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A systematic review: the use of botulinum toxin A for the treatment of masseter hypertrophy and masticatory myofascial pain associated with bruxism

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BOSTON UNIVERSITY
HENRY M. GOLDMAN SCHOOL OF DENTAL MEDICINE

Thesis

**A SYSTEMATIC REVIEW: THE USE OF BOTULINUM TOXIN A FOR
THE TREATMENT OF MASSETER HYPERTROPHY AND
MASTICATORY MYOFASCIAL PAIN ASSOCIATED WITH BRUXISM**

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DEDICATION

I would like to thank and dedicate this work to all those who have supported and shaped me into the person I am today. I would like to thank my parents, Dr. Tariq Mehmood and Aasmi Tariq, for their endless sacrifices in giving me the opportunities that I have today. I would like to thank my husband, Dr. Arsalan Khawaja – my biggest supporter, the one person who will push me to be the best version of myself. Without him, this achievement would not be possible. And finally, my son and the light of my world, Zaydaan Zaeem Khawaja – my true purpose in life. It is he who has taught me the true meaning of strength.

A SYSTEMATIC REVIEW: THE USE OF BOTULINUM TOXIN A FOR THE TREATMENT OF MASSETER HYPERTROPHY AND MASTICATORY MYOFASCIAL PAIN ASSOCIATED WITH BRUXISM

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ABSTRACT

Benign masseter hypertrophy causes swelling at the angulus mandibulae and may be associated with masticatory myofascial pain due to hyperfunction from bruxism. The aim of this research was to use the systematic review process to investigate the true or reliable scientific evidence contained in four major databases pertaining to the efficacy and safety of intra-muscular injections of botulinum toxin A (BTX-A) for the treatment of masticatory myofascial pain and benign masseter hypertrophy associated with bruxism, compared with placebo or other traditional treatments prescribed for bruxism such as occlusal splints, pharmacotherapy, or lifestyle modification. Using the PICO format, a research question was formulated, MeSH terms were derived, and an electronic literature search was conducted in PubMed, Embase, Web of Science, and Cochrane. This sequence was followed by a screening and selection of articles by two independent reviewers according to defined inclusion and exclusion criteria. The selected studies were then evaluated and assessed based on study quality and identification of biases, and the results were summarized and reported. This review highlighted the lack of well-designed, randomized controlled trials to evaluate the efficacy and safety of botulinum toxin A for reducing the size/volume of the masseter muscles and for improving masticatory myofascial pain in patients who present with bruxism. Thus, the results were inconclusive.

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LIST OF ABBREVIATIONS

BOTULINUM TOXIN A.....	BTX-A
CALCITONIN GENE RELATED PEPTIDE.....	CGRP
COMPUTED TOMOGRAPHIC.....	CT
ELECTROMYOGRAPHIC.....	EMG
FOOD AND DRUG ADMINISTRATION.....	FDA
MAGNETIC RESONANCE IMAGING.....	MRI
POPULATION, INTERVENTION, COMPARISON, OUTCOME.....	PICO
PRESSURE PAIN THRESHOLD.....	PPT
PRESSURE PAIN TOLERANCE.....	PPtol
SOLUBLE- N-ETHYLMALEIMIDE SENSITIVE FACTOR ATTACHMENT PROTEIN RECEPTOR.....	SNARE
TEMPOROMANDIBULAR JOINT DYSFUNCTION.....	TMD
VISUAL ANALOGUE SCALE.....	VAS

Introduction

With a heightened interest in maintaining a stable dental structure and functional occlusion while simultaneously optimizing orofacial esthetics, the use of botulinum toxin A (BTX-A) injections has emerged as a treatment option to combat the consequences of bruxism, such as masseter hypertrophy and masticatory myofascial pain. Bruxism is defined as a diurnal or nocturnal parafunctional activity, characterized by repetitive masticatory muscular activity, which results in clenching or grinding of the teeth. If this activity results in overload of the stomatognathic system, bruxism may lead to muscle hypertrophy, myofascial pain, limitation in functional movement of the mandible, tooth surface loss, fracture of restorations, hypersensitive teeth, or loss of periodontal support.¹ The prevalence of bruxism in the adult population is reported to be about 8-20% and is more common in females.¹ Bruxism can occur as a result of psychological and neurological diseases, as well as temporomandibular joint problems, sleep apnea, gastroesophageal reflux disorder, and psychosocial factors.² Previously, it was believed that one of the primary etiologies of bruxism was occlusal discrepancies, with the presence of tooth wear confirming diagnosis. This occlusal concept was popularized in an article by Ramfjord in 1961. A more recent hypothesis, proposed by Klass et al, supports the role of the central and autonomic nervous systems in oromandibular activity. In their view, the influence of certain neurotransmitters such as dopamine may increase motor activity of the masticatory muscles which precedes the characteristic tooth grinding.³ A diagnosis of bruxism may be derived by patient report and clinical interview, clinical examination, recording of muscle activity using electromyography, and polysomnography in the case of nocturnal bruxism.²

Benign masseter hypertrophy is a relatively uncommon clinical phenomena that causes swelling at the angle of the mandible and can be a consequence of masticatory muscle

hyperfunction associated with bruxism or emotional stress. Clinical examination may reveal a soft tissue swelling near the angle of the mandible which becomes prominent upon clenching of the teeth.⁴ This may present as a unilateral phenomena. According to Arpa et al, it has been shown that muscular activity during maximum clenching is increased more on the hypertrophied side than on the non-hypertrophied side. Furthermore, a study by Kitagawa et al has shown that hypertrophied masseter muscle with bruxism is uniformly or predominantly composed of type 1 fibers. These results are consistent with muscular enlargement due to “work hypertrophy.”

Diagnosis should not solely be based on patient report or clinical findings but also should include a computed tomographic (CT) scan, a magnetic resonance imaging (MRI) scan, ultrasonographic measurement, or electromyographic measurement. The hypertrophic muscles may be associated with myofascial pain on occasion and may be prominent enough to be considered cosmetically disfiguring by the patient. Moreover, limitation of mouth opening, midline deviation, and masseteric spasm have also been reported in some cases. Various treatment methods have been introduced, ranging from a partial resection of masseter muscle with simultaneous mandible angle ostectomy, radiofrequency volumetric reduction, or intra-masseteric BTX-A injections.⁴

Masticatory myofascial pain is a type of acute or chronic facial pain that is characterized as a soft tissue inflammatory condition causing myogenous pain or stiffness. Masticatory myofascial pain can be considered a primary ailment, or secondary to an underlying condition, such as bruxism, temporomandibular joint dysfunction (TMD), occlusal abnormalities, sleep disturbances, depression, and others. It has been reported in the literature that 65% of patients suffering from myofascial pain have sleep disturbances, which also is a strong predictor of developing clinical depression.⁷

The clinical hallmark of myofascial pain is the trigger point. Myofascial pain consists of two components: sensory and motor. The sensory component contains sensitized nociceptors that give sensations of pain, twitching, and referred pain. The motor component is caused by excess acetylcholine from multiple dysfunctional endplates which results in hyper-irritated contracted nodules accumulating in the muscle fibers. These are palpable bands of taut muscle, known as trigger points, which are a clinical hallmark of myofascial pain. Trigger points can be latent, existing asymptotically until palpated, or active, occurring independent of stimulation and causing a pain that radiates across overlying subcutaneous and cutaneous tissue. The pain is often described as sharp, numbing, radicular, visceral, or deep, and can cause muscle stiffness which in turn may reduce the range of motion and mouth opening and cause disturbed motor function. Diagnosis is based on clinical examination and thorough review of the patient's pain history. Different examination guidelines are available for diagnosis, one being the Diagnostic Criteria for TMDs. A cornerstone of all guidelines is palpation, which, due to a compressive force, locates trigger points as taut bands and reproduces familiar pain and/or localized twitching. Once diagnosed, myofascial pain can be quantified subjectively based on various pain scales. The main management strategies include cognitive behavior therapy, non-pharmacological interventions such as occlusal splints, and pharmacological intervention, including nonsteroidal anti-inflammatory drugs, muscle relaxants, and others. The goals of treatment include providing empathetic reassurance to the patient, as well as deactivating trigger points which aid in pain relief and regain of range of motion.⁸

When managing masseter hypertrophy and masticatory myofascial pain, the clinician is presented with a variety of therapeutic modalities, each with varying degrees of risk/benefit and concerns of patient compliance. The disadvantages/risks of surgical management, for example,

include the risks of general anesthesia, postoperative hemorrhage, edema, hematoma, scarring, facial nerve damage, and others. Occlusal splints for managing the complications of bruxism have shown conflicting reports of success in the literature. This may be due to the heterogeneity of occlusal splints, i.e. maxillary vs mandibular, different materials, different designs of splints, as well as poor patient compliance due to reports of discomfort, xerostomia, and failure to form a habit of wearing the splint.⁸ Pharmacological management is limited in therapeutic success due to the short-term treatment usage recommendations of the drugs compared with the chronic nature of the aforementioned conditions. Cognitive behavioral therapies and lifestyle modifications such as stress management, soft food diets, physical therapies may show unpredictable degrees of success based on patient compliance and recurrence after the modifications have been abandoned. Intra-muscular injections of botulinum toxin A may offer a useful, efficient, and minimally invasive alternative without the required patient compliance.

