The topographic anatomy of the masseteric nerve: A cadaveric study with an emphasis on the effective zone of botulinum toxin A injections in masseter

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Summary Introduction: Botulinum toxin injections are previously reported to be a noninvasive alternative method for treating masseteric hypertrophy. However, there is a debate on finding an ideal place for injection. The aim of this study is to document the anatomical landmarks for defining the motor nerve entry points (MNEPs) of the masseteric nerve in the masseter for effective botulinum toxin injections.

Materials and methods: Twelve sides from six adult fixed cadavers were used for this study. The MNEPs of the masseteric nerve were defined according to standard landmark lines including the orbitomeatal line (OML) and the line (VL), which intersects the mid-distance of the OML to the tip of the angle of the mandible.

Results: All MNEPs were located 4.4 cm inferior to the OML. In addition, the average anterior distance of the MNEPs to the VL was 1.4 cm and the average posterior distance was 0.6 cm.

Conclusion: The ideal site of Botox injection into the masseter is a rectangular area: 5 cm inferior to the OML, 1 cm anterior and posterior to the VL, and just above the periosteum. Based on the data of our study, injections to the parotid gland and branches of the facial nerve such as the marginal mandibular and buccal can be avoided. The masseteric nerve can easily be found

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Introduction

The masseter is one of the four principal muscles of mastication which covers the angle of the mandible and mandibular ramus. Its main function is elevation of the mandible and it has a small effect on side-to-side movements. It also has an important morphological role in delineating the lower facial contour, which is aesthetically important. Masseteric hypertrophy is a rare disorder that may present as either a unilateral or bilateral swelling in the region of the angle and the ramus of the mandible. Patient complaints are usually aesthetic in nature, but there may also be pain in the region and limitation of mouth opening.

The surgical treatment of this anomaly involves resection of a portion of the masseter muscle. However, there are several complications associated with surgery. These complications include severe bleeding, hematoma, prolonged edema, facial nerve injury, trismus, and bad scarring. To avoid these complications, noninvasive modalities such as occlusal adjustment, splint therapy, tranquilizers, and antidepressants have been used for treatment. Botulinum toxin type A (BTX-A) injection is an alternative, relatively noninvasive, and effective treatment for masseter muscle hypertrophy.

BTX-A is a potent bacterial neurotoxin produced by an anaerobic bacterium Clostridium botulinum. It causes muscle paralysis, atrophy, and weakness by chemical denervation. In muscle, it blocks the neuromuscular transmission through rapid and strong attachment to the presynaptic nerve membrane. It inhibits the release of vesicle-bound acetylcholine at the neuromuscular junction.

Previous studies indicate the importance of defining the motor nerve entry points (MNEPs) of a muscle for an effective, selective, and safe BTX injection. In 2010, Hu et al. established the safest injection sites for BTX injection into the masseter muscle by a well-designed study. They showed that the safest injection point is the central compartment of the muscle so as to avoid injections into the parotid gland or into the marginal mandibular branch of the facial nerve; however, they did not investigate the MNEPs to the muscle.

There are numerous studies targeting the different muscles in the body investigating MNEPs; however, the MNEPs of the masseter muscle have not been well defined according to standard anatomical landmarks. The aim of this study, therefore, is to document the anatomical landmarks for defining the MNEPs of the masseteric nerve in the masseter for effective botulinum toxin injections.

Materials and methods

Twelve sides from six adult fixed cadavers were used for this study. Of these, four were male and two were female and ranged in age from 56 to 69 years (mean 63 years) at death. None of the cadavers showed any gross pathology or signs of surgical procedures in the examined area. Each cadaver was placed prone and the skin and the subcutaneous tissue over the masseter were cut and removed completely. The branches of the facial nerve were carefully preserved.

Using a surgical microscope, the massetteric nerve was exposed at the mandibular notch and its branches were followed to its entrance into the masseter muscle. The dorsal part of the inferior attachment of the muscle to the mandible was detached in order to observe the entry site of each branch.

