Sebum Production Alteration after Botulinum Toxin Type A Injections for the Treatment of Forehead Rhytides: A Prospective Randomized Double-Blind Dose-Comparative Clinical Investigation

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Abstract

Background: Research has investigated the decrease in human skin sebum after the application of botulinum toxin. Few studies of the mechanism and objective assessments of this phenomenon have been conducted and the correlation between the sebum production and injection dosages or techniques remains unclear.

Objectives: We prospectively investigated the sebum regulation and its gradient around the injection site in patients who received intramuscular injections of botulinum toxin A (BTX-A) for forehead rhytides, comparing two injection doses.

Methods: Forty-two female volunteers with rhytides on the forehead region were randomly assigned to receive 10 or 20 units of BTX-A, which was administered in five standard injection sites. The baseline and post-treatment sebum production was measured using a Sebumeter.

Results: Treatment with BTX-A exhibited significant sebum alteration at the injection site of both groups, with a sebum gradient surrounding the injection point. The efficacy did not improve at higher injection doses, with the four-unit regimen generally not being more potent than the two-unit regimen. The sebum production recovered to normal levels at the 16 week follow-up for both treatment groups, indicating that a higher dosage (four units) did not result in a longer duration until relapse compared with the two-unit dose.

Conclusions: We determined that the sebum production has a positive correlation with the distance away from the injection point. Intramuscular injection of BTX-A significantly reduces sebum production at the injection site but increases the sebum production of the surrounding skin at a radius of 2.5 cm at the 2, 4, and 8 week follow-ups.

Level of Evidence: 2

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Botulinum toxin was first reported for glabellar folds associated with corrugators and/or procerus muscle dynamics in 1992. The high efficacy and patient satisfaction quickly moved the indications from glabellar wrinkles to more global and sophisticated indications, such as crow’s feet, lower eyelid wrinkles, nasolabial lines, horizontal lines, and masseter hypertrophy. A lack of studies and experiments in vitro or in vivo results in physicians’ applying botulinum toxin type A (BTX-A; Botox, Allergan, Irvine, CA) empirically, which causes adverse events, such as injection pain, erythema, hematoma, headache, and asymmetry. Recent studies have demonstrated that botulinum toxin will affect skin texture and sebum production at the injection site, leading to local skin dryness. Sebum contributes to the delivery of fat-soluble antioxidants to the skin surface and has antimicrobial activity, which represents the ultimate barrier of the human body against exogenous insults. Undesirable sebum blocks the pores, provides nourishment to bacteria, and results in acne. Patients who suffer from forehead lines are suggested to obtain a standard treatment of botulinum toxin injection in the forehead region. As part of the so-called “T-zone” (forehead, nose and chin), the forehead region normally has higher sebum production than other regions because of a higher density of sebaceous follicles in the face (300–900/cm²). Alterations in sebum production will appear obvious in this region compared with other regions. Because the majority of female patients regularly use makeup to prevent their skin from drying, few reports or studies have been conducted regarding this issue. Recently, insights into the effect of botulinum toxin on sebum production have been published. The results indicated a significant reduction in sebum production and demonstrated a correlation between sebum production and injection techniques, although the dosage(s) used is unknown. In this study, we conducted a prospective randomized dose-comparative clinical trial to investigate the sebum regulation in patients who received intramuscular injections of botulinum toxin A.

**METHODS**

**Patient Selection and Characteristics**

From April of 2013 to September of 2013 a total of 42 female patients (Fitzpatrick skin types II to IV) with forehead rhytides were enrolled in this study and treated with intramuscular injection of BTX-A with a 16 week follow-up period. The exclusion criteria included any previous treatment with botulinum toxin or fillers; a history of chemical peeling, lasers, visible scars or abrasions in the treatment area; a history of facial nerve palsy, keloid formation, or human immunodeficiency virus; or a state of immunocompromise. Pregnant or breast-feeding patients were ineligible for the study.

