Intramuscular nerve distribution of the masseter muscle for botulinum toxin injection

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Intramuscular nerve distribution of the masseter muscle for botulinum toxin injection

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감사합니다.

2008 년 12 월

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ABSTRACT

Intramuscular nerve distribution of the masseter muscle for botulinum toxin injection

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(Directed by professor Jong-Hoon Choi, D.D.S., M.S.D., Ph.D.)

To verify the conventional botulinum toxin (BTX) injection technique by demonstrating the anatomic intramuscular distribution pattern of the masseteric nerve, thereby providing the efficient and safe BTX injection site to intervene the masseteric hypertrophy.

Twelve masseter muscles were dissected and observed the pattern of innervation of the masseteric nerve on each layer (superficial, middle, deep layer). We also analyzed ten muscles which stained by modified Sihler's staining technique.

The nerve branches from the posterosuperior and the posteroinferior groups were distributed to the deep and middle layer of the masseter muscle, respectively. Among the nerve twigs originated from the anteroinferior nerve group, 2~3 nerve twigs perforated the superficial layer of the muscle. As a result of observation of stained

specimens, all perforating branches innervating the superficial layer were mainly confined and distributed at the area V or VI.

2 to 4 perforating branches supplying the superficial layer of the masseter muscle. In addition, the richer arborization of the perforating masseteric nerve branches were mostly confined at the area V, approximately in accordance with the BTX injection point that has been clinically applied. Therefore, area Ⅴ is strongly recommended as an accurate and safe injection area for treatment of masseteric hypertrophy.

Key words: botulinum toxin; masseter muscle; masseteric hypertrophy; masseteric nerve

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Ⅰ. INTRODUCTION

The masseter muscle has a clinical significance because it bears a relation to masseteric hypertrophy, bruxism, and disorders of temporomandibular joint. In the case of masseteric hypertrophy, surgical operation was mainly applied for treatment in the past. Recently, however, botulinum toxin (BTX) is preferred as a conservative and reversible treatment. Botulinum toxin is a neurotoxin protein produced by the bacterium *Clostridium botulinum*. It is one of the most poisonous naturally occurring substances, and it is the most toxic protein (Montecucco and Molgó, 2005). Botulinum toxin is used in minute doses to treat many diseases despite its virulence. Among the subtypes of botulinum toxin, BTX type A was first separated by Hermann Sommer in 1920s.

BTX reduces the muscular contractions by temporarily inhibiting the release of acetylcholine at the neuromuscular junction. As a result, there is a palliative effect of many problems occurred by excessive contraction of the masseter muscle. A lot of movement disorders and disorders of the autonomous nerve system can be treated

with this agent and the head and neck region is an interdisciplinary focus in this field (Laskawi, 2008).

In the procedure of BTX injection, a point of needle should be considered. It is more effective to inject BTX in the area where the nerve distributing. Up to now, bulky region of the masseter muscle has been regarded as an innervation point. The study about the course of the masseteric nerve, however, is required because it has no ground in actual fact. The method injecting the part of the zygomatic arch and mandibular angle impartially, like von Lindern, has a possibility of invading the parotid gland. Therefore, the location of the parotid gland also should be thought when we are injecting BTX in this area.

Sihler's staining technique that provide more exact and specific information on nerve distribution compared to manual dissection of the nerve branches used in this study. This staining method recently reintroduced into the research fields makes it possible to study intramuscular distribution of the peripheral nerves in whole-mount specimen without microdissection and thus preserves natural nerve fascicles architecture.

The purposes of this study were to verify the conventional injection technique that has been used in clinical practices by demonstrating the anatomic intramuscular distribution pattern of the masseteric nerve, thereby providing the efficient and safe BTX injection site to intervene the masseteric hypertrophy.

Ⅱ. MATERIALS AND METHODS

Twenty-two embalmed adult hemifaces from cadavers (13 male and 9 female cadavers; average age, 78 years (46~96)) were used in this study. Among the specimens, 12 cases were Korean (from Yonsei University, Seoul, Korea) and 10 cases were French (from the University of Lille II, Lille, France).

