

**Change of distribution and timing of bite
force after botulinum toxin type A injection
evaluated by a computerized occlusion
analysis system**

JI Hee Song

Department of Dental Science

The Graduate School, Yonsei University

**Change of distribution and timing of bite
force after botulinum toxin type A injection
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analysis system**

Directed by Professor Hyung Joon Ahn, D.D.S., Ph.D.

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Ji Hee Song

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**This certifies that the doctoral dissertation
of Ji Hee Song is approved.**

Thesis Supervisor : Hyung Joon Ahn

Thesis Committee Member : Jong-Hoon Choi

Thesis Committee Member : Seong Taek Kim

Thesis Committee Member : Baek Il Kim

Thesis Committee Member : Young Bum Park

The Graduate School

Yonsei University

December 2014

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먼저 지나간 석, 박사 통합 과정 동안 논문의 시작과 끝을 함께 고민해주시고 지도해주신 안형준 지도교수님께 깊은 감사를 드리고 싶습니다. 또한 항상 제게 아버지 같은 관심과 사랑을 주시는 최종훈 교수님, 바쁘신 와중에도 연구의 아이디어를 먼저 제공해 주시고 방향을 잡아주신 김성택 교수님, 제 연구를 본인의 연구처럼 매사 신경써주신 권정승 교수님께도 감사 드립니다. 마지막으로 부족한 저의 논문 심사와 지도를 기꺼이 맡아주신 김백일 교수님과 박영범 교수님께도 깊은 감사의 인사를 드립니다.

논문 작성과 SCI 저널의 투고기간동안 많은 도움을 준 조은애 선생과 바쁜 수련의 생활 중에도 많은 도움을 준 모든 의국원 여러분에게도 진심으로 감사드립니다.

항상 제게 무한한 사랑을 주시는 부모님, 늘 저를 먼저 위해주시는 시부모님, 그 감사의 마음을 말로 다 표현할 수 없을 것 같습니다. 마지막으로 언제나 든든히 저를 옆에서 지켜주고 아껴주는 남편에게 진심으로 감사와 사랑을 전합니다.

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Abstract

Change of distribution and timing of bite force after botulinum toxin type A injection evaluated by a computerized occlusion analysis system

Ji Hee Song

Department of Oral Medicine / Dental Science

The Graduate School, Yonsei University

(Directed by Professor Hyung Joon Ahn, D.D.S., Ph.D.)

Often it has been observed that botulinum toxin type A has analgesic effects and reduces hyperactivity of injected muscle. However in our clinic, there are some patients who have unilateral chewing habits before botulinum toxin type A injection, but after injection, they indicate both masticatory force become similar. However, there were no study about this effect of botulinum toxin type A. So, we studied about relationship of botulinum toxin type A and distribution of masticatory force.

The aim of this study was to determine the force distribution and pattern of mastication after injection of BTX-A into both masseter muscles. The hypothesis to be tested was that the

difference between right and left balance of occlusal force diminishes over time following BTX-A injection.

Fifteen patients were submitted to BTX-A injection therapy for subjective masseter hypertrophy. A total of 25 U of BTX-A (50 U in total) was injected into two points located 1 cm apart at the center of the lower one-third of both masseter muscles. All of the patients were examined using the T-Scan occlusion analysis system before and 4, 8, 12, and 24 weeks after BTX-A injection.

A significant change in force balance was found between the right and left sides over time and the difference between the two sides decreased with the time post-injection, reaching a minimum at 12 weeks. Comparison of the force balance between the anterior and posterior occlusions revealed no significant difference at any of the time points. The occlusion and disclusion times (right and left sides) did not differ significantly with time since BTX-A injection.

Keywords: Botulinum toxins type A, Bite force, Dental occlusion, Masseter muscle, Mastication, Time factors

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I. INTRODUCTION

In dental field, the clinical use of botulinum toxin type A has been expanded for the past few years. It is used in treatment of masticatory and facial muscle spasm, severe bruxism, facial tics, orofacial dyskinesias, dystonias, and idiopathic hypertrophy of the masticatory muscles(Clark, 2003). Also, it is used in the treatment of temporomandibular disorders (Freund *et al.*, 2000), myofascial pain syndrome (Lang, 2002), a headache such as chronic migraine(Lovell and Marmura, 2010), recurrent dislocation on the TMJ, drooling and Frey's syndrome(Bhogal *et al.*, 2006). When we apply botulinum toxin type A in various orofacial regions of patients, we often

inject into masticatory muscle such as masseter muscle and/or temporalis muscle. In case of botulinum toxin type A injected into masticatory muscles, according to the pharmacological property of botulinum toxin type A itself, it cause a temporary muscle paralysis, weakness and atrophy.

