



Multicenter Study: Effectiveness and Efficiency of Prabotulinum Toxin-A Injection on Wrinkles in Forehead, Crow's Feet, and Glabellar

Sinta Murlistyarini^{1*}, Adinda Amalia Dani¹, Lilik Norawati², Rita Maria², Abraham Arimuko², Arie Kusumawardani³, Minna Hasniah³

¹Department of Dermatology and Venereology, Faculty of Medicine, Universitas Brawijaya, Regional Public Hospital dr. Saiful Anwar, Malang, Indonesia; ²Department of Dermatology and Venereology, Presidential Gatot Soebroto Central Army Hospital, Jakarta, Indonesia; ³Department of Dermatology and Venereology, Faculty of Medicine, Universitas Sebelas Maret, Regional Public Hospital dr. Moewardi, Surakarta, Indonesia

Abstract

BACKGROUND: Prabotulinumtoxin A is the newest type A botulinum toxin produced by wild-type *Clostridium botulinum*. Prabotulinumtoxin A has a higher purity level than onabotulinum toxin A, but the literature discussing it is still limited and requires more research on its effectiveness.

AIM: This research aimed to examine the efficacy of Prabotulinumtoxin A on wrinkles in forehead, crow's feet, and glabellar.

METHODS: This research was a pre- and post-test and multicenter study from three center in Indonesia. A total of 121 subjects will be observed at week 0, week 2, week 4, week 8, and week 12. Injection of Prabotulinumtoxin A (INIBO[®], Covaltt, South Korea) was performed intramuscularly on forehead, crow's feet, and glabellar with a dose of 4 IU/0.1 ml. The measurement uses a scoring from the Upper Face Rating Scale. Different statistical test with repeated measurement test was conducted to compare the results between observations. The statistical test uses a confidence level of 0.95 with an error rate (α) = 0.05.

RESULTS: The results of this study showed that Prabotulinumtoxin A injection was effective in reducing wrinkles in the frontal, glabellar, and crow's feet area, with a statistically significant difference between week 0 and week 12.

CONCLUSION: Prabotulinumtoxin A products improve the condition of wrinkles with a fairly good level of satisfaction and efficacy. Observations of more than 12 weeks are needed to determine the duration of Prabotulinumtoxin A products.

Edited by: Ksenija Bogoeva-Kostovska
Citation: Murlistyarini S, Dani AA, Norawati L, Maria R, Arimuko A, Kusumawardani A, Hasniah M. Multicenter Study: Effectiveness and Efficiency of Prabotulinum Toxin-A Injection on Wrinkles in Forehead, Crow's Feet and Glabellar. Open Access Maced J Med Sci. 2022 Jul 25; 10(B):1927-1931. https://doi.org/10.3889/oamjms.2022.9834
Keywords: Crow's feet; Dynamic wrinkle; Forehead wrinkle glabellar wrinkles; Prabotulinumtoxin A
***Correspondence:** Sinta Murlistyarini, Department of Dermatology and Venereology, Faculty of Medicine, Universitas Brawijaya/Regional Public Hospital dr. Saiful Anwar, Malang, Indonesia. E-mail: sintamur@ub.ac.id
Received: 30-Apr-2022
Revised: 17-May-2022
Accepted: 15-Jun-2022
Copyright: © 2022 Sinta Murlistyarini, Adinda Amalia Dani, Lilik Norawati, Rita Maria, Abraham Arimuko, Arie Kusumawardani, Minna Hasniah
Funding: This research was partially supported by INIBO[®], Covaltt, South Korea
Competing Interests: The authors have declared that no competing interests exist
Open Access: This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (CC BY-NC 4.0)

Introduction

Botulinum toxin (BTX) injection is known as one of the therapeutic options in the field of aesthetic medicine. BTX injection is relatively safe and effective, so this procedure is used by many people. BTX injection is widely used for various treatments in the fields of dermatology, esthetic medicine, secretory disorders, ophthalmology, and orthopedics [1].

Botulinum toxin is a neurotoxin derived from the bacterium *Clostridium botulinum* that works by paralyzing muscles by impairing signal transmission at the neuromuscular junction (NMJ) [2]. Botulinum toxin has seven types of neurotoxins, namely, Types A, B, C, D, E, F, and G which have different antigens, but structurally have homologous subunit structures [2]. Onabotulinum toxin A, which has a molecular weight of 900 kDa, was the first BTX toxin to be approved

by the FDA for use in facial esthetic procedures in the United States [3]. In 2002 the FDA approved BTX-A as a treatment for glabellar frown wrinkles [4], [5].

