Botulinum Toxin Type A Injection for Lateral Canthal Rhytids
Effect on Tear Film Stability and Tear Production

Min-Chieh Ho, MD; Wei-Cherng Hsu, MD; Yi-Ting Hsieh, MD

IMPORTANCE Botulinum toxin injection for lateral canthal rhytids has been reported to result in dry eye, but its effect on tear film stability and tear production has not been studied thoroughly.

OBJECTIVE To investigate the effect of botulinum toxin type A on tear film stability and tear production after treatment of lateral canthal rhytids.

DESIGN, SETTING, AND PARTICIPANTS We performed a clinical intervention study at a regional hospital in Taiwan of 58 women 30 to 60 years of age with lateral canthal rhytids from January 1 through December 31, 2011.

INTERVENTIONS Botulinum toxin type A at 2 different preparations and doses (dose A: 3 injections of 2 U in 0.05 mL of normal saline per injection; dose B: 3 injections of 4 U in 0.05 mL of normal saline per injection) was injected at the lateral canthal areas. One eye of each study participant was randomly chosen for dose A, and the other eye received dose B.

MAIN OUTCOMES AND MEASURES Baseline tear film break-up time (TBUT) and Schirmer tests without and with anesthesia were measured before and at 1 week, 1 month, 3 months, and 6 months after botulinum toxin injection. The TBUT and Schirmer test results were compared between different periods and doses.

RESULTS The TBUT decreased significantly at 1 week after botulinum toxin type A injection ($P = .003$), and the effect persisted at 1 month and 3 months after treatment ($P = .01$ and .02, respectively). In younger participants, the TBUT recovered faster than in older patients. The results of the Schirmer tests without and with anesthesia decreased gradually, with significant reduction at 1 month after treatment ($P = .05$ and .02, respectively) and then recovered gradually. Both the TBUT and Schirmer test results decreased more in eyes that received dose B than in those that received dose A; however, none of the differences were statistically significant.

CONCLUSIONS AND RELEVANCE Tear film stability decreased as early as 1 week after botulinum toxin type A treatment for lateral canthal rhytids, and the effect persisted for more than 3 months. Tear production decreased to the trough at 1 month after treatment and then recovered gradually.

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Botulinum toxin type A is widely used in cosmetic treatments. Its main action is to paralyze muscles by blocking acetylcholine release from neuromuscular junctions. It is also used in dystonias, such as blepharospasm and hemifacial spasm. Currently, it is widely used for cosmetic purposes in aging faces where skin rhytids are a concern. Local injection of botulinum toxin results in paralysis of facial expression muscles as a result of its chemodenervation effect and thus reduces the superimposed dynamic cutaneous rhytids. Of all facial rhytids now treated with botulinum toxin, lateral canthal rhytids are of interest because of their proximity to the lacrimal glands. A few case studies have reported dry eye symptoms in patients after receiving botulinum toxin type A for periocular rhytids, whereas one study concluded that botulinum injection for lateral canthal rhytids did not suppress tear production. In this study, we evaluated whether tear film stability and tear production were affected by botulinum toxin type A injection for the treatment of lateral canthal rhytids and analyzed whether the effect differs in varying doses and age groups.

Methods

Study Population
We prospectively recruited women 30 to 60 years of age who had lateral canthal rhytids from January 1 through December 31, 2011, at Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation. Study participants were excluded if they had a history of systemic immune disease, previous eyelid or ocular surgery, or previous botulinum injections for facial rhytids in the past 6 months; were pregnant; were receiving anticholinergics or antihypertensive medications or hormone therapy; were using eye drops other than artificial tears; or wore contact lenses. This research followed the tenets of the Declaration of Helsinki. Institutional review board approval was obtained prospectively from the institutional review board of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation. Written informed consent was obtained from all participants at the time of recruitment.

All study participants received examinations in the following order: tear film break-up time (TBUT), Schirmer test without anesthesia, and Schirmer test with anesthesia. One eye was randomly selected to be tested first followed by the other eye. The interval between tests for the 2 eyes of the same participant was at least 10 minutes.

