

EFFICACY OF BOTULINUM TOXIN TYPE A IONTOPHORESIS IN THE MASSETER MUSCLE FOR TREATMENT OF MYOFASCIAL PAIN DYSFUNCTION SYNDROME (A RANDOMIZED CONTROLLED CLINICAL STUDY)

Alaa A. Mubarak¹*BDS, Riham M. Eldibany² PhD,, Lydia F. Melek³ PhD.,

Hoda M. Abdel-Naby⁴ PhD.,

ABSTRACT

BACKGROUND: Masticatory myofascial trigger points (TrPs) represent a major contributor to non-dental orofacial pain. The primary treatment for myofascial TrPs involves intramuscular injections, which can be painful and anxiety-inducing for patients. An alternative, pain-free treatment approach for myofascial pain is iontophoresis.

AIM OF THIS STUDY: This study aimed to assess the efficacy of Botulinum toxin type A (BTX-A) Iontophoresis in managing muscle activity, Visual Analogue Scale (VAS) pain scores, mouth opening range, and Electromyography in individuals diagnosed with myofascial pain dysfunction syndrome (MPDS).

MATERIALS AND METHODS: Twenty-two patients exhibiting symptoms of MPDS in the masseter muscle were included. These patients were randomly divided into two equal groups: the study group (n=11) received BTX-A iontophoresis, while the control group (n=11) received saline iontophoresis as a placebo. Muscle activity at rest and during function was assessed using VAS, the range of mouth opening was measured, and electromyography assessments were conducted. These assessments were performed pre-operatively and during all follow-up visits.

RESULTS: There analysis revealed no significant differences between the two groups at any of the measured timepoints (preoperative, immediate postoperative and 3-month follow-up) concerning pain levels, mouth opening or muscle activity.

Conclusion: The current study revealed no significant differences between the BTX-A and saline groups in the treatment of MPDS by iontophoresis.

KEYWORDS: Myofascial pain, Masseter muscle, trigger points, Botulinum toxin type A, iontophoresis.

RUNNING TITLE: Efficacy of botulinum-toxin iontophoresis in masseter myofascial pain treatment

1 BDS Faculty of Dentistry Alexandria University

2 Professor of Oral Maxillofacial Surgery Oral & Maxillofacial Surgery Department Faculty of Dentistry, Alexandria University.

3 Associate Professor of Oral & Maxillofacial Surgery Oral & Maxillofacial Surgery Department Alexandria University, Faculty of Dentistry

4 Lecturer of physical Medicine, Rheumatology and Rehabilitation Physical Medicine, Rheumatology and Rehabilitation Department. Alexandria University, Faculty of Medicine

* Corresponding Author:

E-mail: triangle999@yahoo.com

INTRODUCTION

Myofascial pain (MP) is a neuromuscular disorder and a painful condition that affects the facial muscles. It manifests as local muscle tenderness and pain, primarily marked by the presence of trigger points (TrPs). These TrPs are hyperirritable spots situated in the taut bands of skeletal muscles, commonly referred to as myofascial trigger points (MTrPs) (1).

In all cases, MTrPs are linked to regions within the muscle that exhibit firm, sensitive nodules when touched. It is believed that this firmness may result from excessive contraction of the sarcomere within this region (2). These TrPs are characterized by a

limited blood supply and an inflammatory milieu (3). MTrPs elicit pain when subjected to palpation. If the pain is solely induced by palpation, they are termed latent TrPs. Conversely, if pain exists without external manipulation, they are classified as active TrPs (4). Pain caused by TrPs can be managed by non-invasive procedures including occlusal guides, muscle relaxants, spraying and stretching, massage, transcutaneous electrical stimulation, cold laser therapy and physical therapy (5).

Trigger point injections (TrPi) serve as a therapeutic approach for managing MTrPs, particularly in patients experiencing symptoms. Their effectiveness in deactivating TrPs has been well-documented. It is

believed that they induce a temporary relaxation of the tense muscle band. Different types of medications can be employed for injection, including local anesthetics, saline solutions, Botulinum toxin, or corticosteroids (6).