Prabotulinumtoxin A (PRA) is the newest Type A botulinum toxin produced by wild-type *Clostridium botulinum*, first produced by Daewoong Pharmaceutical Co., Ltd., South Korea [6]. Prabotulinumtoxin A had a higher level of purity than Onabotulinum toxin A, which was confirmed by chromatographic analysis (>98% for Prabotulinumtoxin A compared to 95% for Onabotulinum toxin A). A multicenter, double-blind, randomized, and active-controlled trial by Won *et al.* showed that PRA has satisfactory results for wrinkles in the glabellar area [6].

This research is a multicenter study on Prabotulinumtoxin A products on wrinkles on the forehead line, crow's feet, and glabellar frown in Indonesia. This research was conducted at (Regional Public Hospital dr. Saiful Anwar Malang/Faculty of Medicine Universitas Brawijaya Malang, Regional

Public Hospital dr. Moewardi Surakarta/Faculty of Medicine Universitas Sebelas Maret Surakarta, and Presidential Gatot Soebroto Central Army Hospital Jakarta). The aim of this study was to examine the efficacy of the product Prabotulinumtoxin A (INIBO®, Covaltt, South Korea) for dynamic wrinkle. There was no control group because this research was a pre- and post-test study.

Methods

This research was a pre- and post-test and multicenter study (Regional Public Hospital Dr. Saiful Anwar Malang/Faculty of Medicine Universitas Brawijaya Malang, Regional Public Hospital Dr. Moewardi Surakarta/Faculty of Medicine Universitas Sebelas Maret Surakarta, and Presidential Gatot Soebroto Central Army Hospital Jakarta). This study was approved by The Health Research Ethic Committee of Regional Public Hospital dr. Moewardi Surakarta no. 808/IX/HREC/2021. Subjects will be observed at week 0, week 2, week 4, week 8, and week 12.

Included in the inclusion criteria were 121 women aged 30–50 years, have wrinkles (on the forehead line, crow’s feet, and glabellar frown) with an Upper Face Rating Scale score (0–4) [7], and were willing to sign informed consent sheet for Prabotulinumtoxin A injection and willingness to be a research subject. Meanwhile, the exclusion criteria were having received BTX-A injection therapy within 6 months, suffering from severe systemic disease or malignancy.

Injection of the glabellar frown with Prabotulinumtoxin A (INIBO®, Covaltt, South Korea) was performed intramuscularly, as much as 5 points on M. Procerus, M. Corrugator supercillii, and M. Orbicularis Oculi, at a dose of 4 IU/0.1 ml. Meanwhile, for the forehead line also left and right crow’s feet, it is carried out according to the state of wrinkles with a dose of 4 IU/0.1 ml. The injection was done by two dermatologists at each center. After the injection, subject is prohibited from excessive facial massage and lying down. If side effects appear, it was suggested to contact the doctor immediately.

The measurement uses a scoring from the Upper Face Rating Scale. The Upper Face Rating Scale is an assessment method for assessing the score for wrinkles in the forehead line, crow’s feet, and glabellar frown areas as well as determining the position of the eyebrows specifically for gender, carried out under conditions of rest and maximum expression [7]. Monitoring by scoring the Upper Face Rating Scale was carried out at weeks 0, 2, 4, 8, and 12.

Statistical test was done using Statistical Package for the Social Sciences (SPSS) version 22.

Difference test with repeated measurement was conducted to compare the results of the Upper Face Rating Scale between observations. The statistical test uses a confidence level of 0.95 with an error rate (α) = 0.05 and $p < 0.001$.

Results

The study was conducted on 121 subjects with details of 42 subjects from Presidential Gatot Soebroto Central Army Hospital Jakarta, 32 subjects from Faculty of Medicine Universitas Sebelas Maret Surakarta and 47 subjects from Faculty of Medicine Universitas Brawijaya Malang, with the average age of the subjects 39.92 ± 6.85 years. Assessment of Upper Face Rating Scale scores by three dermatology and venereology specialists from each research center who were not involved in the injection of Prabotulinumtoxin injection. The scoring results for wrinkles in the forehead area (rest and dynamic) are shown in Figure 1 and Table 1. The difference in scoring for wrinkles in the forehead area, both in conditions of no expression and maximum expression from time to time, is significantly different ($p < 0.001$). However, in the condition of an expressionless face, the results of the LSD test showed that the decrease in the 2nd week to the 4th week was not significant, but significant between the 4th, 8th, and 12th weeks. Figure 2 is a photo of the improvement of wrinkles on the forehead from week 0 to 12.