The massetteric nerve and distribution of its branches were determined in reference to the following bony landmarks:

Determination of the origin of the massetteric nerve through the mandibular notch

The closest distances between the origin of the nerve through the mandibular notch to the most medial part of the temporomandibular joint (TMJ), zygomatic arch, and mandibular notch were measured. The TMJ landmark was located on the medial surface of the mandibular condyle. In addition, the measurements concerning the mandibular notch were made on its distal edge.

Determination of the distribution of the branches of the massetteric nerve

The number of branches of the massetteric nerve at the level of the mandibular notch, as well as the MNEPs of each nerve branch, was recorded. Next, the MNEPs were defined according to standard landmark lines. These standard lines included the orbitomeatal line (OML) and the line (VL) which intersects the mid-distance of the OML to the tip of the angle of the mandible. The OML was classically determined as the line that connects the lateral palpebral commissure to the tragus. The distance of each motor entry point of massetter nerve branches to these lines was measured by taking the OML as X and the VL as Y landmark lines. These landmark lines were expressed in absolute distances, and based on the Y landmark line, the region anterior to it was determined as the (+) Y landmark line.
while the region posterior to it was \((-\) \text{ Y landmark line}) (Figure 1).

All measurements were carried out using digital calipers (Vernier LCD Digital Caliper Measuring, USA). In order to correct for individual examiner variability, each specimen was examined by two members of the team (BK, NA). Each observer made single independent measurements and the mean of all measurements was recorded.

Results

The masseteric nerve was observed to travel through the mandibular notch in all cases. The nerve and its motor branches coursed just above the periosteum in the majority of the specimens (eight cases) and it traveled between the superficial and deep layers of the masseter in the remaining specimens (four cases). The branches of the masseteric nerve were accompanied by branches of the masseteric artery and had no close relation with other nerve branches. The origin of the nerve through the mandibular notch was $13.8 \pm 4.5 \text{ mm inferior to the zygomatic arch}$, $10.6 \pm 2.7 \text{ mm medial to the TMJ capsule}$, and $7.8 \pm 2.0 \text{ mm superior to the mandibular notch}$ (Figure 2).

The mean length of the OML was measured as $8.4 \pm 1.8 \text{ cm}$ and the VL as $8.6 \pm 1.5 \text{ cm}$. There were three to seven branches (mean five) of the masseteric nerve separately innervating the deep and superficial layers of the masseter. It was also observed in all cases that the upper half of the muscle mass received more fibers than the lower half of the muscle.

The evaluation of the location of the MNEPs showed that all MNEPs were located inferior to the OML (X landmark line). The average distance on the X landmark line for all regions of the MNEPs was $4.4 \text{ cm}$ (minimum: $3.1 \text{ cm}$, maximum: $7.5 \text{ cm}$). The average distance of the MNEPs on the \((+) \text{ Y landmark line})$ was measured to be $1.4 \text{ cm}$ (minimum: $1.3 \text{ cm}$, maximum: $2.0 \text{ cm}$) whereas on the \((-) \text{ Y landmark line})$, the average distance was $0.6 \text{ cm}$ (minimum: $0.2 \text{ cm}$, maximum: $1.1 \text{ cm}$) (Figure 3).

Discussion

BTX-A is commonly used for treating a variety of neuromuscular disorders such as strabismus, blepharospasm, hemifacial spasm, and torticollis.\(^{13,15}\) It has also been used as a noninvasive treatment for patients with masseteric hypertrophy.\(^{8,9}\) The anatomy of the masseter muscle has been well defined in classical anatomy textbooks\(^1\) and in recent reports.\(^2\) However, the innervation patterns and the distribution of nerve branches into the muscle are not well investigated. Hence, there is still debate in determining the ideal site of injection into the masseter muscle.
In a study by Hu et al., \[\text{16}\] the surface of the masseter was associated with the risk of invading the parotid gland. However, this method was suggested to be applied on part of the zygomatic arch and mandibular angle. Nevertheless, this method of harvesting it as a donor nerve for facial reanimation has been investigated by Brenner and Schoeller, \[\text{17}\] and it was found in a so-called "masseteric area" which is located to the parotid gland, marginal mandibular nerve, and other branches of the facial nerve such as its buccal branch.

