Efficacy of Botulinum Toxin in Treating Asian Indian Patients with Masseter Hypertrophy: A 4-Year Follow-Up Study

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Background: Asian Indians usually have wide lower faces because of masseter hypertrophy. The authors evaluated the efficacy of botulinum toxin type A in long-term management of bilateral masseter hypertrophy in Asian Indian patients.

Methods: Fifty patients were enrolled in the study and injected with 30 U of botulinum toxin type A to each side of the face, at baseline. Based on masseter muscle thickness and response to the injections, 25 patients underwent a second injection session at week 12, and the other 25 patients underwent additional third sessions, at week 24 after the first injection, respectively. Standardized photography and ultrasonography were performed to assess facial contour and masseter muscle thickness at baseline and at 1-, 2-, 3-, and 4-year follow-ups. A p value < 0.05 was considered statistically significant.

Results: The authors observed 12 percent ($p < 0.0001$) average masseter muscle size reduction at week 12. The maximum reduction (26.6 percent; $p < 0.0001$) was observed at week 24 for the patients who received two injections and maintained an average 24.43 percent ($p < 0.0001$) reduction until follow-up at year 4. Patients who received three injections exhibited very high reduction (42.52 percent; $p < 0.0001$) of masseter volume at week 36 and maintained an average 40.64 percent ($p < 0.0001$) reduced volume until year 4.

Conclusions: Botulinum toxin type A treatment is effective for long-term management of bilateral masseter hypertrophy. Doses repeated at 12-week intervals accentuate masseter volume reduction and also help maintain reduced masseter volume for 4-year follow-up, with satisfactory facial contour. (*Plast. Reconstr. Surg.* 144: 390e, 2019.)

Perception of facial beauty is influenced by culture. Asians have a wider lower third of the face compared with Caucasians, which is aesthetically less acceptable to women,[1] who prefer an oval or almond-shaped face as the epitome of beauty, compared with a round or a square jaw.[2] The contour of the lower face is determined by the thickness of the mandibular bone, the soft tissues, and the masseter muscle. A square face (wide lower third of the face) appearance is commonly caused by a prominent mandibular angle or muscle enlargement (i.e., masseter hypertrophy).[1] A square-shaped face, caused by symmetric or asymmetric increase in the masseter muscle (masseter hypertrophy), is most common in Asians aged between 20 and 40 years.[3,4] The cause of masseter hypertrophy is

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still unknown, although several causes are postulated.[3] To achieve a more aesthetically pleasing ovoid facial shape, Asian patients frequently choose aesthetic alteration through surgical resection of the mandibular angle or the masseter muscle.[5] Although aesthetic surgery provides success in reshaping of the lower face, many patients prefer effective minimally invasive alternative therapy.[5]

OnabotulinumtoxinA (Botox; Allergan, Inc., Irvine, Calif.), or botulinum toxin type A, was first approved by the U.S. Food and Drug Administration for treatment of blepharospasm and later approved for treatment of cervical dystonia, strabismus, pain syndrome, severe primary axillary hyperhidrosis, and muscle spasm. Botulinum toxin type A also received U.S. Food and Drug Administration approval for upper face rejuvenation (glabella frown lines and crow's feet lines).[6,7] Injection of botulinum toxin type A into the masseter muscle is now extensively used as an off-label alternative noninvasive treatment for masseteric hypertrophy.[2] Various studies demonstrate that use of botulinum toxin type A treatment provides a long-term effect on cosmetic reduction of masseter muscle volume, in a dose-dependent manner, to narrow the width of the lower face in various ethnicities.[1,2,4,8,9]

The effect of botulinum toxin type A is temporary, and repeated injections are required to maintain the reduced masseter volume.[9] A number of studies have been conducted for standardization of botulinum toxin type A dose in effective treatment of masseter hypertrophy, for lower facial remodeling, in various populations,[6,8,9] including the ongoing phase II clinical trial (NCT02010775) of botulinum toxin type A. Data suggest that injection of 30 IU of botulinum toxin type A per side significantly reduces gross masseter size.[7]