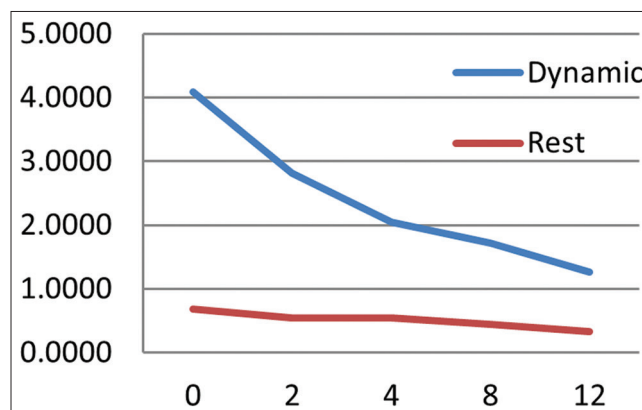


Figure 1: Forehead dynamic and rest wrinkle average score

The scoring results for wrinkles in the crow’s feet (rest and dynamic) are shown in Figure 3 and Table 2. The difference in scores for wrinkles in the crow’s feet area, both in conditions of no expression

Table 1: Different test results repeated measurement of forehead dynamic and rest wrinkle

Time	Dynamic		Rest	
	Mean	p value	Mean	p value
Week 0	4.09 ± 0.96		0.68 ± 1.24	
Week 2	2.81 ± 0.80	0.000	0.54 ± 0.93	0.000
Week 4	2.05 ± 0.86	0.000	0.55 ± 0.95	0.319
Week 8	1.72 ± 0.71	0.000	0.44 ± 0.70	0.000
Week 12	1.26 ± 0.49	0.000	0.33 ± 0.47	0.000

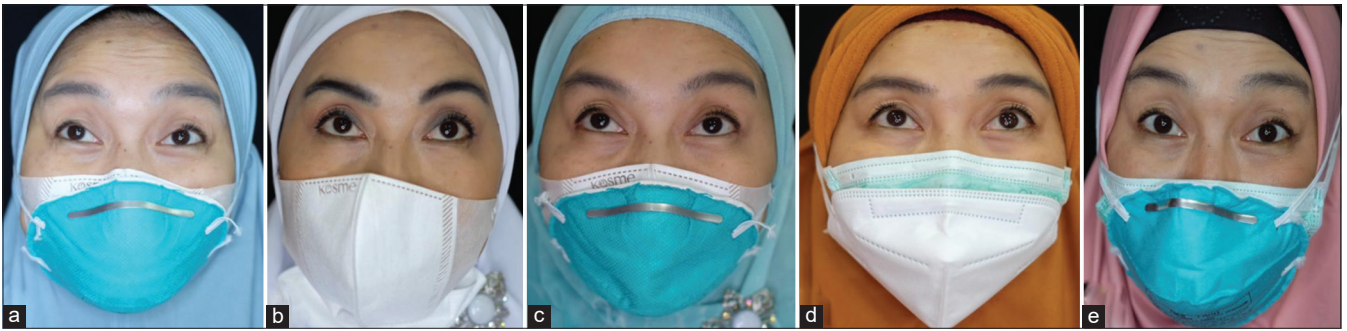


Figure 2: Clinical improvement of wrinkles in the forehead area at the time of maximum expression. (a) Week 0. (b) Week 2. (c) Week 4. (d) Week 8. (e) Week 12

and maximum expression from time to time, differed significantly ($p < 0.001$).

Table 2: Different test results repeated measurement of crow's feet dynamic and rest wrinkle

Time	Dynamic		Rest	
	Mean	p value	Mean	p value
Week 0	4.09 ± 0.96		0.84 ± 1.10	
Week 2	2.81 ± 0.80	0.000	0.71 ± 0.83	0.018
Week 4	2.05 ± 0.86	0.000	0.67 ± 0.79	0.025
Week 8	1.72 ± 0.71	0.021	0.62 ± 0.74	0.416
Week 12	1.26 ± 0.49	0.000	0.24 ± 0.43	0.000

However, in the condition of an expressionless face, the LSD test results showed that the decrease at week 2–week 8 was not significant. Figures 4 and 5 are photos of the repair of wrinkles on the left and right sides of the crow's feet from week 0 to 12.

