Change of the thickness from skin surface to masseter muscle after botulinum toxin injection into human masseter muscle

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Change of the thickness from skin surface to masseter muscle after botulinum toxin injection into human masseter muscle

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논문뿐만이 아니라 3 년간 의국 생활을 하면서 교수님들의 관심 어린 지도와 애정이 제겐 큰 힘이 되었습니다. 함께 학문을 연구해 나가며 좋은 추억도 많이 쌓았던 전공의 선생님들, 진료에 많은 도움을 주신 직원분들께도 진심으로 감사드립니다.

마지막으로 언제나 큰 힘이 되어주고 항상 저를 믿어주고 응원해 주시는 부모님과 동생 건일이에게 사랑과 고마움을 전하고 싶습니다.

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박건우 드림

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Botulinum toxin is widely used to treat masseter muscle hypertrophy. It affects the end of neuron to decrease acetylcholine secretion, causing muscle atrophy which leads to cure. Change of muscle thickness has been reported in many studies. However, there has been no report about change in the thickness from skin surface to masseter muscle. In this study, we aim to measure not only change of muscle thickness but also the change of thickness from skin surface to masseter muscle by using ultrasonography.

A total of 17 volunteers were enrolled in this study. 10 patients were assigned to an experimental group (injected 25 U of botulinum toxin on both masseter muscle) and 7 to a control group (injected normal saline). Thickness was measured before injection and 4 weeks, 8 weeks, 12 weeks after the injection each at rest and during maximum clenching.

Result showed that thickness from skin surface to masseter muscle did not show significant difference by time at rest (p = 0.063) and maximum contraction state (p = 0.392). There was also no significant difference between experimental group and control group at rest (p = 0.392) and during maximum clenching (p = 0.259). Muscle thickness of experimental group showed a significant difference by time. In conclusion, botulinum toxin injection only affects change in muscle thickness and does not affect thickness from skin surface to masseter muscle.

Keywords: Botulinum toxin (BoNT), masseter, ultrasonography

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I. INTRODUCTION

Masseter muscle hypertrophy is an abnormal hypertrophy of unilateral or bilateral masseter. It has been first reported by Legg and has been reported several times since, but there is still a controversy over its etiology (Legg, 1880). In the past, surgical methods such as masseter resection have been chosen as a treatment. However, reversible and preservative treatment methods have been required due to post-operative complications and repulsion of patients.

Botulinum toxin (BoNT) is a substance made by *clostridium botulinum*, causing temporary chemodenervation by suppressing acetylcholine secretion in neuromuscular junctions (Burgen *et al*., 1949; Wheeler and Smith, 2013). As a result, it causes muscle paresis and atrophy in striated muscles. BoNT is being used not only in FDA approved fields such as bladder dysfunction, chronic migraine, upper limb spasticity, cervical dystonia, primary axillary hyperhidrosis, blepharospasm, and strabismus, but also in various neuromuscular disorders as an off-label use (Bigalke, 2013).

Using BoNT to treat masseter muscle hypertrophy was first introduced in 1994 in a field of plastic surgery (Moore and Wood, 1994). Using BoNT in treating masseter muscle hypertrophy patients is being considered as a safer, simpler and noninvasive method than surgical treatment and is widely used in clinic.

Studies that were made in the past using BoNT in masseter muscle hypertrophy measured its effect by photography (Castro *et al*., 2005), computed tomography (CT) (Chang *et al*., 2011; Kim *et al*., 2003; Kim *et al*., 2007; No *et al*., 2015; Park *et al*., 2003; Yu *et al*., 2007), magnetic resonance imaging (MRI) *(Raadsheer et al., 1994; Rijsdijk et al*., 1998), three-dimensional (3D) image (Cha *et al*., 2013; Lee *et al*., 2015; Shim *et al*., 2010), and ultrasonography (Choe *et al*., 2005; Kim *et al*., 2010; To *et al*., 2001; Wei *et al*., 2015). These studies report that BoNT injection causes masseter muscle atrophy which decreases lower facial volume.