Tear Film Break-up Time
We modified the method of measuring TBUT described by Cho and Brown. A standard FUL-GLO strip (Akorn Inc) was wetted with a drop of unpreserved 0.9% sodium chloride solution and then shaken gently to remove excess fluid from the strip. The participants were instructed to look inferonasally. The examiner pulled the upper eyelid up gently and then applied the fluorescein strip to the superior temporal bulbar conjunctiva gently for 1 second. The participants were asked to blink naturally several times without squeezing to distribute the fluorescein and then to stare straight ahead without blinking until told otherwise or until they felt uncomfortable. The cornea was observed with a slitlamp at ×10 magnification by illumination of full-beam cobalt blue light. The time between last blink and the first appearance of black spots or streaks was recorded by a stopwatch. For participants who kept blinking before the first appearance of black spots or streaks, the time between blinks was recorded instead. Three measurements were taken for each eye, and the mean time was recorded as the TBUT. After all measurements were obtained, the participants were instructed to blink freely.

Schirmer Test Without Anesthesia
Participants were instructed to look upward, and the examiner gently pulled the lower eyelid away from the bulbar conjunctiva. A standard strip of filter paper (Alcon Laboratories Inc) was inserted to one-third the width of the eyelid length nasally from the lateral canthus and was placed properly in the cul-de-sac of both eyes of the participants. The participants were instructed to close their eyes and rest in a quiet room for 5 minutes. After 5 minutes, the participants were asked to look upward, and the examiner pulled the lower eyelid and then gently removed the strips from the lower fornix. The length of strip wetting was recorded in millimeters.

Schirmer Test With Anesthesia
Fifteen minutes after the Schirmer test without anesthesia, 2 drops of 0.5% proparacaine hydrochloride (Alcaine; Alcon Laboratories Inc) were applied to both eyes of the participants. The participants were then instructed to close their eyes and rest for 5 minutes. The examiner pulled the lower eyelid and gently swiped away excess tears if present. The paper strip then was inserted and results were recorded in the same way as described for the Schirmer test without anesthesia.

Botulinum Toxin Type A Injection
After completion of the TBUT and Schirmer tests, the patient received botulinum toxin type A (Botox; Allergan) injection by 1 ophthalmologist (W.-C.H.). Two different doses were administered: 3 injections with 2 U in 0.05 mL of normal saline per injection (dose A) and 3 injections with 4 U in 0.05 mL of normal saline per injection (dose B). The injection sites are shown in Figure 1. One eye of each participant was randomly chosen for dose A, and the other eye received dose B. The randomization and preparation of botulinum were performed by the same operator (Y.-T.H.). The examiner who performed the TBUT and Schirmer tests (M.-C.H.), the physician who performed the botulinum injections (W.-C.H.), and all participants were masked to the dosage.

Follow-up Examinations
The participants underwent TBUT and Schirmer tests without and with anesthesia at 1 week, 1 month, 3 months, and 6 months after the botulinum injections.

Statistical Analysis
To capture the correlation between 2 eyes of the same participant, linear mixed models were used for comparison of TBUT and Schirmer test results between baseline and postinjection.
times with the participant and the injected eye as random effects. Linear mixed models were also used for comparison of botulinum toxin effects of different doses with the participant as a random effect. \( P < .05 \) was considered statistically significant. SAS statistical software, version 9.1 (SAS Institute Inc), was used for all statistical analyses.

**Results**

A total of 58 female participants with a mean (SD) age of 46.3 (10.6) years were included in the study. Of the 58 participants, 20 were 30 to 40 years of age, 17 were 40 to 50 years of age, and 21 were 50 to 60 years of age. Baseline TBUT and Schirmer test results are given in **Table 1**. All participants completed the 1-week and 1-month follow-up examinations, 50 (86.2%) completed the 3-month follow-up examinations, and 42 (72.4%) completed the 6-month follow-up examinations.

**Baseline TBUT and Schirmer Tests Results Among Different Age Groups**

Among the 3 age groups, participants 30 to 40 years of age had the longest TBUT and the largest Schirmer test values, both with and without anesthesia, whereas those 50 to 60 years of age had the shortest TBUT and the smallest Schirmer test values without anesthesia at baseline (Figure 2). The results of TBUT and Schirmer tests after botulinum injection for lateral canthal rhytids are given in **Table 2**. The TBUT decreased significantly at 1 week after botulinum injection (\( P = .003 \)) and then persisted through 1 month and 3 months after the injection (\( P = .01 \) and \( .02 \), respectively), with partial recovery at 6 months after the injection. Both Schirmer tests with and without anesthesia decreased to a trough level at 1 month after botulinum injection (\( P = .05 \) for test without anesthesia and \( P = .02 \) for test with anesthesia) and then recovered gradually, with full recovery at 6 months after the injection.

