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Clinical trial

The clinical effect of botulinum toxin on pigmentation

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Abstract

Background Botulinum toxin injection is a common cosmetic procedure often used to treat dynamic wrinkles, but it has also been observed to have a lightening effect on the skin. It is thought that this lightening effect develops due to muscle innervation blockage; however, the change in the amount of melanin levels has not been quantified.

Method Thirty-one patients who presented to the dermatology clinic of a tertiary hospital for botulinum toxin injection for wrinkle treatment were included in the study. A standard dose of botulinum toxin was injected to each patient's forehead, glabellar, and crow's feet region, and then the melanin index (MI) was measured with the Mexameter® MX 18 (Courage + Khazaka Electronic, Köln).

Results After botulinum toxin treatment, a statistically significant decrease was found in the forehead and upper face MI. The upper face total baseline MI was significantly lower in the Glogau 1 group than in the Glogau 2 group ($P = 0.033$). The forehead 15th day MI was significantly lower in the Glogau 1 group than in the Glogau 2, 3, and 4 groups ($P = 0.030$).

Discussion Botulinum toxin application to healthy skin for wrinkle treatment can cause facial skin lightening by reducing MI. It was also remarkable that this decrease was more pronounced in the forehead, which is a region that is particularly vulnerable to sun exposure, compared to other regions. Younger people, who are included in the Glogau type 1 group, may benefit more from this lightening effect.

Background

Botulinum toxin type A is a protein obtained from a microorganism called *Clostridium botulinum*. Botulinum toxin partially blocks the nerve impulses to the injected muscles and reduces the excessive contraction of muscles by creating temporary and reversible paralysis.[1] In addition, it reduces the amount of sweat formed by acting on sweat glands and prevents the development of migraines by preventing pain signals.[2] When used for wrinkle treatment, improvement in the depth of lines usually occurs within the week after treatment. The maximum effect is observed 5–6 weeks after injection. The treatment effect has been shown to persist for up to 4 months after injection.

Botulinum toxin type A injection is a common cosmetic procedure often used to treat dynamic wrinkles, but it has also been observed to have a lightening effect on the skin. Recent publications have also suggested that botulinum toxin type A may have a lightening effect on periorbital skin pigmentation.[3–5] Jung et al. investigated the mechanism of action of botulinum toxin type A on skin pigmentation. Their findings suggest that botulinum toxin type A can decrease tyrosinase activity and melanin amounts. In this way, botulinum toxin type A can suppress epidermal melanogenesis.[6]

Although it is thought that this lightening effect develops as a result of muscle innervation blockage, according to new studies, this effect may also occur through melanin. However, the change in the amount of melanin levels in the skin after botulinum toxin type A treatment has not yet been quantified.

Methods

Patient selection and inclusion

Thirty-one patients who were applied to the dermatology clinic of a tertiary hospital for botulinum toxin injection for wrinkle treatment were included in the study. Patients who had underlying neuromuscular diseases (e.g., myasthenia gravis, amyotrophic lateral sclerosis, or Lambert-Eaton myasthenic syndrome), had been taking any oral aminoglycoside agent, benzodiazepine, or muscle relaxants for 4 weeks preceding the study, had undergone any aesthetic procedure or received botulinum toxin treatment to the face in the preceding 6 months, had an autoimmune disease such as systemic lupus erythematosus, had facial flushing due to menopause, were pregnant or lactating, had a history of allergy to the botulinum toxin, or had tanned after botulinum toxin treatment were excluded from this study.

Patients' ages and genders were recorded. In addition, patients' Fitzpatrick skin types and Glogau classification of photoaging (mild: 1, moderate: 2, advanced: 3, and severe: 4) were evaluated and recorded by a dermatologist.

The Institutional Ethics Committee of the Istanbul Training and Research Hospital approved the study protocol. In addition, written informed consent was obtained from the patients.

Botulinum toxin injection

Each 100 units of onabotulinumtoxinA (BOTOX®, Allergan Phramaceuticals, Mayo, Ireland) was reconstituted with 2.5 ml of bacteriostatic 0.9% sodium chloride. First, the forehead, glabella, and crow's feet were wiped with disinfectant. Then, a standard dose of botulinum toxin type A was applied to each patient by a dermatologist with a 30-gauge needle. On the forehead, 18 units of botulinum toxin type A were injected to 10 points in total, targeting the frontalis muscle. Sixteen units of botulinum toxin type A was injected to five points in total in the glabella region, and the procerus and corrugator supercilii muscles were targeted. Then, a total of 24 units of botulinum toxin type A was injected to the bilateral crow's feet area, targeting three points on each side and the orbicularis oculi muscle (Figure 1).