**Study Design**

With the approval of our Hospital Ethical Research Committee (Shanghai Ninth People’s Hospital, Shanghai Jiao Tong University, School of Medicine, 639 ZhiZaoJu Road, Shanghai, China), 42 patients were enrolled and distributed equally into two botulinum toxin treatment groups in a randomized, double-blind fashion. After giving written informed consent, patients were randomly allocated (Microsoft Windows.NET Framework 3.5, Seattle, WA) to one of the following two groups before injection: Group I included 21 patients who received intramuscular injections with 2 units of toxin at each point and Group II included 21 patients who received intramuscular injections with 4 units of toxin at each point (Table 1). Every patient got a 50% probability to be allocated to either treatment group. BTX-A (Botox; Allergan, Irvine, CA) was administered into the forehead region, divided equally among five injection sites, and the patients were followed up over a four-month period. Patients and providers were blinded to treatment.

**Study Medications and Injection Technique**

Each vial of BTX-A contained 100 units. Lyophilized BTX-A was reconstituted in 2.5 mL of sterile saline and immediately injected through a 30-gauge needle. A final volume of 0.25 mL (10 U) or 0.5 mL (20 U) of botulinum toxin was injected evenly in five injection sites using an intramuscular technique on the forehead region according to the group design.

To minimize individual bias, the injection sites were standardized for all of the subjects. The injection sites were located across the middle of forehead horizontally (Figure 1). The patients of Groups I and II received botulinum toxin injections in the frontalis muscle. The patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Range of Years</th>
<th>Mean Age ± SD (Years)</th>
<th>Injection Doses at Each Point (Units)</th>
<th>Technique</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>25-52</td>
<td>41 ± 8.49</td>
<td>2 U</td>
<td>Intramuscular</td>
<td>20</td>
</tr>
<tr>
<td>Group II</td>
<td>31-58</td>
<td>48.8 ± 9.4</td>
<td>4 U</td>
<td>Intramuscular</td>
<td>21</td>
</tr>
</tbody>
</table>

SD, standard deviation.
were asked to use ice packs after treatment, and no specific medications were to be taken after the injections.

**Outcome Assessments**

**Standardized Digital Photographs and Objective Assessment**

The patients were assessed before the injections (baseline) and at weeks 2, 4, 8, and 16. Digital photographs were taken using one Nikon digital camera at each follow-up visit.

**Sebum Production**

The Sebumeter SM 815 (Courage & Khazaka, CK Electronic, Köln, Germany) is the most commonly used commercial device for the quantification of sebum production.\(^{15-17}\) After the sebum was collected from the skin surface on an opaque plastic strip, the photometry system determined the translucency of the tape. The light permeability of the tape changed after 30 seconds of skin contact with a 10 N constant pressure for measurement. The probe was placed back into the primary unit of the Sebumeter, and the result was evaluated by a microprocessor using an internal standard. The results are shown in units from 0 to 350 µg/cm\(^2\). Taking into account the fact that sebum production strongly varies by location, results up to 70 to 100 µg/cm\(^2\) represented dry facial skin, and results over 180 to 200 µg/cm\(^2\) represented seborrheic facial skin.\(^{14}\)

The vertical distance between the middle eyebrows and the injection horizontal line above the eyebrow was recorded for each patient, and the photographs were compared to enable the physician to locate the same injection site at the follow-up visits. The patients were asked to eat a normal diet during 24 hours before and wash their faces with clean water two hours before each assessment.

We assumed that the diffusion area of the toxin was a circular area with a radius of 1.5 cm. The highest concentration of the toxin should have been found in the central 0.5 cm of the radius area around the injection point; this was termed Area 1. The further 1 cm radius area was assumed to have a lower concentration of the toxin and was termed Area 2. The circular area with a radius of 1 cm outside the diffusion area of the toxin was termed Area 3 (Figure 2). The sebum production of the three areas was assessed at baseline and at each follow-up (Figure 3).

**Statistical Analysis**

The statistical analysis was performed using SPSS software, version 19 (IBM Company, Armonk, NY, USA). The patient characteristics were analyzed using Student’s t-tests. The average sebum production and change rate from baseline to each follow-up visit were analyzed using analysis of variance for repeated measurement data by subgroups of the injection doses and by the age group.