BoNT is generally known to only affect muscle. However, assumptions that it could have an effect on tissues other than muscle are being brought up. Tsai *et al*. reported decrease of cortical thickness, trabecular thickness and bone mineral content in mandible

of adult rats after BoNT injection on unilateral masseter muscle (Tsai *et al.*, 2010). Rafferty *et al*. reported condyle bone loss in adult rabbits after BoNT injection on unilateral masseter muscle (Rafferty *et al*., 2012). Kun-Darbois *et al*. reported alveolar bone and condylar bone loss in adult rats after BoNT injection on unilateral masseter and temporalis muscle (Kun-Darbois *et al*., 2015).

BoNT injection may cause change in thickness from skin surface to masseter muscle (S-M) by diffusing effect to adjacent tissue or by body weight loss caused by masticatory disturbance due to decreased chewing force. However, change in S-M thickness after BoNT injection has not been studied until now.

The purpose of this study is to measure S-M and masster muscle thickness after BoNT injection to see the difference and find out if change in S-M thickness is occur.

II. SUBJECTS AND METHODS

1. Subjects

The study population consisted of 17 volunteers who requested treatment of masseter hypertrophy in Seoul, Korea. The volunteers were randomly divided into 2 groups. Ten volunteers (3 men and 7 women) aged 21 to 40 years (mean age, 29.8 years) received a single BoNT injection (Experimental group), whereas the remaining 7 volunteers (2 men and 5 women) aged 27 to 40 years (mean age, 30.9 years) received saline injections (Control group). The exclusion criteria were pregnancy, a history of any serious medical illnesses including drug allergy and who have had BoNT injection, orthodontic treatment, or plastic surgery within 1 year. During the experimentation several drugs that can affect muscles were prohibited including muscle relaxants, benzodiazepine and anticholinergic drugs.

2. Methods

2.1. Injection of botulinum toxin type A

BoNT (Meditoxin®; Medytox, Ochang, Korea) was supplied as a freeze-dried powder and reconstituted at a concentration of 50 U/mL (100 U in 2 mL of sterile saline) and used immediately. A volume of 25 U of BoNT was injected into the masseter muscle bilaterally using a 1-mL syringe with a 29-G ½-inch-long needle. Injections were performed at 2 points, 1 cm apart at the center of the middle one third of the masseter muscle [Figure 1].

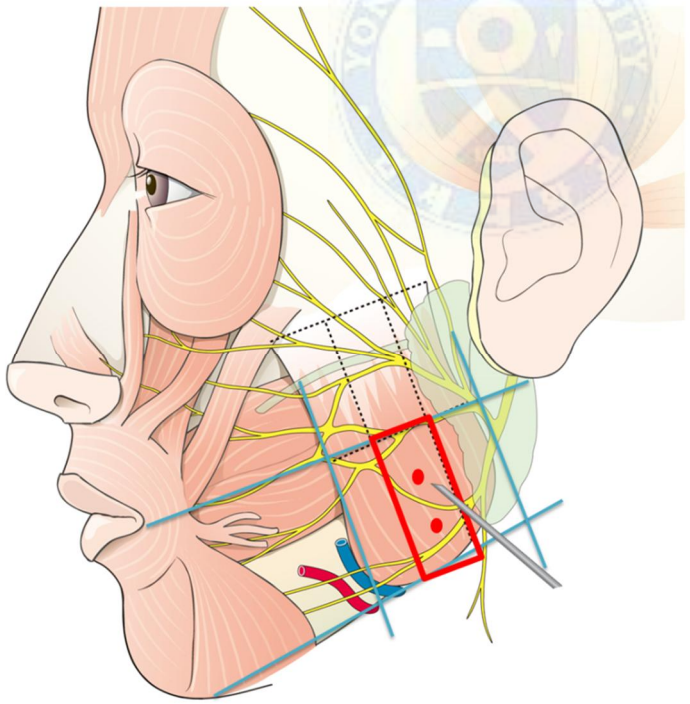

Figure 1. Red point shows the BoNT injection site (Hu *et al.*, 2010).

2.2. Ultrasonography procedure

All scans were performed using a E-cube9 diagnostic ultrasound (Alpinion, Seoul, Korea) using a frequency 3.0–12.0 MHz broadband linear transducer (L3-12H, Seoul, Korea). The characteristics of the preset were: frequency 12.0 MHz; dynamic range 70; gain setting 50; frame rate 59; and depth 3.0. The transmission gel was applied to the probe before the imaging procedure.