Botulinum toxin type A (BTX-A) is a highly potent neurotoxin derived from anaerobic bacteria, namely *Clostridium botulinum*. Its mode of action entails blocking the release of acetylcholine at neuromuscular junctions, leading to muscle paralysis and reduced strength. BTX-A has been used in managing different muscular conditions in the maxillofacial region, including hypertrophy of the masseter and temporalis muscles, muscular dystonia, and temporomandibular joint (TMJ) disorders (7).

While Botox injections can effectively treat various conditions, they may come with side effects like swelling, bruising, and mild discomfort. In rare instances, patients may also encounter symptoms such as nausea, temporary headaches, or flu-like symptoms. Additionally, there is a risk of complications, including excessive muscle relaxation, vision problems, difficulty in speaking or swallowing, and even breathing difficulties. Fortunately, most complications tend to be short-term and typically resolve without the need for medication (8).

Iontophoresis is a technique for delivering drugs transdermally by utilizing electrodynamic principles to drive the drug deep into the tissues for localized application (9). It operates on the concept that within an electric field, positively charged drug ions (known as cations) experience a repulsive force from a positively charged electrode (anode) and move toward the negatively charged electrode (cathode). Conversely, negatively charged ions (anions), repelled by the negative electrode (cathode), move in the direction of the anode. Ideally, the molecules best suited for iontophoresis are those that are small and hydrophilic (10).

There are various medications delivered by iontophoresis such as local anesthetic solutions, opioids, corticosteroids, non-steroidal anti-inflammatory agents, antibiotics, antifungal medications, antiviral drugs, anticancer treatments, fluorides, and multivitamins (11). Botox iontophoresis has been used in the management of hand hyperhidrosis due to the ability of BTX-A to suppress the release of acetylcholine in eccrine sweat glands. This makes it an efficient treatment for primary palmar hyperhidrosis. Traditional intradermal injections in the palms can be painful; however, iontophoresis offers a painless method for delivering BTX-A to the palms (12).

In the pilot study published by Kavanagh and Shams (13), it was reported that they successfully utilized iontophoresis for the delivery of botulinum neurotoxin as a treatment for palmar hyperhidrosis. This method exhibited clear advantages when compared to the conventional injection approach.

The authors noted significant clinical improvements and were able to demonstrate that iontophoresis effectively facilitated the delivery of BTX-A through the intact skin of live rats.

No previous studies were performed on Botox iontophoresis for treatment of TMJ disorders. Thus, this study was designed to evaluate the effectiveness of BTX-A Iontophoresis in reducing pain associated with MTRPs within the masseter muscle.

MATERIALS AND METHODS

Twenty-Two patients with myofascial pain dysfunction syndrome requiring treatment participated in this study between February 2022 and February 2023. Participants were recruited from the Outpatient Clinic of the Alexandria University Teaching Hospital and underwent surgery in the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Alexandria University.

Ethical approval was granted by the Research Ethics Committee, Faculty of Dentistry, Alexandria University. All participants signed an informed consent prior to the commencement of the study.

Inclusion Criteria:

The study included adult patients of both genders who were experiencing unilateral or bilateral myofascial pain, accompanied by other symptoms as headache, pain in the orofacial region, and limited range of mouth opening that necessitated treatment.

Exclusion Criteria:

Patients with TMJ pathology (rheumatoid arthritis, osteoarthritis, condylar resorption) and internal derangement were excluded from this study.

Sample size Calculation

Sample size was planned on 5% alpha error and 80% study power. The mean (SD) maximum mouth opening after 3 months was 44.3 (3.85) mm and 39.2 (4.27) mm for the BTX-A and the control groups, respectively (14). Based on comparison of two independent means using a pooled SD= 4.06 mm, the sample size was calculated to be 10 patients per group, increased to 11 patients to make up for cases lost to follow-up. The total required sample size = number per group × number of groups = 11 × 2 = 22 patients. Sample size was based on Rosner's method (15) and calculated using Brant's sample size calculator (16).

Method of randomization

The randomization sequence was generated using an online software (17), using permuted block technique with variable block sizes (18). Allocation sequence was kept concealed from the individual responsible for assigning participants to the two study groups using opaque sealed envelopes (9). A double blinded approach was implemented where blinding extended to both the participants, and outcome assessors who remained unaware to the group allocation of participants (19).