In 12 cases of the specimen, the skin and subcutaneous tissue of the face were removed and a detailed dissection was performed, with extreme care being taken not to damage the underlying the masseter muscles, facial nerves, and blood vessels. After the dissections, all specimens were sketched to analyze the innervation pattern of the masseteric nerve on each layer (superficial, middle, deep layer) (Fig. 1).

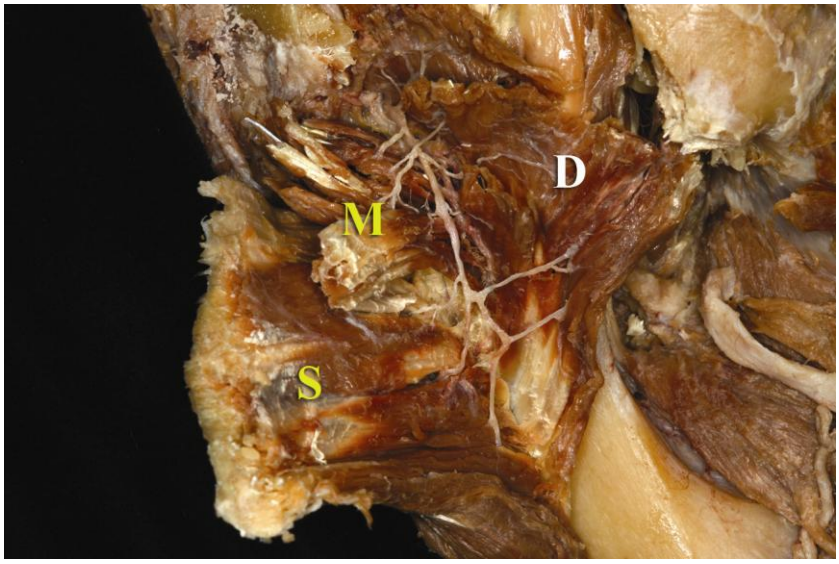

Fig. 1. The photograph showing the innervation of the masseteric nerve on the right side of the dissected specimen (S; superficial layer, M; middle layer, D; deep layer).

From the 10 cases of the specimen, the masseter muscles including each layer; superficial, middle and deep layer were removed. Using the harvested masseter

muscles, a modified Sihler's staining technique was performed to observe the intramuscular nerve arborization patterns. The staining procedures are as follows.

Fixation- Harvested muscles were fixed again in 10% unneutralized formalin for up to a month. The solution was changed when it becomes cloudy.

Maceration and Depigmentation- Fixed muscles were then washed in running water and placed in 3% aqueous potassium hydroxide solution with addition of 0.2 ml of 3% hydrogen peroxide solution for 4 weeks. Through this step, the specimens lost their own color and became transparent (Fig. 2).

Decalcification- Macerated muscles were then transferred into Sihler solution I, composed of 1 part glacial acetic acid, 1 parts glycerin, and 6 parts of 1% aqueous chloral hydrate.

Staining- Adequately decalcified specimens were then stained by immersion in Sihler's solution II, composed of 1 part Ehrlich hematoxylin, 1 part glycerin, and 6 parts 1% aqueous chloral hydrate. Specimens were left in Sihler's solution II until fine intramuscular nerve branches was visibly stained. This step took 3 to 4 weeks.

Destaining- After the staining, muscles were placed into Sihler's solution I again to destain the muscle fibers. This process was stopped when stained fine nerve fibers began to fade. This usually took one to two hours and the solution was changed whenever it turned purple.

Neutralization- Destained muscles were washed in running tap water for half an hour and then immersed into 0.05% lithium carbonate solution for approximately 1 hour.

Clearing- This last step made the neutralized muscle fibers transparent in increasing concentrations of glycerin (40%, 60%, 80%, and 100%). Muscles were kept in each solution for one day. The whole process took approximately four months to complete, after which muscles were stored in a dark place.