Evaluation of the melanin and erythema indices

The melanin index (MI) and erythema index (EI) were measured with the Mexameter® MX 18 (Courage + Khazaka Electronic, Köln) in a closed environment with a constant temperature (21–23°C) and controlled air humidity (40–60%). The Mexameter contains 16 diode lamps located at the periphery of a photodetector. The lamps emit light at 568, 660, and 880 nm, corresponding to green, red, and infrared light. After the emission of green and red light, detecting their reflection from the skin with a photodetector allows the calculation of the content of cutaneous hemoglobin (cutaneous erythema in units of its own). In addition, the reflections from the red and near-infrared wavelengths of light allow for the calculation of skin melanin content values (skin melanin level in units of its own). Melanin and erythema indices were calculated using the formulas MI = log10 (R870nm/R660nm) × 1,000 and EI = log10 (R660nm/R568nm) × 1,000. The results were expressed in arbitrary units (AU).

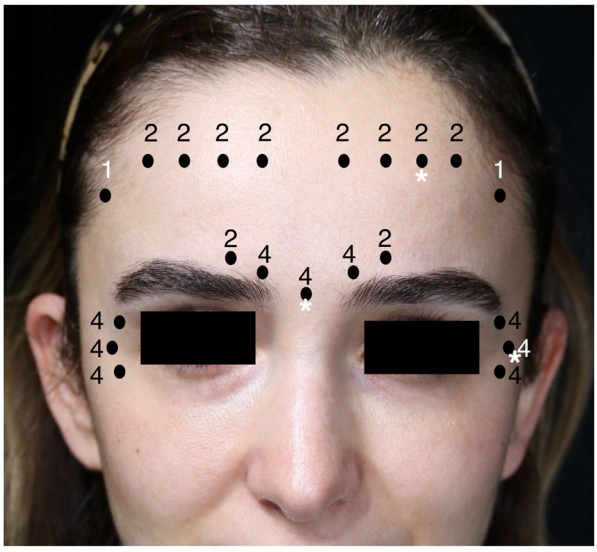

Figure 1 Black spots: Botulinum toxin type A injection points and doses, White stars: MI and EI measurement points

All participants sat quietly in the room for at least 30 minutes before measurement to acclimate their skin to environmental conditions. Participants had been asked to discontinue all topical medications and cosmetics for 24 hours prior to the study. In addition, they had been asked to avoid skincare products, intense exercise, and sweating for at least 2 hours prior to the measurements. The Mexameter® MX 18 (Courage + Khazaka Electronic, Köln) was used to measure melanin and erythema photometrically. For each participant, three measurements were made: on the forehead, glabella, and left crow's feet (periorbital). This was done prior to and on the 15th day after botulinum toxin injection, and average values were used (Figure 1). The upper face total score was calculated by means of forehead, glabella, and periorbital area measurements. Photographs of the patients were taken before and on the 15th day after botulinum toxin injection.

Statistical analysis of the study

Statistical analyses were performed with the help of the SPSS version 23.0 program. The conformity of the variables to the normal distribution was examined using histogram graphs and the Kolmogorov–Smirnov/Shapiro–Wilk test. Mean, standard deviation, and median values were used when presenting descriptive analyses. The Mann–Whitney *U* test was used to evaluate non-normally distributed (nonparametric) variables between the two groups, and the Kruskal–Wallis test was used to evaluate more than two groups. Bonferroni multiple comparison tests were used to investigate the significant differences between the groups. The Wilcoxon signed-rank test was used to investigate the differences between the two dependent groups. While presenting the categorical variables, the frequency and percentage values of the variables were used. Spearman's correlation test was used to evaluate the relationships between the quantitative variables. Cases with a p-value below 0.05 were considered statistically significant.

Results

Thirty-two patients, 30 females and two males, were included in the study. One female patient was excluded from the study because she tanned after a botulinum toxin type A injection. The mean age of the patients participating in the study was 39.81 ± 11.10 years. Fitzpatrick's skin type for the patients participating in the study was 1 for two patients, 2 for 22 patients,

and 3 for three patients. None of the patients included in the study had Fitzpatrick skin types 4, 5, or 6. According to the Glogau Classification of Photoaging (Table 1), seven participants were mild, 16 were moderate, seven were advanced, and one was in the severe group.