Cotrufo et al. \[\text{16}\] have revealed a "masseteric area" where the main trunk of the masseteric nerve can be easily defined. Thus, they have suggested an optimized technique of harvesting it as a donor nerve for facial reanimation. The branching pattern of the masseteric nerve has also been investigated by Brenner and Schoeller, \[\text{17}\] and it was revealed that the masseteric nerve may have variant numbers of branches after leaving the infratemporal fossa. Although our anatomical landmarks are different, we also suggest that the main trunk of the masseteric nerve can be found in a so-called "masseteric area" which is located 13.8 ± 4.5 mm inferior to the zygomatic arch, 10.6 ± 2.7 mm medial to the temporomandibular joint capsule, and 7.8 ± 2.0 mm superior to the mandibular notch.

Previous studies have attempted to define an ideal site of BTX-A injection to the masseter. Injection into the muscle was first defined by von Lindern et al. \[\text{7}\] and the injection was suggested to be applied on part of the zygomatic arch and mandibular angle. However, this method was associated with the risk of invading the parotid gland. In a study by Hu et al. \[\text{16}\] the surface of the masseter was compartmentalized into eight areas and numbered from I to VIII. Areas I, III, V, and VII were assigned to represent the upper four compartments and areas II, IV, VI, and VIII were assigned to represent the lower four compartments. The center of compartment VI was suggested to be the safest and most efficient injection site for BTX-A into the masseter muscle to avoid injury to the parotid gland, which usually occupies compartments I and II, and the marginal mandibular branch of the facial nerve, which was located a mean of 7.4 mm superior to the inferior mandibular margin. Although this study provided information about the location of the nerve branches with respect to the structures that should be avoided during injection, the exact locations of MNEPs were not defined. Additionally, as the patients have masseteric hypertrophy, the compartmentalization of the muscle mass may not be standardized for each individual. We suggest that defining standard anatomical landmarks, which is not dependent on personal characteristics, is mandatory.

The exact localization of MNEPs of the masseteric nerve has been investigated by Kim et al. \[\text{18}\] These authors dissected twelve masseter muscles and observed the innervation pattern of masseteric nerve in the superficial, middle, and deep layers of the muscle. They also divided the muscle into compartments and investigated the distribution of the nerve branches in different compartments. They used the anterior margin of the middle layer of the muscle as a reference, and according to this reference point, they divided the masseteric nerve into four groups: posterosuperior, posteroinferior, anterosuperior, and anteroinferior. Although they were able to show the innervation patterns in each segment and revealed that the masseteric nerve branches were mostly confined to the lower middle third of the whole masseter muscle, they did not suggest an ideal place for injection of the muscle.

In this study, we defined standard and easily definable anatomical landmarks including the OML and the line (VL), which intersects the mid-distance of the OML to the tip of the angle of the mandible. These lines can be used for exact localization of the injection point of BTX-A into the muscle. As these references are individually based, we suggest that they can be used reliably in any patient. These reference lines can be considered as X and Y landmark lines as was defined previously by Lee et al. \[\text{13}\] for the sternocleidomastoid muscle. Accordingly, our results revealed that all MNEPs were located 4.4 cm (minimum: 3.1 cm, maximum: 7.5 cm) below the OML (X landmark line). The average distance of the MNEPs on the (+) Y landmark line was 1.4 cm (minimum: 1.3 cm, maximum: 2.0 cm) whereas on the (−) Y landmark line, it was 0.6 cm (minimum: 0.2 cm, maximum: 1.1 cm). These results were addressing the ideal site of Botox injection into the masseter is an area, approximately 5 cm inferior to the OML (minimum: 3.1 cm, maximum: 7.5 cm), 1 cm anterior and posterior to the VL (Figure 4). This location can also be considered as the half distance of the VL. This region was free of the parotid gland, marginal mandibular nerve, and other branches of the facial nerve such as its buccal branch.