Botulinum toxin is a potent neurotoxin produced by the rod-shaped, gram-positive anaerobic bacterium *Clostridium Botulinum*, which is known to cause food-borne botulism. The first accurate and complete description of food borne botulism was described in 1820 by the German physician Justinus Kerner. He believed that the toxic substance found in inadequately prepared blood sausages caused symptoms of food poisoning. He not only recognized that the toxin is potent and lethal at low doses but he was the first author to propose that the toxin may have therapeutic qualities.⁹ In 1870, Mueller coined a descriptive term for this food-borne illness: botulism. The term is derived from the Latin word *botulus*, which means sausage. In 1895, *Clostridium Botulinum* was isolated by Emile-Pierre van Ermengem during a sausage poisoning outbreak in Ellezelles, Belgium. It was not until fifty plus years later that the

physiologic mechanism of action was discovered by Arnold Stanley Vincent Burgen, a British physician and pharmacologist.¹⁰

Burgen discovered that the paralytic effect of the neurotoxin was due to its effect on the neuromuscular junction through blocking the release of acetylcholine. In 1979, BTX-A was first used therapeutically in the treatment of neuromuscular disorders, when Allen Scott, an ophthalmologist, injected the toxin into non-human primates to correct strabismus. In 1980, he published the results of a clinical trial conducted on 67 patients that showed that BTX-A injection into muscles of the eyes can improve strabismus in human subjects.¹⁰ Over time, the therapeutic uses of BTX-A have spread into other fields of medicine, including dentistry. The use of BTX-A was first approved by the FDA in 1989 for therapeutic use in strabismus, blepharospasm, and cervical dystonias in humans older than 12 years. It has since been approved for the use for glabellar rhytides (2002), for axillary hyperhidrosis (2004), for chronic migraine (2010), and in 2013 for lateral canthal lines. However, the FDA has not approved BTX-A for the treatment of bruxism or masticatory muscle spasms yet, which may be due to the lack of adequate research on the issue. Hence, these uses are considered off-label.⁹

Botulinum toxin exists in seven different serotypes which are structurally similar but immunologically distinct: A, B, C, D, E, F, and G, with type A being the most widely used. The toxin is a double chain polypeptide, consisting of a heavy chain and light chain linked with a disulfide bond and non-covalent interactions.⁹

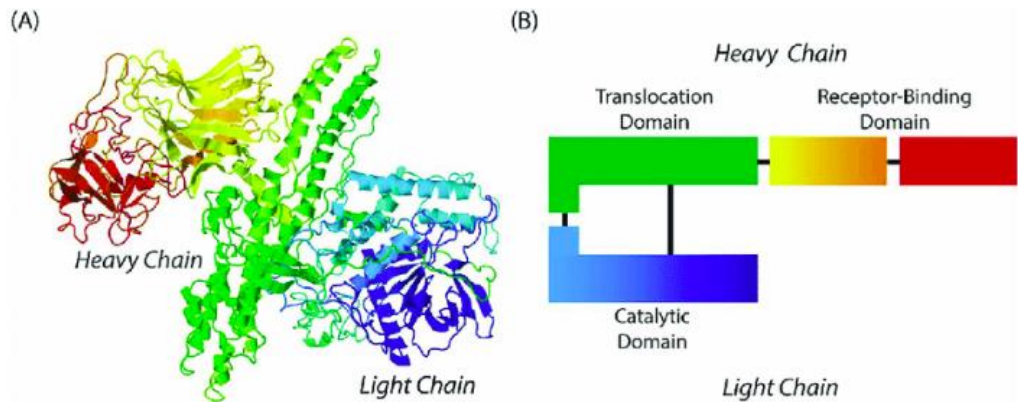


Figure 1.

- A) Crystal structure of botulinum toxin A obtained from the Protein Data Base (PDB), PDB id: 3BTA
 B) Schematic representation of the crystal structure showing the heavy chain and light chain.
 Reference: Lim, C., Granger, J., Porter, M. SERS Detection of Clostridium botulinum Neurotoxin Serotypes A and B in Buffer and Serum: Towards the Development of a Biodefense Test Platform. *Analytica Chimica Acta*: X. (2018).

The carboxy-terminus of the heavy chain mediates the binding of the toxin to cholinergic nerve endings, while the light chain acts as the intracellular toxic portion. In order for muscle contraction to occur, acetylcholine binding to the acetylcholine receptor of the motor endplate is necessary, followed by the acetylcholine exocytosis process at the presynaptic membrane. The light chain is an endopeptidase that cleaves protein components of the SNARE (soluble N-ethylmaleimide sensitive factor attachment protein receptor) protein, which is involved in the neuroexocytosis process of acetylcholine and is essential for membrane fusion. This explains how acetylcholine containing vesicles are prevented from joining with the terminal membrane of the motor neuron, resulting in disruption of the neuromuscular transmission. When injected into muscle, the neurotoxin interferes with the action potential mechanism producing selective paralysis and atrophy.¹¹

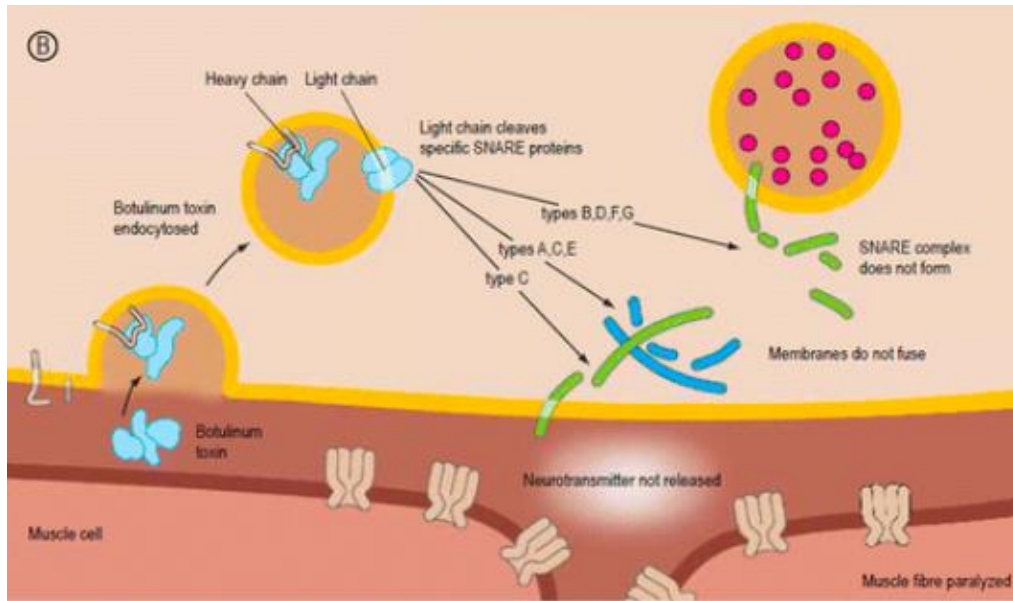


Figure 2.

Figure 2. Mechanism of Action of Botulinum Toxin A.
 Reference: Fromage, G. Science of botulinum toxin: structure, mechanism of action and therapeutic uses. *Journal of Aesthetic Nursing*. (2013). Vol 1. No. 2.

The aim of this research was to employ the systematic review process to investigate the true or reliable scientific evidence contained in four major databases pertaining to the effectiveness and safety of intra-muscular injections of botulinum toxin A for the treatment of masticatory myofascial pain and benign masseter hypertrophy associated with bruxism, compared with placebo or other clinical treatments prescribed for bruxism such as occlusal splints, pharmacotherapy, or lifestyle modification.