**RESULTS**

**Patient Population**

A total of 42 female subjects were randomly included in the study without overlap, and one subject originally included in Group I was lost after the initial follow-up visit at week 2 for a relapse of photosensitive dermatitis. The remaining 41 females were equally distributed in both groups of treatment. The mean age of the subjects was 41 ± 8.49 (range 25-52 years) for Group I and 48.8 ± 9.4 (range 31-58 years) for Group II. Two explicative examples are shown in Figures 4 and 5.
Sebum Production Alteration of the Injection Site

Intramuscular botulinum toxin treatment resulted in sebum production alteration in the forehead region as measured by the Sebumeter at the follow-up visits. We investigated the average sebum production of the injection site (Area 1: radius \( \leq 0.5 \text{ cm} \)) and the areas outside surrounding the injection point (Area 2: 0.6 cm < radius \( \leq 1.5 \text{ cm} \); Area 3: 1.6 cm < radius \( \leq 2.5 \text{ cm} \); Figure 2). The average sebum production in Area 1 of the two treatment groups, as measured by the Sebumeter readings, was assessed and is shown in Figure 6.

Subgroup Analysis by Injection Dosages

The average sebum production in Area 1 is shown in Figure 6. The sebum production change rate from the baseline of the two treatment groups is summarized in Table 2.

The average sebum production in Area 1 for Group I at the baseline, 2, 4, 8, and 16 week follow-ups were 62.31, 47.22, 36.91, 50.69, and 66.98 \( \mu \text{g/cm}^2 \), respectively. The average sebum production in Area 1 for Group II at the baseline, 2, 4, 8, and 16 week follow-ups were 46.22, 23.78, 29.80, 32.98, and 49.82 \( \mu \text{g/cm}^2 \), respectively.

The results demonstrated that the average sebum production changed (percent) for Group I at the 2, 4, 8, and 16 week follow-ups were \(-25.25 \pm 1.94\), \(-45.84 \pm 2.50\), \(-17.59 \pm 2.62\), and \(9.62 \pm 2.29\), respectively, in Area 1; \(0.58 \pm 3.12\), \(12.12 \pm 2.85\), \(9.78 \pm 3.46\), and \(21.05 \pm 2.62\), respectively, in Area 2; and \(36.75 \pm 3.79\), \(45.46 \pm 3.89\), \(47.41 \pm 4.70\), and \(29.93 \pm 2.84\), respectively, in Area 3. The average sebum production change (percent) for Group II at the 2, 4, 8, and 16 week follow-ups were \(-46.95 \pm 2.53\), \(-34.72 \pm 3.36\), \(-27.36 \pm 3.47\), and \(10.30 \pm 2.84\), respectively, in Area 1; \(0.83 \pm 4.40\), \(-1.40 \pm 4.41\), \(2.17 \pm 4.43\), and \(29.36 \pm 3.67\), respectively, in Area 2; and \(45.21 \pm 5.15\), \(47.88 \pm 5.58\), \(42.41 \pm 5.30\), and \(48.88 \pm 4.34\), respectively, in Area 3.

Figure 2. Compartments of the forehead skin after botulinum toxin injection. We assumed that the botulinum toxin resides in the skin in a circular area with a radius of 1.5 cm. The highest concentration of the toxin should have been located in the central 0.5 cm of the radius area around the injection point; this was termed Area 1. The further 1 cm radius area was assumed to have a lower concentration of the toxin and is termed Area 2. The outermost area, with a radius of 1 cm outside the diffusion area of toxin, was termed Area 3.

Figure 3. The hand-held Sebumeter probe was used to detect the sebum production at baseline and at each follow-up time point, as shown on this 27-year-old woman.
Subgroup Analysis by Age

The change in sebum production (percent) by age is summarized in Table 3. The results demonstrated that the average sebum production change (percent) in patients below 40 years old at the 2, 4, 8, and 16 week follow-ups were $-36.63 \pm 3.12$, $-35.84 \pm 1.89$, $-16.80 \pm 2.06$, and $5.89 \pm 2.26$, respectively, in Area 1; $-7.73 \pm 3.06$, $8.90 \pm 2.83$, $6.31 \pm 1.90$, and $13.86 \pm 2.27$, respectively, in Area 2; and $20.80 \pm 3.04$, $37.67 \pm 4.22$, $39.57 \pm 3.89$, and $27.41 \pm 2.54$, respectively, in Area 3. The average sebum production change (percent) for Group II at the 2, 4, 8, and 16 week