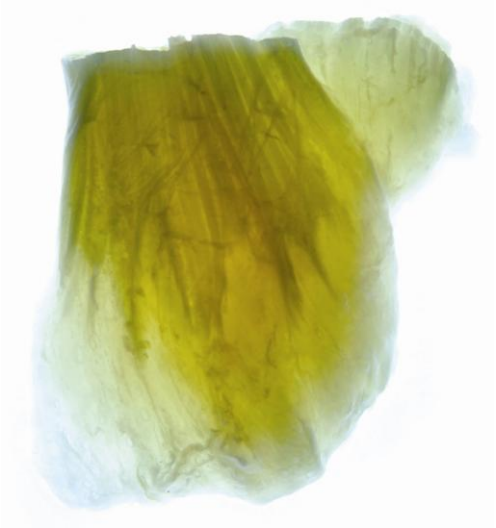

Fig. 2. The specimen of the masseter muscle after the step of maceration.

After all staining procedure were completed, each masseter muscle was observed under the microscope with x40 magnification and photographed (Fig. 3). Photographs of the masseter muscles were taken in transmitted light (YAKE M-180) using Canon EOS 5D with 24-70mm automatic lens and 100mm lens.

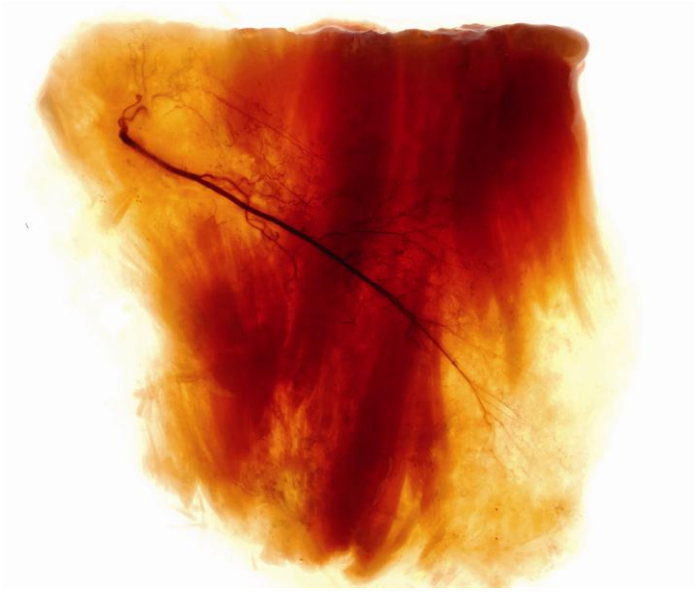

Fig. 3. The masseter muscle after completion of the modified Sihler's staining on the medial surface of the left side of the specimen.

To identify the arborization of the masseteric nerve within the muscle, the specimen was divided into 6 sections from area I to VI from the medial aspect of each muscle. Area I, II and III were designated as the upper posterior 1/3, middle 1/3, and anterior 1/3, respectively. Likewise, area IV, V, and VI were the lower posterior 1/3, middle 1/3, and anterior 1/3 of the masseter muscle, respectively. All the intramuscular nerve branches of the masseteric nerve on 10 stained specimens were traced out with a pencil on the scheme of the muscle outline.

No distinction was made between male and female cadavers. However, the laterality on the innervation patterns was observed with regard to the left or right of all of the specimens.

Ⅲ. RESULTS

Through the detailed dissections, all the masseter muscle was divided into three layers. The zygomaticomandibularis muscle was included the masseter muscle group as a deep layer of the muscle. The masseteric nerve, originating from the mandibular nerve trunk, descended in the plane anteriorly and inferiorly between the middle and deep layer of the masseter muscle, and it supplied all the part of the muscle in every case of the specimen. With a reference of the anterior margin of the middle layer of the muscle, the masseteric nerve divided into four groups of the nerve branches; the posterosuperior, posteroinferior, anterosuperior and anteroinferior nerve groups, respectivley. The nerve branches from the posterosuperior and the posteroinferior groups were originated from the masseteric nerve after penetrating the sigmoid fascia attached to the mandibular notch and each of them distributed to the deep and middle layer of the muscle, respectively. Among the posteroinferior nerve group, it was observed few perforating branches penetrating the middle and superficial layer of the muscle (Fig. 4A).