Materials/Methods

In an effort to explore the use of BTX-A injections in the scope of dentistry, a scoping review was completed with the aid of an institutional librarian. The scoping review was completed to explore which of the therapeutic and/or cosmetic uses of botulinum toxin would fall within the scope of dentistry. The scoping review research question was formulated: “In patients who have facial myopathies, myofascial pain, or esthetic concerns, is botulinum toxin A

a safe and effective intervention?” The aim was to assess the extent of the available evidence, to organize it into groups, and to highlight gaps in knowledge. Through completing a scoping review, the following uses of botulinum toxin were highlighted:

1. Facial myopathies: Bruxism (Injection into masseter and temporalis), Oromandibular dystonia (Injection into masseter, medial or lateral pterygoids), Masseter hypertrophy (related to bruxism, injection into masseter), TMJ dislocation
2. Myofascial pain: Pain from TMD/bruxism, Migraines
3. Esthetics: Gummy smile, wrinkles, asymmetric smiles (injection into depressor labii inferioris)

Using the PICO format (population, intervention, comparison, outcome), a more focused and specific research question was formulated: "Is botulinum toxin A treatment a safe and effective intervention for patients who present with myofascial pain and masseter hypertrophy from bruxism?" To perform this systematic review, it becomes necessary to break down this question into its individual PICO elements and to then develop a search protocol using concepts from the research question, develop inclusion and exclusion criteria, and finally to identify the types of study design that should be included in the review:

P Population	Patients who present with masseter hypertrophy and myofascial pain associated with bruxism
I Intervention	Intra-muscular injections of botulinum toxin A
C Comparison	No botulinum toxin A treatment, alternative treatment, or placebo.
O Outcome	Effectiveness (Reduction of masseter size and improvement of pain) and safety

Table 1. PICO guided question format

The planning and preparation of this systematic review followed the guidelines established by PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). It is an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses. PRISMA consists of a 27-item checklist and a 4-phase flow diagram, which evaluates the quality of the review and allows replication of the review methods.

Inclusion criteria included randomized control trials and controlled clinical trials that studied human subjects aged 18 years and older who presented with unilateral or bilateral benign masseter hypertrophy and or masticatory myofascial pain due to bruxism or a myogenous temporomandibular disorder, where intra-muscular injections of BTX-A were performed as intervention, with control of injections of saline (placebo) or other traditional methods of treatment for bruxism, such as occlusal splints, pharmacotherapy/physiotherapy, or counseling/reassurance. Primary outcomes to be measured will include assessment of myofascial pain (subjective and/or objective), reduction of the size of the hypertrophied masseter (muscle thickness), and safety in the form of reported adverse effects.

Exclusion criteria included animal studies, case series, cohort studies, and other systematic reviews. The following concurrent conditions were excluded:

1. Participants who presented with a comorbidity to bruxism, such as neurological disease or movement disorders were excluded.
2. Participants who presented with masseter hypertrophy from malignancy.
3. Participants a presented with headaches or cervical pain of the myofascial type.
4. Studies that included cosmetic fillers as an adjunctive treatment alongside botulinum toxin A.

Four databases were searched on November 12th, 2021, with the assistance of the academic librarian: PubMed, Embase, Web of Science, and Cochrane. Detailed search strategies were developed for each database. These were based on the search protocol for PubMed but modified appropriately for each additional database.

The steps of search term generation were as follows: 1) Divide research question into component concepts, 2) Match MeSH terms to concepts in research question, and 3) Devise synonymous key words/phrases. (This can include colloquial ways of describing something, alternative spellings, etc. This process is highlighted in Table 2.)

Concept in Research Question	MeSH terms	Synonymous keywords/phrases
TMD/ Bruxism/ Myofascial Pain	"Temporomandibular Joint Disorders"[Mesh] "Bruxism"[Mesh] "Masticatory Muscles, Hypertrophy of" [Supplementary Concept] "Myofascial Pain Syndromes"[Mesh] OR (Hypertrophy"[Mesh] AND "Masseter Muscle"[Mesh])	"TMD" "Temporomandibular Joint Disorder" "masseter hypertrophy" "Bruxism" "teeth clenching" "teeth grinding" "Myofascial Pain"
Botox	"botulinum toxins, type a"[MeSH]	"Botox" "botulinum toxin"

Table 2. Search Term Generation

Data collection and analysis

On December 9th, 2021, all articles that were retrieved from the database searches were downloaded and imported into Zotero, a citation manager, which allows duplicate articles to be removed. On January 6th, 2022, the remaining articles were then exported to Rayyan, a web based tool used to screen and select articles based on the specified inclusion and exclusion

criteria. Two review authors identified potentially relevant articles, blindly and independently, based on the pre-defined inclusion and exclusion criteria. A title and abstract screening was completed first, followed by a full text screening. Rayyan was used to expedite this process by filtering articles into an “include,” “exclude,” and “maybe” category by the reviewers.

After screening and selection was finalized, the data of relevant articles was then manually extracted in the format of an Excel spreadsheet. Extracted data included:

1. Study characteristics: Author, title, type of study, duration
2. Participants: Sample size, gender, average age
3. Intervention: Botulinum toxin
4. Control: Placebo, occlusal splint, pharmacotherapy, lifestyle modification, behavioral therapy
5. Outcome Measures: Primary and secondary outcomes measured
6. Results

The extracted data was used to assess for heterogeneity/homogeneity based on the reviewer’s clinical judgment and visual inspection, which will help to detect outliers, asymmetry, or patterns of heterogeneity within the data.

Assessment of Study Quality

The methodological quality of the individual study designs was assessed for the purpose of limiting bias and guiding the interpretation of the findings of the studies. This assessment was based on the criteria outlined in the Cochrane Collaboration’s tool for assessing risk of bias in randomized trials. The first part of the tool (Table 3) highlights the domains of bias on which the tool is based:

1. Randomization/Concealment of Allocation (Selection bias): This will be graded as adequate, inadequate, or unclear. Adequate methods of randomization/allocation concealment would include approaches that are:
 - a. Centralized (Allocation by a central office unaware of subject characteristics)
 - b. Pre-numbered or coded identical containers which are administered serially to participants
 - c. On-site computer system combined with allocations kept in a locked, unreadable computer file that can be accessed only after the characteristics of an enrolled participant have been entered (or sealed, opaque envelopes).

Approaches to randomization/allocation concealment that are inadequate shall include alternation, the use of case record numbers or dates of birth, or any procedure that is transparent before allocation, such as an open list of random numbers. Approaches to allocation concealment that are unclear shall include studies that don't report on any concealment of allocation.

2. Blinding of assignment status by providers and participants (Performance bias): This will be graded as adequate, inadequate, or unclear. Were the recipients of care unaware of their assigned intervention? Were those providing care unaware of the assigned intervention?
3. Blinding of outcomes assessment (Detection Bias): Were providers assessing the outcomes of care aware of which treatment the participant received? This will be graded as adequate, inadequate, or unclear.
4. Handling of withdrawals and losses (Attrition Bias): There is a certain expectancy of withdrawals and losses that will occur in human studies. Attrition bias can lead to inaccurate results because it affects internal and/or external validity. Reasons for attrition/exclusions should be reported. To avoid attrition, the study design should include measures to help reduce participant dropout. Some of these measures include providing compensation, minimizing the number of follow-ups as much as possible, and recruiting more patients than needed. This will be graded as adequate, inadequate, or unclear.

Domain	Description	Inadequate	Adequate	Unclear
Selection Bias: Random sequence generation	Described the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups	Inadequate generation of a randomized sequence	Random sequence generation method should produce comparable groups.	Not described in sufficient detail
Selection Bias: Allocation concealment	Described the method used to conceal the allocation in sequence in sufficient detail to determine whether intervention allocations could have been foreseen before or during enrollment	Inadequate concealment of allocations prior to assignment	Intervention allocations likely could not have been foreseen in before or during enrollment	Not described in sufficient detail
Performance bias (Blinding participants and personnel)	Described all measured used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provided any information relating to whether the intended blinding was elective.	Knowledge of the allocated interventions by participants and personnel during the study	Blinding was likely effective	Not described in sufficient detail
Detection bias (Blinding outcome assessment)	Described all measured used, if any, to blind outcome assessors from knowledge of which intervention a participant received. Provided any information relating to whether the intended blinding was elective.	Knowledge of the allocated interventions by outcome assessors	Blinding was likely effective	Not described in sufficient detail
Attrition bias (Incomplete outcome data)	Described the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. Stated whether attrition and exclusions were reported, the numbers in each intervention group, reasons for attrition/exclusions were reported.	Attrition bias due to amount, nature, or handling of incomplete outcome data.	Handling of incomplete outcome data was complete and unlikely to have produced bias	Insufficient reporting of attrition/exclusions to permit judgment, i.e. no reasons for missing data provided.