Also, the control of mastication is dependent in large part on sensory feedback, which consists of epithelial mechanoreceptors, periodontal afferents, temporomandibular joint afferents and muscle afferents (Soboleva *et al.*, 2005). Change in afferent input caused by injection of botulinum toxin type A into muscle can cause modify the response of the cortex, modify the motor neuron activity, and even initiate irrelevant muscle activity. Subsequently, injection of botulinum toxin type A into masticatory muscle can directly influence on mastication by muscle weakness and atrophy, also indirectly it can influence on mastication by effect on central pattern generator in brainstem through modifying the sensory feedback from masticatory muscle spindle (Okeson, 1993).

Often it has been observed that botulinum toxin type A has analgesic effects and reduces hyperactivity of injected muscle (Fallah and Currimbhoy, 2012). However in our clinic, there are some patients who have unilateral chewing habits before botulinum toxin type A injection, but after injection, they indicate both masticatory force become similar. However, there were no study about this effect of botulinum toxin type A. So, we studied about relationship of botulinum toxin type A and distribution of masticatory force.

The T-Scan occlusal analysis system is dental equipment for analyzing masticatory force. It was first devised in 1984 to measure occlusal force and contact time to utilize as a prosthodontic adjunction for the improvement of TMD and occlusal diagnoses in the treatment of occlusal problems. And it has been used as a measurement guide during the prosthetic insertion and occlusal adjustment procedures. The all information about occlusal force and

contact time is gathered to the recording sensor in T-Scan system (Kerstein et al., 2006). This information can be showed by movie of T-scan. So, T-scan have definitive diagnostic imaging of the force balance and function of the masticatory muscles(Montgomery et al., 2011).

The aim of this study was to determine the force distribution and pattern of mastication after injection of BTX-A into the bilateral masseter muscles. The hypothesis to be tested was that the difference between right and left balance of occlusal force diminishes over time following BTX-A injection.

II. Material and Methods

1. Patient selection

This study was approved by the institutional review board committee of Yonsei School of Dentistry (No. 2-2011-0024). A total of 15 patients who conducted botulinum toxin injection therapy because of subjective masseter hypertrophy and T-scan examination were selected for this study. Subjects who had normal occlusal conditions(class I) were included in the study. However, subjects who had abnormal occlusal conditions such as missing tooth, severe tooth attrition which could affect the normal occlusion were excluded. And subjects who had dental treatment or had TMJ osteoarthritis, had occlusal interference during eccentric movement, and who had a experience injection of BTX-A in last six months and who got pregnant were also excluded.

2. BTX-A injection

The BTX-A used in this study was Botox (Allergan, Irvine, CA, USA); 100 U of Botox, obtained as a freeze-dried powder, was reconstituted to a concentration of 5 U/0.1 mL using 2 mL of 0.9% sterile, nonpreserved saline, and used immediately after reconstitution. A total of 25 units of BTX-A was injected into both masseter muscle bilaterally using a 1-ml syringe with a 29-gauge, 1/2-inch-long needle. Areas of masseter prominence on clenching were marked, and injected at two points at the center of the lower one-third of the masseter muscle separated by 1 cm. This site was chosen to avoid accidental toxin injection into the parotid gland, parotid duct, or facial artery. The Injection was conducted by a single person in order to reduce error range. (Fig. 1.)(Hu et al., 2010)