After traveling the anterior margin of the middle layer of the masseter muscle, the masseteric nerve divided into the anteroinferior and anterosuperior nerve groups. From the anteroinferior group, 4~5 nerve twigs were divided and they innervated into the most part of the superficial layer of the masseter muscle. Among the nerve twigs originated from the anteroinferior nerve group, 2~3 nerve twigs perforated the superficial layer of the masseter muscle and they reached to the surface of the muscle. Whereas, the 2~3 nerve twigs originated from the anterosuperior nerve group distributed into the deep layer of the masseter muscle (zygomaticomandibularis muscle) (Fig. 4B).

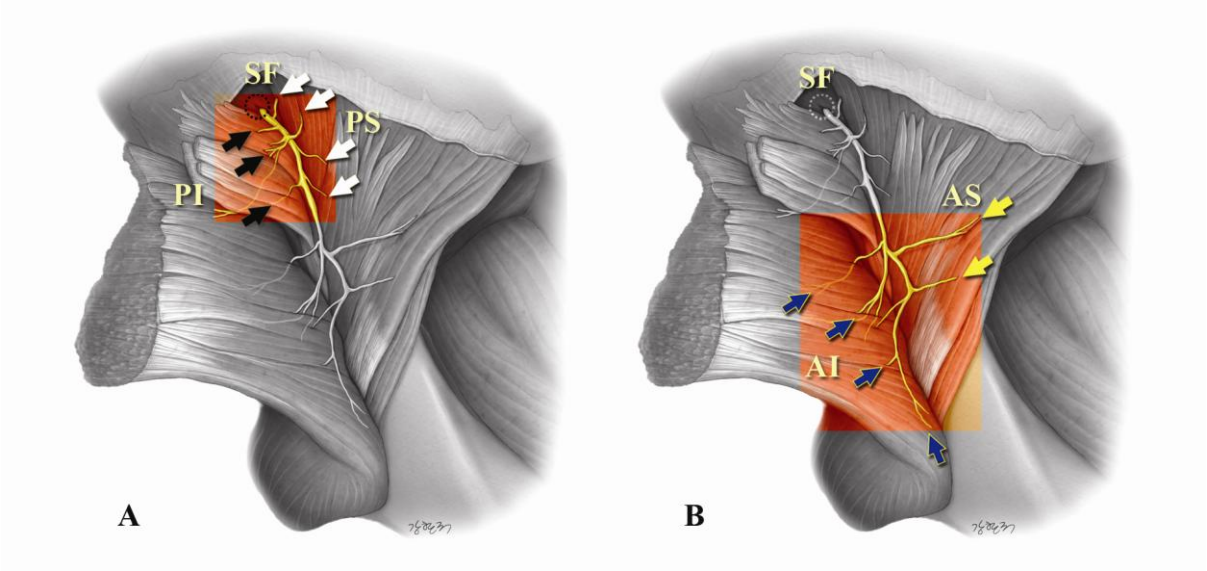

Fig. 4. Distribution pattern of the masseteric nerve on each layer (superficial, middle, deep layer) (SF: Sigmoid fascia, PS: Posterosuperior group (white arrows), PI: Posteroinferior group (black arrows), AS: Anterosuperior group (yellow arrows), AI: Anteroinferior group (blue arrows)).

Observations of the innervation pattern of the masseteric nerve were performed on the stained muscle specimen especially focused on the perforating branches of the anteroinferior nerve group. In all specimens stained with modified Sihler's staining technique, the detailed nerve branches and twigs of the masseteric nerve were revealed in situ within the muscle with the naked eye (Fig. 5, 6).

Based on the observation under the surgical microscope, most of nerve twigs including the perforating branches from the anteroinferior nerve group were originated from the masseteric nerve at the area Ⅱ. All perforating branches innervating the superficial layer of the masseter muscle were mainly confined and distributed at the area Ⅴ or Ⅵ (Fig. 6).

The number of perforating branches from the anteroinferior nerve group was 2 (5 cases, 50%), 3 (3 cases, 30%) and 4 (2 cases, 20%) respectively (Fig. 5). Among all the perforating branches, the nerve twigs distributing at the area Ⅴ were observed ranged from 1 to 3. The nerve twigs from the anteroinferior nerve group were running

through the area V and headed for the area IV. However, relatively few innervation of the masseteric nerve were observed at the mandibular angle region (Fig. 6).