**Botulinum Toxin Effects Among Different Age Groups**

The results of TBUT and Schirmer tests after botulinum toxin type A injection by age group are shown in **Figure 3**. For participants 30 to 40 years of age, TBUT decreased to a trough at 1 week after botulinum toxin injection and then recovered gradually with full recovery at 6 months. For participants 40 to 50 years of age, TBUT decreased throughout the first 3 months, with only partial recovery at 6 months after injection. For participants 50 to 60 years of age, TBUT continually decreased throughout the 6-month postinjection follow-up period. In all age groups, Schirmer tests with and without anesthesia exhibited the lowest values at 1 month after injection and then recovered gradually.

![Figure 1. Three Injection Sites of Botulinum Toxin](https://example.com/figure1)

The 3 sites are 1 cm superotemporally from the lateral bony orbital rim, 1 cm temporally from the lateral bony orbital rim, and 1 cm inferotemporally from the lateral bony orbital rim.

![Figure 2. Baseline Tear Film Break-up Time (TBUT) and Results of Schirmer Tests With and Without Anesthesia in Different Age Groups](https://example.com/figure2)

<table>
<thead>
<tr>
<th>Age Group, y</th>
<th>30-40</th>
<th>40-50</th>
<th>50-60</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBUT, s</td>
<td>8.4 (6.4)</td>
<td>10.7 (7.0)</td>
<td>8.5 (7.4)</td>
</tr>
<tr>
<td>Schirmer test result, mm</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Without anesthesia</td>
<td>20.3 (12.0)</td>
<td>28.9 (9.3)</td>
<td>18.4 (10.3)</td>
</tr>
<tr>
<td>With anesthesia</td>
<td>14.2 (9.5)</td>
<td>19.8 (10.8)</td>
<td>11.1 (7.2)</td>
</tr>
</tbody>
</table>

**Table 1. Baseline TBUT and Schirmer Test Results**

- Abbreviation: TBUT, tear film break-up time.
Botulinum Toxin Effects of Different Dosages
Changes of the results of TBUT and Schirmer tests after injections of botulinum toxin of different dosages are given in Table 3. Both TBUT and Schirmer test results decreased more in eyes that received dose B than in those that received dose A; however, none of the differences were statistically significant.

Discussion
Botulinum toxin is an exotoxin produced by Clostridium botulinum. It works as a neurotransmitter blocker by acting on cholinergic nerve terminals. The terminals of postganglionic neurons of parasympathetic nerves have synaptic vesicles that contain acetylcholine. After fusing with the membrane, the acetylcholine in vesicles is released into the neuromuscular junction and acts on the target muscle. The fundamental mode of action of the botulinum is to inhibit extracellular release of acetylcholine to the neuromuscular junction. By injecting botulinum toxin in the area of the target muscle, the muscle can be paralyzed.5

Several studies1,2,6 have reported that dry eye may occur after botulinum A injection in the periocular area. On the other hand, Arat and Yen3 reported that botulinum injection for lateral canthal rhytids did not significantly suppress tear production, but significant decrease in tear production was noted in certain cases (5 of 26 eyes). In that study, however, only 13 patients (ranging in age from 31 to 58 years) were included, and thus the results may have been affected by the small number of cases. In our study, we prospectively recruited 58 women 30 to 60 years of age to receive botulinum toxin type A injection for lateral canthal rhytids and compared their Schirmer test and TBUT results before and after injections. We found that both Schirmer tests with and without anesthesia decreased to the trough level at 1 month after the injection and then recovered gradually, and TBUT decreased significantly at 1 week and the decrease persisted through 3 months after the injection.