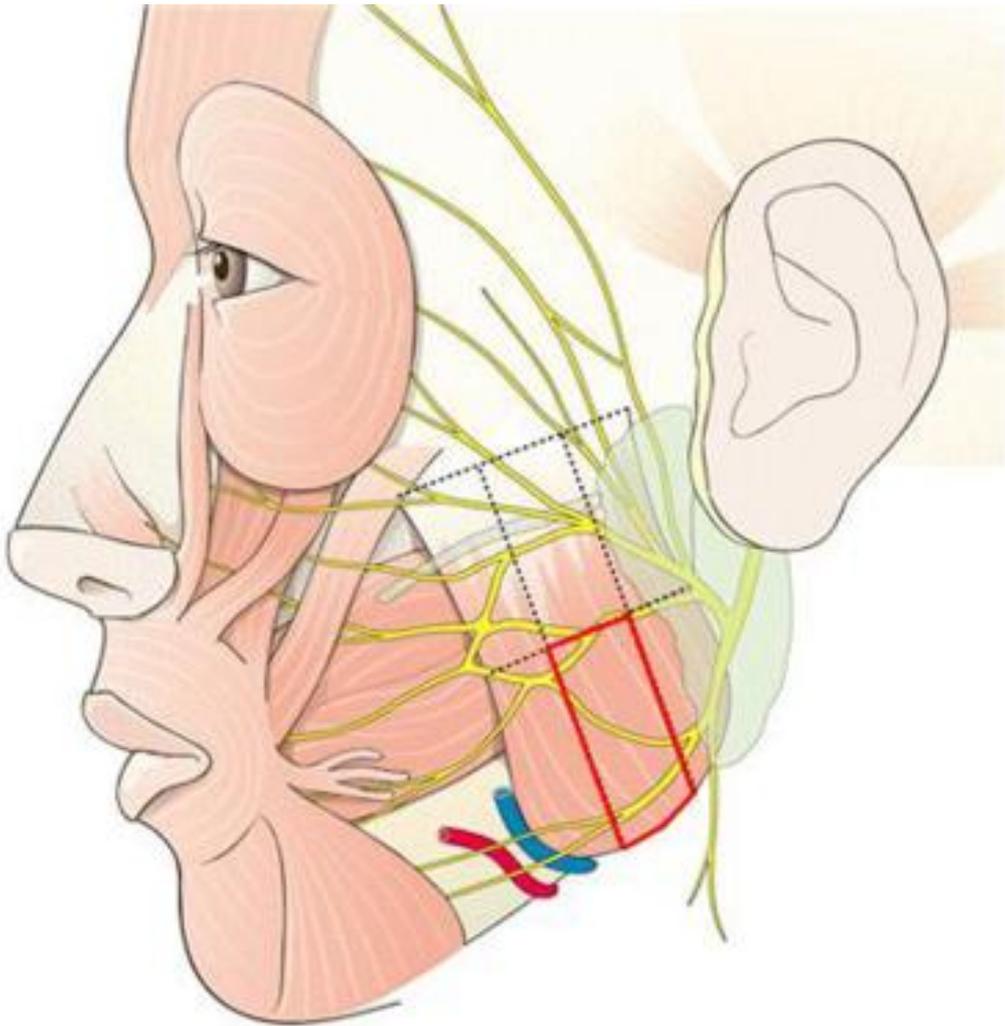


Figure 1. The red boxed area shows the botulinum toxin type A (BTX-A) injection site (Hu KS, Kim ST, Hur MS, Park JH, Song WC, Koh KS, et al.: Topography of the masseter muscle in relation to treatment with botulinum toxin type A. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 110(2): 167-171, 2010.)

3. T-Scan analysis

Also, all the patients were examined through T-scan occlusal analysis system(T-scan III, Yours Dental Co., Korea) before and 4, 8, 12, 24 weeks after injection. This T-scan system is comprised of Microsoft Windows-based software, the associated hardware, and patented paper-thin disposable sensors(Montgomery et al., 2011).(Fig.2) The information provided on T-Scan movies is listed in Table 1.

The sensor consists of two layer of mylar(reinforced polyester film) laminated pressure sensitive ink grid. The film is covered by a silver thread grid, the intersectioning points of which are bathed by conductive ink. When a patient closes firmly on the sensor, the resultant reduction on electric resistance will be converted to an 8-bit digital values and translated into an image on the screen.(Kerstein, 2004)

The patient was seated in an upright position with because supine position can alter contact position. (Makofsky et al., 1991) The T-SCAN III sensor and sensor support assembly were inserted intraorally and positioned correctly. Then T-Scan force-movie mode is activated manually by pushing the button on the handle. The patients were instructed to bite in the habitual intercuspal position, and then made excursive movements which were not guided by the investigator. The right and left excursions were recorded separately. Between the records, patient had few minutes of resting time. All these procedures were repeated three times and we used third record when analyzing the data because when the patient bite the record sensor in fist time, it may not reflect the wonted chewing pattern due to unfamiliarity.