On the 15th day after the botulinum toxin type A treatment, a decrease was observed in the average MI in all regions, but a statistically significant difference was detected in the forehead and upper-face MI ($P = 0.029$ and $P = 0.040$). No statistically significant change was observed in EI values on the 15th day after the botulinum toxin type A treatment (Table 2).

In terms of the Glogau Classification of Photoaging, the baseline and 15th-day MI were compared. Forehead 15th day MI was significantly lower in the Glogau 1 group than in the Glogau 2 group or Glogau 3–4 groups ($P = 0.030$). Periorbital baseline MI was significantly lower in the Glogau 1 group than in the Glogau 2 and Glogau 3–4 groups ($P = 0.035$). The upper-face baseline MI was significantly lower in the Glogau 1 group than in the Glogau 2 group ($P = 0.033$) (Table 3) (Figures 2 and 3).

The relationship between age and baseline MI was examined (Table 4). There were moderate positive and significant relationships between age and forehead, periorbital, and upper face MI ($P < 0.05$). When the relationship between age and MI on the 15th day after botulinum toxin type A treatment was examined, there were moderate, positive, and significant relationships between age and forehead MI ($P < 0.05$). As age increased, the measurement levels that had a significant relationship with age also increased. As age increased, the total level of MI also increased ($r = 0.425$; $P = 0.017$). In the measurements 15 days after the botulinum toxin type A injection, it was determined that there was a relationship between age and MI only in the forehead region ($r = 0.376$; $P = 0.037$).

Table 1 Sociodemographic and clinical characteristics of the patients

	***n* (%)**
Age, $\overline{x} \pm$ SD	39.81 ± 11.10
Sex	
Female	29 (93.55)
Male	2 (6.45)
Fitzpatrick skin type	
1	6 (19.35)
2	22 (70.97)
3	3 (22.58)
Glogau classification of photoaging	
1- Mild	7 (22.58)
2- Moderate	16 (51.61)
3- Advanced	7 (22.58)
4- Severe	1 (3.23)

Table 2 Comparison of baseline and 15th-day MI and EI

	Mean (±SD), Median		
	Baseline	**15th day**	***P***
Glabella			
MI	170.15 (±79.89), 161.67	157.30 (±37.38), 154.67	0.550
EI	420.67 (±175.76), 361.00	367.13 (±127.79), 348.33	0.232
Forehead			
MI	236.54 (±161.31), 202.00	193.49 (±67.70), 179.00	0.029
EI	345.62 (±118.00), 311.00	325.26 (±89.36), 316.67	0.597
Periorbital			
MI	216.97 (±129.84), 182.67	183.76 (±76.26), 184.33	0.290
EI	302.40 (±98.66), 262.67	336.59 (±144.77), 288.00	0.193
Upper face total			
MI	207.89 (±86.06), 192.67	178.19 (±44.84), 181.22	0.040
EI	356.23 (±104.90), 320.78	342.99 (±78.18), 313.00	0.875

Discussion

Human skin color is affected by skin chromophores, including melanin, hemoglobin, and various carotenes. The amount and epidermal distribution of melanin is the distinguishing feature of skin color.[7,8] The association of each melanocyte with keratinocytes is known as an epidermal melanin unit. Melanin biosynthesis takes place within the melanosome, the metabolic unit of the melanocyte. Within this pathway, tyrosinase is the key regulatory enzyme. Then, the melanosomes are transferred from the melanocyte to the neighboring keratinocytes of the epidermis. Racial and ethnic differences in skin color result from differences in the number, size, and clustering of melanosomes within melanocytes and keratinocytes. There are no racial differences in the number of melanocytes. The actual melanocyte count may differ from person to person and from one anatomical region of the body to another; for example, the head and forearm have the highest number of melanocytes.[9] Botulinum toxin type A is commonly injected to the face, which is currently the area most exposed to UV damage and prone to pigmentation. Therefore, in this study, we aimed to evaluate the effect of botulinum toxin type A on pigmentation by measuring MI from the facial area.