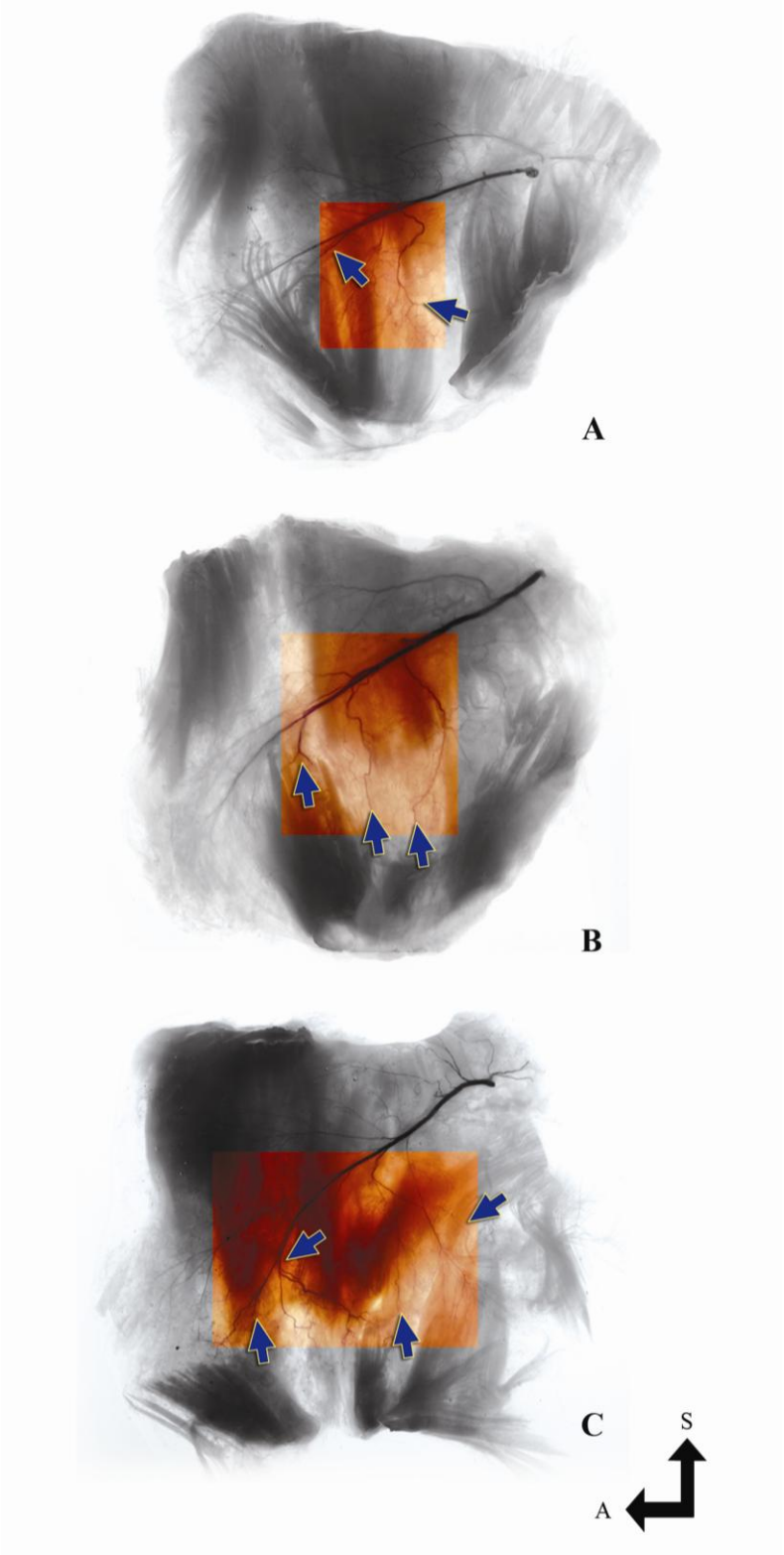

Fig. 5. Perforating branches (blue arrows) innervating to the superficial layer of the masseter muscle from the anteroinferior nerve group. The number of perforating branches from the anteroinferior nerve group was 2 (panel A, 5 cases), 3 (panel B, 3 cases) and 4 (panel C, 2 cases), respectively.