The disclusion time is defined as a time, in seconds, required to disclude the working and nonworking molar interferences and non-working premolar interferences from the habitual centric closure position to the completion of a mandibular excursion and the occlusion time

defined as the time from the first contact of teeth to the maximal intercuspation, and the occlusal contact area. (Kerstein and Wright, 1991)

In this study, we analyzed about the distribution of forces between the left and the right side , the distribution of forces between the front and the back side in clenching and the change of occlusion time and disclusion time after botulinum toxin type A injection.(Fig. 2.,3.)

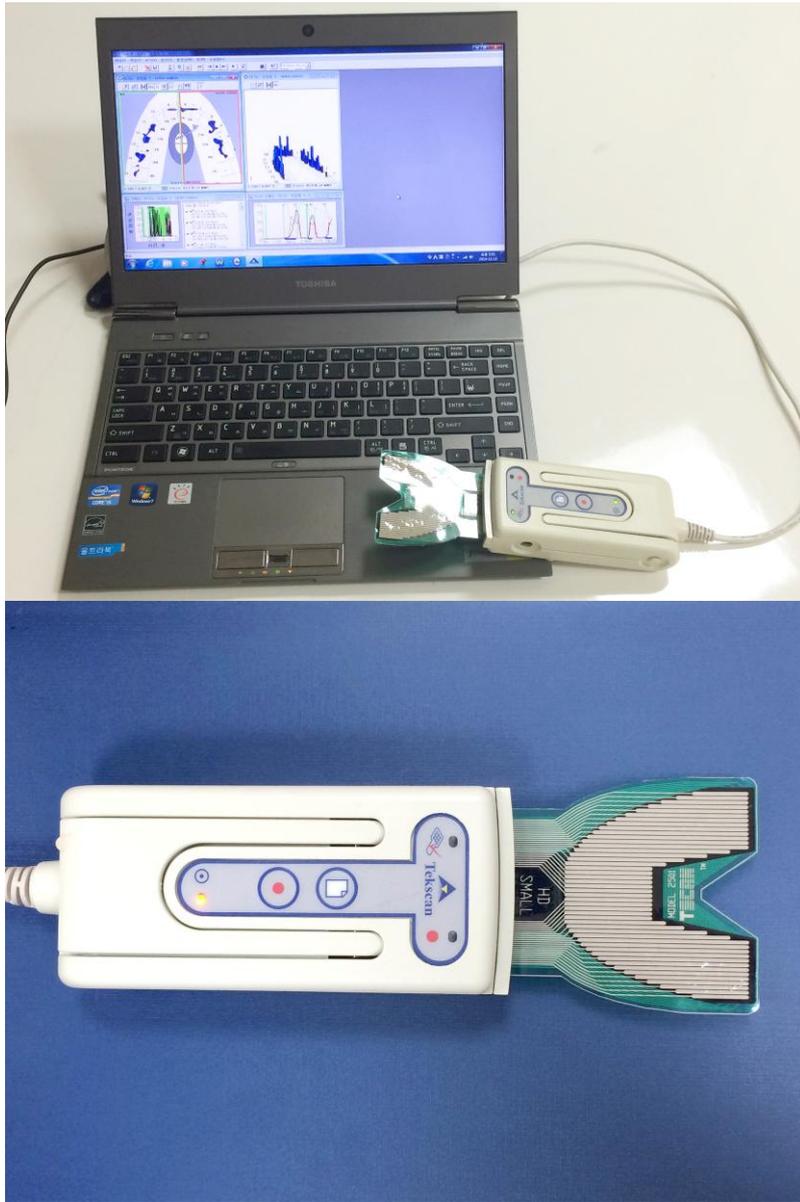


Figure 2. T-Scan appliance

Table 1. The information provided on T-Scan movies

The distribution of forces (as percentages) around the arch

The timing of the forces – early or late

The presence of interference during closure

The balance of forces left to right and/or anterior to posterior at any point during closure