The width of the lower face in Asian Indians is less than in Southeast Asians but greater than in Caucasians. Therefore, masseter hypertrophy is also less common in Asian Indians than in other Asians.[10] Although the botulinum toxin type A treatment for masseter hypertrophy and facial remodeling is reported in various populations, limited studies have been conducted to evaluate the efficacy of masseter hypertrophy reduction in the Indian population with botulinum toxin type A injections. In a study by Sharad, 25 Indian patients with bilateral masseter hypertrophy were treated with botulinum toxin type A and followed-up for as long as 1 year. This study demonstrated that single injections of 25 to 30 U for women and 30 to 35 U for men are adequate to maintain cosmetically acceptable reduction of the masseter muscle size for 1 year.[11] In a single case study of a male patient, Bhattacharjee et al. observed that 25 U of botulinum toxin type A injection maintains appropriate facial contour until 24 months.[12] Therefore, botulinum toxin type A treatment can help to narrow the lower face of Asian Indians too, but the dose and its long-term efficacy need to be further evaluated. In this study, we assessed the effects of injection of 30 U of botulinum toxin type A in long-term management (4-years follow-up) of bilateral masseter hypertrophy in Indian patients.

PATIENTS AND METHODS

We conducted a prospective interventional trial of patients treated for lower face reshaping at The Esthetic Clinics from May of 2014 to May of 2018. Subjects younger than 18 years, pregnant and lactating women, patients with local trauma, mental stress, dental problems, bruxism, bony mandibular prominence, cow's milk protein allergy, myasthenia gravis, amyotrophic lateral sclerosis, history of receiving botulinum toxin type A treatment for lower face reshaping in the preceding 12 months, and patients of non-Indian origin were excluded from this study. Finally, a total of 50 (29 men and 21 women; mean age, 40.5 ± 19.5 years) bilateral masseter hypertrophy patients of Asian Indian origin wanting a smaller, more aesthetic lower face were enrolled in this study. Written consent was obtained from all the enrolled patients. Institutional review board approval was obtained for this trial.

Dilution and Dosage

One vial of onabotulinum toxin A (botulinum toxin type A), containing 100 U of lyophilized botulinum toxin type A, was reconstituted in 2 ml of nonpreserved normal saline, yielding a final concentration of 5 U/0.1 ml.

Botulinum Toxin Type A Injection

First, a safe area for the injections was established by delimiting a line between the mouth angle and the lower implantation of the ear, with patients strongly grinding their teeth. Anterior and posterior edges of the muscles were also outlined, with the ramus of the mandible being the lower border of the area. This is considered the safety zone, because there are no important anatomical structures under the mouth corner/

earlobe line. Using a 26-gauge 1-inch tuberculin syringe, a total 30 IU of botulinum toxin type A was injected on each side of face. Ten international units of botulinum toxin type A was injected at the center of the muscle and 10 IU each at the upper and the lower points, 1 cm away from the initial point, as shown.[8,9] [**See Figure, Supplemental Digital Content 1**, which shows the safe area (marked with black marking) for application of botulinum toxin type A injection, ***http://links.lww.com/PRS/D637***.]

Sessions

The injections were performed at baseline (session 1), week 12 (session 2), and week 24 (session 3), and the patients were followed up for 4 years. The number of injection sessions required for patients was determined based on the initial thickness of the masseter muscle.