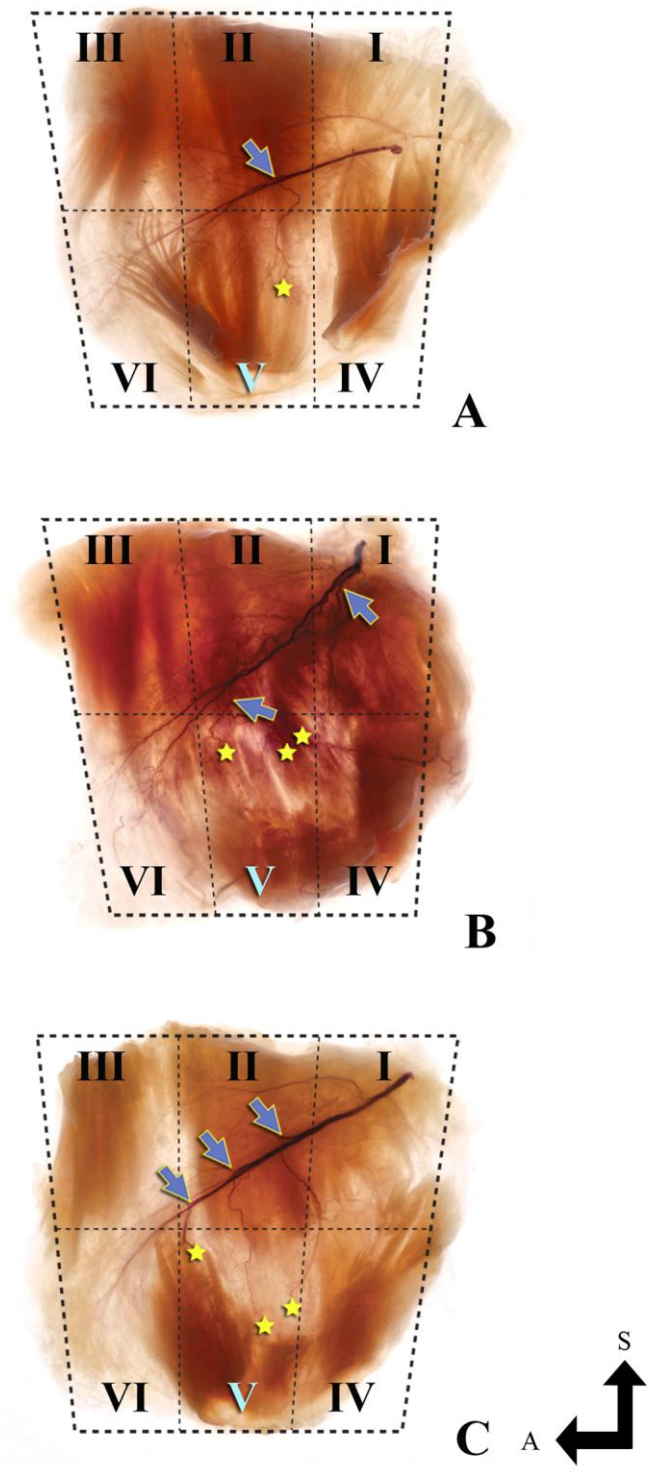

Fig. 6. The perforating branches (blue arrows) distributing at the area V. It was observed ranged from 1 (panel A), 2 (panel B), and 3 (panel C), respectively. In the panel B, the two perforating branches (blue arrows) were originated from the masseteric nerve divided into three nerve twigs (yellow stars) at the area V.

Ⅳ. DISCUSSION

Masseteric hypertrophy is a benign condition characterized by an enlargement of the masseter muscle and was first described by Legg in 1880 (Smyth, 1994). The condition has its highest incidence in the second and third decades of life and there is no sex predilection (Smyth, 1994). Etiology of the state is obscure; however nocturnal bruxism and daytime clenching can be a reason of masseteric hypertrophy (To et al., 2001).