The masseter muscle was scanned bilaterally on Cheilion-Obi (Otobasion inferius) line which is horizontal reference [Figure 2]. During imaging, the transducer was held perpendicular to the surface of the skin and care was taken to avoid excessive pressure. To be perpendicular to the ramus the transducer was tilted until the ramus was depicted on the screen as a sharp white line. The thickness was defined as the largest distance between ramus and masseter muscle surface, perpendicular to the underlying ramus.

The imaging and measurements were performed with the subjects in an upright position under two different conditions [Figure 3]:

(A) When teeth are not contact with muscle in a relaxed position: physiologic resting position

(B) During maximum clenching with the masseter muscle contracted

The measurements were made directly from the image at the time of scanning. The thickness of S-M and masseter muscle was measured 4 times using an ultrasonography; before the injection, 4, 8, and 12 weeks after the injection.

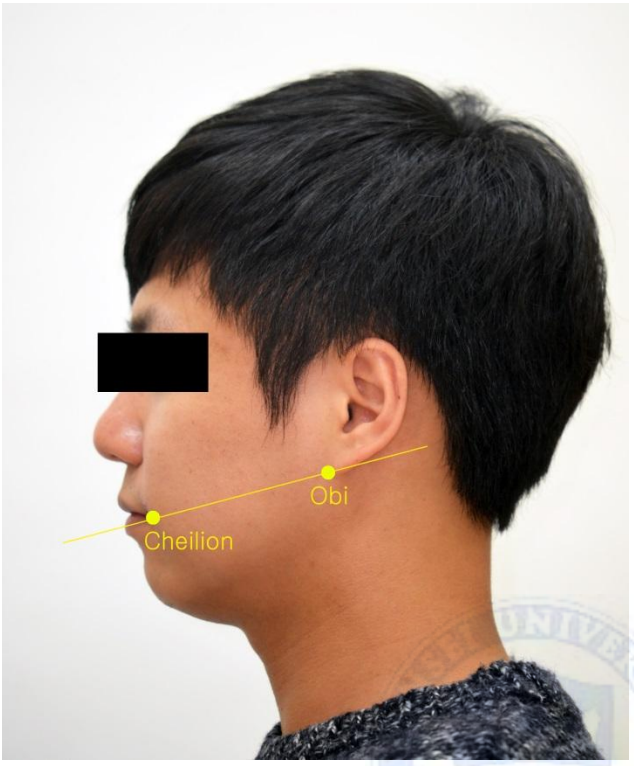

Figure 2. Horizontal reference of ultrasonography: Cheilion (a cephalometric point located at the corner of the mouth) - Obi (a cephalometric point of attachment of the ear lobe to the cheek)

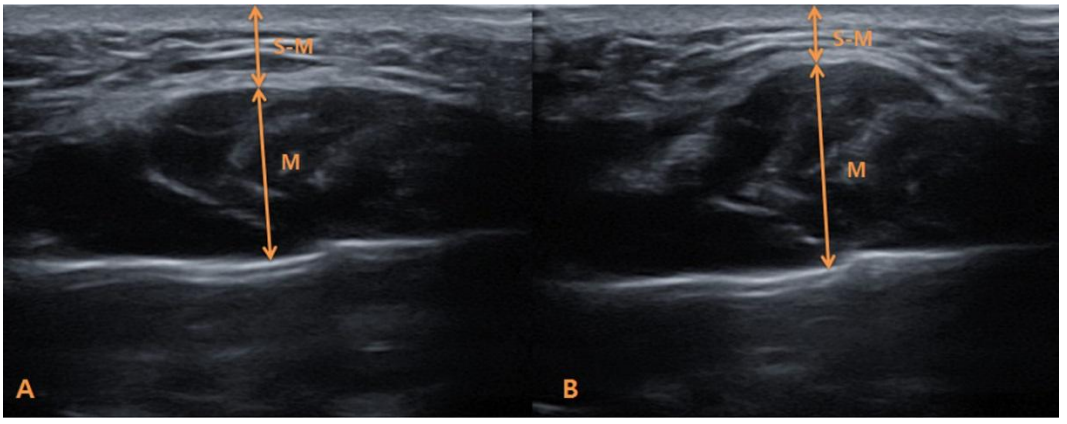

Figure 3. (A) Ultrasound image at rest, (B) Ultrasound image during maximum clenching. The orange color arrow indicate the thickness of the S-M and masseter muscle (M).