Efficacy Measurements

Subjective and objective scales were used for measurement. Under objective assessment, the volume of the masseter muscle was measured at baseline; at 1, 3, and 6 months; and at 1, 2, 3, and 4 years after treatment, using B-scan ultrasonography (8 MHz), and the mean thickness reduction of masseter muscle for both sides of the face was calculated. Clinician assessment scores and patients' self-assessment scores were used under subjective assessment. Standardized photographic documentation was obtained at baseline; at 1, 3, 6, 12 months; and at every yearly follow-up visit thereafter until year 4, using identical camera settings, lighting, and patient positioning. Independent clinical assessments of masseter reduction were evaluated by three blinded plastic surgeons, by comparing the photographs. [**See Figure, Supplemental Digital Content 2**, which shows the change of facial contour after botulinum toxin type A injection. (*Above, left*) Baseline photograph of a 29-year-old woman. (*Above, second from left*) Twelve weeks after the first session. (*Above, second from right*) At 12 weeks after the second session. (*Above, right*) Twenty-four weeks, after the third session. (*Second row, left*) Baseline photographs of a 28-year-old man. (*Second row, second from left*) Twelve weeks after the first session. (*Second row, second from right*) Twelve weeks after the second session. (*Second row, right*) Four years after the second session. Note the regain in bulk of muscle. (*Third row, left*) Baseline photograph of a 21-year-old woman. (*Third row, second from left*) Twelve weeks after the first session. (*Third row, second from right*) Twelve weeks after the second session. (*Third row, right*) Four years after the third session. Note the sustained effect of botulinum toxin type A in maintaining the reduction of muscle after three injection sessions. (*Below, left*) Baseline photograph of a 28-year-old woman. (*Below, second from left*) Twelve weeks after the first session. (*Below, second from right*) Twelve weeks after the second session. (*Below, right*) Four years after the second session. Note the regain in muscle volume after two sessions of botulinum toxin type A injection, ***http://links.lww.com/PRS/D638***.] For patients' self-assessment scores, questionnaires for the assessment of pain, adverse effects, and satisfaction were used on a scale of 0 to 5, as shown in Tables 1 and 2.

Table 1. Patient Satisfaction Assessment Scores

Score	Improvement
0	None or worsening; no improvement
1	If improvement present, then how much is the improvement in percentage from 1–20%?
2	21–40%
3	41–60%
4	61–80%
5	81–100%

Statistical Analysis

For each patient group, mean thickness of masseter muscle and percentages of masseter reduction were calculated using descriptive statistics. The mean differences of masseter muscle volume reduction between each follow-up visit within one group or between two groups were calculated using paired-samples t test. All statistical analyses were performed using IBM SPSS Version 24.0 (IBM Corp., Armonk, N.Y.). A value of $p < 0.05$ was considered statistically significant.

RESULTS

We observed that both of our patient groups showed an average 8.4 percent ($p < 0.0001$) and 12 percent ($p < 0.0001$) masseter muscle reduction from the baseline, at weeks 4 and 12, respectively. Although we observed a 26.6 percent ($p < 0.0001$) masseter muscle reduction from baseline at week 24 for the group of patients who received the second injection at week 12, it was 19.81 percent ($p < 0.0001$) for the other patient group.

However, at week 35, in the group of patients who received the third dose of injection at week 24, we observed a dramatic reduction (42.52 percent; $p < 0.0001$) of masseter volume (Table 3 and Fig. 1). The maximum effect of botulinum toxin

Table 2. Pain Assessment Scores

Score	Pain Level
0	No pain
1	Very mild pain
2	Mild pain
3	Moderate pain
4	Severe pain
5	Very severe pain

Table 3. Average Percentage of Masseter Volume Reduction in Two Groups of Patients at Various Follow-Up Times

Follow-Up Duration	Mean % of Masseter Muscle Volume Reduction ± SD	*p*
Two sessions		
Wk 0		
Wk 4		
Wk 12		
Wk 24		
Wk 36	14.472 ± 11.2847	<0.0001*
Yr 1		
Yr 2		
Yr 3		
Yr 4	17.745 ± 2.9093	>0.0001
Three sessions		
Wk 0		
Wk 4		
Wk 12		
Wk 24		
Wk 36		
Yr 1		
Yr 2		
Yr 3		
Yr 4	26.87333 ± 16.79286	<0.0001*

*$p < 0.0001$ is considered significant.

type A in reducing the masseter volume was 26.6 percent ($p < 0.0001$) at week 24 for the patients who received the second dose of botulinum toxin type A at week 12.