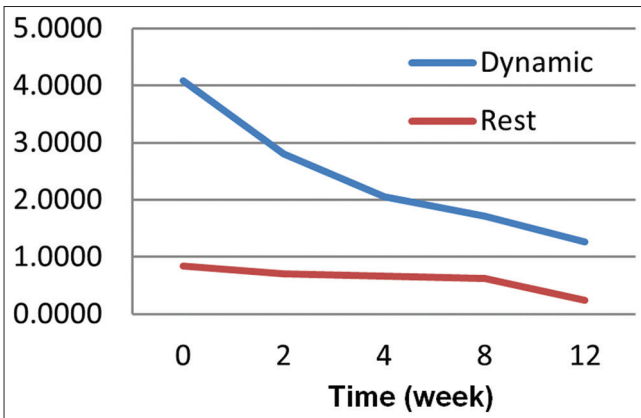


Figure 3: Crow's feet dynamic and rest wrinkle average score

The scoring results for glabellar wrinkles (rest and dynamic) are shown in Figure 6 and Table 3. The difference in scores for wrinkles in the crow's feet area, both in the non-expressive condition and in maximal expression from time to time, was significantly different ($p < 0.001$). However, in the condition of an expressionless face, the LSD test results showed that the decrease at week 4–week 12 was not significant. Figure 7 is a photo of the repair of wrinkles on the forehead from week 0 to 12.

Table 3: Different test results repeated measurement of glabellar dynamic and rest wrinkle

Time	Dynamic		Rest	
	Mean	p value	Mean	p value
Week 0	3.33 ± 0.80		0.46 ± 0.68	
Week 2	2.49 ± 0.67	0.000	0.33 ± 0.47	0.000
Week 4	1.29 ± 0.51	0.000	0.02 ± 0.13	0.000
Week 8	0.96 ± 0.61	0.000	0.00 ± 0.00	0.158
Week 12	0.90 ± 0.65	0.008	0.00 ± 0.00	1.000

Discussion

This research is the first multicenter research on the product Prabotulinumtoxin A (INIBO®, Covaltt, South Korea) conducted in Indonesia involving three hospitals (Regional Public Hospital dr. Saiful Anwar Malang/Faculty of Medicine Universitas Brawijaya Malang, Regional Public Hospital dr. Moewardi Surakarta/Faculty of Medicine Universitas Sebelas Maret Surakarta, and Presidential Gatot Soebroto Central Army Hospital Jakarta). The results of this study showed that Prabotulinumtoxin A injection was effective in reducing wrinkles in the frontal, glabellar and crow's feet area, with a statistically significant difference between week 0 and week 12.

Prabotulinumtoxin A (PRA) is a botulinum toxin Type A preparation with a molecular weight of 900 kDa produced by Clostridium botulinum. The final formulation of PRA is vacuum-dried, in contrast to other BTX preparations which are freeze-dried [3]. Prabotulinumtoxin A has a similar mechanism of action to BTX-A. The cellular action of BTX-A occurs as a three-step mechanism, namely: binding, internalization, and blockade [8].

Kim et al. (2015) conducted a study comparing two types of BTX-A injection (Botox®, Allergan Inc., and DWP450®, Daewoong Pharmaceutical Co., Ltd) on rat's gastrocnemius muscle. DWP450® is a lyophilized form of Prabotulinumtoxin A. From this study, it was found that based on changes in CMAP (compound muscle action potential), both BTX-A were able to trigger a paralytic effect on the tibialis anterior muscle in rat, but there was no significant difference in effectiveness and did not cause a delay in conduction velocity [9]. Toxicological studies comparing DWP450® and Onabotulinum toxin A (OBoNT) showed that DWP450® to be twice as safe as OBoNT; NOAEL (no observed adverse effect level) for DWP450® is at a dose of 60 U/KgBW for female rats, while OBoNT is 30 U/KgBW [6].