The botulinum toxin is taken up by the peripheral nerve terminals and locally blocks the release of acetylcholine neurotransmitters, leading to muscle paralysis. Melanocytes, like neurons, are of ectoderm origin. Melanosomes that provide pigmentation are transmitted to keratinocytes via dendritic connections similar to those in neurons. Jung et al. investigated the intracellular penetration of botulinum toxin type A in cultured human epidermal melanocytes, human epidermal keratinocytes, neuroblastoma cells (as a positive control), and human dermal fibroblasts (as a negative control). The study found that Botulinum toxin type A was taken up into cells by melanocytes and keratinocytes. They found that melanocyte dendricity and melanin content decreased after botulinum toxin type A treatment. In the same study, the effects of botulinum toxin type A injected after ultraviolet B

Table 3 Comparison of baseline and 15th-day MI in terms of the Glogau Classification of Photoaging

	Glogau Classification of Photoaging									
	1 (n = 7)			2 (n = 16)			3–4 (n = 8)			
MI	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	*P*
Glabella Baseline	181.76	±155.72	127.67	171.83	±51.16	165.83	156.63	±24.74	164.67	0.199
Glabella 15th day	152.19	±61.51	128.67	157.02	±31.14	151.50	162.33	±24.75	156.83	0.505
Forehead Baseline	164.90	±39.26	146.00	206.79	±43.59	209.00	358.71	±286.20	218.83	0.055
Forehead 15th day	134.81	±53.95	138.00[a]	215.50	±70.64	192.67[b]	200.83	±42.88	201.33[b]	0.030
Periorbital Baseline	140.86	±40.25	128.33[a]	227.60	±135.81	184.67[b]	262.29	±151.47	199.33[b]	0.035
Periorbital 15th day	144.29	±58.55	117.67	206.85	±90.62	185.17	172.13	±38.18	178.83	0.151
Upper face Baseline	162.51	±73.47	136.56[a]	202.08	±55.77	199.39[b]	259.21	±123.69	220.17[a,b]	0.033
Upper face 15th day	143.76	±49.48	119.89	193.13	±43.15	188.28	178.43	±28.88	182.72	0.124

Figure 2 Photographs of a 29-year-old, Glogau type 1 female patient before (a–c) and after (d–f) botulinum toxin type A injection

irradiation in a mouse were examined on skin pigmentation, and it was found that tyrosinase activity and melanin content were also reduced. In other words, in vivo, botulinum toxin type A has been reported to suppress skin pigmentation and might have a protective effect against ultraviolet-induced skin pigmentation.[6] In our study, we found a decrease in MI most prominently and significantly in the forehead region. However, according to the wrinkle treatment protocol, the amount of botulinum toxin type A per square centimeter in the forehead region was lower than in the periorbital region. We attribute this result to the fact that the forehead is a more susceptible area to UV rays and to the UV-protective effect of botulinum toxin type A.

Any inflammation or injury to the skin can cause changes in pigmentation, often through post-inflammatory hyperpigmentation. There are studies showing the effect of botulinum toxin type A on wound healing and preventing post-inflammatory hyperpigmentation. This effect could be mediated mainly by the anti-inflammatory effects of botulinum toxin type A. Botulinum toxin type A injection decreases basic fibroblast growth factor, interleukin-1α, prostaglandin E2, substance P, transforming growth factor beta1 levels, and inflammatory cell infiltration.[6,10–12] A few exceptional publications in the literature report that skin color changes after botulinum toxin application. Friedland et al. reported that porcelain-like whitening of the skin with

Figure 3 Photographs of a 42-year-old, Glogau type 3 female patient before (a–c) and after (d–f) botulinum toxin type A injection

Table 4 The relationship between age and baseline MI

	Age (Baseline)	Age (15th day)
Glabella MI		
r	0.304	0.335
P	0.096	0.066
Forehead MI		
r	0.409	0.376
P	0.022	0.037
Periorbital MI		
r	0.375	0.057
P	0.037	0.760
Upper face MI		
r	0.425	0.224
P	0.017	0.225