2.3. Statistical analysis

Data were analyzed using SPSS 20 software (SPSS, Inc., Chicago, IL, USA). Measurement of thickness of S-M and muscle was made both rest and maximum clenching state by time, calculating mean value and standard deviation. To evaluate statistical significance of change in thickness by time and group, we used two-way repeated measures Analysis of variance (ANOVA). Mauchly's sphericity test was used to verify the independent variable. When p-value of Mauchly's sphericity test was lower than 0.05, Greenhouse-Geisser (epsilon < 0.75) and Huynh-Feldt (epsilon > 0.75) was used to modify degrees of freedom. P-value was considered statistically significant when it is less than 0.05. Values are presented as mean ± standard deviation.

III. RESULTS

Table 1 shows that change of S-M and masseter muscle thickness at rest. There was no significant difference of S-M thickness by time (p = 0.063) or group (p = 0.392). No significant interaction between time and group was seen (p = 0.823) in S-M thickness. Significant interaction between time and group was seen in masseter muscle thickness (p = 0.024), and large amount of decrease in thickness was seen in experimental group compared to control group [Figure 4].

Table 1. Mean changes in the thickness of S-M and masseter muscle at rest[a].

		Pre-injection	4 weeks	8 weeks	12 weeks	Time	Group	Time*Group
		m ± σ	m ± σ	m ± σ	m ± σ	*p*	*p*	*p*
S-M[b]	Exp	5.79 ± 0.97	5.55 ± 1.12	5.73 ± 1.10	5.91 ± 1.43	0.063	0.392	0.823
	Con	5.41 ± 0.68	5.20 ± 0.80	5.15 ± 0.62	5.55 ± 0.92			
M[c]	Exp	14.19 ± 2.13	12.51 ± 3.23	11.76 ± 2.67	11.27 ± 2.85	0.001*	0.121	0.024*
	Con	14.84 ± 2.93	14.60 ± 2.75	14.28 ± 2.31	14.30 ± 2.50			

[a] Main effects and interactions were tested by two-way repeated measure ANOVA
[b] No significant interaction and main effect of time and group.
[c] Significant interaction of time and group (with Greenhouse-Geisser correction, epsilon = 0.650, F = 4.279, $p = 0.024$) and significant main effect of time (with Greenhouse-Geisser correction, epsilon = 0.650, F = 9.677, $p = 0.001$). No significant main effect of group.
* $p < 0.05$
S-M: thickness from skin surface to masseter muscle (mm), M: thickness of masseter muscle (mm), Exp: experimental group, Con: control group, m: mean, σ: standard deviation

