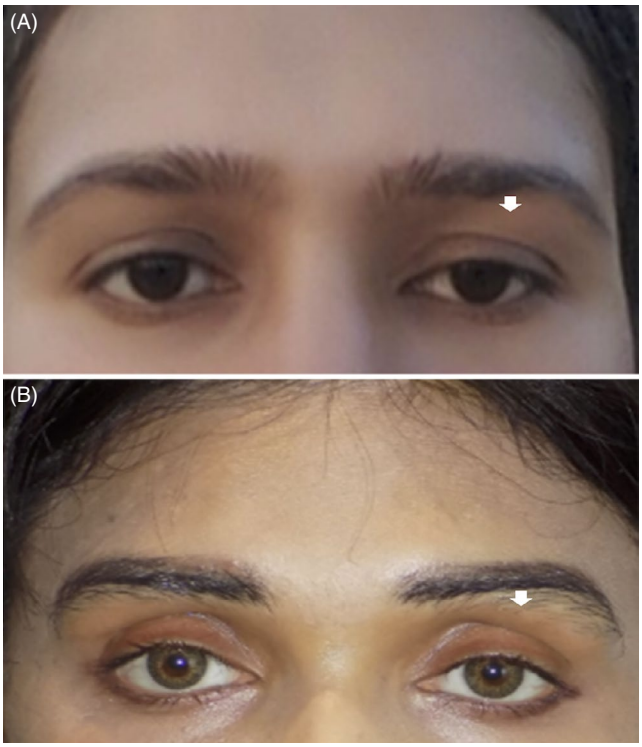

FIGURE 6 Mild Ptosis on the Left Side of the Eye After IncobotulinumtoxinA Injection Into the Frontalis

The temperature, which is stably maintained in a polystyrene (thermocol) box over a 24-hour period, is typically between 5 and 8°C, as assessed by us in this trial. It appears that these higher ambient temperatures of storage impact the longevity and the efficacy of OnabotulinumtoxinA more than IncobotulinumtoxinA.

It is also known that IncobotulinumtoxinA contains only the 150 kDa toxin purified from the fermentation of *C botulinum* and is free from complexing proteins (hemagglutinins and a nontoxic non-hemagglutinating protein).[15] It thus has a low foreign protein content[16]; any failure of secondary therapy may be attributable to the administered foreign protein.

Shome et al[17] studied the effect of vigorous agitation on reconstituted vials of botulinum toxin type A and demonstrated that OnabotulinumtoxinA is stable enough to retain its efficacy for up to 6 weeks after reconstitution. Garcia and Fulton were pioneers who observed that the clinical efficacy of diluted OnabotulinumtoxinA that was stored for 30 days was not impaired.[18] Hexsel and colleagues conducted a study with 85 patients using OnabotulinumtoxinA diluted and stored for up to 6 weeks. They showed a reduction in the motility of the glabellar area, with no loss of therapeutic efficacy.[19] Studies by Thomas and Parsa examined the refreezing of OnabotulinumtoxinA for later use and concluded that it could be reconstituted and refrozen for 8 weeks to 6 months without loss of therapeutic efficacy and safety.[20,21] All of these studies were conducted with reconstituted BoNT-A.

However, two patients developed mild ptosis: one at 9 days after IncobotulinumtoxinA injection in the frontalis and a second patient at 12 days postinjection. Both patients were managed well with apraclonidine eye drops. The cause of this mild ptosis remains unknown; however, we hypothesize that with its comparatively smaller molecular size and fewer aggregating proteins, IncobotulinumtoxinA may diffuse beyond the intended area of treatment more than OnabotulinumtoxinA and lead to ptosis. To facilitate positive patient outcomes, injectors should bear this in mind and adjust their chosen injection sites appropriately.

Confusion previously resulted as a consequence of comparing diffusion characteristics between different type A botulinum toxins.

TABLE 5 Comparison of Patient and Physician Assessment Scores

	BOTOX	XEOMIN
Helped in improvement of forehead lines	100%	100%
Duration of reappearance of forehead lines has increased comparatively from previous times	20%	80%
Are the lines lesser on one side of the forehead compared to the other, currently? If Yes, please specify which side.	0%	100%