The effectiveness of guidance patterns that provide somatosensory muscle control

Evidence of muscle balance or imbalance during function

Evidence of abnormal dental forces secondary to injury, pain, or inflammation

Presence and timing of forces that are above or below the average for this patient

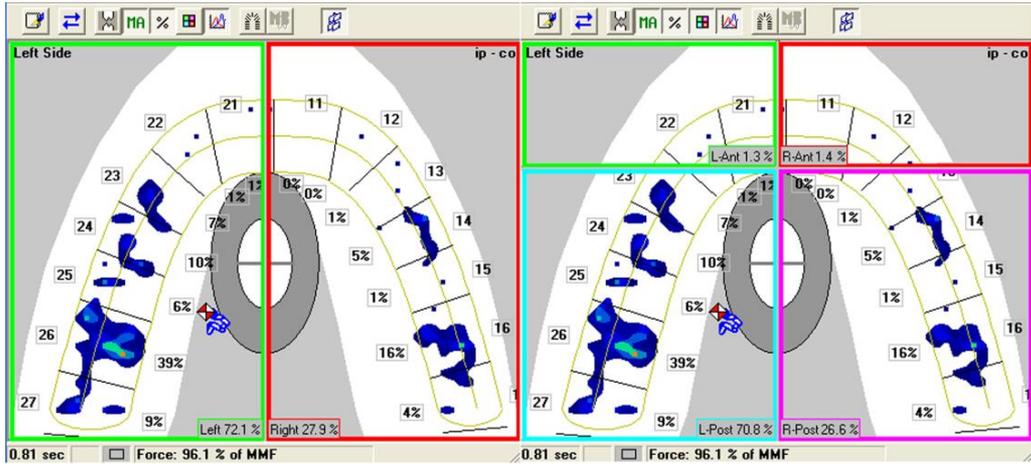


Figure 2. Balance of clenching force in a T-Scan movie

Left: divided into two parts: left and right sides

Right: divided into four parts labeled in clockwise direction : anterior left, anterior right, posterior right, posterior left