There was significant regain in volume of masseter muscles at 1, 2, 3, and 4 follow-up years for patients who underwent two sessions of botulinum toxin type A injections. However, patients who had undergone three sessions of botulinum toxin type A injections showed a statistically significant ($p < 0.0001$) sustained effect of botulinum toxin type A until year 4 from the baseline. Thus, three injections help in long-term management of bilateral masseter hypertrophy, compared with two injections (Table 3 and Figs. 1 and 2).

No significant side effects were noted after the injections. After injection, there was mild pain noted at the injection sites in 20 percent of patients, especially on mastication. This pain lasted for approximately 24 hours after injection. Headaches were noted in two patients after the first injection, but these patients did not note headaches after the subsequent injections.

DISCUSSION

The cause of masseter muscle hypertrophy is unknown. Painless enlargement of the angle of the jaw between the ages of 20 and 40 years without any gender specification is the most common presentation, but the literature also describes some patients with localized pain and trismus.[13]

Botulinum toxin type A produces its therapeutic effect by acting selectively on peripheral cholinergic motor nerve endings to inhibit the release of the neurotransmitter acetylcholine at the neuromuscular junction.[14] The effectiveness of botulinum toxin type A as an off-label drug in reducing lower facial masseter muscle has been proven by several studies,[1,2,4,8,9] and 20- to 50-U intramuscular injection of botulinum toxin type A is recommended, depending on the masseteric muscle thickness.[15]

However, the efficacy of botulinum toxin type A in reducing masseter muscle volume varies in different studies. Whereas Kim et al. observed a 22 percent reduction, Yu et al. reported 31 percent reduction in masseter volume after botulinum toxin type A treatment.[16,17] A very low percentage of reduction (11.9 percent) was observed by Klein et al. in the Brazilian population.[8] Similarly, data suggest that the effect of botulinum toxin type A is temporary and is diminished within 4 to 6 months after injection.[9] Therefore, repeated injections are required to maintain the reduced masseter volume. A detailed meta-analysis[18] was unable to identify any randomized clinical trials or controlled clinical trials assessing the efficacy and safety of intramasseteric injections of botulinum toxin for people with bilateral benign masseter hypertrophy. The authors emphasized the absence of high-level evidence for the effectiveness of this intervention and the need for well-designed, adequately powered trials to prove the efficacy.

These facts suggest that a standardized dose and duration of the effect of botulinum toxin type A in treatment of masseter hypertrophy is yet to be determined.[6,8,9] An ongoing phase II clinical trial (NCT02010775) is aimed at evaluating the safety and efficacy of a range of doses of botulinum toxin type A toward the dose standardization in treatment of masseter hypertrophy.

As repeated injections are required to maintain reduced masseter volume and lower facial symmetry, we performed the botulinum toxin