Materials

Botulinum Toxin type A (Nabota - Daewoong, Seoul, South Korea) was used in the current study.

The vial was diluted at a ratio of 100-IU vial mixed with 3 mL of unpreserved saline. (Figure 1)

Preoperative evaluation

Patient complaints, incidence and location of the disease were documented, along with detailed personal information. Patients' previous dental and medical histories were recorded, and a thorough assessment of their general health condition was performed. Clinical examinations included evaluation of joint clicking, assessment of masticatory muscles tenderness, and identification of TrPs through digital palpation. Muscle activity was measured during both rest and function using the Visual Analogue Scale for pain (VAS), mouth opening range assessment, and Electromyography. (Figure 2).

Operative procedure (Figure 2)

1. The patient was seated on a reclined chair and the TrPs of the Masseter muscle were identified and marked.
2. A small piece of gauze, soaked with the liquid medication was placed beneath the electrode patch that corresponded to the drug charge. This patch was then applied on the patient's facial skin on areas corresponding to the masseter muscle TrPs.
3. Subsequently, a plastic device was attached to the patch for half an hour. During this period, the device generated electrical charges; facilitating the transdermal delivery of the medication into the muscle.
4. The voltage of the device was carefully adjusted to a level that the patient could comfortably tolerate, ensuring safety and comfort during the procedure.



Figure (1): Photos showing: Materials and Devices, (A) Botulinum toxin type A, (B) Iontophoresis device.

Study Group

In this group, patients were treated with BTX-A iontophoresis on the Masseter muscle. BTX-A is most effectively administered when diluted, with a recommended ratio of a 100-IU vial mixed with 3 mL of unpreserved saline (13). Since BTX-A is negatively charged (13), it was placed beneath the negative patch of the device. The session lasted for half an hour.

Control Group

Patients of this group underwent a similar procedure to that of the study group. However, instead of BTX-A, they received a placebo treatment involving 0.9% normal saline iontophoresis applied on the Masseter muscle for the same duration.



Figure (2): Figure showing: preoperative patient preparation, (A) palpation of the trigger points, (B) marking of the trigger points, (C) operative phase frontal view of the patient with iontophoresis device patches during the session. (D) lateral view of the patches during the session.

Postoperative Phase

Participants were instructed to rest the masticatory muscle for seven days, adhere to a soft diet and apply moisturizing ointment to prevent potential skin dryness resulting from the iontophoresis procedure.

Clinical Follow-up Phase

Several indicators were used to assess the effectiveness of the treatment. These indicators were measured preoperatively, immediately after the procedure, and during a 3-month follow-up visit. These outcomes were compared to evaluate the treatment outcomes.

The study outcomes included the following:

a) Pain evaluation using Visual Analogue Scale (VAS)

Pain level was assessed preoperatively, immediately after the procedure, and 3 months after the operation. A 10 points VAS was used, where "0" signified the absence of pain, and "10" indicated the most severe pain (20).

b) Mouth opening range measurement

The mouth opening range is the distance between the incisal edge of the maxillary central incisor to the incisal edge of the mandibular central incisor when the mouth is opened as wide as possible without discomfort. This measurement was recorded during the follow-up visits at one week, two weeks, four weeks, and 3 months postoperatively using a ruler (21).

c) Electromyography (EMG) for muscle activity assessment

Patients underwent electromyography assessment at rest and in a functional (clenching) position. These assessments were performed preoperatively, immediately after the procedure, and during the 3-month follow-up visit. Surface electrodes were strategically positioned on the masseter muscle to capture data on muscle activity during both rest and

function. These surface EMG electrodes were positioned parallel to the muscle's long axis, equidistant from its origin and insertion points. Muscle tone was recorded both at rest and during maximal clenching (22). (**Figure 3, 4**)