Conventional treatment involves a surgical resection and reduction of the masseter muscle or mandibular angle. Gurney (1947) suggested the extraoral access to the hypertrophic masseter muscle for resection however; the postoperative complications such as bleeding, swelling and trismus can be encountered by the surgical treatments (Kim et al., 2005; Park et al., 2003).

To avoid the complications followed from the surgical interventions, BTX injection into the masseter muscle can be used as an alternative noninvasive treatment for masseteric hypertrophy (Mandel and Tharakan, 1999; Moore and Wood, 1994; Smyth, 1994; To et al., 2001; von Lindern et al., 2001). Once BTX is injected into the muscle, it is immediately diffused in the muscle within a few centimeters of the needle tip (Kinnett, 2004). When a higher volume is injected, the areas of diffusion appear to increase (Hsu et al., 2004). Inaccurate injection and excessive diffusion of toxin can lead to systemic side effects or unwanted weakness of neighboring muscles (Park and Rha, 2006).

General anatomy textbooks have been described the layer of the masseter muscle as two parts; the superficial and deep layer (James and Leslie, 2001). In the present study, the masseter muscle was depicted as three layers including the zygomaticomandibularis (ZM) as a deep layer of the masseter muscle group. According to our observation on the distribution of branches on each layer (superficial, middle, deep layer), the superior branches innervate into the zygomaticomandibularis

muscle as a deep layer of the masseter muscle group. The posteroinferior and anteroinferior nerve groups pierced the middle and superficial layer of the masseter muscle, respectively.

From the present study, we tried to find out the more efficient injection point by analyzing the perforating branches from the anteroinferior nerve group innervating to the lower portion of the superficial layer of the muscle, based on the conventional injection technique used in clinical practices. On the stained specimens by the modified Sihler's method, the richer arborization of the anteroinferior branches of the masseteric nerve was observed at the area Ⅴ and Ⅵ. However, the area Ⅵ at the anterior part of the masseter muscle far from the site of the masseteric hypertrophy revealed, has no clinical implication. On the other hand, there were poor arborization of the masseteric nerve and the parotid gland partially covered the posterior part of the area I and IV. At the superior portion (area Ⅰ, Ⅱ, Ⅲ), it was ineligible for BTX injection due to the location of the parotid duct and the absence of perforating branches supplying the superficial layer of the masseter muscle. Therefore, area Ⅴ is strongly recommended as an accurate and safe injection area for treatment of masseteric hypertrophy.

Summarizing the present study, we could observe 2 to 4 perforating branches supplying the superficial layer of the masseter muscle based on our anatomical examinations. In addition, the richer arborization of the perforating masseteric nerve branches were mostly confined at the area V (lower middle 1/3 of the whole masseter muscle), approximately in accordance with the BTX injection point that has been clinically applied. Therefore, through our findings, we could provide the theoretical evidence of the BTX injection practically used.

Ⅴ. CONCLUSION

In the present study, the analyses on the pattern of innervation of the masseteric nerve were performed from twenty-two embalmed masseter muscles (twelve masseter muscles were dissected and modified Sihler's staining technique was applied on ten muscles) for the purposes to provide the guideline in the injection of BTX.

1. As a result of observation on dissected masseter muscles, anteroinferior branches innervating the superficial layer could be the target of injection of BTX.

2. In the specimens stained by modified Sihler's staining technique, the number of perforating branches existed from 2 to 4 out of the anteroinferior branches. The branches innervating on the area Ⅴ were ranged from 1 to 3 among the perforating branches. The other areas could not be recommended as an injection point because of some anatomical structures such as parotid gland and duct.

For efficient injection of BTX, understanding of distribution pattern of the masseteric nerve is important. The method that injects BTX into the area which connecting the anterior and posterior margin of the masseter muscle, the inferior margin of the mandible and the line interlinking the mouth angle and the subaurale is adequate. Injection into the midportion makes possible that more effective treatment in the aspect of anteroposterior length.

