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ORIGINAL ARTICLE



Prospective study of the aesthetic effect of botulinum toxin A when injected into the muscles of facial expression

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ABSTRACT

Botulinum neurotoxin A (BoNT-A) causes an anticholinergic effect on neuronal fibers, which control muscle contraction and autonomic disorders. Thus, it has been widely used in facial aesthetics, decreasing the action of motor muscles and consequent wrinkles. This preliminary study evaluated the effect of BoNT-A in 77 patients, the treatment satisfaction index was defined in percentage (from 0% to 100%). The evaluation was carried out on 15th, 30th, 60th, 90th, and 180th days after BoNT. The data were analyzed using the Friedman, Student t, Mann-Whitney test with t (alpha=0.05). The results showed that at 15th and 30th days the scores were similar in all muscles with high level of satisfaction and until 90th days the scores decreased significantly for *Corrugator supercilii* 79.38%, *Occipitofrontalis* 71.46%, *Orbicularis oculi* 70.43%; but the satisfaction was good. At 180 days, there was a drop in effectiveness in all treated muscles since the scores decreased significantly, showing low satisfaction by the participants. This study demonstrated that the BoNT-A had attested satisfaction effect by participants for up to 90th days, but at 180th days the satisfaction was low. In the comparative analyzes between women under 40 and over 40 years of age, there was no significant differences.

KEY WORDS

Botulinum toxin; Muscles of expression; Facial aesthetic; Gummy smile.

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INTRODUCTION

Botulinum neurotoxin A (BoNT-A) is a product derived from the anaerobic bacterium *Clostridium botulinum*⁽¹⁾. At low concentrations, BoNT-A enters the nerve endings, cleaving and inactivating the synaptosomal associated proteins 25 (SNAP-25) that are essential for the acetylcholine release, a neurotransmitter that acts on muscle contraction^(2,3). By temporarily reducing muscular contraction, BoNT-A prevents the formation of lines and rhytids. It also decreases the hyperactive muscle behavior. This aspect composes BoNT-A as a good option for facial aesthetic rejuvenation. BoNT-A is currently the most used material to reduce hyperkinetic facial lines, with techniques that injects this toxin into the muscles of facial expression⁽⁴⁾.

The duration of the BoNT-A effect is one of the main determinants of efficacy in an aesthetic treatment. BTX-A duration and efficacy depends on several factors, most of them can be controlled by the professional in order to obtain better results. Factors such as dosage, patient's metabolism, anatomical characteristics of the treated muscle, point and angle of injection, dilution techniques, and injection protocols may affect the duration of the BoNT-A effect⁽⁵⁾. Although the molecular mechanism underlying the duration of BoNT-A remains unknown, the toxin remains in the nerve terminal for months⁽⁶⁾ and this aspect may determine the duration of its effect⁽⁷⁾.

The clinical effects of BoNT-A can last for 30 days up to 6 months⁽⁸⁾. However, data available in the literature regarding the efficacy and duration of BoNT-A effects on the muscles of facial expression are still controversial, and they do not address experimental criteria that define its usage protocol, especially regarding to the patient's satisfaction as a function of the duration of action⁽⁹⁾. Thus, this study estimated the patient satisfaction index in relation to the aesthetic effect of BoNT-A as a function of its duration of action. Furthermore, the possible influence of age was also considered.

MATERIALS AND METHODS

This preliminary prospective study was conducted in accordance with the recommendations of the International Council for Harmonisation and the principles of the Helsinki Declaration, under the approval by the Research Ethics Committee of FOP/UNICAMP #60121116.1.0000.5418. All subjects that agreed to participate in the study signed an informed consent form. This study consisted of a prospective longitudinal clinical study conducted at the Clinic of Specialization Courses in the Faculdade de Odontologia de Piracicaba – FOP, in 2016 and 2017.

Inclusion criteria were female patients, aged from 18 to 72 years (mean age 45±12.6 years), presenting hyperkinetic facial lines from moderate to severe indicated for correction by BoNT-A injection and/or gummy smile

with muscular origin, without any systemic alteration or contraindication to the use of botulinum toxin. Pathologies that compromised the stomatognathic system, trigeminal neuralgia, signs of ptosis, pathological decrease in muscle activity, facial surgeries, aesthetic treatments in the past 12 months with botulinum neurotoxin type A, patients who received plastic or orthognathic surgery were considered as exclusion criteria. The final sample was composed of 77 patients.