Once BTX is injected into the muscle, it is known to immediately diffuse into the muscle within a few centimeters of the needle tip. When a higher volume is injected, the area of diffusion seems to increase; however, inaccurate injection and excessive diffusion of the toxin can lead to systemic adverse effects or unwanted weakness of the
neighboring muscles.\textsuperscript{18,19} We suggest that a single injection to an ideal site would be enough for diffusion of the toxin through MNEPs of other branches, and thus systemic adverse effects or unwanted weakness of neighboring muscles may avoided. Concerning the dosage, there is no standardized dose of BTX-A for effective treatment of masseteric hypertrophy. Although Lee et al.\textsuperscript{20} have stated that 30 IU BTX-A injection is effective for masseteric hypertrophy, Kim et al.\textsuperscript{21} had proposed that >30 IU BTX-A may be required to achieve an adequate result. In addition to this, Andrade and Deshpande\textsuperscript{22} have stated they had successfully managed masseter muscle hypertrophy by injecting 40 IU of BTX-A.

The branches of the masseteric nerve are increasingly being used for facial reanimation procedures.\textsuperscript{17} However, it may not always be very easy to locate the nerve due to a variety of described branching patterns and variability of facial measurements on which some surgical approaches are based upon. Borschel et al.\textsuperscript{23} have studied the relation of the masseteric nerve to some surgical landmarks such as the auricular tragus and the zygomatic arch on eight cadavers and found the nerve to lie 3 cm anterior to the tragus at a level 1 cm inferior to the zygomatic arch. Collar et al.\textsuperscript{24} dissected 10 cadavers and identified the masseteric nerve in the “subzygomatic triangle” which was formed by the zygomatic arch superiorly, the TMJ posteriorly, and the frontal branch of the facial nerve inferiorly and anteriorly. Cheng et al.\textsuperscript{25} presented a technique to identify the nerve in a 1.5 cm\textsuperscript{2} area defined by constant anatomical landmarks such as the zygomatic arch, condyle, coronoid process, and mandibular notch. Our study revealed that the nerve was 1.4 cm inferior to the zygomatic arch, which is consistent with these studies. Therefore, we also suggest that the masseteric nerve can easily be found approximately 1.0–1.5 cm inferior to the zygomatic arch, 1 cm medial to the TMJ capsule and 1 cm superior to the mandibular notch which makes its use for facial reanimations more efficient.

The masseter muscle has significant applications for head and neck reconstruction and for muscle transposition flap for facial nerve paralysis.\textsuperscript{26–28} Therefore, it is important to know the course and the location of branches of the masseteric nerve in relation to standard anatomical landmarks not only for BTX injections but also to provide a safe and efficient approach for these applications. Hontanilla and Qui\textsuperscript{27} had reported a constant course with a distribution pattern that shows a tree-like anatomy in six cadavers. Hwang et al.\textsuperscript{28} examined the course of the masseteric nerve in relation to the masseteric muscle and found that the nerve ran anteriorly and inferiorly between the deep and the middle layers. The nerve was observed at 33 ± 5.6 mm from the inferior border of the muscle on the anterior third vertical line of the masseter muscle and at 47 ± 5.5 mm in the posterior third. The results of our study also revealed that the nerve was coursing between the superficial and deep layers of the muscle. However, we found its motor branches to distribute widely among muscle fibers and the upper half of the muscle mass received more fibers than the lower half of the muscle.

The limitation of this study is the fact that it is an experimental study conducted on cadavers. All cadaver studies have the limitation that it is difficult to reproduce. However, a clinical study would need to be undertaken to properly determine the effects of this procedure.

**Conclusion**

According to the results of our study, the ideal site of Botox injection into the masseter is a rectangular area, 5 cm inferior to the OML, 1 cm anterior and posterior to the VL, and just above the periosteum. These landmarks will avoid injection into the parotid gland, marginal mandibular nerve, and other branches of the facial nerve such as its buccal branch. We also suggest that the masseteric nerve can easily be found approximately 1.0–1.5 cm inferior to the zygomatic arch, 1 cm medial to the TMJ capsule and 1 cm superior to mandibular notch which makes its use for facial reanimations more efficient.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**References**