Table 3. Cochrane Collaboration’s tool for assessing of risk of bias in randomized trials – Part 1

The second part of the Cochrane Collaboration’s tool (Table 4) involves summarizing the validity of the studies based on the risk of bias according to the grading system shown in Table 4.

Risk of Bias	Interpretation	Relationship to Individual Criteria
A. Low risk of bias	Plausible bias unlikely to seriously alter the results	All of the domains were graded as adequate
B. Moderate risk of bias	Plausible bias that raises some doubt about the results	Two or more domains were graded as adequate.
C. High risk of bias	Plausible bias that seriously weakens confidence in the results	Three or more domains were graded as inadequate or unclear.

Table 4. Cochrane Collaboration’s tool for assessing risk of bias in randomized trials – Part 2

Clinical heterogeneity will be assessed by examining the characteristics of the studies and the similarity between types of participants, interventions, and outcomes.

Results

The finalized search for each corresponding database can be seen in Table 5

Database	Search
Pubmed	("TMD" OR "Temporomandibular Joint Disorder" OR "masseter hypertrophy" OR "Bruxism" OR "teeth clenching" OR "teeth grinding" OR "Temporomandibular Joint Disorders"[Mesh] OR "Bruxism"[Mesh] OR "Masticatory Muscles, Hypertrophy of" [Supplementary Concept] OR "Myofascial Pain" OR "Myofascial Pain Syndromes"[Mesh] OR ("Hypertrophy"[Mesh] AND "Masseter Muscle"[Mesh])) AND ("botulinum toxins, type a"[MeSH Terms] OR "Botox" OR "botulinum toxin")
Embase	('temporomandibular joint disorder'/exp OR 'temporomandibular dysfunction' OR 'temporomandibular joint disorder' OR 'temporomandibular joint disorders' OR 'temporomandibular joint dysfunction' OR 'masseter hypertrophy'/exp OR 'masseter hypertrophy' OR 'bruxism'/exp OR 'teeth clenching' OR 'teeth grinding' OR 'bruxism' OR 'myofascial pain'/exp OR 'myofascial pain' OR 'myofascial pain syndrome' OR 'myofascial pain syndromes') AND ('botulinum toxin a'/exp OR 'botox' OR 'botulinum a toxin')
Web of Science	TS=("temporomandibular joint disorder" OR "temporomandibular dysfunction" OR "temporomandibular joint disorder*" OR "temporomandibular joint dysfunction*" OR "TMD" OR "masseter hypertrophy" OR "bruxism" OR "teeth clenching" OR "teeth grinding" OR "myofascial pain" OR "myofascial pain syndrome*") AND TS=("botulinum toxin a" OR "botox" OR "botulinum a toxin" OR "Botulinium toxin")
Cochrane	"TMD" OR "Temporomandibular Joint Disorder" OR "masseter hypertrophy" OR "Bruxism" OR "teeth clenching" OR "teeth grinding" MeSH descriptor: [Temporomandibular Joint Disorders] explode all trees MeSH descriptor: [Myofascial Pain Syndromes] explode all trees MeSH descriptor: [Bruxism] explode all trees MeSH descriptor: [Masseter Muscle] explode all trees MeSH descriptor: [Hypertrophy] explode all trees {OR #1-#4} #5 AND #6 #7 OR #8 MeSH descriptor: [Botulinum Toxins, Type A] explode all trees "botulinum toxin a" OR "botox" OR "botulinum a toxin" OR "Botulinium toxin"

Table 5. Finalized database searches

The databases were searched on November 12, 2021, and the search yielded the following number of articles per database: PubMed (487), Embase (705), Web of Science (173), and Cochrane (90), for a total of 1455 search results. The search results were downloaded from the respective database and exported to Zotero on December 9, 2021. The process of removing duplicate articles was completed on Jan 6th, 2022, for an updated total of 978 articles. The articles were then imported into Rayyan, where a primary screening was completed based on the title and abstract of articles. The primary screening was completed on February 9th, 2022 and yielded the following results: Include (186), Exclude (700), and Maybe (90). On February 15th, 2022, the secondary screening, or full-text screening was initiated for those articles that were placed in the “include” and “maybe” category as part of the abstract screening. The full-text screening was completed on April 3rd, 2022 and yielded 2 final results for the outcomes of improvement in masseter hypertrophy and 11 final results for the outcome of improvement in myofascial pain. However, there were only 12 final articles, since one of the articles included both outcomes for masseter hypertrophy and myofascial pain (Canales 2020). The excluded studies were removed due to failure to meet inclusion criteria for study design, population, and outcome (page 9). Starting on April 11, 2022, an excel template was created to be used as a data report sheet and data was extracted as mentioned in the “Methods” section (page 11). See Table 6A, 6B, 6C (page 18-20). It was determined that a meta-analysis could not be completed. Since a meta-analysis requires homogenous quantitative data in order to develop a conclusion that has greater statistical power, it is not possible to do a meta-analysis with the available data.