The BoNT-A used was Botox® 100U (Allergan do Brasil, Guarulhos, São Paulo, Brazil), reconstituted in 2 mL of saline solution at 0.9%⁽²⁾. The injection procedure followed the manufacturer's instructions presented in the package insert. The injections were performed by a professional duly trained in these procedures. Facial regions, dosages, and injection techniques adopted were in agreement with the literature, as presented in Table 1.

Patient follow-up was carried out at 15, 30, 60, 90, and 180 days after BoNT-A injection. At each follow-up meeting, patients reported their self-perception to the outcome of the treatment. To measure the rate of satisfaction with the treatment in this period, patients issued their answers using a verbal analog scale measured in percentage numbers ranging from 0% to 100%, with 0% representing the worst possible result, and 100%, the best possible result.

The questions were formulated by the same rater who sought to inquire aspects related to the presence of wrinkles, quality of the skin regarding elasticity, softness, brightness, and firmness. At the end, patients indicated a percentage value for their satisfaction index. These parameters were observed equally throughout the evaluation period, considering the facial regions treated.

After data collection, the information was compiled, inserted in tables, and submitted to statistical analysis. The analysis was performed by comparing linked sample data, that is, when the same individual is evaluated more than once. First, the data from the total sample were compared between the evaluation days. In addition, two age groups were formed to verify for possible differences due to physiological reasons, the first consisting of women under 40 years of age and the other of women over 40 years of age. Moreover, the scores were correlated with age.

The Sigmaplot 14.0 software (Systat Software Inc., San Jose, CA, USA) was used for all statistical analysis. A 0.05 alpha significance level was considered. Friedman test for comparisons between evaluation days was applied, as well as T-test and Mann-Whitney test for comparisons between groups. Moreover, the correlation between age and patients' satisfaction scores was calculated using Spearman coefficient.

RESULTS

According to the data analyses, significant differences occurred between patients' satisfaction scores during the follow-up periods

($p < 0.0001$), considering each facial region treated. From 15 to 30 days post-operation, patients provided very good percentages of treatment satisfaction for all facial regions, with values above 90%. However, the effect decreased significantly at 60 days for the treatments performed in the upper third of the *Corrugator supercilii*, Procerus, Orbicularis oculi, and *Occipitofrontalis* muscles, although satisfaction scores were still high. At that time, values of *Occipitofrontalis*, Levator labii superioris, and Levator labii superioris alaeque nasi muscles were similar to 60 days. In the period of 90 days, there was a significant decrease in the scores of *Corrugator supercilii*, *Occipitofrontalis* and Orbicularis oculi in relation to 60 days, but the values showed a good level of satisfaction. Moreover, at 90 days the scores for Procerus, Levator labii superioris, and Levator labii superioris alaeque nasi muscles remained similar to the scores at 60 days, also demonstrating good satisfaction of the participants. At 180 days, the scores decreased significantly in relation to other evaluation days, demonstrating low satisfaction in relation to the clinical effects of BoNT-A for all muscles. The satisfaction scores are described in table 2.

On comparing each evaluation day between the two age groups (Table 3), no statistical significant differences were found ($p > 0.05$). However, significant negative correlations were observed between age and patient satisfaction on day 180 in *Corrugator supercilii* ($rs = -0.298$, $p = 0.040$) and age and patient satisfaction on day 60 in *Occipitofrontalis* ($rs = -0.287$, $p = 0.048$), meaning that the younger the age, the greater the satisfaction with the treatment in respective days and muscles. The correlations between other days and muscles were not significant.

(TABLE 3)

DISCUSSION

The results of this study indicate that BoNT-A is a good option for aesthetic facial treatments, including gummy smile with high patients' satisfaction until 90 days after application, but at 180 days, the effect decreased, as demonstrated by low satisfaction scores.

