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Technical Report

Analysis of the Intramuscular Innervation of the Lateral Pterygoid Muscle

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Abstract: The lateral pterygoid muscle has unique anatomical, physiological and functional properties. Since it is attached to the temporomandibular joint (TMJ) disc, its pathologies are closely related to TMJ disorders, which affect many people worldwide. Muscle structures and units are characterized by their morphological appearance, nerve distribution and function. In the present study, we examined the intramuscular innervation pattern of the lateral pterygoid muscle using modified sihler's method. Two types of innervation pattern were evident. In type I, representing the majority of the samples, a total of three branches arising from the main trunk of the mandibular nerve and the buccal nerve innervated the inferior head of the muscle, while branches from the buccal nerve innervated the superior head. In type II, divisions of the lateral pterygoid nerve branched from the buccal nerve located between the heads of the lateral pterygoid muscle and innervated each head separately. Interestingly, muscle bundles with a stronger tendineous structure showed much more innervation than other parts of the muscle. Future studies including quantitative analysis of the nerve distribution to the muscle bundles are warranted.

Key words: Lateral pterygoid muscle, Intramuscular innervation, Modified sihler's method, Mandibular nerve, Temporomandibular joint

Introduction

Within the masticatory muscles, the lateral pterygoid muscle (LPM) takes more attention due to its morphological and functional relation with the temporomandibular joint (TMJ). Thus many studies involving morphological, physiological and functional aspects of this muscle have been reported. LPM originates from the lateral pterygoid plate and the infratemporal surface of the greater sphenoid wing and inserts into the pterygoid fossa and TMJ capsule and disc [1-3)]. In majority of the cases, LPM is composed of two divisions namely superior and inferior heads [2, 4-9)]. Both heads play a key role in movement of the TMJ. The superior head closes the jaw whereas the inferior head acts in opening, protraction and eccentric lateral movements [10-15)].

From the viewpoints of morphology and physiology, LPM consists of a specific and complex structure. Thus its classification as a functional unit is not clear. Though 2 heads of LPM are generally described as being two distinct structures [16-22)], there are also some authors who consider it as a single unit [23,24)]. However considering the nerves entering the muscle units, it is difficult to classify LPM into 2 independent muscles [4,6,25-27)]. Even sometimes the muscle shows the 1-head or 3-head pattern other than the classical 2-head pattern [28-30)]. Moreover LPM was also reported to be composed of 5 to 6 independent functional musculo-aponeurotic layers according to findings of the nerve distribution [1)].

Studies based on electromyographic activities (EMG) have also demonstrated controversial results. Though few researchers reported similar integrated activities of each of LPM heads [31,32)], most investigators mentioned a reciprocal activity of superior and inferior heads of LPM [10-15,33)]. However, insertion of the EMG electrodes is highly difficult due to deep location of the muscle heads in the infratemporal fossa. Thus interpretation of the results from EMG recordings should be commented with caution. Nevertheless morphologically the TMJ disc is placed at its position by reciprocal movements of LPM heads, supporting the majority of the researchers, who showed reciprocal movement of LPM heads.

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Innervation patterns of skeletal muscles can be examined using various methods including anatomical dissection both morphological observation and microscope-based, three-dimensional reconstruction of the serial anatomical sections and use of Modified Sihler's method[30]. Each method has advantage and drawbacks for observing the skeletal muscles. Within these methods, Sihler's staining is best to analyze intramuscular distribution of the nerves within the muscles especially for thin skeletal muscles[34]. To date, comprehensive analysis of intramuscular innervation patterns of the LPM has not been performed. In the current study, we tried to examine intramuscular innervation patterns of the mandibular nerve by applying Modified Sihler's method in quite a lot number of LPM specimens from Japanese cadavers. By this way, we also tried to contribute for identification of the physiological and functional structures of LPM heads whether they function as one unit or the LPM heads have reciprocal relation.

Materials and Methods

Thirty head halves of 15 Japanese cadavers (10 males, 5 females) obtained from practical morphology laboratory of Tokyo Dental College were used in the current study. The average age of the cadavers was 74.4 years old (62-85 years old). The Etthics Committee of Tokyo Dental College, Ethical clearance Number 309, approved the experiment. The cadavers were fixed in 10% formalin and preserved in 30% alcohol. To examine the lateral pterygoid muscle, the muscle bundles, which are attached to the TMJ disc in situ, the bony elements including parietal bone, frontal bone, squamous part of the temporal bone and the greater wing of the sphenoid etc. were completely removed from inside of the cranium according to the method reported by Pinto (1962) [35].