Figure 4. (A) Photograph of a 47-year-old woman with moderate forehead lines enrolled in Group I (2 units per point) at baseline (prior to treatment), (B) at the 2 week follow-up, (C) at the 4 week follow-up, when she demonstrated an obvious effect of the botulinum toxin type A, (D) at the 8 week follow-up, and (E) at the 16 week follow-up, when the forehead lines relapsed.
follow-ups were $-36.69 \pm 2.49$, $-41.90 \pm 3.07$, $-25.73 \pm 3.09$, and $11.86 \pm 2.45$, respectively, in Area 1; $4.61 \pm 3.65$, $3.21 \pm 3.81$, $5.53 \pm 4.10$, and $30.77 \pm 3.02$, respectively, in Area 2; and $50.62 \pm 4.07$, $50.92 \pm 4.58$, $47.19 \pm 5.30$, and $45.62 \pm 3.75$, respectively, in Area 3.

**Safety**

All 42 patients involved received the treatment, and 1 patient was lost to follow-up after 4 weeks. There was no statistically significant difference between the two dose
groups. Only one serious adverse event (relapse of photosensitive dermatitis) was found, and it was not related to the treatment.

**DISCUSSION**

Sebum excretion of human skin results from four different components, which are sebum production, storage, surface output, and stratum corneum permeation. The pilosebaceous follicles play essential roles during the entire process. Human pilosebaceous follicles can be subdivided into terminal hair follicles, vellus hair follicles, and sebaceous follicles according to their volume and the size of the associated hair.15 The sebaceous follicle, which is located intradermally with a miniature hair shaft, is the typical type of pilosebaceous follicle observed on the chest, shoulders, back, and face. There are two mechanisms thought to affect sebum excretion: physicochemical regulation18-21 and hormonal regulation.22-27 The complex interaction of androgens results in a higher signal of 5α-reductase type 1, thereby increasing the releasing of sebum. Oestrogens have the opposite effect, with a much weaker potency by inhibiting gonadotropin-releasing activity and 5α-reductase activity.25 Because growth hormone increases sebum production, sebum excretion is believed to differ with gender, age, climacteric, and pregnancy.18-27

Shah11 first reported the intradermal injection technique of botulinum toxin type A for the treatment of oily skin in 2008. The present investigation confirmed that botulinum toxin type A can effectively reduce sebum production. Rose

![Figure 6. Average sebum production at Area 1 of the two treatment groups. Intramuscular injections of BTX-A significantly reduced the average sebum production of Area 1 at 2, 4, and 8 week follow-ups in both treatment groups. At the 16 week follow-up, the average sebum production of Area 1 recovered and showed no statistical differences compared with the baseline. 2u, two-unit dose; 4u, four-unit dose.](image)

**Table 2. Sebum Production Change Rate of at each Follow-Up visit**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Area 3</th>
</tr>
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<tr>
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<tr>
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<tr>
<td>Change Rate</td>
<td>I</td>
<td>−25.25 ± 1.94*</td>
<td>0.58 ± 3.12</td>
<td>36.75 ± 3.79</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>−46.95 ± 2.53</td>
<td>0.83 ± 4.40</td>
<td>45.21 ± 5.15</td>
</tr>
<tr>
<td>Visit 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change Rate</td>
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<td>−45.84 ± 2.50</td>
<td>12.12 ± 2.85</td>
<td>45.46 ± 3.89</td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>−34.72 ± 3.36</td>
<td>−1.40 ± 4.41</td>
<td>47.88 ± 5.58</td>
</tr>
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<td>Visit 4</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Change Rate</td>
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<td>−17.59 ± 2.62</td>
<td>9.78 ± 3.46</td>
<td>47.41 ± 4.70</td>
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<td>II</td>
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<td>Visit 5</td>
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<tr>
<td>Change Rate</td>
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<td>21.05 ± 2.62</td>
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<td></td>
<td>II</td>
<td>10.30 ± 2.84</td>
<td>29.36 ± 3.67</td>
<td>48.88 ± 4.34</td>
</tr>
</tbody>
</table>