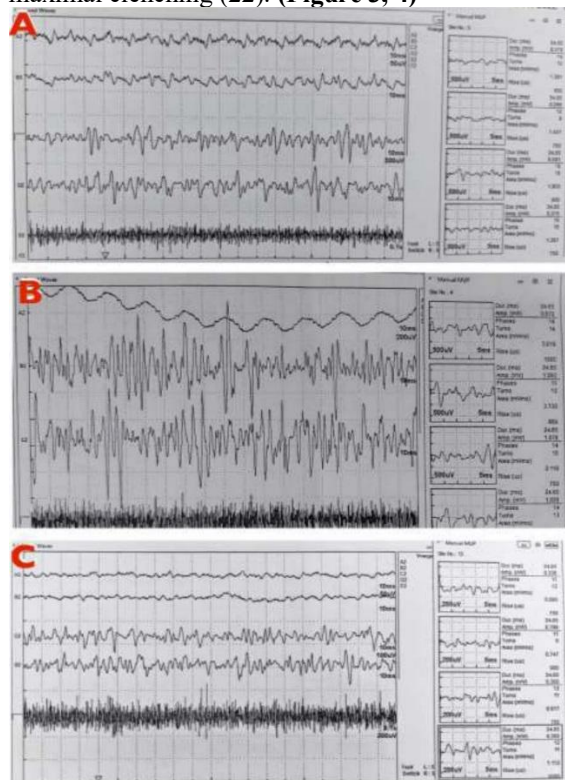


Figure 3: Photograph showing: Electromyography for Botulinum toxin type A patient. (A) preoperative EMG. (B) immediate postoperative EMG, (C) after 3-month EMG.

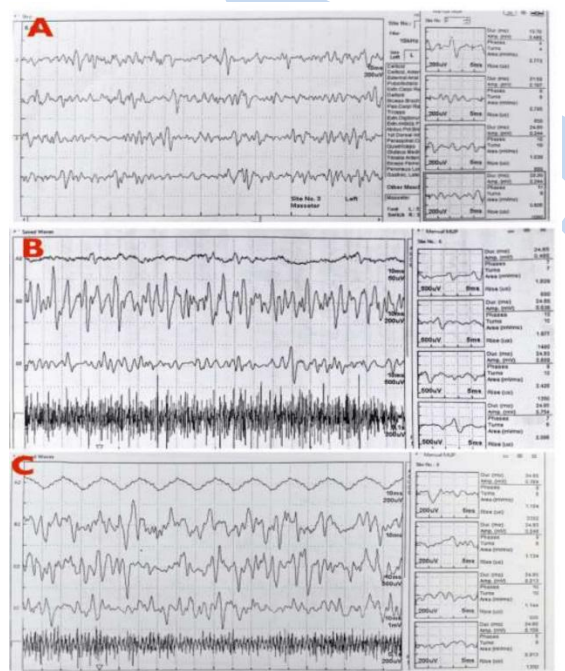


Figure 4: Photograph showing: Electromyography for control patient. (A) preoperative EMG. (B) immediate postoperative EMG. (C) 3-month EMG.

Statistical analysis

Data were analyzed using SPSS software for Windows (Version 25) (23). The Kolmogorov-Smirnov test of normality indicated significant deviations from normal distribution for most variables, so non-parametric statistical methods were adopted (24). Data were summarized by calculating the range (minimum and maximum), median and 95% confidence interval (CI) of the median. Mann-Whitney U test (25), Wilcoxon Signed Ranks test (26), and Friedman's test were used for the comparisons (27). Statistical level was set at p value $< .05$ (28).

RESULTS

The sociodemographic characteristics of the 22 included patients were described. Age ranged from 20 to 45 years for the BTX-A group and from 21 to 42 years for the control group. Among the 22 patients, there were 15 females and 7 males. In the BTX-A group ($n=11$), males constituted 3 out of 11 (27.27%), while females made up 8 out of 11 (53.33%). In the control group ($n=11$), males accounted for 4 out of 11 (36.36%), and females comprised 7 out of 11 (63.64%).

VAS for pain:

In both groups, repeated measures analysis showed a significant decrease in VAS scores when comparing preoperative and immediate postoperative measurements (Table 1).