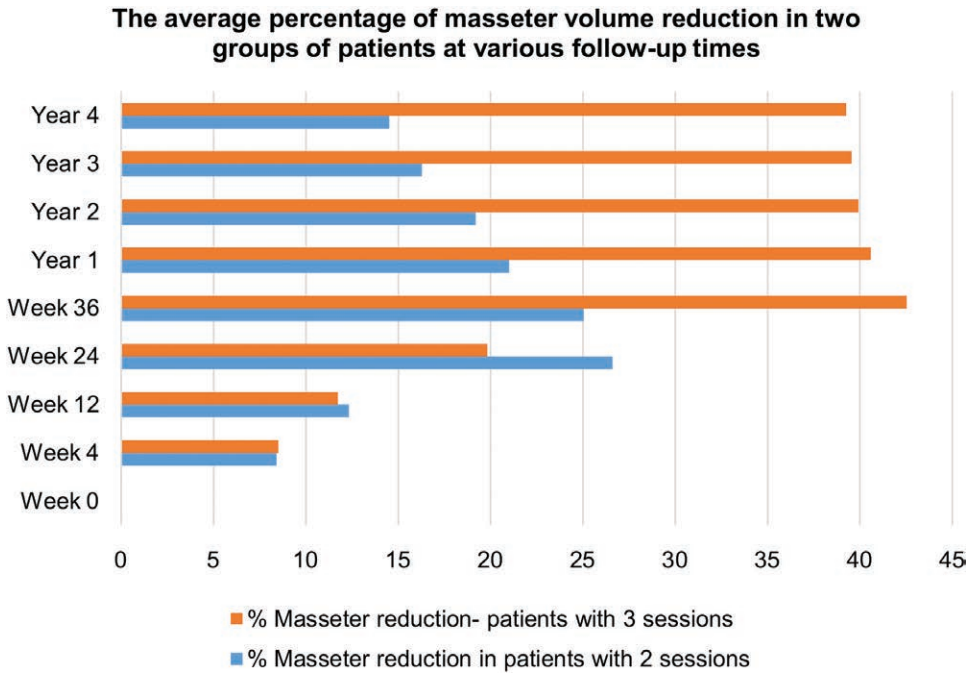

Fig. 1. Average percentage of masseter volume reduction in patients at various follow-up times.

type A injections at 12-week intervals, in two groups of patients: one group received two injections and the other received three injections. Similar to previous reports,[8] we observed a statistically significant ($p < 0.0001$) reduction of masseter muscle volume after 12 weeks after initial injection of 30 U of botulinum toxin type A per side. In our study, the observed reduction at week 12 was approximately 12 percent from the baseline, which is similar to the findings in Brazilian women who received 90 U of botulinum toxin type A.[8]

Yu et al. achieved a 31 percent reduction of masseter volume in Asian women at month 6 after injection of a single comparable dose of botulinum toxin type A; however, they reported a gain in muscle bulk after a 9-month period.[17] We achieved maximum reduction of masseter volume of 26.6 percent ($p < 0.0001$) at month 6 after the second injection (Table 3 and Fig. 1).

However, we achieved a 42.52 percent ($p \leq 0.0001$) reduction in masseter volume from the baseline at week 36 in patients who received the third dose of botulinum toxin type A at week 24. In contrast, patients who received the second dose of botulinum toxin type A at week 12 exhibited a 24.03 percent ($p < 0.0001$) reduction at week 36 (Table 3 and Fig. 2). These results suggest that a low but repeated dose of botulinum toxin type A can reduce masseter volume more effectively than a single dose, as reported by Lee et al.[19]

In an Indian study, a 35-year-old male patient exhibited a 22 to 29 percent reduction of masseter thickness and maintains a satisfactory facial contour at 24 weeks after a single botulinum toxin type A injection of 25 IU.[12] However, the sustained effect after 24 months was not reported in this case. Kim et al.,[16] who followed up patients until 52 months, reported an increased frequency of repeated injections sustained long-term botulinum toxin type A effect.

We followed our patients until 4 years and, similar to the observation of Kim et al., found that the patients who received three injections maintain better mean reduction (40.64 percent; $p < 0.0001$) of masseter volume, compared with patients who received two injections (24.43 percent; $p < 0.0001$) until year 4. The difference of the reduction between these two groups is also highly significant ($p < 0.0001$) (Fig. 2), indicating that botulinum toxin type A can effectively be used for long-term management of bilateral masseter hypertrophy in Asian Indian patients, where three injections is a better, more efficacious approach than a single dose.

How did the effect of botulinum toxin type A for masseter hypertrophy last so long? The answer to this may be that the mechanism of action of botulinum toxin type A in masseter hypertrophy may be different from the mechanism of action of botulinum toxin type A for hyperkinetic lines and wrinkle treatment. Botulinum toxin type A,