Scale of correlation coefficient	Value
$0 < r \leq 0.19$	Very Low Correlation
$0.2 \leq r \leq 0.39$	Low Correlation
$0.4 \leq r \leq 0.59$	Moderate Correlation
$0.6 \leq r \leq 0.79$	High Correlation
$0.8 \leq r \leq 1.0$	Very High Correlation

concomitant edema developed in three patients after applying very high doses of botulinum toxin to the eye area.[4] Carruthers et al. designed a split-face study and compared the efficacy of whole-face broadband light treatments administered alone and in combination with botulinum toxin type A. They reported that a more successful treatment response was obtained in lentigines with broadband light therapy combined with botulinum toxin.[5] Yamauchi et al. combined laser skin resurfacing and botulinum toxin treatment for periorbital wrinkles in their study. They reported that the combined approach resulted in significantly greater improvement in texture, pigmentation, and wrinkle reduction.[3] Although positive effects of botulinum toxin on pigmentation in combination treatments were reported in these studies, as far as we know, no study has evaluated the effects of the sole use of botulinum toxin on pigmentation. In our study, we evaluated pigmentation in the areas where botulinum toxin type A was applied for the treatment of upper facial wrinkles by measuring MI. Although MI was decreased in forehead, glabella, periorbital region, and upper face measurements, a statistically significant difference was found only in forehead region and upper face measurements. As we mentioned, we attribute this result to the fact that the forehead is a more sensitive area to UV rays and to the UV-protective effect of botulinum toxin type A.

The number of active melanocytes in the skin decreases almost 10–20% per decade with age. The melanocyte density is approximately twice as high in chronically sun-exposed skin as in protected skin at any age.[13,14] The main effects of chronic sun exposure on melanocytes have been claimed to be activation and proliferation.[15] To our knowledge, there are not enough

clinical studies to explain age-related changes in melanocytes. In this study, we investigated the relationship between age and baseline MI and found moderate, positive, and significant relationships between age and forehead, periorbital area, and upper-face total MI ($P < 0.05$). Our study found an increase in MI with increasing age. We also compared the MI of patients at baseline and on the 15th day after botulinum toxin type A injection according to the Glogau Photoaging Classification. The upper face total baseline MI was lower in the Glogau 1 group than in the Glogau 2 group. The forehead 15th day MI was significantly lower in the Glogau 1 group than in the Glogau 2 and Glogau 3–4 groups. This revealed that the effect of a reduction in melanin on the forehead after botulinum toxin application was more pronounced in the Glogau 1 group than in the other groups. Younger people who are included in the Glogau type 1 classification may benefit more from this lightening effect.

Microdermal injections of botulinum toxin type A have been shown to be effective in rosacea by reducing redness, erythema, and inflammation.[16] The possible mechanism by which botulinum toxin type A can ameliorate rosacea might be the inhibition of acetylcholine release from the peripheral autonomic nerves of the cutaneous vasodilator system. Other mechanisms could be the inhibition of the release of neurotransmitters such as substance P, glutamate, and calcitonin-related peptide, or the reduction of non-nociceptive stimuli by the alteration of postganglionic cholinergic nerve fibers with blood vessels.[17,18] Our study found no significant decrease in EI after botulinum toxin type A injection. The erythema-reducing effect in rosacea could be due to its anti-inflammatory effect, as in post-inflammatory hyperpigmentation. The reason why there was no decrease in EI after botulinum toxin type A injection in our study might be because the procedure was performed on healthy skin.

After botulinum toxin type A treatment, the general opinion is that patients should be evaluated after 14 days to see the treatment response, which allows touch-ups to be made if necessary.[19] For this reason, we evaluated the patients to whom we applied wrinkle treatment on the 15th day and made post-treatment melanin and erythema measurements. Delaying measurement of melanin values after botox injection can bring more confounding factors such as UV rays, sunscreen use, and relocation, among other things. Furthermore, it is unclear how long the lightening effect that we detected on the 15th day lasts. In further studies, the long-term effects of the botulinum toxin on pigmentation might be examined.

Another controversy is that a smoother skin surface after botulinum toxin injection might reduce the cutaneous light absorption in reflectance spectrophotometers, such as the Mexameter® MX 18 (Courage + Khazaka Electronic, Köln). According to the working principle of Mexameter® MX 18 (Courage + Khazaka Electronic, Köln), the probe makes direct contact with the skin surface and measures with constant pressure (the application pressure on the skin is 91 g/cm^2). Ensuring adequate and standard surface tension with the probe may increase the probability of correct measurement with the Mexameter® MX 18 (Courage + Khazaka Electronic, Köln).

Conclusion

Botulinum toxin type A injection to healthy skin for treatment of dynamic wrinkles can also cause facial lightening by reducing MI. We observed that this decrease was more pronounced in the forehead, a region that is particularly vulnerable to sun exposure. Younger people, who are included in the Glogau type 1 classification, may benefit more from this lightening effect.

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