Group I: n = 20; Group II: n = 21; Visits 2, 3, 4, and 5 are at the 2, 4, 8, and 16 week follow-ups, respectively. *Values are the means ± SE (standard error).
and Goldberg\textsuperscript{12} assessed the treatment for oily skin quantitatively with unknown mechanisms and relevant factors. Since Bulstrode and Grobbelaar\textsuperscript{10} first reported a decrease in localized sebum production after forehead line treatment with botulinum toxin as an adverse event, few studies were carried out regarding this issue. With increasing numbers of patients being treated by botulinum toxin for aesthetic purposes, thousands of patients are suffering from skin sebum disorder every day without recognizing it. Our study is the first prospective, randomized, double-blind, dose-related trial to provide an insight into this situation in patients who received botulinum toxin treatment for forehead rejuvenation.

The results of the present study confirmed that intramuscular injections of botulinum toxin type A significantly reduced sebum excretion in Area 1 (the high concentration area) for both treatment groups (Figures 6 and 7). The efficacy did not improve at higher injection doses, with the four-unit regimen generally not being more potent than the two-unit regimen. The sebum production recovered to normal levels at the 16 week follow-up for both treatment groups, indicating that a higher dosage (four units) did not result in a longer duration until relapse compared with the two-unit dose. We determined that the sebum production reduced to a nadir at week 2 for Group II and at week 4 for Group I, which was mainly because of the high product concentration in Group II. We did not conduct any other dosage groups other than the two-units and four-units in each injection site according to the injection dosage recommended by the consensus on the treatment of horizontal forehead lines in 2010.\textsuperscript{28} In the results of our study, the efficacy of botulinum toxin type A in sebum excretion was dosage-dependent, but it showed no significant differences between the two doses used in this investigation.

According to our study, the average sebum production decrease in the high concentration area (Area 1) for Group I and II ranged from $-17.59 \pm 2.62\%$ to $-46.95 \pm 2.53\%$, which was less effective than the intradermal technique reported by Rose and Goldberg.\textsuperscript{12} The intradermal injection resulted in an average decrease in sebum production of 75\%, 80\%, 73\%, and 59\% at one week, one month, two month, and three month follow-ups, respectively. The mechanism leading to the decrease in sebum excretion is not entirely clear. Rose and Goldberg attributed this phenomenon to the neuromodulatory effects on the erector pili muscles and local muscarinic receptors in the sebaceous glands. The blockade of local acetylcholine receptors alters sebocyte differentiation and reduces sebum production. Li et al\textsuperscript{29} demonstrated that the nicotinic acetylcholine receptor $\alpha_7$ (nAchR$\alpha_7$) was expressed in human sebaceous

<table>
<thead>
<tr>
<th>Variables</th>
<th>Age</th>
<th>Area 1</th>
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<td>$3.21 \pm 3.81$</td>
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<td>Visit 4</td>
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<td></td>
<td>&gt;40</td>
<td>$-25.73 \pm 3.09$</td>
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<td>Visit 5</td>
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<td>$5.89 \pm 2.26$</td>
<td>$13.86 \pm 2.27$</td>
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<td>$11.86 \pm 2.45$</td>
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</tbody>
</table>

Visits 2, 3, 4, and 5 are at the 2, 4, 8, and 16 week follow-ups, respectively. \textsuperscript{a}Values are the means ± SE (standard error).
glands in vivo, and Ach signal increased lipid synthesis in vitro in a dose-related manner. It is plausible that botulinum toxin diffuses into the dermal layer after being injected with an intramuscular technique into the frontal muscle, and the neurotoxin inhibits the Ach signals in human sebaceous glands, resulting in a skin sebum production decrease with a complex interaction. This finding explains the reason why injections with an intradermal technique may exhibit a higher efficacy than that observed in the intramuscular group.