After removal of the pterygoid muscle en bloc with the mandibular nerve and its branches, innervation patterns of the mandibular nerve were examined.

Then intramuscular distribution of the lateral pterygoid nerve was observed using Modified Sihler's method and classified.

Observation Items

1) Classification of the muscle head number
2) States of the circumference of the lateral pterygoid muscle as well as the nerve piercing the muscle
3) Course as well as intramuscular distribution state of the lateral pterygoid nerve

Modified Sihler's Method

The Modified Sihler's staining method according to Liu et al.[30] was shown as follows:

1. Fixation and Maceration

1) The removed neuromuscular samples were exposed to experimental antiseptic spray. The samples were preserved while utmost care was taken keeping them from drying.
2) The samples were washed in the tap water for 30 minutes.
3) The samples then were soaked in 3%KOH aqueous solution (0.2 ml hydrogen peroxide solution added for 100 ml solution) while stirring several times daily until color of the soft tissues fades either becoming transparent or semi-transparent state for 3 weeks. During this period, the solution was changed twice at the 3rd and 10th days due to its dirty condition.

2. Decalcification

1) The samples were washed in tap water for 30 min.
2) The samples were soaked in Sihler I solution (glacial acetic acid: glycerin: 1% chloral hydrate = 1: 1: 6 = 250 mL : 250 mL : 1500 mL) while stirring several times daily until softening of the calcified cartilage occurred during the course of 2-4 weeks. One week later, the solution was changed once.

3. Staining

The samples were again washed in tap water for 30 min. The samples were soaked in Sihler II solution (Ehrlich's hematoxylin : glycerin : 1% chloral hydrate = 1 : 1 : 6 = 250 mL : 250 mL : 1500 mL) while stirring several times daily until the color became deep purple over the course of 3 weeks. One week later, the staining solution was changed once.

4. Destaining

The samples were soaked again in Sihler I solution. Within 2-3 hours, the color of the fine nerves began to fade. When the color had changed from deep purple to purple, the solution was changed. This procedure was performed until the muscle had become light purple and the nerve had become purple.

5. Neutralization

1) The samples were washed in tap water for 30 min.
2) Then the samples were soaked in 0.05% lithium carbonate, which changed the color of the nerve from purple to blue. The reaction was stopped after one hour.

6. Clearing

1) The samples were soaked in graded glycerin solutions of 40%, 60%, 80% for 3 days, 2 days and 1 day, respectively, then finally in 100% glycerin solution.
2) A small amount of thymol crystals was added to the 100% glycerin solution, and the samples were preserved in a cool, dark place.

7. Trimming

The course and distribution of the nerves in the samples preserved in glycerin were then examined under a binocular

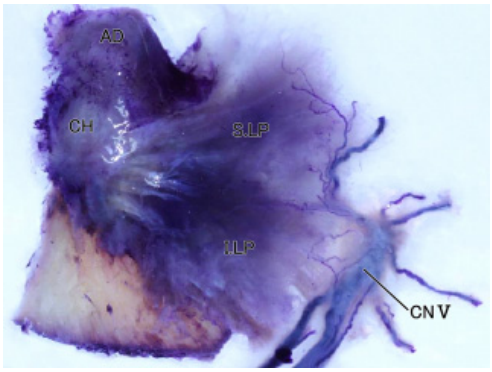

Figure 1. Results of staining by Sihler's method. Medial view of the left temporomandibular joint. After processing of all the staining steps, the nerve fibers become dark blue-purple in color, whereas the muscle as well as connective tissue are almost transparent. Under this condition, the intramuscular course and distribution of the nerves were recorded.