Range of mouth opening:

For both groups, repeated measures analysis showed a significant increase in the mouth opening across the different timepoints. (Table 1)

Muscle activity (μV):

Regarding the muscle activity there was no statistically significant difference between the two groups when measured preoperatively, immediately postoperatively, and at the 3-month follow-up (Table 2)

Table (1): Comparison of Mouth opening (mm) between the two studied groups.

Mouth opening (mm)	Group		Test of significance p -value
	Botulinum toxin type A ($n=11$)	Saline ($n=11$)	
Preoperative			
- Min-Max	26.00-46.00	30.00-46.00	$Z_{(MW)}=0.789$ $p=.430$ NS
- Median	35.00	35.50	
- 95.0% CI of the median	27.00-46.00	32.00-43.00	
Immediate postoperative			
- Min-Max	24.00-50.00	35.00-50.00	$Z_{(MW)}=1.587$ $p=.113$ NS
- Median	35.00	38.00	
- 95.0% CI of the median	32.00-50.00	36.00-45.00	
Three months postoperative			
- Min-Max	30.00-50.00	34.00-50.00	$Z_{(MW)}=1.149$ $p=0.250$ NS
- Median	35.00	38.00	
- 95.0% CI of the median	30.00-50.00	36.00-45.00	
Friedman Test of significance p -value	$\chi^2_{(df=6)} = 18.094$ $p=.006^*$	$\chi^2_{(df=6)} = 11.015$ $p=.088$ NS	

Table (2): Comparison of Muscle activity between the two studied groups.

Muscle activity (μ V)	Group		Test of significance <i>p</i> -value
	Botulinum toxin type A (n=11)	Saline (n=11)	
Preoperative - Min-Max - Median - 95% CI of the median	7.60-15.75 11.50 8.50-12.50	8.00-15.50 11.25 9.25-13.00	$Z_{(MW)}=0.560$ $p=.576$ NS
Immediate postoperative - Min-Max - Median - 95% CI of the median	5.60-18.00 11.87 8.00-13.50	2.50-13.75 8.00 5.25-10.25	$Z_{(MW)}=1.938$ $p=.056$ NS
Three months postoperative - Min-Max - Median - 95% CI of the median	4.00-14.25 10.50 6.70-13.25	2.30-12.00 6.75 4.60-10.50	$Z_{(MW)}=1.708$ $p=.088$ NS
Friedman Test of significance <i>p</i>-value	$\chi^2_{(df=2)}=3.455$ $p<.178$ NS	$\chi^2_{(df=2)}=14.279$ $p<.001^*$	

In the BTX-A group, repeated measures analysis revealed a statistically significant decrease in muscle activity (μ V) at different measurement points. Similarly, in the control group, repeated measures analysis showed a statistically significant decrease in muscle activity across various measurement points.

DISCUSSION

In the current study, there was no statistically significant difference regarding age distribution between the BTX-A and control groups. Additionally, the majority of the patients in both groups were females, aligning with findings from a study conducted by Ernberg et al. (2011) (29), which also observed a predominance of female patients. Furthermore, when examining the preoperative and immediate postoperative VAS scores, the present study showed no statistically significant differences between the BTX-A and control groups. Nevertheless, within each group, the repeated measures analysis showed a statistically significant decrease in VAS scores when comparing the preoperative and immediate postoperative measurements, both in the BTX-A and control groups.

In line with our results, Ernberg et al. (2011) (29), conducted a study involving 21 patients suffering from myofascial pain, who had not experience sufficient pain relief from conventional treatments. In this study, the participants received random injections of either 50 U of BTX-A (test) or saline (control) into the masseter muscles experiencing pain. Assessments were performed at the 1- and 3-months follow-up visits. The study found no significant difference in pain reduction between the

Alexandria Dental Journal. Volume 50 Issue 2A

group that received BTX-A injections and the group that received saline injections into the painful masseter muscles.

Conversely, our findings contradicted the results of randomized controlled trials conducted by Hatice Hosgor et al. (2020) (30), and by Guarda-Nardini et al. (2008) (31). In both of these studies, patients were treated using BTX-A injections, and the results indicated that the BTX-A group exhibited significantly greater pain relief compared to the saline group.