The innervation of the lacrimal gland is somewhat complex. For reflex tearing, irritation of the ocular surface activates the afferent pathway of the reflex tear arc through the trigeminal nerve. The parasympathetic fibers originate from the superior salivary nucleus of the pons and exit the brainstem with the facial nerve. The lacrimal nerve then leaves the facial nerve as the greater superficial petrosal nerve, passes to the sphenopalatine ganglion, and finally enters the lacrimal gland. Although still debated, the innervation of the main lacrimal gland is mainly sympathetic, and the parasympathetic innervation is limited to the puncta and the tear lake.7

Table 2. TBUT and Schirmer Test Results After Botulinum Toxin Type A Injection

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline, Mean (SD)</th>
<th>1 Week</th>
<th>1 Month</th>
<th>3 Month</th>
<th>6 Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBUT, s</td>
<td>8.4 (6.4)</td>
<td>6.2 (4.7)</td>
<td>6.6 (5.2)</td>
<td>6.6 (5.6)</td>
<td>7.3 (5.7)</td>
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<tr>
<td>Schirmer test result, mm</td>
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<tr>
<td>Without anesthesia</td>
<td>20.3 (12.0)</td>
<td>19.4 (11.3)</td>
<td>17.6 (11.5)</td>
<td>18.5 (11.3)</td>
<td>20.9 (11.8)</td>
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<tr>
<td>With anesthesia</td>
<td>14.2 (9.5)</td>
<td>13.3 (9.2)</td>
<td>11.5 (8.2)</td>
<td>13.4 (9.9)</td>
<td>14.8 (9.8)</td>
</tr>
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</table>

Abbreviation: TBUT, tear film break-up time.
*P values are for comparison with the baseline value.

Figure 3. Tear Film Break-up Time (TBUT) and Results of Schirmer Tests With and Without Anesthesia Before and After Botulinum Toxin Type A Injection in Different Age Groups

Age Group, y
- 30-40
- 40-50
- 50-60
Flynn and Clark reported that the area of wrinkle reduction was bilateral septums. Hsu et al injected botulinum toxin type A into mal glands by passing through the orbicularis muscles and or-al cases. This infers that the botulinum can diffuse into the lacri-mal glands. Our study also found similar results, but the depth of botulinum injection was limited to the layer of orbicularis oculi at lateral canthal areas. These studies confirmed the inhibitory effect of botulinum toxin in lacrimal glands. Our study also found that the parasympathetic fibers not the sympathetic fibers, the result implies that both basal and reflex tearing may be at least partially innervated by parasympathetic nerves.

Keegan et al reported that direct injection of 20 U of botu-linum toxin into the lacrimal glands in cases of crocodile tears significantly reduces Schirmer test values by 7 to 9 mm without food or exercise stimulation. The amount of reduction may be up to 26 to 55 mm with stimulation. Hofmann also demonstrated complete resolution of gustatory epiphora after intraglandular and subcutaneous injection of botulinum toxin around lateral canthal areas. These studies confirmed the inhibitory effect of botulinum toxin in lacrimal glands. Our study also found similar results, but the depth of botulinum injection was limited to the layer of orbicularis oculi at lateral canthal areas in our cases. This infers that the botulinum can diffuse into the lacri-mal glands by passing through the orbicularis muscles and or-bital septums. Hsu et al injected botulinum toxin type A into the frontal muscle and defined the diffusion area by observ-ing the paralyzed area of the frontalis. The mean diffusion area after an injection of 5 U of botulinum toxin was 4.12 to 6.05 cm². Flynn and Clark reported that the area of wrinkle reduction was approximately 17 cm² after 1 injection of 5 U of botulinum toxin type A at the frontalis. These studies indicate that botulinum toxin may diffuse transversely as far as 2 to 4 cm from the injection site. However, no studies have demonstrated the diffusion depth of botulinum toxin. We hypothesize that the injected botulinum toxin must diffuse through the orbicularis muscles and orbital septums to react with the lacrimal glands.