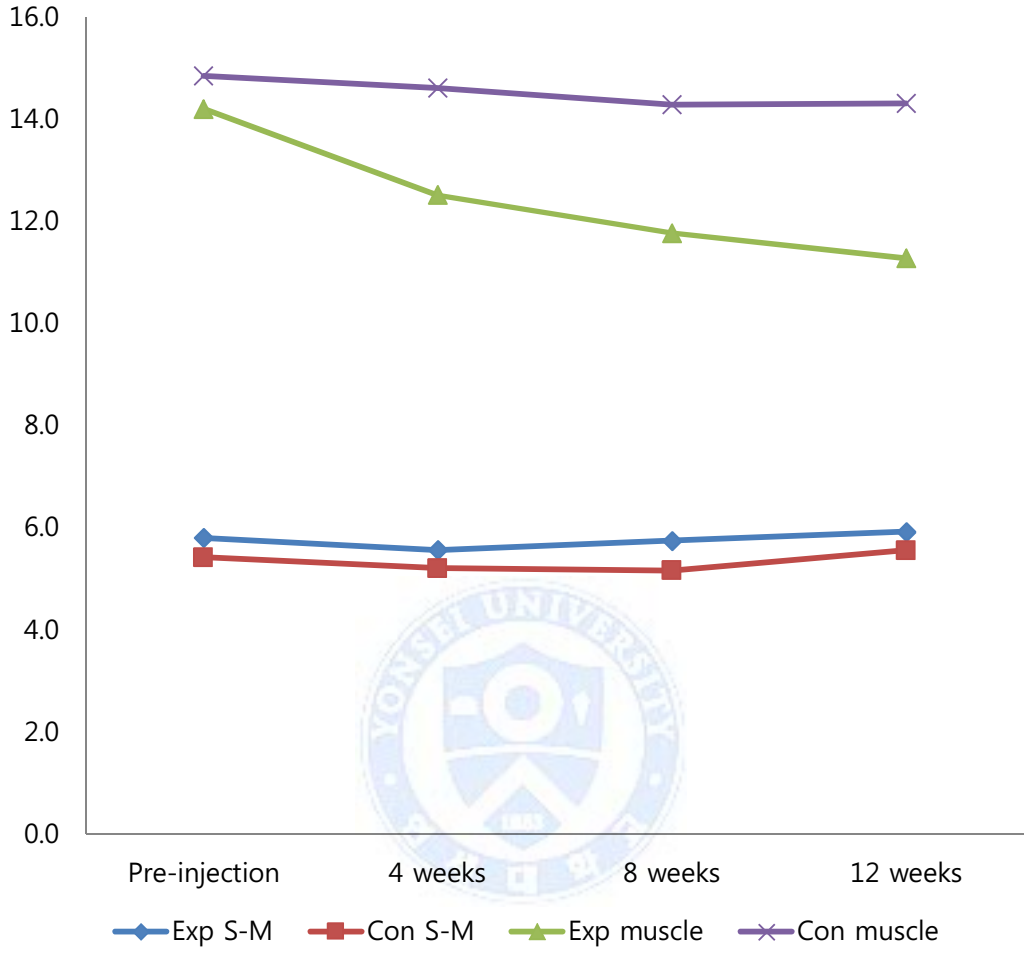

Figure 4. Mean changes in the thickness of S-M and masseter muscle at rest. Exp: experimental group, Con: control group

Table 2 shows that change of S-M and masseter muscle thickness during maximum clenching. There was no significant difference of S-M thickness by time (p = 0.166) or group (p = 0.259). No significant interaction between time and group was seen (p = 0.115) in S-M thickness. Significant interaction between time and group was seen in masseter muscle thickness (p = 0.025), and large amount of decrease in thickness was seen in experimental group compared to control group [Figure 5].

Table 2. Mean changes in the thickness of S-M and masseter muscle during maximum clenching[a].

		Pre-injection	4 weeks	8 weeks	12 weeks	Time	Group	Time*Group
		m ± σ	m ± σ	m ± σ	m ± σ	*p*	*p*	*p*
S-M[b]	Exp	4.84 ± 0.92	5.42 ± 1.19	5.14 ± 1.12	5.30 ± 1.24	0.166	0.259	0.115
	Con	4.75 ± 0.66	4.69 ± 0.70	4.45 ± 0.58	4.70 ± 0.86			
M[c]	Exp	16.58 ± 2.17	14.04 ± 3.48	13.72 ± 2.87	13.24 ± 2.97	0.002*	0.082	0.025*
	Con	17.01 ± 2.25	16.56 ± 2.09	14.46 ± 2.13	16.47 ± 1.84			

[a] Main effects and interactions were tested by two-way repeated measure ANOVA
[b] No significant interaction and main effect of time and group.
[c] Significant interaction of time and group (with Greenhouse-Geisser correction, epsilon = 0.484, F = 4.956, $p = 0.025$) and significant main effect of time (with Greenhouse-Geisser correction, epsilon = 0.650, F = 9.907, $p = 0.002$). No significant main effect of group.
* $p < 0.05$
S-M: thickness from skin surface to masseter muscle (mm), M: thickness of masseter muscle (mm), Exp: experimental group, Con: control group, m: mean, σ: standard deviation