De la Torre Canales et al.'s (2020) (32), study revealed that even with the administration of lower doses of BTX-A, there was a noticeable reduction in subjective pain intensity (measured by VAS) over a period of 24 weeks (33).

Regarding the mouth opening, there were no significant differences between the BTX-A and the control groups before treatment, or during the entire follow-up duration. In the BTX-A group, repeated measures analysis demonstrated a statistically significant increase in mouth opening across the different measurement timepoints.

Nagy et al. (2020) (14), found that the study group exhibited significantly higher values for maximum pain-free mouth opening at two, three, and four months, while the control group had significantly greater values only at one week and six months. This study's findings revealed a significant improvement in maximum pain-free mouth opening. The study also noted that the most significant reduction in maximal mouth opening occurred during the initial two weeks following the injection. However, starting from the third week onward, there was a gradual increase in mouth opening, which aligns with the results reported by Kim et al. (2009) (34).

Chaurand et al. (2017) (35), reported that the average interincisal mouth opening at the beginning of the study for the initial eight patients was 42.3 ± 6.09 mm (ranging from 31 to 50 mm). After one month, patients who received BTX-A therapy exhibited an improvement in mouth opening, with an average measurement of 43.4 ± 6.25 mm. The average change in mouth opening, two months following both treatments, was 1.1 ± 6.5 mm, which is equivalent to 2.5% of the initial measurement. Notably, there were no significant changes detected in mouth opening between the initial and conservative therapy measurements, which remained at 42.3 ± 5.2 mm.

In terms of muscle activity, the preoperative, immediate postoperative, and three months postoperative muscle activity revealed no significant difference between the BTX-A and control groups. However, in the BTX-A group, repeated measures analysis demonstrated a statistically significant reduction in muscle activity (μ V) across the different measurement points. Similarly, in control group, the repeated measures analysis indicated a significant

decrease in muscle activity across the different timepoints.

In the study conducted by De La Torre Canales et al. (2021) (32), one month after the treatment, the BTX-A group exhibited a significant decrease in EMG activity. When comparing the groups at the one-month follow-up, a remarkable reduction in masseter muscle activity was observed in the BTX-A group in comparison to both the acupuncture and saline groups. Notably, the EMG findings for both the masseter and anterior temporal muscles revealed a significant decrease in EMG activity one month following treatment, but this effect was observed exclusively in patients treated with BTX-A. When comparing the groups, the BTX-A group demonstrated a significant reduction in muscle activity for both muscles when compared to the acupuncture and saline groups.

De la Torre Canales et al. (2020) (32), highlighted that BTX-A injections resulted in a decrease in EMG activity during the initial month. Interestingly, it was observed that only the low dose of BTX-A demonstrated a recovery in EMG activity after 3 months, ultimately returning to the baseline values after six months. These results can suggest a dose-duration effect for the toxin (36).

Nagy et al. (2020) (14), assessed the efficacy of botulinum toxin injections in the masseter and temporalis muscles as compared to treatment with hard maxillary occlusal splints for managing myofascial pain in a group of forty adults, who were divided into two groups. The test group received bilateral BTX-A injections in the masseter and temporalis muscles, while the control group was treated with hard maxillary occlusal splints. After three months, it was observed that the control group displayed significantly greater EMG values when compared to the study group.

Nagy et al. (2020) (14), found that the EMG readings of the masseter and temporalis muscles exhibited a significant reduction after 3 months, followed by a significant increase after 6 months. This finding aligns with the results reported by Lee et al. (2010) (37), who observed a reduction in EMG activity in cases of bruxism following the injection of 80 U of botulinum toxin into the masseter muscle alone, over a 3-month period. Additionally, another study by Tan et al. (2000) (38) showed that the administration of 62 U of Botox into each masseter muscle provided a safe and effective treatment for severe bruxism for a duration of 5 months following the injection.

The findings of Sitnikova et al. (2022) (39) contradicted our own regarding the significant reduction in EMG values after BTX-A injection into the masseter muscle when compared with saline. This study compared botulinum toxin injection to saline injection in terms of muscle activity measured by EMG and showed a decrease in muscle activity in the patients treated with BTX-A injection.