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ABSTRACT(IN KOREAN)

보툴리눔 독소 주사를 위한 깨물근의 근육내 신경분포 양상

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<지도교수 최 종 훈>

Botulinum toxin type A (BTX)는 현재 미용성형뿐 아니라 근육긴장이상 (dystonia)이나 강직 (spasticity)의 치료 및 관자아래턱장애 (temporomandibular disorders)의 치료 등 다양한 분야에서 널리 이용되고 있다. 지금까지 이에 따른 BTX 관련 연구가 활발히 진행되어 왔으나, 실제 임상 적용 시 고려해야 할 시술 부위에 관한 연구는 부족한 실정이다. 본 연구는 깨물근신경의 분포 양상을 분석하여 정확하고 안전한 주사점을 찾아 깨물근비대 (masseteric hypertrophy) 치료 시 BTX의 효과를 최대화하는데 목적을 두었다.

모두 22 쪽의 고정된 깨물근 중 12 쪽을 해부하여 깨물근의 각 층 (얕은층, 중간층, 깊은층)에 분포하는 깨물근신경의 주행 양상을 관찰하였고, 나머지 10 쪽은 실러염색법 (Sihler's staining)을 이용하여 근육내 신경분포 양상을 자세히 관찰하였다.

한국인 시신의 맨눈해부 결과, 깨물근신경은 깨물근의 중간층과 깊은층 (광대아래턱근) 사이를 앞아래 방향으로 주행하였다. 깨물근신경은 턱뼈패임근막 (sigmoid fascia)을 뚫고 나와 시작 지점과 근접한 위치에서 위가지와 뒤아래가지가 거의 동시에 나와 깨물근의 깊은층과 중간층에 각각 분포하였다. 좁은 간격으로 일정하게 유지되던 이러한 신경 분포 양상은 중간층의 앞모서리를 기준으로 변화하여 아래 방향으로는 2-3 개의 앞아래가지가 여러 개의 가지로 나누어지며 얕은층에 분포하였고, 위가지는 뒤쪽에 비하여 넓은 간격으로 깊은층에 분포하고 그 수도 적었다. 깨물근 표본의 영역은 위와 아래, 그리고 앞, 중간, 뒤의 6 부분 (Ⅰ~Ⅵ)으로 나누어 관찰하였다. 표본의 신경염색 (modified Sihler's

staining)을 통한 연구 결과, 모든 표본의 Ⅰ, Ⅱ, Ⅲ 부분에서 일어나는 가지가 있었으나 깨물근 얕은층에 분포하는 관통가지는 모두 Ⅴ 또는 Ⅵ 부위로 분포하고 있었다. 깨물근신경 앞아래가지의 주행 방향이 뒤아래쪽을 향하는 경향을 보였으나, Ⅳ 부분까지 분포가 나타나는 경우는 드물었다.

효율적인 BTX 주사를 위해서는 깨물근의 얕은층에 분포하는 신경 가지의 분포 양상을 분석하여 주사점을 정해야 한다. 앞아래가지의 운동종말판 (motor end plate)이 가장 치밀하게 모여있는 지점은 Ⅴ와 Ⅵ 부분이었으나, Ⅵ 부분은 깨물근비대의 증상 발현 부위와 거리가 있으므로 임상적인 의미가 없다. Ⅳ 부분은 상대적으로 신경 분포가 적음과 동시에 귀밑샘에 의해 깨물근 뒤부분이 덮여 있는 경우가 있으므로 적절하지 않다. 따라서 본 연구의 결과를 토대로, 깨물근비대의 치료 시 가장 정확하고 안전한 주사점으로 추천할 수 있는 부분은 Ⅴ로 생각한다. 현재 임상에서 추천되고 있는 방법은 촉진을 통하여 깨물근의 앞뒤 경계를 확인하고 아래턱모서리, 그리고 입꼬리와 귀밑을 연장한 선을 연결한 사각형의 공간에 BTX 를 자입하는 것이다. 깨물근의 앞뒤 경계선을 확인한 후 그 중간 지점에 BTX를 주사할 경우 보다 안전하고 효과적인 치료가 가능할 것으로 생각한다.

핵심되는 말: 보툴리눔 A형 독소, 깨물근, 깨물근비대, 깨물근신경