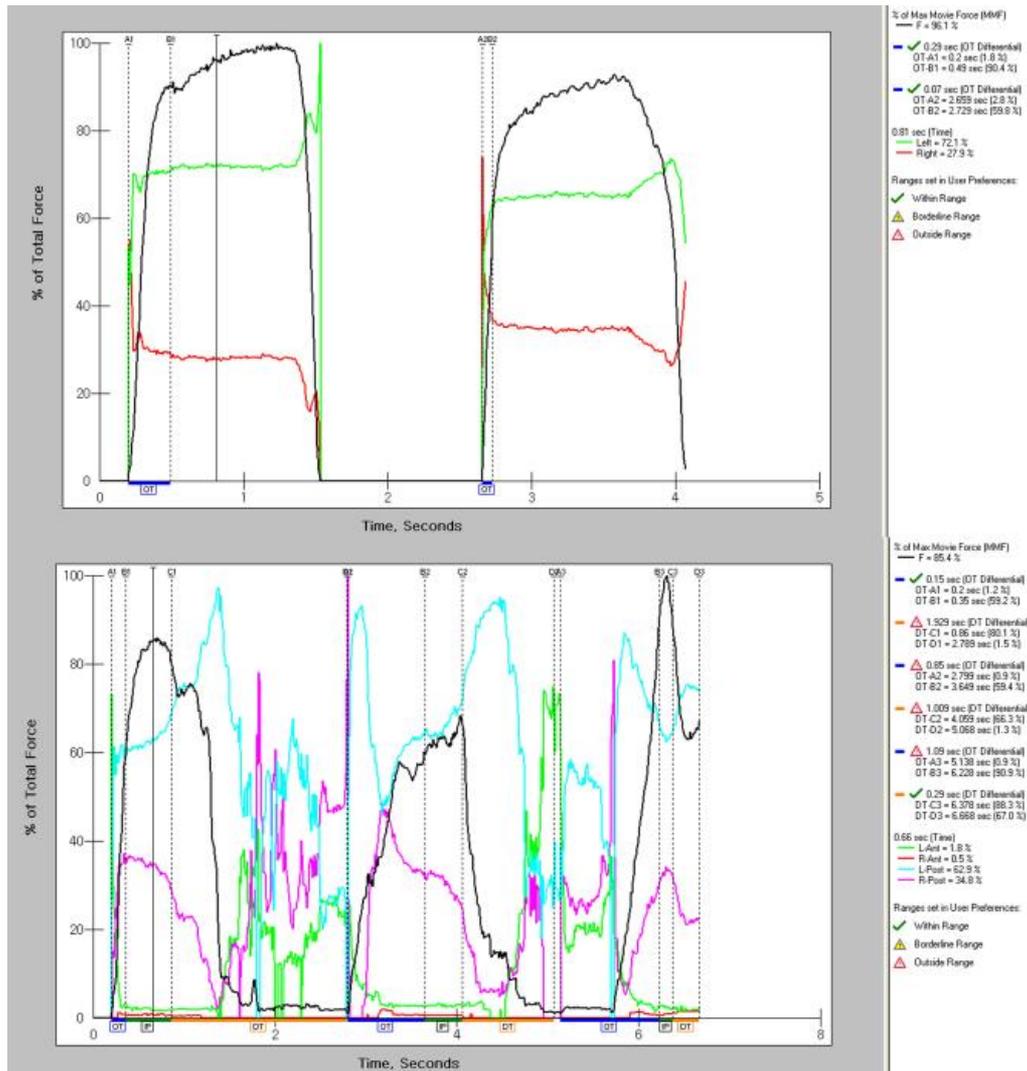


Figure 3. the upper diagram: Occlusion time, the lower diagram: disclusion time

In the upper diagram, A1 is the point the teeth start to occlude, B1 is the point the dentition is fully occluded, and the difference of A1 and B1 is occlusion time, marked as OT.

In the lower diagram, C1 is the point lateral movement starts, D1 is the point the dentition is fully separated and the difference is disclusion time, marked as DT.

4. Statistical analysis

Linear mixed models for longitudinal data was carried out to analysis the data in process of time. The interaction between times and groups was evaluated by compound symmetry covariance structure or banded toeplitz or autoregressive covariance structure. A Bonferroni's correction was used for post hoc analysis. A p-value of < 0.01 was considered statistically significant. All statistical analyses were performed using SAS® Version 9.2 Windows Statistics Program (SAS Institute Inc., Cary, NC, USA).

III. RESULTS

1. Changes in the distribution of forces

A significant change in force balance was found between the right and left sides over time (i.e., preinjection and 4, 8, 12, and 24 weeks post-BTX-A injection) and at each measurement time point ($p < 0.0001$). The difference between the two sides decreased with the time postinjection, reaching a minimum at 12 weeks (Figure 4). These findings reflect the well-established time course of the actions of BTX-A (Aoki et al., 2006). In other words, according to this result, the left and right clenching force becomes more balanced with the increasing effect of botulinum toxin injection.

Comparison of the force balance between the anterior and posterior occlusions revealed no significant difference at any of the time points (i.e., preinjection and 4, 8, 12, and 24 weeks postinjection; $p > 0.01$ for all). Furthermore, comparison of the preinjection force balance with those measured at 4, 8, 12, and 24 weeks postinjection revealed no significant difference at any time point ($p > 0.05$ for all; Table 2). In other words, there is no correlation between the balance of anterior and posterior masticatory force and the increasing effect of botulinum toxin injection.