Figure 3. PRISMA flow chart

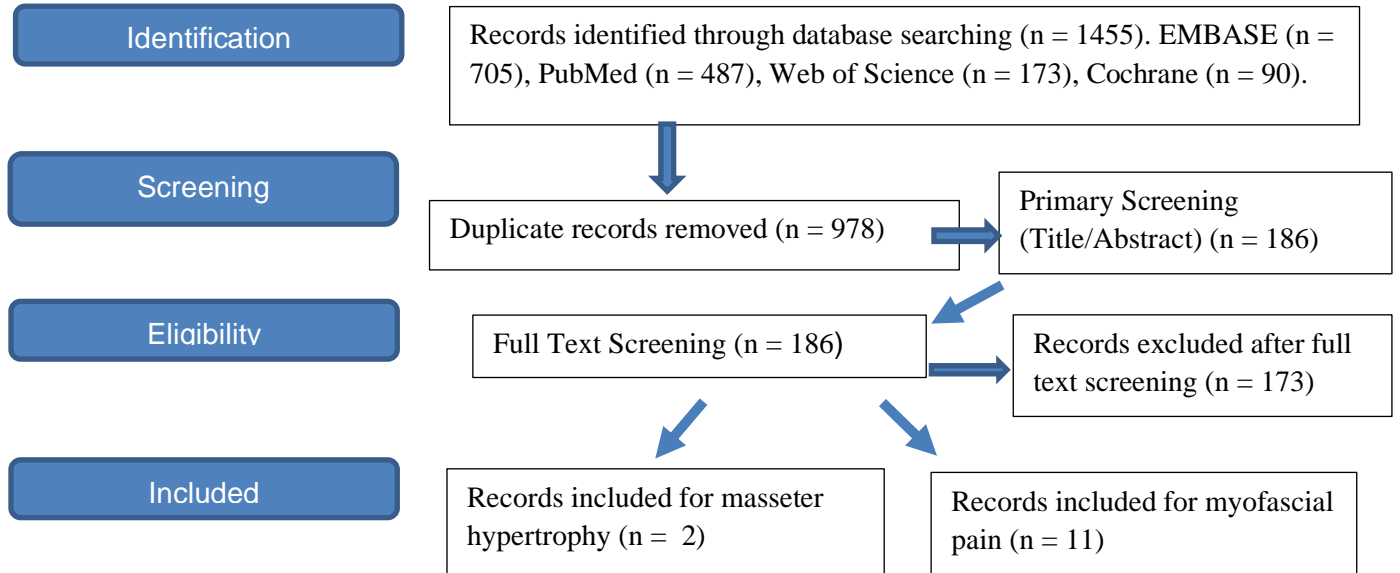


TABLE 6. Extracted Data for Final Analysis and Comparison

Article	Author	Title	Study Design
1	Park et al 2018	Does Botulinum Toxin Injection into Masseter Muscles Affect Subcutaneous Thickness?	Double-blind, randomized placebo-controlled trial
2	Canales et al 2020	Efficacy and Safety of Botulinum Toxin Type A on Persistent Myofascial Pain: A Randomized Clinical Trial	Double-blind, randomized placebo-controlled trial
3	Al-Wayli 2017	Treatment of chronic pain associated with nocturnal bruxism with botulinum toxin. A prospective and randomized clinical study	Randomized, controlled, parallel group design study
4	Kaya et al 2021	Botulinum toxin treatment of temporomandibular joint pain in patients with bruxism: A prospective and randomized clinical study	Randomized controlled trial
5	Al-Wayli et al 2021	Does botulinum toxin have any role in the management of chronic pain associated with bruxism?	Double-blind, randomized, placebo- controlled trial
6	Nixdorf et al 2002	Randomized controlled trial of botulinum toxin A for chronic myogenous orofacial pain	Double-blind, randomized, placebo-controlled trial
7	Yurttutan et al 2019	Which Treatment Is Effective for Bruxism: Occlusal Splints or Botulinum Toxin?	Single-blind, randomized controlled trial
8	Jadhao et al 2017	Efficacy of botulinum toxin in treating myofascial pain and occlusal force characteristics of masticatory muscles in bruxism	Double-blind, randomized, placebo-controlled trial
9	Patel et al 2017	IncobotulinumtoxinA Injection for Temporomandibular Joint Disorder: A Randomized Controlled Pilot Study	Double blind, randomized, placebo-controlled trial
10	Guarda-Nardini et al 2012	Myofascial pain of the jaw muscles: comparison of short-term effectiveness of botulinum toxin injections and fascial manipulation technique.	Randomized controlled trial
11	Ernberg et al 2011	Efficacy of botulinum toxin type A for treatment of persistent myofascial TMD pain: A randomized, controlled, double-blind multicenter study	Double-blind, randomized, placebo-controlled trial
12	Guarda-Nardini et al 2008	Efficacy of botulinum toxin in treating myofascial pain in bruxers: A controlled placebo pilot study	Double-blind, randomized, placebo-controlled trial

Table 6a. Author, Title, Design

Article	Population	Intervention	Comparison
1	20 participants (65% female, 35% male; aged 20-40 years)	Bilateral, intra-masseteric injections of BTX-A: 50 units total.	Normal saline injections
2	100 participants (All female; mean age 36.8)	Bilateral, intra-masseteric injections of BTX-A: 60 units (low dose group), 100 units (medium dose group), 150 units (high dose group)	Positive control group: Oral appliance Negative control group: Normal saline injections
3	50 participants (all female; mean age 45.5)	Bilateral, intra-masseteric injections of BTX-A: 40 units total	Oral appliance and behavioral strategies
4	40 participants (7 males, 33 females; mean age 36.3)	Unilateral intra-masseteric injections of BTX-A: 24 units total	Oral appliance
5	40 participants (24 females, 16 males)	Bilateral, intra-masseteric injections of BTX-A: 40 units total	Normal saline injections
6	10 participants (all female; mean age 33)	Bilateral, intra-masseteric injections of BTX-A: 100 units total	Normal saline injections
7	73 participants	Bilateral, intra-masseteric injections of BTX-A: 60 units total	Oral appliance
8	24 participants (mean age 20-35)	Bilateral, intra-masseteric injections of BTX-A: 60 units total	Placebo group: Normal saline injections Control group: No treatment
9	20 participants	Bilateral, intra-masseteric injections of BTX-A: 100 units total	Normal saline injections
10	30 participants	Bilateral, intra-masseteric injections of BTX-A: 60 units total	Fascial manipulation techniques
11	21 participants	Bilateral, intra-masseteric injections of BTX-A: 100 units total	Normal saline injections
12	20 participants (10 males, 10 females; aged 24-45)	Bilateral, intra-masseteric injections of BTX-A: 60 units total	Normal saline injections

Table 6b. Population, Intervention, Outcome

Article	Outcome measures	Follow-up	Results
1	Masseter muscle thickness using ultrasound	Baseline, 4, 8, 12 weeks.	Masseter muscle thickness significantly decreased at all 4 periods of measurements in experimental group
2	-Masseter muscle thickness using ultrasound -Myofascial pain using VAS and PPT.	Baseline, day of treatment, 7, 14, 21, 28, 90, 180 days.	-Masseter muscle thickness significantly decreased in experimental group in all evaluations. -Significant decrease in pain in BTX-A group throughout experiment (no difference between oral appliance group)
3	Myofascial pain using VAS	Baseline, 3 weeks, 2 months, 6 months, 1 year	Significant decrease in pain in the BTX-A group starting at 3 weeks until last follow up.
4	Myofascial pain using VAS	Baseline, 2 weeks, 6 weeks, 3 months, and 6 months	Significant decrease in pain in both groups at each follow up
5	Myofascial pain using VAS and subjective efficacy of treatment	Baseline, 2, 4, 8, 12, 16, 18, 20, 24 weeks.	Significant decrease in pain in BTX-A group, with pain returning at 12 weeks.
6	Myofascial pain using VAS, range of interincisal jaw opening, palpation tenderness	Baseline and 8 weeks	No significant differences was found in pain intensity. Statistical significance in maximum opening without pain achieved in placebo group.
7	Myofascial pain using Temporomandibular Disorder Pain Screener, Graded Chronic Pain Scale, Oral Behavior Checklist, Jaw Function Limitation Scale, VAS	Before treatment and 6 months after treatment	Significant decrease in pain achieved in BTX-A group compared to oral appliance group alone.
8	Myofascial pain using VAS and subjective efficacy of treatment	Baseline, 1 week, 3 months, 6 months	Significant decrease in pain in BTX-A group compared to placebo and no treatment group from baseline to 6 months.
9	Myofascial pain using VAS and decrease in pain medication usage	Baseline and 4 week intervals until 16 weeks.	Significant decrease in pain in BTX-A group compared to placebo group at 4 weeks.
10	Myofascial pain using VAS	Baseline and 3 months	Both groups provided significant improvement of pain
11	Myofascial pain using VAS, jaw opening capacity, palpatory pain, PPT, PPTol	Baseline, 1 month, 3 months	Significant pain reduction in both groups at 1 and 3 months
12	Myofascial pain using VAS, subjective efficacy of treatment	Baseline, 1 week, 1 month, 6 months	Decrease in pain in BTX-A group compared to placebo, however, not significant

Table 6c. Outcome, Follow-ups, Results

Article	Selection Bias	Performance Bias	Detection Bias	Attrition Bias	Overall Bias
1	Adequate	Adequate	Adequate	Inadequate	Moderate
2	Unclear	Adequate	Adequate	Adequate	Moderate
3	Unclear	Unclear	Unclear	Unclear	High
4	Unclear	Unclear	Unclear	Unclear	High
5	Inadequate	Adequate	Unclear	Adequate	Moderate
6	Unclear	Adequate	Unclear	Inadequate	High
7	Unclear	Inadequate	Inadequate	Inadequate	High
8	Unclear	Adequate	Unclear	Unclear	High
9	Unclear	Inadequate	Unclear	Adequate	High
10	Unclear	Inadequate	Inadequate	Unclear	High
11	Adequate	Adequate	Unclear	Adequate	Moderate
12	Unclear	Adequate	Unclear	Unclear	High

Table 7. Bias Grading of Articles

Effectiveness

1. Reduction of masseter hypertrophy

A) Type of study, population characteristics, follow-up period, and bias

Both studies were designed as double-blind, randomized, placebo-controlled trials. While Park 2018 included only 20 participants, Canales 2020 included 100 participants. In both studies, majority of the participants were female and had a mean age around 30 years old. The follow-up periods ranged from 12 weeks to 180 days.^{24, 25} Both articles were graded as having a moderate risk of bias according to the aforementioned grading criteria. Please refer to table 7.

B) Intervention

The interventions described in both included studies included bilateral, intra-masseteric injections of BTX-A. Park 2018 utilized 50 units of BTX-A, while Canales 2020 utilized a range from 60 units to 150 units.^{24, 25}

C) Comparison/Control

The control group in Park 2018 was injected with normal saline.²⁴ The positive control group in Canales 2020 included an oral appliance, while the negative control group received normal saline injections.²⁵

D) Outcome Measures

Using ultrasonography, Park 2018 measured masseter muscle thickness and the thickness from the skin surface to the masseter muscle under the following 2 conditions: 1) with the teeth not clenched, and hence with the masseter muscle in a relaxed position, and 2) with the teeth clenched to elicit the maximum contraction of the masseter muscle. The measurements were taken before the injection, and at 4, 8, and 12 weeks after the injection.²⁴ In Canales 2020, the thickness of both masseters and anterior temporalis muscles during maximum contraction was calculated using ultrasonography by a single calibrated operator. Subjects were evaluated 8 times throughout the 6 months of study: baseline, at day of injection, and 7, 14, 21, 28, 90, and 180 days after.²⁵

E) Results

Park 2018 reported that the masseter muscle thickness was consistently decreased at rest and during maximum contraction at all 4 periods of measurements in the experimental group. However, the distance between skin to the masseter muscle did not differ significantly over time or by group at any of the 4 periods when measurements were

taken.²⁴ Canales 2020 reported a significant decrease in the thickness of all muscles treated with BTX-A injections in all evaluations. However, when botulinum toxin A group was compared to the sterile saline group, only the group which received low dose of BTX-A injection showed no significant differences at any evaluation.²⁵

2. Decrease in masticatory myofascial pain

A) Type of study, population characteristics, follow-up period, and bias

All studies were randomized controlled trials. Seven of the 11 included studies were designed as double-blind, randomized, placebo-controlled trials. Four studies (Al-wayli 2017, Kaya 2021, Yurtuttan 2019, and Guarda-Nardini 2012) could not be double-blinded, due to the inherent difference in treatment groups (BTX-A injections vs oral appliance and BTX-A vs fascial manipulation techniques). The number of participants ranged from 10 participants in Nixdorf 2002 to 100 participants in Canales 2020. The majority of the participants were female, with three studies having exclusively female participants.^{25, 26, 29} Five studies did not provide information on the characteristics of the participants.³⁰⁻³⁴ The follow-up periods ranged from 8 weeks to 1 year. All studies included baseline measures. Eight out of the 11 included studies were graded as having a high risk of bias, while three were graded as having a moderate risk of bias according to the aforementioned grading criteria.