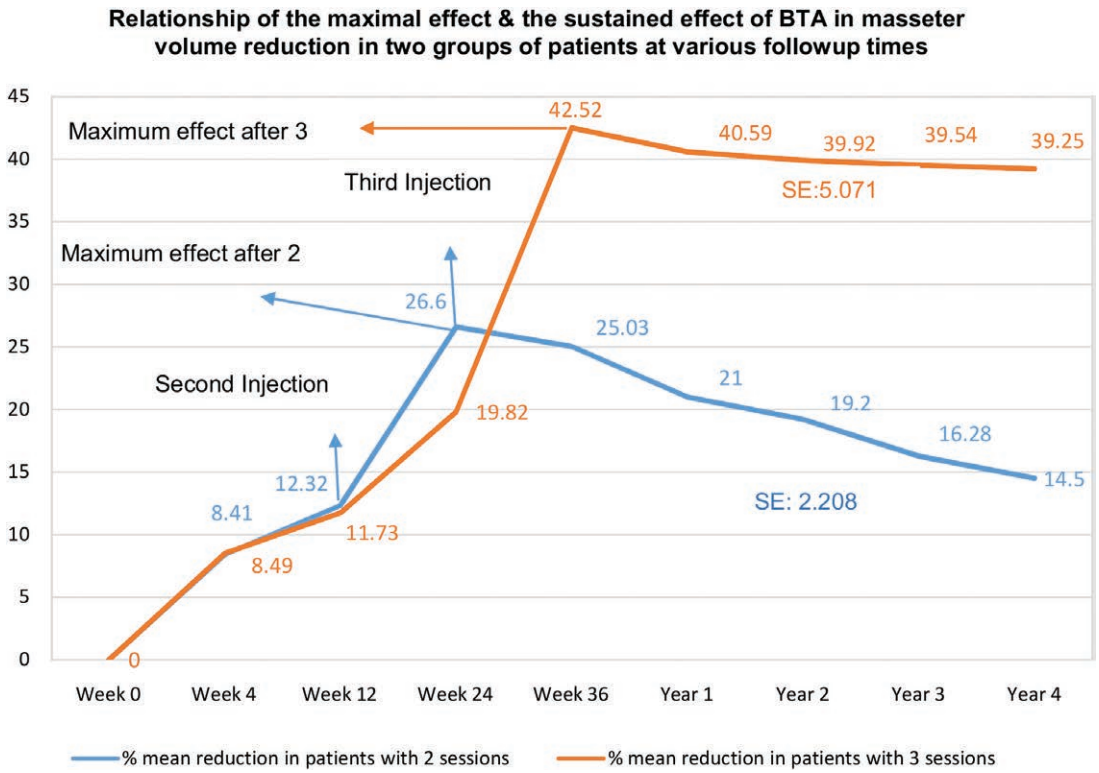

Fig. 2. Relationship of the maximal effect and the sustained effect of botulinum toxin type A in masseter volume reduction in two groups of patients.

when injected in high concentrations, may cause cell apoptosis, leading to atrophy of the masseter muscle. Repeated injections may prevent the muscle fibers from regenerating and therefore the muscle atrophy may be semipermanent or even permanent. This has been proven in multiple animal studies.[20,21] It has also been shown that human jaw muscles are very different from other skeletal muscles and that, after atrophy, the regeneration of these muscles may be limited.[22] The combination of cell apoptosis, occurring in a muscle that has limited regenerative capacities, makes botulinum toxin type A for masseter hypertrophy work very well, without any impact on chewing or mastication activities.

CONCLUSIONS

Botulinum toxin A is equally effective in treating bilateral masseter hypertrophy of Indian patients irrespective of age or sex. Our long-term follow-up analysis of 4 years demonstrates that standard dosages, but administered as three injections at 12-week intervals, gives better reduction of mean masseter volume compared with two injections. The repeated injection is also found highly beneficial in long-term management of bilateral masseter hypertrophy, with maintenance of desired facial contour and patient satisfaction. No significant side effects were noted following the botulinum toxin type A injections. This may be attributable to our injection technique, deep and into the mass of the masseter muscle. To the best of our knowledge, this is the first clinical study evaluating and analyzing the long-term effects of botulinum toxin type A injections for masseter hypertrophy.

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PATIENT CONSENT

Patients provided written consent for the use of their images.

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