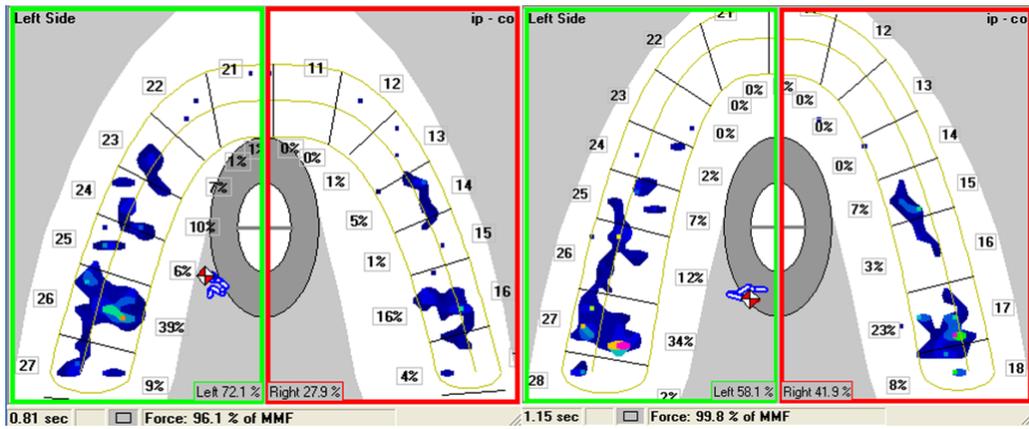


Figure 4. Force distribution on right and left sides before BTX-A injection (left) and 12 weeks thereafter (right)

In the left figure, before botulinum toxin injection the distribution of the left and right force was uneven, 72.1% and 27.9%. 12 weeks post injection, the right figure, the distribution evened as left 58.1% and right 41.9%.

Table 2. Changes in the force distribution (%; mean±SD) after injection of BTX-A (n=15)

	<i>Preinjection</i>	<i>After 4 weeks</i>	<i>After 8 weeks</i>	<i>After 12 weeks</i>	<i>After 24 weeks</i>
Difference between left and right sides (%)	25.5±10.1	15.7±10.3*	11.9±6.0*	7.1±5.6*	16.4±11.4*
Difference between anterior and posterior (%)	91.0±7.5	90.7±10.1	88.2±12.8	87.6±10.5	87.8±8.7

* $p < 0.01$ (compared to preinjection)

Table 3. Change in occlusion and disclusion times (in seconds) with time after BTX-A injection ($n=15$)

	<i>Preinjection</i>	<i>After 4 weeks</i>	<i>After 8 weeks</i>	<i>After 12 weeks</i>	<i>After 24 weeks</i>
Occlusion time	0.32±0.26	0.39±0.51	0.22±0.11	0.20±0.10	0.25±0.10
Disclusion time (right)	1.52±1.32	2.54±2.17	2.01±1.82	2.69±2.35	1.82±1.79
Disclusion time (left)	1.30±1.53	1.66±1.94	2.63±2.24	3.24±2.38	3.05±2.30

* $p<0.01$ (compared to preinjection)

2. Changes in occlusion and disclusion times

The occlusion and disclusion times (right and left sides) did not differ significantly with time after BTX-A injection ($p > 0.01$; Table 3).

IV. DISCUSSION

Benign masseter hypertrophy is a relatively uncommon condition that can occur unilaterally or bilaterally. Unilateral or bilateral hypertrophy of the masseter muscle is characterized by an increase in the volume of the muscle mass. This condition is benign, asymptomatic, and must be differentiated from parotid gland disease odontogenic problems, and rare neoplasms of muscular tissue. The reasons why patients request a medical consultation are predominantly related to aesthetics, especially if the hypertrophy is unilateral due to a noticeable asymmetry of the lower third of the face. (Addante, 1994; Rispoli et al., 2008). This study was intended for the patients who complained only about masseter hypertrophy, and not other symptoms.

Botulinum toxin is a potent biological toxin produced by *Clostridium botulinum* (Simpson, 1981). And it is a pre-synaptic neurotoxin which causes dose-dependent weakness or paralysis in skeletal muscle by blocking the calcium-mediated release of acetylcholine from motor nerve endings (Drachman, 1964), (Melling et al., 1988). Botulinum toxin type A, one of seven subtypes, is a dichain protein consisting of a light chain (50 kD) linked to a heavy chain (100 kD) by a disulfide bond (Lang, 2002). This functionally denervates the affected portions of the muscle. The primary effect is on α -motor neuron function but may also affect the γ motor neurons in the muscle spindles (Filippi et al., 1993). Local paralysis is reversed chiefly by neural sprouting with re-innervation of the muscle (Holds et al., 1990). Long-term reduction of α -, γ -, and Ia-neuronal activity may have indirect effects on the central nervous system. This was demonstrated when Moreno-Lopez et al. showed that single injections of botulinum toxin type A into the lateral rectus muscle of cats caused inhibition of abduction, altered electro myographic signals of the contralateral ocular muscles, and a disruption of

abducens motor neuron discharge patterns that lasted longer than 2 months (Moreno-Lopez et al., 1994). Botulinum toxin type A may also act directly or indirectly on nociceptors that affect transmission of sensory signals through α - δ and C- γ fibers, and impact detection of sensory signals through mechanoreceptors and chemonociceptors (Priori et al., 1995). These reports may explain the changes of the distribution in clenching forces after botulinum toxin type A injection in our study. We think that redistribution of clenching force after botulinum toxin type A injection is due to the changes of chewing pattern in central nervous system level as well as atrophy and weakness of peripheral muscle region. But, it will be in need of more detailed study about the exact mechanism of chewing pattern change occurred in central nerves system.