Won et al. (2015) conducted a Phase III, multicenter, randomized, double-blinded, and active-controlled study comparing DWP450® and

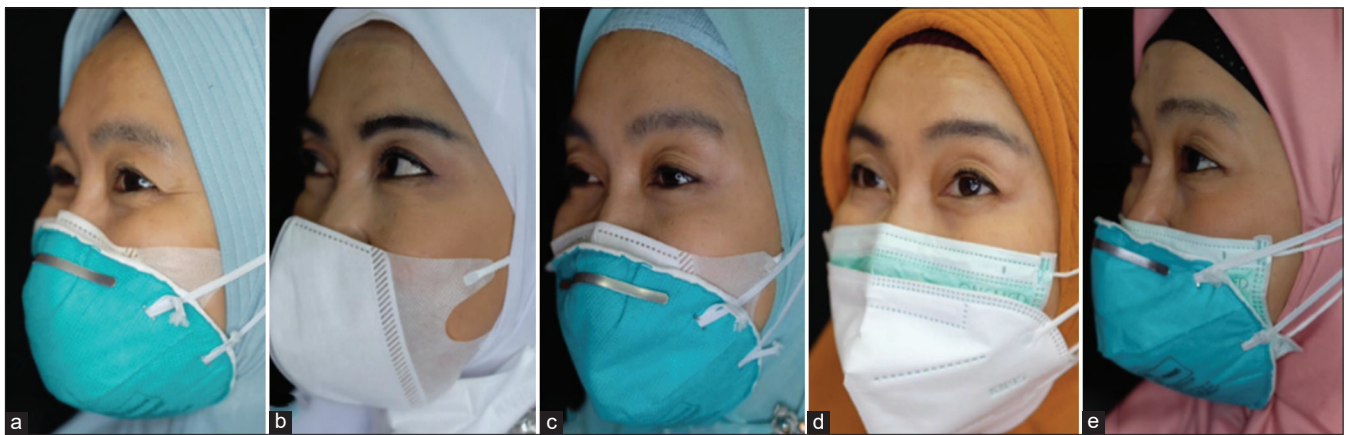


Figure 4: Clinical improvement of wrinkles in the left crow's feet area at the time of maximum expression. (a) Week 0. (b) Week 2. (c) Week 4. (d) Week 8. (e) Week 12

Botox® on glabellar lines in 268 subjects. Each vial contains 100U of BoNT-A, 0.5 mg of human serum albumin, and 0.9 mg of sodium chloride; and diluted with 2.5 ml of 0.9% NaCl to obtain a final dilution of 4U/0.1 ml. Observations were made in the 4th, 8th, 12th, and 16th weeks. Responder rates using a physician-rating severity measuring maximal contraction at the 4th week were 93.98% in the DWP450® group and 88.64% in the Botox group. The satisfaction rating by

subjects at the 16th week for the DWP450® group was 84.68% while for the Botox® group, it was 82.64%. The safety assessment between the two groups showed statistically insignificant results ($p = 0.68$), and the side effects were mild [6].

Beer *et al.* (2019) conducted a PRA study for Phase III, double-blind, placebo-controlled, and single-dose glabellar wrinkles on 654 subjects (divided into two different studies, namely, EV-001 and EV-002)



Figure 5: Clinical improvement of wrinkles in the right crow's feet area at the time of maximum expression. (a) Week 0. (b) Week 2. (c) Week 4. (d) Week 8. (e) Week 12

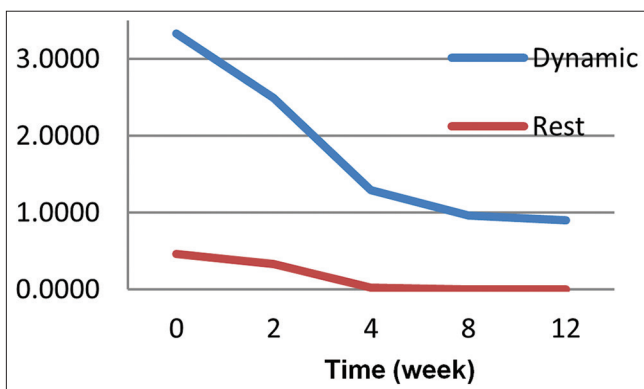


Figure 6: Glabellar dynamic and rest wrinkle average score

and observed for 150 days. Efficacy was measured using the Glabellar Line Scale (GLS), Global Aesthetic

Improvement Scale (GAIS), and 5-point Subject Satisfaction Scale (SSS). A total of 492 subjects received PRA injections (246 subjects in the EV-001 study, and 246 subjects in the EV-002 study), while 162 subjects received a placebo injection (84 subjects in the EV-001 study, and 78 subjects in the EV-002 study). Measurement of GLS on day 30 between the two groups in the EV-001 and EV-002 studies based on the investigator's assessment and the study subject's assessment showed $p < 0.001$. GLS measurements on day 90 for each study EV-001 and EV-002 had $p < 0.001$; The 120th day GLS for EV-001 and EV-002 had $p = 0.023$ and < 0.001 , respectively; and on the 150th day for EV-001 is $p = 0.041$ and EV-002 $p = 0.047$. Finally, it could be concluded that PRA injection gave significant results in reducing glabellar wrinkles up to 150 days [3].