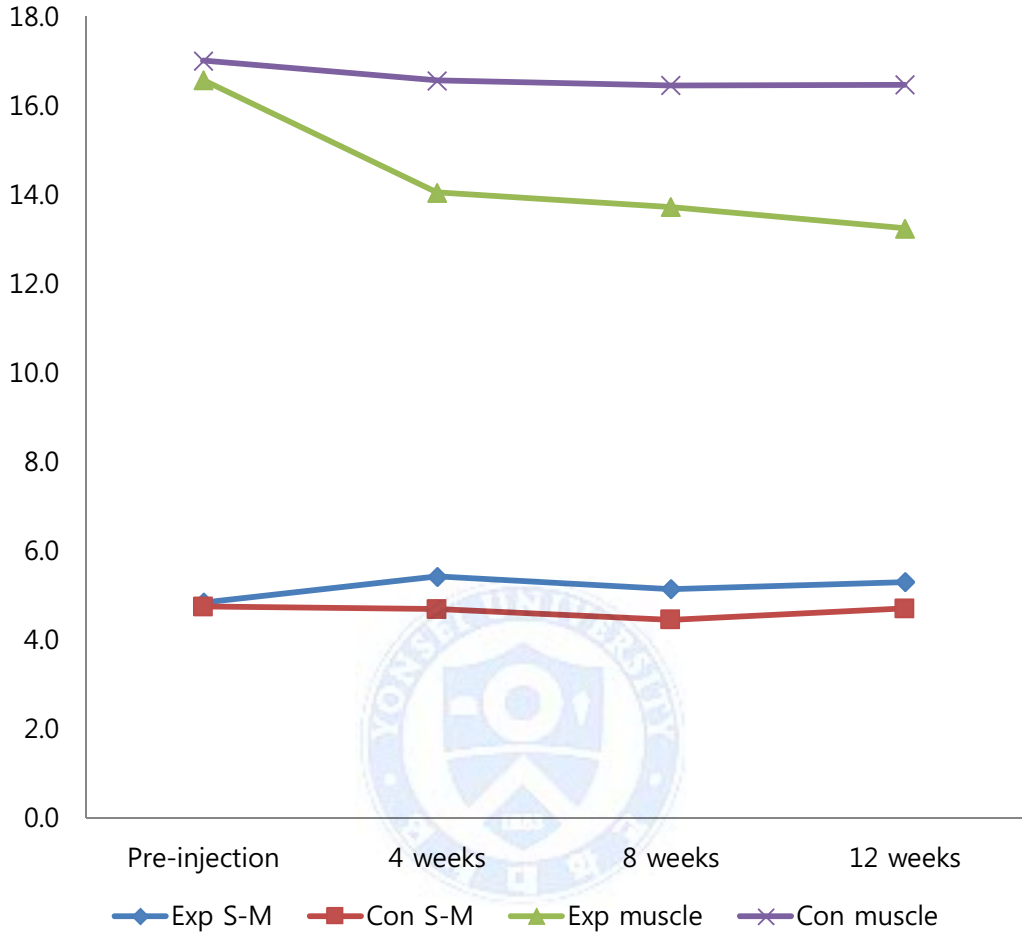

Figure 5. Mean changes in the thickness of S-M and masseter muscle during maximum clenching. Exp: experimental group, Con: control group

IV. DISCUSSION

Square-faced is caused complexly by protrusion of mandibular angle and hypertrophy of masseter muscle (Baek *et al*., 1994). Koreans have wide mandible because they are classified as a Mongolian race. Moreover, traditional Korean food is mostly rough and hard, promoting muscular development of masseter muscle. In Asian women, standard of beauty is related with slim face and soft looking feature, making square-faced patients as problem that needs to be treated (Ahn *et al*., 2004; Yuan *et al*., 2013).

Masseter muscle hypertrophy is a common cause of square-faced patients. It is occurred mostly by parafunction such as bruxism or clenching and also by dietary habit. Various treatments were introduced to reduce masseter muscle volume. It can be treated surgically but side effects such as post-operative pain, delayed healing time, bleeding, and nerve injury can be caused (Huang *et al*., 2003).