Its mechanism of action is at peripheral cholinergic synapses; cleaves the protein associated with the synaptosome, 25 kDa (SNAP-25), which is present in the neuronal plasma membrane and inhibits the release of acetylcholine (ACh) at the neuromuscular junction, preventing muscle activity by chemical denervation. The BoNT-A acts on cholinergic neurons that is it inhibits the secretion of the neurotransmitter ACh from somatic and parasympathetic nerves, as well as some sympathetic nerves. In somatic nerves, the effects of paralysis and muscle atrophy by injections of BoNT-A used cosmetically to treat facial wrinkles with skin rejuvenation and to contour the lower part of the face, calf, neck and shoulders. In the parasympathetic nervous system, BoNT-A is also used to bypass the lower and mandibular lines using atrophy of the salivary glands⁽¹⁰⁾.

Thus, the predilection for females in this study occurs because of the

Table 1. Description of the treated regions, muscles involved, BoNT-A dose, needle characteristics and injection angles.

	Reference to the Puncture Points	Bont-A Dose (U)	Tri-Bevel 32g Needle	Angle of Injection
Gummy smile <i>Levator labii superioris</i> (n=15) <i>Levator labii superioris alaeque nasi</i> (n=12)	Horizontal – 10mm from the ala nasi; vertical – 30 mm of the labial commissure of mouth (Yonsei point) with the lips at rest	4 U in each point	6 mm	Perpendicular to the skin surface
Frontal rhytids <i>Occipitofrontalis</i> (n=48)	Rhytids identified when expressing "fright." V-shaped injection, based on the limited area between vertical lines passing through the distal of the iris and 10 mm above the eyebrows	Five to eight points within the security area, 2 U in each point	4 mm	Perpendicular to the skin surface
Glabella <i>Procerus</i> (n=27) <i>Corrugator supercilii</i> (n=45)	Muscle identified when expressing "anger."	From 3 to 4 U in each point	6 mm	Perpendicular to the skin surface
Periorbital lines <i>Orbicularis oculi</i> (n=46)	Muscle identified when expressing a "fake smile," keeping 10 mm of distance from the lateral of the <i>rima palpebrarum</i> , above the upper margin of the zygomatic bone	1 U in each point	4 mm	45° angle to the skin surface, towards the lateral side of the face

Table 2. Satisfaction scores on the effects of the treatment with BoNT-A on the muscles of expression and number of patients who received the treatment, according to the evaluation periods. Values are expressed as Mean (standard deviation).

	Satisfaction scores (%) in the evaluation periods				
	15 Days	30 Days	60 Days	90 Days	180 Days
<i>Corrugator supercilii</i> (n=48)	97.92 (6.83) ^A	96.46 (8.63) ^A	89.58 (22.40) ^B	79.38 (33.29) ^C	8.54 (19.02) ^D
<i>Procerus</i> (n=28)	98.57(5.91) ^A	96.79 (8.63) ^A	86.43 (27.78) ^B	86.43 (27.78) ^B	9.64 (20.27) ^C
<i>Occipitofrontalis</i> (n=48)	96.04 (15.67) ^A	94.38 (16.62) ^A ^B	88.13 (26.07) ^B	71.46 (39.68) ^C	10.00 (22.41) ^D
<i>Orbicularis oculi</i> (n=46)	95.00 (16.43) ^A	93.91 (16.93) ^A	83.04 (31.82) ^B	70.43 (40.11) ^C	10.22 (22.26) ^D
<i>Levator labii superioris</i> (n=15)	97.33 (7.99) ^A	97.33 (7.99) ^A	88.67 (26.42) ^A ^B	81.33 (34.82) ^B	10.00 (26.46) ^C
<i>Levator labii superioris alaeque nasi</i> (n=13)	93.85 (15.57) ^A	91.54 (23.04) ^A	88.46 (23.04) ^A ^B	86.15 (28.44) ^B	11.54 (28.24) ^C

*Friedman test ($P < 0.001$); Durbin-Conover for pairwise comparisons ($P < 0.05$).*Values followed by different letters represent statistically significant difference ($P < 0.05$).**Table 3.** Descriptive data on the perceived efficacy of aesthetic treatment with BoNT-A in women under 40 years old and over 40 years old on follow-up days.