Figure 7: Clinical improvement of wrinkles in the Glabellar area at the time of maximum expression. (a) Week 0. (b) Week 2. (c) Week 4. (d) Week 8. (e) Week 12

Theoretically, although Botox can be used for both dynamic and static wrinkle, it gives more satisfactory results for dynamic wrinkle. It is caused of Botox's mechanism of action that affect acetylcholine receptors when the muscles contract. If we want to improve appearance for static wrinkle, it is recommended to combine Botox with another modalities, such as filler.

In this study, the side effect felt by all subjects was discomfort during the injection procedure (Visual Analog Scale 3/10), but disappeared <30 min after the procedure. There were no other side effects such as hematoma, paralysis, and ptosis during observation. The limitation of this study is the length of observation time which is only 12 weeks. A longer research period is needed to determine the duration of this PRA effect to wrinkle using The Upper Face Rating Scale. In addition, this study was only conducted on female subjects, so the exact dose of PRA for men has not been confirmed.

Conclusion

Prabotulinumtoxin A products improve the condition of wrinkles on the forehead, crow's feet, and glabellar frown areas with satisfying result and efficacy. Observations of more than 12 weeks are needed to determine the duration of Prabotulinumtoxin A products.

Acknowledgment

This research was partially supported by INIBO®, Covaltt, South Korea. We thank our colleagues from Universitas Brawijaya/Regional Public Hospital dr. Saiful Anwar Malang, Indonesia Presidential Gatot Soebroto Central Army Hospital Jakarta and Universitas Sebelas Maret/Regional Public Hospital dr. Moewardi

Surakarta who provided insight and expertise that greatly assisted the research.

References

1. Sundaram H, Huang PH, Hsu NJ, Huh CH, Wu WT, Wu Y, et al. Aesthetic applications of botulinum toxin A in Asians: An international, multidisciplinary, pan Asian consensus. *Plast Reconstr Surg Glob Open*. 2016;4(12):e872. <https://doi.org/10.1097/GOX.0000000000000507> PMID:28293488
2. Rohrer TE, Beer K. Background to Botulinum Toxin. United States: Elsevier Inc.; 2005. p. 9-18.
3. Beer KR, Shamban AT, Avelar RL, Gross JE, Jonker A. Efficacy and safety of prabotulinumtoxinA for the treatment of glabellar lines in adult subjects: Results from 2 identical phase III studies. *Dermatol Surg*. 2019;45(11):1381-93. <https://doi.org/10.1097/DSS.0000000000001903> PMID:30893162
4. James WD, Elston D, Berger T, Neuhaus IM. *Andrew's Diseases of the Skin E-book: Clinical Dermatology*. New York: Elsevier Health Sciences; 2011.
5. Benedetto AV. *Botulinum Toxin in Clinical Dermatology*. Boca Raton: CRC Press; 2006.
6. Won CH, Kim HK, Kim BJ, Kang H, Hong JP, Lee SY, et al. Comparative trial of a novel botulinum neurotoxin Type A versus onabotulinumtoxin A in the treatment of glabellar lines: A multicenter, randomized, double-blind, active-controlled study. *Int J Dermatol*. 2015;54(2):227-34. <https://doi.org/10.1111/ijd.12627> PMID:25311357
7. Flynn TC, Carruthers A, Carruthers J, Geister TL, Görtelmeyer R, Hardas B, et al. Validated assessment scales for the upper face. *Dermatol Surg*. 2012;38(2 Spec No):309-19. <https://doi.org/10.1111/j.1524-4725.2011.02248.x> PMID:22316187
8. Flávio A. *Botulinum Toxin for Facial Harmony*. Batavia: Quintessence Publishing Co.; 2019.
9. Kim CS, Jang WS, Son IP, Nam SH, Kim YI, Park KY, et al. Electrophysiological study for comparing the effect of biological activity between Type A botulinum toxins in rat gastrocnemius muscle. *Hum Exp Toxicol*. 2013;32(9):914-20. <https://doi.org/10.1177/0960327113479044> PMID:23475433