BoNT is one of the neurotoxin protein (A, B, C_1, C_2, D, E, F and G) produced by *Clostridium botulinum*, which is an anaerobe that causes food poisoning. *Clostridium botulinum* was first extracted in 1897 by Van Ermengen from a dead body and salted pork, and BoNT was separated and refined in 1946 by Edward J. Schantz. In 1949, Burgen *et al*. reported that BoNT suppressed contraction of muscle by depressing acetylcholine secretion at the end of cholinergic nerve (Burgen *et al*., 1949; Jankovic and Brin, 1997). By this function, when injected into masseter, reversible decrease in contraction force and volume of muscle has been observed. In the 1990s, BoNT started to replace surgical methods in many clinics (Jankovic and Brin, 1991).

In previous studies, BoNT injection was proven to be effective in decreasing size of masseter muscle. In these studies, photography, CT, MRI, 3D image, ultrasonography were used to detect changes (Castro *et al*., 2005; Cha *et al*., 2013; Chang *et al*., 2011; Choe *et al*., 2005; Kim *et al*., 2003; Kim *et al*., 2007; Kim *et al*., 2010; Lee *et al*., 2015; No *et al*., 2015; Park *et al*., 2003; Raadsheer *et al*., 1994; Rijsdijk *et al*., 1998; Shim *et al*., 2010; To *et al*., 2001; Wei *et al*., 2015; Yu *et al*., 2007). Among them, CT, MRI and ultrasonography can be used to measure S-M thickness. Repeated measurement by taking CT can cause problem by increase in patient's radiation exposure rate. MRI does not have such problems but it is expensive and difficult to acquire subdivided image, which makes it hard to expect exact value. In clinic, ultrasonography is favorable for its fast, easy, and harmless feature.

Published paper that measures thickness of masseter with ultrasonography has different measuring reference. Volk *et al*. placed the probe transversely at middle of zygomatic bone and mandibular angle (Volk *et al*., 2014). Other studies just measured at maximum thickness or had no indication about reference point.

Emshoff *et al*. reported that masseter muscle seems to be thickest in the middle area when divided into 5 from origin to insertion (Emshoff *et al*., 2003). Raadsheer *et al*. reported that measurement value of masseter using MRI and ultrasonography is highly related, and it was most highly related when measured at the middle of masseter (Raadsheer *et al*., 1994). Suh *et al*. reported that maximal thick point of masseter muscle from the mandibular angle in Koreans was 27.77mm in male and 25.79mm in female. Maximal thick point between mandibular angle and zygomatic arch was located in the area of 53% and 59% from mandibular angle each in male and female (Suh *et al*., 2011).

Therefore, in this study, we tried to minimize the errors from change of reference point while repeating the measurement of maximum thickness of S-M and masseter muscle by setting Cheilion-Obi line as a reference.

BoNT injection can directly affect adjacent tissue other than masseter muscle by drug diffusion or indirectly affect S-M thickness by weakening chewing force. S-M layer consists of epidermis, dermis, subcutaneous tissue. Subcutaneous tissue is mainly composed of fat tissue, which has a very close relationship with weight difference (Deurenberg *et al*., 1998). If BoNT injection decreases food intake due to weakness of masticatory function, weight loss and body fat decrease can occur. This can lead to decrease of subcutaneous tissue thickness. However, in this study, we did not measure body weight and body fat difference, which should be supplemented on following studies.

BoNT diffusion potency was studied by several authors. Studies in experimental animals have shown that 5 – 10U of BoNT has biologic activity 4.5 cm from the injection site within the targeted muscle (Borodic *et al*., 1994), that muscle fascia is an ineffective barrier for diffusion of the toxin (Shaari *et al*., 1991), and that doses of 10 U have biologic effects that spread across fascial planes to adjacent, noninjected muscles for 2.5 – 4.5 cm from the injection site (Borodic *et al*., 1994; Borodic *et al*., 1990). However, muscles were not included in S-M layer. Therefore, even if BoNT diffuses to this layer, it may not have a direct effect.