B) Intervention

All included studies had interventions of intra-masseteric injections of BTX-A, ranging from 24 units to 150 units. All injections were bilateral, except Kaya 2021, in which injections were only administered unilaterally. Nixdorf 2002, Patel 2017, and Ernberg

2011 administered neurotoxin with correct placement confirmed by electromyographic (EMG) machine. All other studies administered neurotoxin via palpation of muscles and apparent knowledge of anatomical landmarks, although landmarks were often not specified.

C) Comparison/Control

Canales 2020 included a positive control group, which was instructed to wear an oral appliance, and a negative control group which received injections of normal saline. Three of the 11 studies had a control group that was instructed to wear an oral appliance (Al-Wayli 2017, Kaya 2021, Yurttutan 2019), while one study had a control group who received fascial manipulation therapy.³³ All other studies were placebo-controlled with normal saline injections.

D) Outcome Measures

All included studies measured subjective masticatory myofascial pain using a visual analogue scale (VAS). Canales 2020 measured subjective masticatory myofascial pain, as well as a more objective pain measure using pressure pain threshold (PPT). While in a relaxed position, a digital algometer 1cm² circular point was used to exert pressure on the muscles bilaterally. An increasing and constant pressure of 0.5kg/cm² was applied perpendicular to the skin. Participants were advised to indicate when the pressure became painful. PPT sites were aligned with the treatment injection sites. In Ernberg 2011, the outcome measures also included pressure pain threshold (PPT) and tolerance (PPTol).

E) Results

Canales 2020 reported a significant decrease of subjective pain in all BTX-A groups seven days after injections and throughout the experiment. In addition, no significant

differences were found among the BTX-A groups. In comparison to the placebo group, subjective pain of BTX-A groups significantly lowered after 14 days and up to the end of the study. When compared to the oral appliance group, no statistical differences were found after 14 days and up to the end of the study. In the BTX-A groups, PPT significantly increased after 14 and 21 days in the masseter and anterior temporalis respectively and was maintained up to the end of the study. BTX-A significantly improved PPT of both muscles after 21 days when compared to the placebo group. Compared to the oral appliance group, all BTX-A groups presented with significantly higher values on the 21st and 28th day for the anterior temporalis and on the 14th, 21st, 28th, and 90th day for the masseter. However, no significant differences were found between BTX-A and oral appliance at the last follow up.²⁵ Al-wayli 2017 reported mean pain scores that decreased significantly in the BTX-A group starting at the 3 week follow up until the last follow-up. However, in the control group, mean pain score did not show improvement with time.²⁶ Kaya 2021 reported statistically significant decrease in pain in both groups at each follow up.²⁷ Al-wayli 2021 demonstrated significantly decreased VPS scores initially, with mild pain returning after 12 weeks. Subjective efficacy of the treatment was rated mostly as good or excellent by the participants.²⁸ The results of Nixdorf 2002 showed that no statistically significant difference was found in pain intensity or palpation muscle tenderness. However, statistical significance was achieved for maximum opening without pain with the test group having a decreased opening.²⁹ In Yurttutan 2019, the VAS and questionnaire scores decreased significantly in the BTX-A group and the occlusal splint and BTX-A group, compared to the occlusal splint group alone.³⁰ Jadhao 2017 showed that when compared to placebo (saline) and no treatment,

intra muscular injections of BTX-A resulted in a significant decrease in pain from baseline to 6 months.³¹ In Patel 2017, pre-injection scores were similar between the test group and placebo group. At 4 weeks, both groups displayed a statistically significant decrease in pain scores from baseline, with the BTX-A group showing statistically significant reduced scores compared to the placebo group. Similar results were seen when examining the composite masticatory muscle tenderness scores. There was no significant change in usage of pain medication.³² The results from Guarda-Nardini 2008 showed that both objective (range of mandibular movements) and subjective (pain at rest, pain at chewing) outcome variables were higher in the test group than in the placebo group, although these results were not statistically significant.³⁵ Guarda-Nardini 2012 showed that both BTX-A injections and fascial manipulation techniques provided significant improvement over time for pain symptoms.³³ Ernberg 2011 showed that for pain at rest, there was no significant difference in pain intensity between BTX-A or saline injections. However, a time effect was observed in that there was significant pain reduction at 1- and 3-months follow ups in both groups. There were no significant changes after treatment in any other outcome measure, with the exception of pain on palpation, which decreased 3 months after saline injection.³⁵

Safety

1. Adverse Effects

The majority of the included studies did not specify if the BTX-A injections had adverse effects or reported that they were transient or absent, both locally and systemically.

Canales 2020 reported a transient decline in masticatory performance and muscle contraction and a decrease in muscle thickness and coronoid and condylar process bone volume as dose related

adverse effects. These were associated with higher doses of the toxin; however, with lower doses of the toxin, adverse effects were not found or easily overcome.²⁵ Nixdorf 2002 reported on a subset of participants who experienced a difficulty in smiling, with an onset during the first week after injections. The experience was a unilateral presentation with an inability to activate the zygomaticus major muscle, resulting in an asymmetrical smile. Several participants also reported increased pain after administration of the injections, with comments ranging from tenderness for 2 days to pain for 8+ weeks. The paralyzation of the zygomaticus major muscle was thought to be from diffusion of the BTX-A from the masseter muscle via the needle puncture site – direct deposition of the toxin into the muscles of facial expression is unlikely since electromyographic guidance was used to confirm needle placement into the masseter muscle.²⁹ An objective of the study conducted by Patel 2017 was to assess the safety of treatment with BTX-A compared to placebo in the form of adverse events. Participants were asked for adverse event occurrence such as pain at injection site, local reaction to the injection, and difficulty chewing, however, no such adverse effects were noted.³² Guarda-Nardini 2012 reported no relevant side effects with the exception of some minor discomfort during chewing reported by participants in the two to three weeks following injections. These effects disappeared and were not rated as relevant by the participants.³³ In the study conducted by Ernberg in 2011, patients reported frequent side effects with varying intensity the first week after injections. However, these side effects seemed to be unrelated to BTX-A. The most frequently reported side effect was headache, which 7 patients reported after botulinum toxin injections and 9 patients reported after saline injections. Two patients reported tiredness or fatigue after botulinum toxin injections, i.e. symptoms that could be interpreted as muscle weakness. Four patients reported this same symptom after receiving saline injections. Three patients reported jaw pain after botulinum toxin injections, compared to one

after saline injection. One participant reported dry mouth after botulinum toxin A injection. Influenza like symptoms were reported by two patients after botulinum toxin injections and one patient after saline. All side effects had resolved at the 1-month follow up.³⁴

Use of Meta-Analysis

Quantitative analysis

The included studies presented with considerable heterogeneity. There were differing dosages of botulinum toxin A (24-150 units), differing injection sites (based on different landmarks vs. EMG-guided injections), and different control groups (occlusal splint vs. saline injections). Moreover, each study had a differing method of measuring variables and outcomes. Since a meta-analysis requires homogenous quantitative data in order to develop a conclusion that has greater statistical power, it is not possible to do a meta-analysis with the available data.