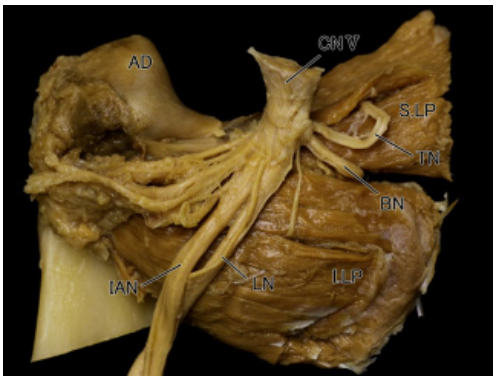

Figure 3. Branching of the mandibular nerve running around the lateral pterygoid muscle (medial view).

microscope.

8. Photography and drawing

Images were taken using a 100-mm lens, and tracings of the intramuscular nerve distribution were made.

After all the staining steps, the color of the nerve fibers became dark blue-purple, and the muscle and connective tissues were almost transparent (Figure 1). This allowed the intramuscular course and distribution of the nerves to be recorded.

Results

All the lateral pterygoid muscles were transparent and the nerves were counterstained dark blue. The nerve course was clearly visible as far as the terminal branches.

Table 1. Numbers of heads of the lateral pterygoid muscles (n=30)

1 head muscle	8 (26.7%)
2 head muscle	22 (73.3%)

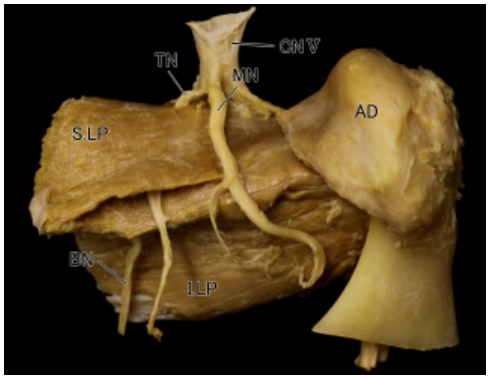

Figure 2. Branching of the mandibular nerve running around the lateral pterygoid muscle (lateral view).

1. Number of heads of the lateral pterygoid muscle

Among the 30 specimens, 8 muscles had a single head unit that could not be differentiated into upper and lower heads. Twenty-two of the thirty lateral pterygoid muscles were composed of the standard superior and inferior heads. There was no difference between the muscles on the right and left sides (Table 1). Type I is described in Figure 4 in which 1 muscle head was observed in 6 cases and 2 muscle heads in 18 cases. Type II is described in Figure 5 in which 1 muscle head was observed in 2 cases and 2 muscle heads were observed in 4 cases.

2. Running state of the nerves innervating the lateral pterygoid muscle and its circumference (Figures 2,3)

1) Main trunk of the mandibular nerve descended through posterior aspect of the lateral pterygoid muscle in close proximity with it. Then it immediately gave the lingual branch and descended through inner surface of ramus of mandible. And it gave the auriculotemporal branch through facial side. Course of the mandibular nerve just after its leave from foramen ovale was similar in all specimens.

2) The masseteric nerve and the posterior deep temporal nerve formed a common trunk and ran through anterior aspect of the temporomandibular articular disc. The masseteric nerve entered between anterior process of the temporomandibular articular disc and upper head of the lateral pterygoid muscle. And then it passed through the mandibular notch and turned to the masseter muscle. The posterior deep temporal nerve ascended through anterior process of the temporomandibular articular disc and gave branches after entering into the temporal muscle. Course of the masseteric nerve and posterior deep temporal nerve was almost same in all specimens. The middle deep temporal nerve ran through anterior aspect of the anterior deep temporal nerve as a single unit and entered into the temporal muscle and gave branches. The anterior deep temporal nerve and the buccal nerve formed a common trunk and ran through between superior and inferior heads of the lateral

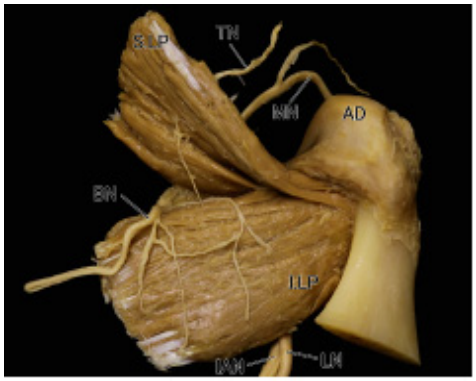

Figure 4. Left lateral pterygoid muscle.The nerve branch (lateral pterygoid nerve) arising from the buccal nerve forms ansa and widely innervated the inferior head. Moreover, a different branch was confirmed between the superior and inferior heads.