In this study, we used snapshot to capture the video when using ultrasonography and measured thickness. Reference point was set on rest and then clenching motion was made. Thickness of maximal thick point of muscle and S-M was measured on maximum

clenching state, than the thickness of same reference area was measured at rest. Ideally, same pressure should be loaded when measuring on maximum clenching and rest state. However, replacing the probe to acquire same pressure can cause change of measuring point from reference point. Also, maximum clenching time of a patient can only last few seconds making it technically impossible to replace the probe at the right time. Thus, the technique that has been used in this study can be considered clinically appropriate. Masseter muscle tends to push the probe away on clenching motion because of muscle contraction, which causes an increase in thickness and volume. Therefore, maintenance of an even pressure by a skilled researcher is crucial.

In this study, average of S-M thickness of total 17 people before BoNT injection was 5.63mm at rest and 4.80mm at maximum clenching, showing a decrease at maximum clenching compared to rest state. This may be due to pressure of masseter muscle on subcutaneous tissue during contraction or stretching of subcutaneous tissue during clenching. We could not find a statistic significance in relationship of change in masseter muscle thickness and S-M thickness due to small number of experimental group, but this would be worth being studied in further experimentations.

The possibility of S-M thickness becoming thinner by pressure from thickened muscle during muscle contraction cannot be excluded. To measure this precisely, CT or MRI, which causes no pressure on the probe during clenching, would be the best choice to minimize measurement errors. However, maintaining consistent maximum clenching state during CT or MRI taking seems to be clinically hard to perform. This should be taken into consideration in following studies.

Suh *et al*. reported that depth of masseter muscle from the skin surface using CT is 6.15 ± 2.90mm in male and 7.37 ± 1.93mm in female, making female measurements larger than male, statistically significant (Suh *et al*., 2011). This seems to be related with difference in body fat of male and female. In our study, subcutaneous tissue thickness at rest before injection showed 5.63 mm in average, 8.04 mm in maximum. It is obvious that needle length should be over 8 mm when injecting BoNT into masseter muscle. Therefore, using 29-G ½-inch-long needle is recommended.

V. CONCLUSION

This study measured S-M and masseter thickness after BoNT injection and analyzed difference between experimental group and control group by time. As a result, masseter muscle thickness of experimental group decreased significantly by time, whereas S-M thickness of experimental group and control group showed no significant difference. As a conclusion, BoNT is only effective on muscles and does not affect subcutaneous tissues around.

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ABSTRACT (in Korean)

교근부 보툴리눔 독소 주사 전후의 교근의 피부표면으로부터의 두께 변화

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박 건 우

보툴리눔독소 주사는 교근비대증의 치료를 위해 널리 사용되고 있다. 작용 기전은 보툴리눔독소가 신경의 말단에 작용하여 아세틸콜린의 분비를 억제함으로서 근육의 위축을 일으켜 치료효과를 나타낸다. 많은 연구에서 근육 두께의 변화에 대해 보고하였지만 피부 표면에서 교근까지 두께 변화에 대해서는 알려진 바가 없다. 본 연구에서는 초음파를 이용하여 근육의 두께 변화뿐만이 아니라 피부 표면에서 교근까지 두께 변화량을 측정하여 피하조직의 변화에 대해 관찰하고자 한다.

10명의 실험군과 7명의 대조군을 모집하여, 실험군에는 보툴리눔 독소를 양측 교근에 각각 25유닛씩 주사하였고 대조군은 식염수를 주사하였다. 주사 후 교근 및 피부 표면에서 교근까지 두께 변화량은 안정시와 최대 수축시에 주사 전, 4주 후, 8주 후, 12주 후에 측정하였다.

실험 결과 피부 표면에서 교근까지 두께는 주사 후 안정시 (p = 0.063)와 최대 수축시 (p = 0.166) 시간의 변화에 따라 유의한 차이를 보이지 않았다. 실험군과 대조군 간에도 안정시 (p = 0.392)와 최대 수축시 (p = 0.259) 유의한 차이를 보이지 않았다. 실험군의 근육 두께량은 시간에 따라 유의한 차이를 보였다(p < 0.05). 따라서 보툴리눔독소 주사는 근육의 두께변화만을 일으키고 피부 표면에서 교근까지 두께변화에 영향을 미치지 않는다.

핵심되는 말: 보툴리눔 독소, 교근, 초음파