Discussion

Botulinum toxin A, a purified exotoxin of *Clostridium botulinum*, has been used for the last few decades for numerous neuromuscular disorders. The toxin has been shown to inhibit neuromuscular transmission, which may explain its clinical application in the treatment of bruxism. Bruxism has a multifactorial etiology mediated by the central nervous and autonomic systems, which regulate the motor activity of the muscles of mastication.¹³ Bruxism may present with secondary conditions, such as masseter hypertrophy and masticatory myofascial pain which effect the patient's esthetic appearance and quality of life. Systematic reviews and controlled clinical trials have been completed addressing the use of BTX-A injections for reducing the frequency of bruxism episodes. Very few systematic reviews have addressed the therapeutic and

cosmetic use of botulinum toxin injections for the sequelae of bruxism, which include but are not limited to masseter hypertrophy and masticatory myofascial pain.

Patients often favor conservative treatments, such as behavioral modification and occlusal splints, which are non-invasive, relatively painless, and economical, but they do require high levels of compliance to be effective. Pharmacotherapy, such as anti-inflammatory drugs and muscle relaxants, may also be effective and is generally accepted by patients, however, it requires an acceptable level of health and awareness of drug interactions. Intramuscular injection of BTX-A requires little compliance and is a quick treatment. However, due to the transient nature of the toxin, injections are required every few months which result in a high cost of treatment and can be a barrier as an option for treatment. In addition, the intangible costs of BTX-A include the risk of side effects, albeit transitory and reversible.

The United States Food and Drug Administration (FDA) approved BTX-A for the treatment of muscle disorders due to its ability to inhibit synaptic exocytosis of acetylcholine and thus inhibiting neural transmission.¹⁵ Moreover, experimental studies have demonstrated an analgesic effect of BTX-A, which is independent of its motor effect, leading to new potential indications for pain conditions including chronic myofascial pain.¹⁵⁻¹⁷ The majority of the included studies studied the outcome of pain subjectively, using a visual analogue scale (VAS).

THE VAS

The VAS is a 100mm horizontal scale anchored by word descriptors at each end, with the left end usually representing “no pain” and the right end representing “worst pain imaginable.”¹⁸ The patient places a mark at a point on the line corresponding to the patient’s rating of pain intensity. While substantial evidence exists to support its validity and reliability for measuring chronic and acute pain intensity, it does possess some limitations. It can be difficult to administer

to patients with perceptual-motor problems, which may be common in chronically painful conditions. Also, because a VAS is generally scored with a ruler (the score is the number of centimeters or millimeters from the end of the line), this can make scoring more time consuming and introduce more error. Finally, relative to other rating scales, a VAS produces more noncompletion rates among certain populations, mostly those with cognitive limitations.³⁷

THE PPT

Canales 2020 showed that even low doses of BTX-A not only reduces subjective pain intensity, but can also increase pain pressure threshold (PPT) values over 24 weeks. PPT is determined using a digital algometer which is used to exert pressure on the muscles. An increasing and constant pressure is applied to the site and subjects are instructed to determine when the pressure becomes painful. Canales 2020 thus demonstrated a potential analgesic effect independent of the accompanying dose-dependent motor activity.²⁵ This analgesic effect has been shown in different in vivo and in vitro studies where BTX-A had peripheral (decreasing levels of substances P, calcitonin gene-related peptide (CGRP), and glutamate)^{19, 20} and even central (axonal transport to sensory regions of trigeminal ganglia)^{21, 22} action on sensitive neurotransmitters and sensory nerves, or both. It should be noted, however, that decreased motor activity, i.e. decreased muscle contractions due to the paralytic effects of BTX-A may also contribute to pain relief. When compared to an occlusal splint, no statistical differences of subjective pain were found with botulinum toxin A at day 14 or until the end of the study. However, when measuring pain using PPT, compared to the oral appliance group, all BTX-A groups presented with significantly higher values on the 21st and 28th day for the anterior temporalis and on the 14th, 21st, 28th, and 90th day for the masseter.²⁵ Although the PPT is regarded as a “more objective” measure of pain because it involves external stimulation in the

form of increasing pressure, it still a highly subjective measure as the subject is still the ultimate determinant of pain.

Ernberg 2011 also included PPT as a part of the outcome measures for pain, as well as VAS, use of analgesics, pain-free jaw opening, and pain on palpation.³⁴ In contrast to Canales 2020, the results from Ernberg 2011 did not indicate a clinically relevant effect of botulinum toxin A in patients with persistent myofascial pain: the study showed no significant difference in pain reduction between botulinum toxin A and saline injection into the masseter muscle with regards to a VAS, frequency of analgesic use, pain-free jaw opening or PPT. However, the study demonstrated a considerable placebo effect, with both groups demonstrating a net pain reduction at 1 month. The study utilized a crossover design, in which participants would be receiving both botulinum toxin A and saline injections but in different orders.³⁴ While the crossover design is a strong design due to the participants being their own controls, it may elicit potential patient bias resulting from expectations of treatment effect: a patient who experiences pain reduction after the first injection might expect the second injection to also be effective, etc. Another explanation for the pain reduction after saline could be a needling effect.²³ The differences in results between the two studies may be attributed to differences in sample sizes (100 participants vs 21 participants), injection dosages and techniques, as well as differences in measuring PPT. Moreover, pain is an enigmatic phenomena that is challenging to understand, study, and treat. One challenging attribute is the subjective nature of pain. Even relying on measures such as PPT to assess and infer the pain experienced by other people may be considered subjective.

Pain is defined as a subjective experience, which means it cannot be directly observed by those who are not experiencing it.³⁶ Regardless, researchers and clinicians attempt to observe, measure, and assess the pain experienced by people. This raises a question of how the

subjectivity of pain should be integrated within its assessment. Different approaches to assessing pain include direct observations of expressions of pain (such as words or behaviors used by the subject) and administering quantitative measures of pain such as self-report questionnaires. In fact, most assessments of pain are focused on quantitative methodologies, i.e. pain intensity ratings or pain threshold levels. One criticism of this type of assessment is that it overlooks important attributes of the subjective experience, such as personal context and meaning. Because patients with pain often do not feel understood or listened to by their providers, it highlights the need to assess pain through more qualitative methods such as talking, observing, and listening. Nonetheless, objective measures of pain such as assessment of range of motion (i.e. maximum interincisal opening) are valued for their usefulness in more standardized, mechanism-based management. Thus, a pain assessment model should include both objective and subjective pillars.³⁶ The subjective pillar should include self-report measures, and it is beneficial to use a multidimensional measure of pain, i.e a model that uses verbal descriptors and a numerical scale. In addition, pain relief should be measured using sequential ratings (changes from pre-treatment to post-treatment), rather than a retrospective impression. In trials of pain treatments, daily diaries are gradually becoming the standard for assessing pain in order to minimize memory biases that may compromise the validity of retrospective ratings of pain.³⁷

Al-wayli 2017, Yurttutan 2019, and Kaya 2021 also compared intra-muscular botulinum toxin A injections to occlusal splints, with conflicting results. Al-wayli 2017 reported mean subjective pain scores that decreased significantly in the BTX-A group starting at the 3 week follow up until the last follow-up. However, in the control group, mean pain score did not show improvement with time.²⁶ Kaya 2021 reported statistically significant decrease in pain in both groups at each follow up.²⁷ In Yurttutan 2019, the VAS scores decreased significantly in the

BTX-A group and the occlusal splint and BTX-A group, compared to the occlusal splint group alone.³⁰ Again, these differences may be attributed to differences in sample sizes, differences in injection techniques and dosages, as well as the subjective nature of pain.

Fascial Manipulation Technique

Of note is the Guarda-Nardini 2012 study that compared BTX-A injections and physiatric treatment by means of a fascial manipulation technique for the relief of myofascial pain of the masticatory muscles. This physiatric technique provides a deep digital pressure over specific “centers of coordination” points defined by the method. Therapists use their elbows, knuckles, or fingertips to exert pressure on these areas. Both treatments seemed to be almost equally effectively, with fascial manipulation being slightly superior to reduce subjective pain perception and BXT-A being slightly superior to increase jaw range of motion.³³ According to Guarda-Nardini, fascial manipulation has been successfully used to manage signs and symptoms of muscular disorders in several body areas, however, this is the first application to the jaw and masticatory muscles. A number of treatment options have been proposed over the years of bruxism-related pain, but evidence of superiority of one treatment modality over the others is inconclusive. It seems to make sense that conservative therapies that don’t require high patient compliance and that do not provide irreversible changes to the dental occlusion are considered the best options, and thus, the fascial manipulation technique may provide an implication for further research in the relief of myofascial pain of the masticatory musculature.