The results of the present study indicated that there was a sebum gradient surrounding the injection point (Figure 2). We investigated the sebum production outside the injection point at a radius from 0 to 2.5 cm. We determined the sebum production has a positive correlation with the distance away from the injection point. The sebum production change rates of Areas 1, 2, and 3 for the two treatment groups are shown in Figure 8A and B. The average sebum production of Area 3 was higher than Area 2, and Area 2 was higher than Area 1 for both treatment groups, with a significant difference at the 2, 4, and 8 week follow-ups. This phenomenon is first reported in our study, and the mechanism is not entirely clear. We presume that there is a compensatory mechanism for human skin sebum control. Kligman and Shelley30 termed this the “positive feedback theory,” and Downing et al31 measured sebum secretion over a period of 24 hours and found the sebum secretion decreased over a period of 12 hours and then remained constant for the remaining 12 hours. These studies indicated that skin sebum is likely regulated by complex interactions that keep the local lipid delivery constant. More studies in vivo or in vitro should be performed to clarify the mechanism.

We also performed the subgroup analysis by age. The results indicated that the average sebum production for the patients younger than 40 years old was greater than in patients older than 40 years (Figure 9). The change rate of the two age groups showed no significant difference (Table 3).

Roh and colleagues32 recruited 60 patients to investigate the relationship between the pore sizes and sebum output
The authors analyzed magnified images of pores taken with a dermoscopic video camera and demonstrated that the sebum output level affects the size of facial pores. Li et al. found a marked decrease in pore size on the botulinum-treated side 4 weeks after injection in his split-face study. Shah enrolled 20 consecutive patients and, with a single application of intradermal BTX-A, noted a down-regulation in sebum production and a decrease in pores size at 1 month after injection in 17 patients. Although in our investigation we did not focus on the changes of facial pore sizes, we agree with previous authors that suggested that a treatment focused on reducing sebum production may be beneficial in decreasing enlarged pore sizes. This pharmacologic efficacy on pore size indicates a potential therapy of BOTX-A for enlarged pores.

For this investigation we designed a double-blind randomized trial comparing two different dosages of BOTX-A for the following main reasons. Even if the aim of the study was the evaluation of sebum production alteration, the patients were enrolled for the treatment of the forehead wrinkles. The decision to not to provide a controlled group treated with saline only was made because we wanted to avoid any bias in the eventual control group due to the ineffectiveness of saline to treat wrinkles. In addition, although the actual fundamental mechanism in sebum production alteration is not entirely clear, a placebo-controlled, split-face study has already demonstrated that saline does not provide a real pharmacologic action on local acetylcholine receptors, erector pili muscles, or local muscarinic receptors in the sebaceous gland. The complex interaction induced by botulinum toxin—that is, the interruption of sebocyte differentiation and lipid synthesis—cannot be carried out by saline injection. So we did not provide a blank control group with only saline treatment and focused our attention on how the different dosages could influence the sebum production. In this way, as much as possible we designed a study closer to the routine clinical practice, including three main aspects: intramuscular injection, forehead wrinkles, and two- or four-unit treatments.

Our study provides insight into the sebum excretion conditions in patients who received botulinum toxin treatment in the forehead region, and provided reliable results collected by an objective measurement method. The potential implications of our present research include some recommendations that should be taken in mind in the common cosmetic practice when the treatment of facial wrinkles with BTX-A injection is performed. Although there is a lack of declarations regarding this issue, physicians should be aware of this drug reaction when using BTX-A as a modern medicine, especially through a micro-injection technique in cases of skin dryness. The compensatory sebum production, increasing at a radius of 2.5 cm from the injection point, will be an annoyance to the patients and might be improved by cosmetic products or medications. A larger, randomized, blinded, placebo-controlled, dose-response trial is necessary to substantiate our findings.

This study has some limitations. Although it is well known that sebum production varies according to the hormonal status (age, gender) and also the menstrual cycle, the present investigation did not take into account the stratification for gender and age of the sebum production in the treated patients. In addition, only female subjects were included in our study. The average age of the patients in Group II was much higher than the patients in Group I (in both average age and age range). A narrower age range would have likely provided more trustworthy results. Larger, randomized, blinded, placebo-controlled, dose-response trials are necessary to substantiate our findings.

CONCLUSIONS

The results indicate that intramuscular injections of BTX-A will significantly reduce sebum production at the injection site but will increase the sebum production in the surrounding skin with a radius of 2.5 cm at 2, 4, and 8 week follow-ups.

Disclosures

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