	15 days		30 days		60 days		90 days		180 days	
	<40	>40	<40	>40	<40	>40	<40	>40	<40	>40
<i>Corrugator supercilii</i> < 40 n=16; > 40 n=32	98.75 (5.00)	97.50 (7.62)	97.50 (6.83)	95.94 (9.46)	88.13 (27.13)	90.31 (20.08)	86.25 (27.29)	75.94 (35.82)	18.13 (28.34)	3.75 (9.42)
<i>Procerus</i> < 40 n=6; > 40 n=22	100.00 (0.00)	98.18 (6.65)	96.67 (8.17)	96.82 (8.94)	88.33 (20.41)	85.91 (29.87)	88.33 (20.41)	85.91 (29.87)	8.33 (13.29)	10.00 (22.04)
<i>Occipitofrontalis</i> < 40 n=12; > 40 n=36	98.33 (5.77)	95.28 (17.81)	98.33 (5.77)	93.06 (18.80)	94.17 (15.05)	86.11 (28.71)	80.00 (31.04)	68.61 (42.17)	11.67 (23.68)	9.44 (22.29)
<i>Orbicularis oculi</i> < 40 n=13; > 40 n=33	88.46 (28.24)	97.58 (7.51)	88.46 (28.24)	96.06 (9.33)	76.92 (37.50)	85.45 (29.59)	72.31 (36.32)	69.70 (42.02)	12.31 (24.21)	9.39 (21.79)
<i>Levator labii superiores</i> < 40 n=8; > 40 n=7	96.25 (10.61)	98.57 (3.78)	96.25 (10.61)	98.57 (3.78)	93.75 (11.88)	82.86 (37.29)	80.00 (35.05)	82.86 (37.29)	8.75 (24.75)	11.43 (30.24)
<i>Levator labii superioris alaeque nasi</i> < 40 n=9; > 40 n=4	91.11 (18.33)	100 (0)	87.78 (27.28)	100 (0)	85.56 (26.98)	95.00 (10.00)	82.22 (33.46)	95.00 (10.00)	7.78 (23.33)	20.00 (40.00)

Student t test or Mann-Whitney test, when indicated, $P > 0.05$

SD – Standard deviation

anatomical muscle difference, which could lead to a difference in the self-perception scale (score from 0 to 100%). The anatomy is substantially different between sexes: the male skull is larger, with a different shape, it is heavier, and robust; whereas the female skull is lighter and more delicate. Men have wider forehead, with prominent supercilial arch, wider glabella, square orbit and quadrangular jaw, as well as larger skeleton and muscle structure – including muscles of the facial expression. These anatomical differences should be considered in any facial aesthetic study^(11,12).

The effect of BoNT-A in patients with gummy smile in this study was effective for 60 days, with a significant decrease in effectiveness after 90 days, but the scores showed good satisfaction. However, according to Mario Polo, 2008⁽¹³⁾; who treated 30 Hispanic individuals with gummy smile, injecting 2.5 U into each muscle with BoNT-A, reported that the gingival display gradually increased, from two weeks after injection to 24 weeks, but at 24 weeks, the average gingival display had not yet returned to baseline values. In a 2018 systematic review and meta-analysis study, the authors state that a significant effect of the treatment tends to be stable up to at least eight weeks of follow-up and the effect can remain up to 12 weeks⁽¹⁴⁾. Another alternative for gummy smile would be a surgical procedure, however, Gregnanin and Aulestia-Viera, 2017; concluded in their study that the surgical procedure – besides being more invasive – does not promote the result expected by the patient, and BoNT-A is a fast and effective option for correcting the gummy smile, as long as the correct amount of BoNT-A is respected according to the type of smile⁽¹⁵⁾.

On the other hand, the longevity of BoNT-A in the occipitofrontalis muscle is indicated in the literature to last on average from three to four months^(16,17). The glabella region – comprising the procerus, corrugator supercilii, and orbicularis oculi muscles. In a 2016 a study⁽¹⁸⁾ composed of women aged from 18 to 64 years, with six months of follow-up, received a total dose of 50 U and two different volumes of injection, and both the double volume of BoNT-A injection and the marked volume of injection provide excellent efficacy, with early onset of the effect and durability up to six months. Other studies indicate that the effect of BoNT-A can be perceived two weeks after injection and it can last some three or four months, rarely exceeding six months^(19,20). Also, other studies indicate that the BoNT-A effect hardly lasts up to six months⁽²¹⁾. Thus, patients can expect from the treatment with BoNT-A – for hyperkinetic facial lines – three months of efficiency; effects after the fourth month, depend on the facial area, dose, and formulation. Age, gender, and combined, repeated and previous treatments also affect the efficacy of the procedure⁽²²⁾. Nevertheless, in the present study a negative significant correlation between age and the effectiveness of BoNT-A in the occipitofrontalis muscle at 90 days was found, meaning that after three months of application younger women were more satisfied with the treatment.