While masseter muscle hypertrophy seldom presents as a health problem, it can be esthetically displeasing to patients. The hypertrophic muscles may be associated with myofascial pain on occasion, as well as limitation of mouth opening, midline deviation, and masseteric spasm. Various treatment methods have been introduced, ranging from a partial resection of

masseter muscle with simultaneous mandible angle ostectomy, radiofrequency volumetric reduction, and intra-masseteric BTX-A injections.⁴ A number of case reports and several cohort studies have sought to illustrate the effectiveness of BTX-A injections for masseter hypertrophy. However, there is a general lack of randomized controlled trials or controlled clinical trials to support or refute the effectiveness of the aforementioned intervention. Only 2 studies were included in this review: Park 2018 and Canales 2020. Both studies were designed as double-blind, randomized controlled trials, and both studies used ultrasonography to measure the thickness of the muscles. Park 2018 reported that the masseter muscle thickness was consistently decreased at rest and during maximum contraction at all 4 periods of measurements in the experimental group vs the control group (sterile saline injections).²⁴ Canales 2020 reported a significant decrease in the thickness of all muscles treated with BTX-A injections in all evaluations. When compared to sterile saline however, the medium dose and high dose group showed statistically significant differences in muscle thickness, but the low dose group did not.²⁵ This is interesting to note, since the low dose group in Canales 2020 received 30 units per masseter, and the test group in Park 2018 received 25 units per masseter. This minor variation in results could again be due to sample size differences (20 participants in Park 2018 vs 100 participants in Canales 2020) or differences in injection technique. Although further research is required, it may be difficult to conduct randomized controlled trials for this intervention. This will present challenges in terms of the willingness of patients to participate in a trial where they may be allocated to an intervention which may result in unilateral facial hypertrophy or deformity for the length of the trial. Outcome assessments should include both subjective and objective evaluations, such as ultrasound measurements, physical measurements of changes in facial contour, cephalometry, and electromyographic studies of masseter function.

The side effects of botulinum toxin A injections are transient and are largely limited to the site of injection. A review by Idhe and Konstantinovic showed that adverse effects of BTX-A injections may be local (sensitivity of mild cutaneous reactions at the injection site), systematic (headache or nervous atrophy), or specific and reversible (dysphonia, dysphagia, and dry mouth).¹⁴ Of the included studies in this review, Nixdorf 2002 reported on a subset of participants who experienced a difficulty in smiling, with an onset during the first week after injections. The experience was a unilateral presentation with an inability to activate the zygomaticus major muscle, resulting in an asymmetrical smile. The paralyzation of this muscle was thought to be from diffusion of the botulinum toxin A from the masseter muscle via the needle puncture site – direct deposition of the toxin into the muscles of facial expression is unlikely since electromyographic guidance was used to confirm needle placement into the masseter muscle.²⁹ Also, Canales 2020 reported a transient decline in masticatory performance and muscle contraction and a decrease in muscle thickness and coronoid and condylar process bone volume as dose related adverse effects. However, due to the short follow up period, it could not be determined if these effects had a long-term impact on the wellbeing of the participants.²⁵ Guarda-Nardini 2012 reported no relevant side effects with the exception of some minor discomfort during chewing reported by participants in the two to three weeks following injections. These effects disappeared and were not rated as relevant by the participants.³³ The majority of the included studies either did not specify if the botulinum toxin injections had adverse effects or reported the absence of such adverse effects, both locally and systemically. In a review by Yeh et al in 2018, the authors found that majority of complications appear within 2-4 weeks of injection and disappear within 12 weeks. The most common reported side effects after masseteric injections of botulinum toxin A were local swelling, bruising, and hematoma, pain

around injection site, and chewing weakness. Despite the transient nature of these complications, patient satisfaction and confidence often may decrease as a result, and thus, it is recommended that clinicians familiarize themselves with regional anatomy and injection safety zones. In addition, awareness of possible complications and incidence rates and recovery times may aid the clinician in pre-procedural consultations.

The results of the present review are inconclusive about the effects of intramuscular injections of botulinum toxin A in reducing muscular hypertrophy as well as in improving masticatory myofascial pain, when compared to other treatment options such as occlusal splints, placebo, behavioral, pharmacology, or psychiatric treatments. The included studies were not exempt from limitations, as most sample sizes were small and were predominantly female. Furthermore, despite being randomized control trials, all studies were graded as moderate or high risk of bias due to inadequate methods of randomization/concealment of allocations, blinding, and attrition bias. It should be noted that in some instances it is impossible for a double-blind protocol due to the nature of the intervention and/or control, i.e. occlusal splints vs. intramuscular injections. In addition, due to the heterogeneity of dosages, injection sites, and injection techniques, it is difficult to determine a consensus on optimal dosages, injection sites, and techniques. It must be added that in all the included studies, the follow-up periods were less than 2 years, necessitating long-term controlled studies which allow the assessment of therapeutic and cosmetic effect over the years, as well as the relative safety in the form of adverse effects with prolonged use.

Suggestions for Future Design

A well designed trial should be conducted as a double-blind, randomized, placebo-controlled clinical trial. The population must be large and diverse, who have both subjectively

been found to have a diagnosis of bruxism (through self or partner reported nighttime-grinding or daytime clenching and masticatory myofascial pain associated with these parafunctional habits), as well as objectively through a polysomnography evaluation, electromyographic recording of muscle activity, and a comprehensive, validated, dental screening tool. Injections of botulinum toxin A injections should be standardized as much as possible, with correct placement confirmed with the use of an electromyographic machine. The dosages must also be standardized according to recent, peer-reviewed guidelines. The injector needs to have received adequate training in neurotoxin injections. Reduction of masseter size should be measured as objectively as possible, including the use of ultrasonography before and after treatments, physical measurements of changes in facial contour, and cephalometry. Patient-reported subjective measure of reduction of masseter size is important as well, as this is a patient-driven procedure and ultimately the patient should be satisfied. Reduction of myofascial pain should also be measured as objectively as possible, utilizing tools such as electromyographic recordings of muscle activity and measurements of range of motion. Subjective pain using a visual analogue scale may be utilized as a patient-reported outcome. In order to reduce selection bias, adequate methods of allocation concealment must be utilized, such as allocation by a central office unaware of subject characteristics, pre-numbered or coded identical containers which are administered serially to patients, or an on-site computer system combined with allocations kept in a locked, unreadable computer file. In order to reduce performance bias, it's critical that the recipients of care and providers of care be blinded to the assigned intervention. This is impossible to do in cases where the intervention and control treatments are inherently different, such as the comparison of botulinum toxin A injections with an occlusal guard. In order to reduce detection bias, it's important that the provider that is assessing the outcome of care be a separate individual from the

provider of care. Finally, in order to lessen attrition bias, the study design should include measures to help reduce participation dropout, such as providing compensation, minimizing the number of follow-ups as much as possible, and recruiting more patients than needed.

Conclusion

This review highlights the lack of well-designed, randomized controlled trials to evaluate the efficacy and safety of BTX-A for reducing the size/volume of the masseter muscles and for improving masticatory myofascial pain in patients who present with bruxism. At this time, due to the lack of high quality evidence, the results are inconclusive.

LIST OF JOURNAL TITLE ABBREVIATIONS

Aesthet Surg J.....	Aesthetic Surgery Journal
Ann Otol Rhinol Laryngol.....	Annals of Otolaryngology, Rhinology, and Laryngology
Arch Oral Bio.....	Archives of Oral Biology
Biochem Pharmacol.....	Biochemical Pharmacology
Br J Oral Maxillofac Surg	British Journal of Oral and Maxillofacial Surgery
Cochrane Database Syst Rev.....	Cochrane Database of Systematic Reviews
Cranio.....	The Journal of Craniomandibular and Sleep Practice
Electromyogr Clin Neurophysiol	Electromyography and Clinical Neurophysiology
Eur J Pain.....	European Journal of Pain
Indian J Dent Res.....	Indian Journal of Dental Research
J Can Dent Assoc	Journal of the Canadian Dental Association
J Clin Exp Dent.....	Journal of Clinical and Experimental Dentistry
J Endod	Journal of Endodontics
J Oral Maxillofac Surg.....	Journal of Oral and Maxillofacial Surgery
J Pain Res.....	Journal of Pain Research
Jpn J Oral Maxillofac Surg	Japanese Journal of Oral and Maxillofacial Surgery
Niger J Clin Prac.....	Nigerian Journal of Clinical Practice
Oral Surg Oral Med Oral Pathol Oral Radiol Endod	Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology
Pharmacol Biochem. Behav.....	Pharmacology Biochemistry and Behavior
Prog Neurobiol.....	Progress in Neurobiology

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