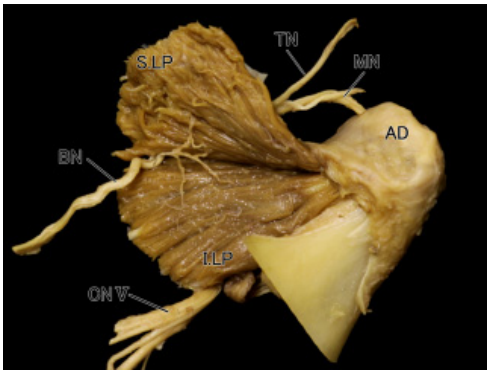

Figure 5. Left-side lateral pterygoid muscle. Many fine twigs from the buccal nerve running between the superior and inferior heads innervated both of the heads.

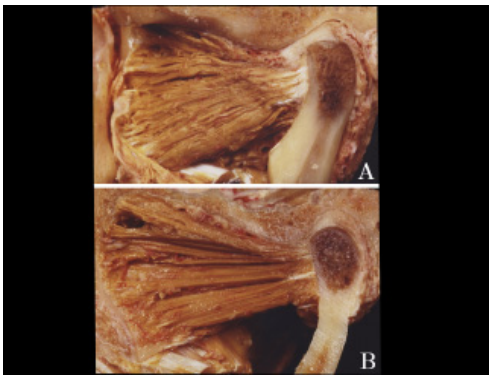

Figure 6.Upper: lateral about 1/3 adhesion form of the lateral pterygoid muscle. Strong tendinous adhesion is observed. Lower: medial about 2/3 adhesion form of the lateral pterygoid muscle Weak tendinous adhesion is observed.

pterygoid muscle. Then it ascended and entered the temporalis muscle, into which the nerve branches were distributed. Courses of both the middle deep temporal nerve and the anterior deep temporal nerve were almost same in all specimens.

3) After division from the mandibular nerve, it passed through superior and inferior heads of the lateral pterygoid muscle. During this course, it gave branches of the anterior deep temporal nerve and the lateral pterygoid nerve. Course of the buccal nerve was almost same in all specimens.

3. Course as well as intramuscular distribution of the lateral pterygoid nerve

After fixing the entrance point of the lateral pterygoid nerve into the muscle, observation of the nerve running in the muscle was done to be ready using Sihler's method. Then sketch for the muscle and its distributed nerve was prepared.

The lateral pterygoid nerve clearly showed the following two types:

Type I:

The lateral pterygoid nerve arose from the buccal nerve together with the anterior deep temporal nerve entered into superior head of the muscle bundle. The nerve entered into the muscle distributed in superior head of the muscle bundles. A part of the nerve fibers also entered into the inferior head and distributed inside it (Figure 4, Table 2).

In this type, the inferior head of the pterygoid muscle was innervated by two branches coming from main trunk of the mandibular nerve and one branch arising from the buccal nerve. Within these nerves, the branches from main trunk of the mandibular nerve also sent twigs to superior head of LPM while innervating the inferior head. And the branches divided from the buccal nerve formed between superior and inferior heads of LPM and widely innervated the inferior head but also sending some branches into the superior head.

In the one single muscle head specimens, nerve distribution of this type was similar to the two heads pattern. Entrance point of the nerve and nerve distribution at the expected separation region of the muscle heads was noticed. Finally three nerve branches of the lateral pterygoid nerve entered into the lateral pterygoid muscle. This type of distribution was classified as type I, and 12 heads (24 sides, 80%) demonstrated almost the same this pattern. Six within these 24 specimens was one headed and the rest 18 specimens had 2 heads. And the lateral pterygoid nerve distributed so much at the surface of the muscle bundle (about 1/3 area, lateral part).