The parasympathetic fibers in pterygopalatine ganglion originate from the superior salivatory nucleus and regulate the function of lacrimal glands and meibomian glands. Botulinum toxin chemodenervates the release of acetylcholine from parasympathetic terminals and therefore inhibits the lipid production from meibomian glands. Meibomian glands are surrounded by the pretarsal orbicularis muscle and the muscle of Riolan. The bundles of Riolan muscle are arranged around the ducts that lead to the orifice of the meibomian glands; contraction of the muscle of Riolan leads to expression of meibomian gland secretions. Paralysis of these muscles by botulinum toxin will decrease the driving force for meibomian oil excretion. Therefore, botulinum toxin injection may result in lipid insufficiency of the tear film layer. The TBUT is a simple index for tear film stability. Insufficient aqueous tear production or an incomplete lipid layer that leads to early evaporation of aqueous tear will reduce the TBUT. Our study revealed a significant decrease of TBUT at 1 week after botulinum injection at lateral can-thal areas. This may be related to suppression of meibomian gland function because the orbicularis oculi and muscles of Riolan were paralyzed. For participants older than 50 years, the TBUT continued to decrease through the entire follow-up period. We think this might be related to laxity of the lower eyelid after the orbicularis oculi was paralyzed in older people, which would hinder tear film redistribution by blinking and further affect the TBUT. However, we did not record whether there was lid laxity in our cases. On the other hand, botulinum toxin treatment for blepharospasm has been found to increase tearing. Sahlin et al reported that injection of botulinum toxin into the medial parts of the lower eyelids could paralyze the nasal orbicularis muscles that surround the canaliculi and decrease the pumping force for draining tears into the nasolacri-mal system. We only injected botulinum at crow’s feet areas, so the lacrimal drainage system should be only minimally involved. In this study, however, we did not measure meibo-mian gland function or the lacrimal drainage function directly, and this merits further study.

<table>
<thead>
<tr>
<th>Table 3. Changes of TBUT and Schirmer Test Results From Baseline Values Between Different Dosages of Botulinum Toxin Type A Injection</th>
<th>Change From Baseline</th>
<th>1 Week</th>
<th>Months</th>
<th>1</th>
<th>3</th>
<th>6</th>
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<tr>
<td>Mean (SD)</td>
<td>P Value</td>
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<td>TBUT, s</td>
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<td>Schirmer test, mm</td>
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<td>Without anesthesia</td>
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<td>Dose A</td>
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<td>Dose B</td>
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<td>Dose B - dose A</td>
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<td>Dose A</td>
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<td>Dose B</td>
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<td>Dose B - dose A</td>
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| Abbreviation: TBUT, tear film break-up time.
Both the Schirmer tests and TBUT decreased after botulinum injection, but the patterns of decrease were different. The Schirmer test results decreased gradually to a trough at 1 month, whereas the TBUT exhibited a sharp decrease during the first week after botulinum injection (Table 2), especially in the youngest age group (30-40 years, Figure 3). We think that after botulinum toxin type A was injected into the orbicularis oculi muscle fibers near the lateral canthus, it directly blocked the function of orbicularis, which might result in disruption of blinking reflex and even temporary lower eyelid laxity. It then diffused quickly to nearby meibomian glands to block the secretion and delivery of meibomian oil. Therefore, TBUT was affected as early as 1 week after treatment because meibomian oil and blinking function are important for maintenance of tear films. As to the lacrimal gland, the distance for the injected botulinum toxin to diffuse around the orbital rim and through the orbital septum to reach the lacrimal gland was longer; therefore, it took a longer time for the aqueous production to decrease. This finding could explain the gradual decrease in Schirmer test results but the sharp decrease in TBUT after botulinum toxin injection.

Although the Schirmer test and TBUT are widely used in clinical practice, their results represent only a broad view of the ocular surface rather than mechanisms in detail. The secretion, excretion, and drainage of tear and lipid influence the stability of the ocular surface. The Schirmer test and TBUT are indirect methods to measure the influence of botulinum toxin on lacrimal glands and meibomian glands. Nevertheless, these results are closely related to clinical symptoms and can reflect the clinical effect of botulinum toxin. According to our study results, botulinum injection for lateral canthal rhytids will decrease Schirmer test and TBUT results. Patients who are going to receive botulinum treatment should be informed about the possible complications of dry eye symptoms.

Our study has some limitations. We did not evaluate the extent of cornea surface erosions or administer questionnaires for subjective dry eye symptoms because we did not limit artificial tear use in the study participants for ethical concerns; therefore, the examinations for corneal erosions or subjective questionnaires might not reflect the real effect of botulinum toxin. In addition, meibomian gland function and lacrimal drainage function were not evaluated in this study. Further study should focus on them.

In conclusion, we found that tear film stability decreased as soon as 1 week after botulinum toxin type A treatment for lateral canthal rhytids, and the effect persisted for more than 3 months. Younger participants tended to have faster TBUT recovery. Tear production decreased to a trough level at 1 month after treatment and then recovered gradually. Higher doses of botulinum toxin seemed to be related to greater effects.

REFERENCES