Age must also be taken into account, as aging causes flaccidity⁽²³⁾. Thus, comparing women under 40 and over 40, significant negative correlations between age and patient satisfaction at 180 days were observed in *Corrugator supercilii* ($p = 0.040$) and at 60 days in *Occipitofrontalis* (p

= 0.048), demonstrating that women under 40 have a better voluntary perception of these muscles.

Therefore, anatomical changes, skin, soft and bone tissues, and soft tissue descent contribute to the appearance of facial aging, in addition to this physiological and anatomical perception. Bone changes with aging seem to contribute to changes in facial aging, enlargement of the skull and frontal sinuses can increase the superciliary arch. The lack of bone support can contribute to the loss of volume under the eyebrow and descent of the eyebrow tissues. Thus, an understanding of the anatomical changes of aging and the perception of these changes can lead to more appropriate therapy⁽²⁴⁾.

Our study suggests that treatment with BoNT-A for aesthetic facial treatments was effective for up to 90 days, depending on the treated muscle, and at 180 days all muscles differed significantly from other days, showing low satisfaction with the treatment. In the comparative analyzes between women under 40 and over 40 years of age, there was no significant difference. However, the muscles *Corrugator supercilii* and *Occipitofrontalis*, showed a negative correlation in women under 40 years. Reinforcing, the importance of the patient to follow up with the clinician so that he can observe the correct dose in order to avoid overdosing or unnecessary recurrent injection.

A comprehensive understanding of the aesthetic indications related to the BoNT-A cholinergic nervous system, in combination with associated physiological factors and conditions such as dosage, volume and dilution, distribution characteristics and injection techniques can influence the effectiveness of BoNT-A. In this way, more sophisticated treatment techniques and management policies to obtain the best effectiveness can be achieved, minimizing effects and helping to expand new advanced clinical indications for the cosmetic use of BoNT-A⁽¹²⁾.

Although this study demonstrated the effectiveness of BoNT-A, we have as limitation the method of satisfaction rate of the treatment reported by the patient, with the responses on a verbal analog scale, ranging from 0 to 100%, being a subjective evaluation method. However, further studies regarding optimal doses of BoNT-A are necessary, for each specific muscle, as well as for the time required for the next application, so that the patient receives a new dose only when necessary.

However, our study contributes with its data compilation, its way of systematizing knowledge on the injection of BoNT-A for facial aesthetic treatments – including gummy smile – demonstrating the period of effectiveness of the BoNT-A based on the ideal minimum dosages in the muscles of facial expression.

CONCLUSION

The BoNT-A was applied in muscles of facial expression, with attested satisfaction effect by participants for up to 90 days, but at 180 days the satisfaction was low. In the comparative analyzes between women under 40 and over 40 years of age, there was no significant differences.

CLINICAL RELEVANCE

This study contributes to the way of systematizing and compiling data in aesthetic treatments with BoNT-A in the muscles of facial expression, including gummy smile, demonstrating its period of effectiveness with the minimum dosage injected into the patients' muscles. Despite the effectiveness of BoNT-A being consolidated and the wide use of it, we know that its effect is not long-lasting and its duration varies, depending on the patient's metabolism, the injection protocols followed, the muscle, and the toxin reconstitution. By injecting the ideal dose of BoNT-A, for each specific muscle and period of effectiveness, it is possible to preserve the patient so that they receive a new dose only when necessary.

DISCLOSURE OF INTEREST

The authors declare no conflicts of interest.

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DATA CONFIDENTIALITY

The authors declare that they followed the workplace protocols on the publication of patient data and that all participants received sufficient information and provided their written informed consent.

RIGHT TO PRIVACY AND INFORMED CONSENT

All patients and/or subjects in this study provided a written informed consent to the authors.

This document is in the possession of the correspondence author.

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