Type II:

The lateral pterygoid nerve arose from the buccal nerve

∗Indication in figure 1-8: AD : articular disc, CH : condyle head, S.LP : superior head of lateral pterygoid muscle, I.LP : inferior head of lateral pterygoid.muscle, CNV : trigeminal nerve, V3 (CNV3) : cranial(cerebral) nerve, MN : masseteric nerve, BN : buccal nerve, TN : deep temporal nerve, IAN : inferior alveolar nerve, LN : lingual nerve

between the superior and inferior heads entered into the both muscle bundles. The nerve entering the lateral pterygoid muscle from the main trunk of the mandibular nerve was not seen in this type (Figure 5, Table 2). That is, two branches of the lateral pterygoid nerve entered into the pterygoid muscle as superiorly and inferiorly. This type of nerve distribution was classified as type II, and 3 heads (6 sides, 20%) demonstrated this pattern. Two within these 6 specimens was one headed and the rest 4 specimens had 2 heads.

And the lateral pterygoid nerve distributed so much at the surface of the muscle bundle (about 1/3 area, lateral part) similar to type I.

In the current study, there was almost no difference between the left and right sides of types of the lateral pterygoid muscle innervation in halves of the same specimen.

Table 2. Classification of the Course of the Lateral Pterygoid Nerve (n=30)

Type I	24 Specimens (80%)
	(1 head: 6 specimens, 2 heads: 18 specimens)
Type II	6 Specimens (20%)
	(1 head: 2 specimens, 2 heads: 4 specimens)

Discussion

In order to evaluate jaw movement in detail, the specific relationship between the masticatory muscles and the temporomandibular joint disc needs to be clarified. The lateral pterygoid muscle is a specific and important muscle with unique anatomical, physiological and functional properties, being attached to the TMJ disc and playing a role in jaw movement. For these e have reached conflicting conclusions, some considering the heads to be separate units with reciprocal movement, and others reporting a single and cooperative unit. However, no studies have obtained functional or morphological data, such as the EMG and nerve distribution characteristics, for each head of the LPM that would clearly indicate its structure to comprise either a single unit or separate ones.

Therefore, in the present study, we attempted to address this controversial issue by obtaining novel information on the function of the LPM through clarification of its intramuscular nerve distribution. Precise anatomical information was obtained from examination of branches of the relationship of the mandibular nerve to the LPM in 30 sides of 15 Japanese cadavers. Most of the cadavers we examined showed a two-head LPM pattern. Some showed a single-head pattern, but none showed a three-head pattern. Because single-head and three-head patterns are considered to be minor anatomical variants, only those muscles with the two-head pattern were included for further analysis.

There are three major methods for investigating the innervation course of muscles: anatomical dissection, computer-based three-dimensional reconstruction, and staining with Sihler's method[28]. To our knowledge, no reported study has investigated the intramuscular distribution of the nerves supplying the LPM using Sihler's method. Anatomical dissection has limited applicability for tracing the terminal distribution of nerve branches[31-33]. Furthermore, data obtained from computer-based reconstruction of serial sections may not be accurate because of possible distortion during tissue cutting, staining, orientation and reconstruction[34,35].

Most studies have reported that the LPM is innervated mainly by a branch of the buccal nerve division of the mandibular nerve. Also in our present specimens, the nerve that entered the LPM was derived from the buccal nerve. We were able to clearly identify the intramuscular nerve distribution in the LPM using Sihler staining. We observed two types of innervation patterns in the LPM heads. In the majority of cadavers, the inferior head of the muscle was innervated by three branches originating from the main trunk of the mandibular nerve and the buccal nerve, while the superior head was innervated by branches from the buccal nerve, and this form of innervation was named type I. However in this type, a proportion of the nerve terminals innervated each head of the LPM reciprocally. Therefore this type of innervation supported some of the reports suggesting reciprocal action of the each head of LPM in harmony. On the other hand in 20% of the specimens, no innervation from the main trunk of the mandibular nerve was observed and classified as type II. Instead the lateral pterygoid nerve originating from the buccal nerve divided into two branches that innervated each head of the LPM. Interestingly, both in type I and typeII, the muscle bundles with stronger tendineous structure were innervated much more than other parts of the muscle, suggesting strong contraction function of these bundles of LPM (Figure 6). Thus intramuscular innervation course of LPM provides clues about its complex and unique various functions.

In conclusion, we have examined the intramuscular pattern of nerve distribution in the lateral pterygoid nerve using Sihler's method. Our data revealed two types of LPM innervation. Our future research will include quantitative analysis of the nerve distribution in the muscle bundles using staining of the nerve terminals in histopathological sections.

Acknowledgements

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