As above, with increasing medicinal effects of botulinum toxin type A, there is decline in clenching force difference of left and right side after botulinum toxin type A injection. But there was no significant change of occlusal time and disclusion time after botulinum toxin type A injection. Decline in difference of clenching force is because of that botulinum toxin can influence on transmission of sensory signal by α - δ and C- γ fiber directly or indirectly as mentioned above. Our results seem to be able to support this opinion. The duration of occlusion time and disclusion time are determined by interference of teeth but botulinum toxin cannot influence on interference of teeth so there might not be significant change in occlusion time and disclusion time after botulinum toxin type A injection. Also, because the contact between upper and lower anterior tooth is much more light than contact between upper and lower posterior tooth in normal occlusion, botulinum toxin cannot change of anterior teeth contact so there was no significant difference between front and back side balance according to time. But when all the subjects have full-contact arch, it may be possible to change of difference between front and back side balance after injection.

There was several limitations in this study. First, actually some patients were acclimatized to T-scan examination compared with the first time so velocity of clenching or lateral movement was quickened as following the examination repeatedly. This phenomenon seems to have low probability on distribution of clenching force but have slight effect on occlusion and disclusion time. In spite of this limitation, we used the third data instead of mean data of three measured values. Because we thought that first and second data was inaccurate.

Second, we have no control group. So we could not consider about any placebo effect. If later similar study is performed, we recommend to supplement these limitations.

V. CONCLUSION

Fifteen patients were examined using the T-Scan system before and after BTX-A injection into the masseter muscles. A decline in the difference in the clenching force between the left and right sides was found with increasing time (and hence increasing medicinal effect) up to 12 weeks following BTX-A injection.

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ABSTRACT (in Korean)

전산화 교합 분석 시스템을 이용하여 분석한 보틀리눔 독소 주사 전후의 교합력 분포와 시간의 변화

<지도 안 형 준 교수>

연세대학교 대학원 치의학과

송 지 희

보틀리눔 독소가 주사된 근육에 진통효과 및 과활성을 줄이는 효과를 가지고 있다는 것은 종종 관찰되어왔다. 그러나 본 진료실에서 보틀리눔 독소 주사를 시행하기 전에 한쪽으로 씹는 습관이 있었으나 주사후에는 양쪽의 씹는 힘이 비슷해졌다고 주장하는 몇몇의 환자들이 있었다. 하지만 보틀리눔 독소 주사의 이러한 효과에 대한 연구는 그동안 없었다. 그리하여 우리는 보틀리눔 독소와 저작력의 분포와의 관계에 대한 연구를 시행하였다.

이 연구의 목적은 보틀리눔 독소를 양측 교근에 주사한 후 저작력의 분포와 패턴을 분석하는 것이다. 본 연구의 가설은 보틀리눔 독소 주사 후에 좌, 우측의 교합력의 차이가 줄어들 것이라는 것이다.

주관적인 교근 비대를 호소하는 15명의 환자가 본 연구에 등록 되었고 보툴리눔 독소 25U(총 50U)을 양측 교근의 하방 1/3 의 가운데에서 약 1cm의 간격을 두고 지정한 두개의 자입점에 나누어 주사하였다. 모든 환자들은 주사전, 주사후 4,8,12,24주에 T-Scan 교합분석시스템을 이용하여 검사를 받았다.

연구 결과 좌우측 교합력의 분포에서 유의한 변화가 관찰되었는데 주사후 시간이 지날수록 좌우측 교합력의 차이는 감소하였으며 주사후 12주에서 최소치를 보였다. 전후방 교합력의 차이에서는 유의한 변화가 관찰되지 않았으며 occlusion time 과 disclusion time 에서도 유의한 변화는 없었다.