Our study has several limitations. First, the relatively sample size might have restricted our ability to detect significant differences between the treatment groups. A larger sample size would be desirable to increase the statistical power and reliability of our findings. Second, the follow-up period in this study was relatively brief. It is necessary to investigate the long-term effects of BTX-A and saline iontophoresis on muscle activity and pain relief in order to assess the sustained efficacy.

CONCLUSION

Despite the limitations of this study, our findings indicate an improvement in pain, mouth opening and muscle activity in both the Botox and control groups. These results suggest that saline iontophoresis can produce effects similar to Botox in treating myofascial pain, making it a potentially more cost-effective alternative.

CONFLICT OF INTEREST

The authors announce that they have no conflicts of interest.

FUNDING STATEMENT

The authors received no specific funding for this work.

REFERENCES

1. Leite FM, Atallah AN, El Dib R, Grossmann E, Januzzi E, Andriolo RB, et al. Cyclobenzaprine for the treatment of myofascial pain in adults. *Cochrane Database Syst Rev*. 2009;2009:Cd006830.
2. Dommerholt J, Bron C, Franssen J. Myofascial trigger points: an evidence-informed review. *J Man Manip Ther*. 2006;14:203-21.
3. Ahmed S, Subramaniam S, Sidhu K, Khattab S, Singh D, Babineau J, et al. Effect of Local Anesthetic Versus Botulinum Toxin-A Injections for Myofascial Pain Disorders: A Systematic Review and Meta-Analysis. *Clin J Pain*. 2019;35:353-67.
4. Alvarez DJ, Rockwell PG. Trigger points: diagnosis and management. *Am Fam Physician*. 2002;65:653-60.
5. Venâncio Rde A, Alencar FG, Zamperini C. Different substances and dry-needling injections in patients with myofascial pain and headaches. *Cranio*. 2008;26:96-103.
6. Wong CS, Wong SH. A new look at trigger point injections. *Anesthesiol Res Pract*. 2012;2012:492452.
7. Dhanrajani PJ, Jonaidel O. Trismus: aetiology, differential diagnosis and treatment. *Dent Update*. 2002;29:88-92, 4.
8. Yiannakopoulou E. Serious and long-term adverse events associated with the therapeutic and cosmetic use of botulinum toxin. *Pharmacology*. 2015;95:65-9.
9. Ebisawa T, Nakajima A, Haida H, Wakita R, Ando S, Yoshioka T, et al. Evaluation of calcium alginate gel as electrode material for alternating

current iontophoresis of lidocaine using excised rat skin. *J Med Dent Sci*. 2014;61:41-8.