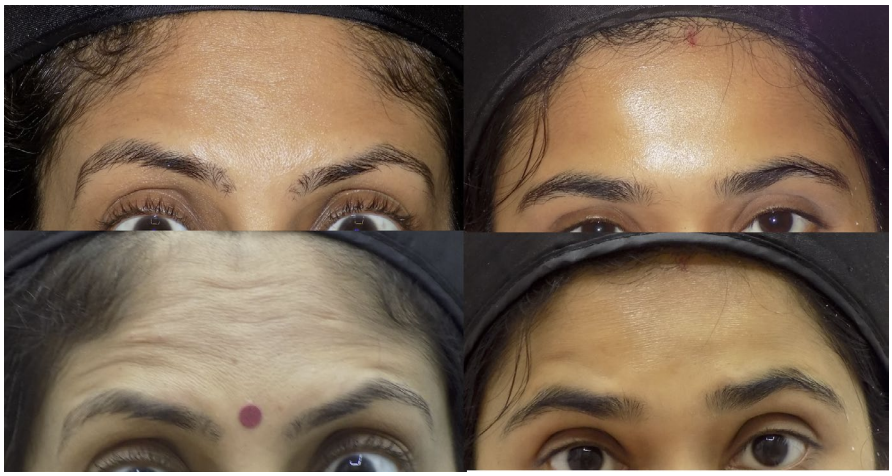

FIGURE 7 Recurrence of wrinkles. Upper Row: Patient at 8 wk post-treatment, dynamic (upper left) and at rest (upper right). Lower Row: Patient at 16 wk post-treatment, dynamic (lower left) and at rest (lower right)

It was hypothesized that because of the larger size of the toxin compound containing the complexing proteins, toxin diffusion from the injection site (and its resulting adverse events) may be minimized.[22] It was thought that the smaller IncobotulinumtoxinA might more easily diffuse away from target tissues into adjacent tissues to produce an adverse event profile different from other BoNT-A products.[22]

Clinical studies do not support this hypothesis; Dodd et al[23] showed that there was no difference in diffusion from the injection site among the three preparations. Furthermore, Tang-Liu et al[24] showed no difference in the diffusion of the free or complexed form of BoNT-A after injection into muscle, even at high doses. While it is conceivable that complexing proteins are involved in stabilizing the botulinum toxin and in restricting its diffusion from the injection site (thereby minimizing adverse events), comparisons of the complexing protein-free IncobotulinumtoxinA product with conventional type A botulinum toxins suggest that this is not the case. It was further proved in a mouse study where the diffusion of different type A botulinum toxins was investigated using a high-sensitivity test called as NCAM for assessing diffusion in the muscle. Injection of OnabotulinumtoxinA and IncobotulinumtoxinA (in a 1:1 ratio) led to a limited diffusion of type A botulinum toxins into adjacent muscles, with no significant differences between the formulations.[25]

This study was limited by low patient numbers. However, a subsequent clinical trial in a larger number of patients is planned. In future, a randomized controlled trial should be carried out to compare the efficacy of reconstituted OnabotulinumtoxinA against reconstituted IncobotulinumtoxinA and abobotulinumtoxinA preparations.

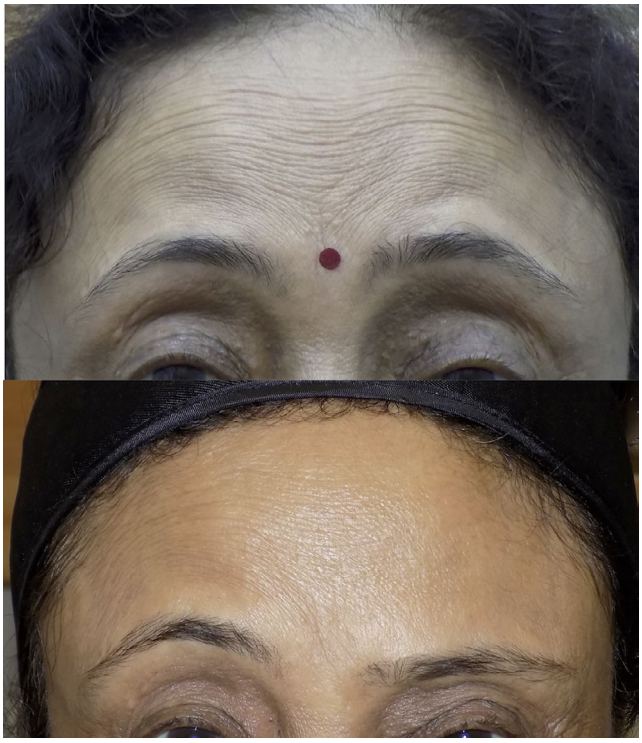

FIGURE 8 Improvements in facial wrinkling at 14-Wk long-term follow-up. (Top) Pretreatment. (Bottom) 14 Wk post-treatment

4 | CONCLUSION

We have detailed our experience in comparing the longevity and efficacy of two types of BoNT-A, namely onabotulinumtoxin A and incobotulinumtoxin A. We found the results of IncobotulinumtoxinA to be longer-lasting than OnabotulinumtoxinA when these were injected after 24 hours of storage in a cold box. These results confirm the usefulness of incobotulinumtoxin A over onabotulinumtoxin A in tropical countries and in clinics with multiple setups, where transportation of BoNT-A is essential and cold boxes are frequently used. IncobotulinumtoxinA may be a better clinical option for successful esthetic outcomes due to its stability at higher temperatures. We consider this finding to be important, and it adds to the quantum of knowledge elucidated in the global consensus recommendations for the use of Botulinum toxin A.[26]

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