10. Dhote V, Bhatnagar P, Mishra PK, Mahajan SC, Mishra DK. Iontophoresis: a potential emergence of a transdermal drug delivery system. *Sci Pharm*. 2012;80:1-28.
11. Karpiński TM. Selected Medicines Used in Iontophoresis. *Pharmaceutics*. 2018;10.
12. Pikal MJ. The role of electroosmotic flow in transdermal iontophoresis. *Adv Drug Deliv Rev*. 2001;46:281-305.
13. Kavanagh GM, Oh C, Shams K. BOTOX delivery by iontophoresis. *Br J Dermatol*. 2004;151:1093-5.
14. Nagy Fathy AI. Botulinum injection in the masticatory muscles for management of myofascial pain. *Al-Azhar Journal of Dental Science*. 2020;23:375-81.
15. Rosner B. Fundamentals of biostatistics. Nelson Education. 2015. .
16. Sample size calculator. Available at: <https://www.stat.ubc.ca/~rollin/stats/ssize/n2.html>.
17. Dallal. GE. Randomization Plans: Randomizing subjects to a single treatment 2020 [updated 12/22/2020 23:23:56.4/15/2021]. Available at: <http://www.randomization.com>.
18. Schulz KF, Grimes DA. Generation of allocation sequences in randomised trials: chance, not choice. *Lancet*. 2002;359:515-9.
19. Karanicolas PJ, Farrokhyar F, Bhandari M. Practical tips for surgical research: blinding: who, what, when, why, how? *Can J Surg*. 2010;53:345-8.
20. Yilmaz O, Sivrikaya EC, Taskesen F, Pirpir C, Ciftci S. Comparison of the Efficacy of Botulinum Toxin, Local Anesthesia, and Platelet-Rich Plasma Injections in Patients With Myofascial Trigger Points in the Masseter Muscle. *J Oral Maxillofac Surg*. 2021;79:88.e1-e9.
21. Li XY, Jia C, Zhang ZC. The normal range of maximum mouth opening and its correlation with height or weight in the young adult Chinese population. *J Dent Sci*. 2017;12:56-9.
22. Attia S, Farmawy M, Nemmat AH, Shalash M. The use of botulinum toxin injection in the management of myofascial pain dysfunction syndrome. *Egypt Dent J*. 2017;63:3069-75.
23. SPSS I. IBM Corp. Released, Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. 2017.
24. Field A. Discovering Statistics Using IBM SPSS Statistics. 4th ed. London, California, New Delhi: SAGE Publications Ltd; 2013.
25. Mann HB, Whitney DR. On a test of whether one of two random variables is stochastically larger than the other. *Ann Math Statist*. 1947:50-60.
26. Wilcoxon F. Individual comparisons by ranking methods. *Biometrics bulletin* 1945;1:80-3.
27. Friedman M. The use of ranks to avoid the assumption of normality implicit in the analysis of variance. *J Am Stat Assoc*. 1937;32:675-701.
28. Curran-Everett D. Evolution in statistics: P values, statistical significance, kayaks, and walking trees. *Adv Physiol Educ*. 2020;44:221-4.
29. Ernberg M, Hedenberg-Magnusson B, List T, Svensson P. Efficacy of botulinum toxin type A for treatment of persistent myofascial TMD pain: a randomized, controlled, double-blind multicenter study. *Pain*. 2011;152:1988-96.
30. Hosgor H, Altindis S. Efficacy of botulinum toxin in the management of temporomandibular myofascial pain and sleep bruxism. *J Korean Assoc Oral Maxillofac Surg*. 2020;46:335-40.
31. Guarda-Nardini L, Manfredini D, Salamone M, Salmaso L, Tonello S, Ferronato G. Efficacy of botulinum toxin in treating myofascial pain in bruxers: a controlled placebo pilot study. *Cranio*. 2008;26:126-35.
32. De la Torre Canales G, Alvarez-Pinzon N, Muñoz-Lora VRM, Vieira Peroni L, Farias Gomes A, Sánchez-Ayala A, et al. Efficacy and Safety of Botulinum Toxin Type A on Persistent Myofascial Pain: A Randomized Clinical Trial. *Toxins (Basel)*. 2020;12.
33. Pellett S, Yaksh TL, Ramachandran R. Current status and future directions of botulinum neurotoxins for targeting pain processing. *Toxins (Basel)*. 2015;7:4519-63.
34. Kim KS, Byun YS, Kim YJ, Kim ST. Muscle weakness after repeated injection of botulinum toxin type A evaluated according to bite force measurement of human masseter muscle. *Dermatol Surg*. 2009;35:1902-6.
35. Chaurand J, Pacheco-Ruiz L, Orozco-Saldivar H, López-Valdés J. Efficacy of botulinum toxin therapy in treatment of myofascial pain. *J Oral Sci*. 2017;59:351-6.
36. Barnes M. Botulinum toxin--mechanisms of action and clinical use in spasticity. *J Rehabil Med*. 2003;56-9.
37. Lee SJ, McCall WD, Jr., Kim YK, Chung SC, Chung JW. Effect of botulinum toxin injection on nocturnal bruxism: a randomized controlled trial. *Am J Phys Med Rehabil*. 2010;89:16-23.
38. Tan EK, Jankovic J. Treating severe bruxism with botulinum toxin. *J Am Dent Assoc*. 2000;131:211-6.
39. Sitnikova V, Kämpfi A, Teronen O, Kemppainen P. Effect of Botulinum Toxin Injection on EMG Activity and Bite Force in Masticatory Muscle Disorder: A Randomized Clinical Trial. *Toxins (Basel)*. 2022;14.