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Morphological Changes in the Mandibular Condyle Following Botulinum Toxin Injection into the Human Masseter Muscle

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Purpose: To determine the effects of botulinum toxin (BoNT) injection into the human masseter muscle on the morphology of the mandibular condyle bone using cone-beam computed tomography (CBCT).

Methods: Twenty volunteers were randomly assigned to one of two groups. Group I received a single BoNT injection; Group II received two injections, with the second being administered 4 months after the first. CBCT scans of both temporomandibular joints (TMJs) were performed before and 6 months after the first injection. Bony changes in the cortical layers of the condyle heads were evaluated and the long and short axes of both mandibular condyles were measured. The thickness at the thinnest part of glenoid fossa was also quantitatively measured.

Results: There was no significant difference between pre- and post-injection CBCT images. Furthermore, no changes in the cortical layers of the condyle heads were observed among the subjects who exhibited mild degenerative TMJ changes. The quantitative measurements (long axis, short axis, and the thickness of thinnest part of glenoid fossa roof) did not differ significantly between pre- and post-injection except for the long axis in Group I.

Conclusions: Within the limitations of this study, it appears that BoNT injections into human masseter muscles do not alter the morphology of the mandibular condyle bone in healthy adults.

Keywords: Botulinum toxins; Cone-beam computed tomography; Mandibular condyle; Masseter muscle

INTRODUCTION

Botulinum toxin (BoNT), a neurotoxin derived from *Clostridium botulinum* bacteria, temporarily blocks acetylcholine from being released to the neuromuscular junction, which causes muscle paralysis and atrophy [1]. In humans, the effect of muscle paralysis lasts for 2 to 4 months and muscle strength begins to reappear after 3 to 4 months [2]. BoNT has the advantages of reversible therapeutic effects, simple application methods, and small side effects, so many clinicians in the dental field currently use BoNT injection to treat masseter muscle hypertrophy, oromandibular motor disorders, and orofacial pain [3-5]. However, some studies have raised concern that localized BoNT injection into the masseter muscle may affect the growth and development of craniofacial disorders [6].

In particular, mandibular condyles are the areas where the pressure generated by the masticatory muscle is distributed, and the occlusal force and mandibular condyle morphology are related [7]. Therefore, there is a possibility that local masseter muscle atrophy due to BoNT may cause a change in the mandible condyle. An animal experimental study conducted on adult rodents observed changes in the mandibular condyle after injecting BoNT into the masseter muscle and a decrease in bone mineral density, a decrease in cortical bone thickness, and a decrease in trabecular bone was found [8-12]. The results of these animal studies suggested that the use of BoNT in the masseter muscle may lead to changes in the mandibular condyle, although humans and animals differ in metabolic rates, anatomical structures, and relative doses of BoNT.

To our knowledge, there are only five previous human studies that have reported the effect of BoNT injection in the masticatory muscle on condylar changes. Four of five studies mentioned condyle changes when BoNT was injected into the masticatory muscle [13-16], and one reported that no clinically significant change was observed [17]. However, there is a limitation in that all five studies observed changes in the condyle after BoNT injection in a temporomandibular joint disorder (TMD) patient. In addition, only two of the five studies compared changes in the condyle before and after BoNT injection [15,16]. One of the two studies limited the number of participants to women [15], and the other included treatment with BoNT and a splint [16]. Therefore, there have been insufficient investigations into the changes in mandibular conditions caused by injecting BoNT into the masseter muscle.

In this study, we evaluated the effects of BoNT injection in the masseter muscle on mandibular condyle changes in healthy adult humans using cone-beam computed tomography (CBCT) images. We compared the pre-treatment and post-treatment images as well as the groups receiving either single or repeated BoNT injections.

MATERIALS AND METHODS

This study was performed in accordance with the Fortaleza (2013) revision of the 1964 Declaration of Helsinki. This study was approved by the institutional review board committee of Yonsei University Dental Hospital (approval no. 2-2014-0004). All participants gave their written informed consent to participate in the study.

1. Participants

The study sample comprised 20 healthy volunteers who requested lower facial contouring at Yonsei Dental Hospital in Seoul, Korea. Twenty study participants were randomly assigned to one of two groups to investigate whether repeated injections of BoNT causes changes in the morphology of the mandibular condyle compared to a single injection. In Group I, 10 applicants (4 males and 6 females) aged 23 to 40 years (average age, 28.5 years) received a single BoNT injection, and in Group II, 10 applicants (2 males and 8 females) aged 22 to 48 years (average age, 28.5 years) received two BoNT injections. The second injection was given at 4 months after the first injection (Group II; Fig. 1) The exclusion criteria were the presence of a notable facial asymmetry, severe malocclusion, present subjective TMD symptoms, pregnancy, and a history of any serious medical illness or drug allergy.

2. BoNT Injection

BoNT was supplied as a freeze-dried powder (Neuronox; Medytox Inc.) and was reconstituted immediately before use at a concentration of 50 U/mL (100 U in 2 mL of sterile saline). A 25-U aliquot of BoNT was injected into the masseter muscle bilaterally using a 1-mL syringe with a 29-G,

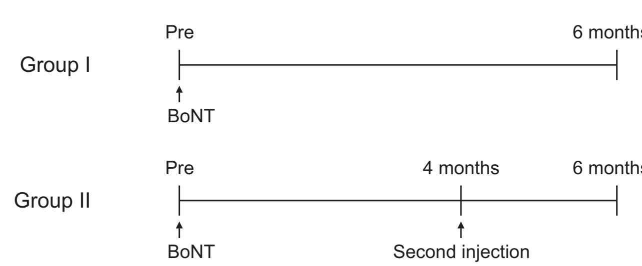

Fig. 1. Injection time schedule for Group I (i.e., a single BoNT injection) and Group II (i.e., two BoNT injections administered 4 months apart). BoNT, botulinum toxin; Pre, pre-injection.

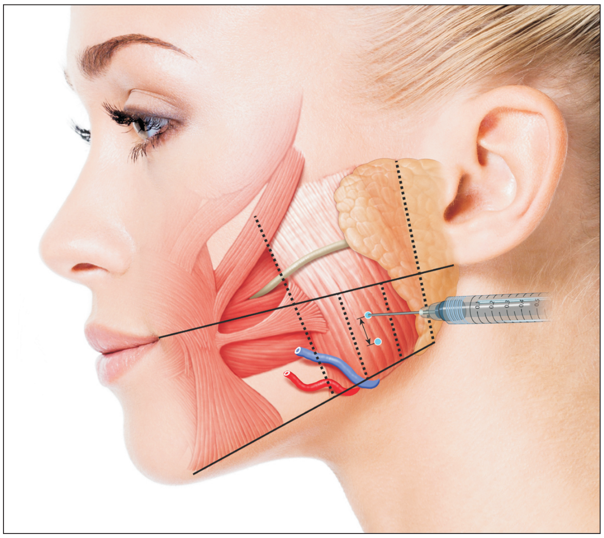

Fig. 2. Blue points indicate botulinum toxin injection sites.

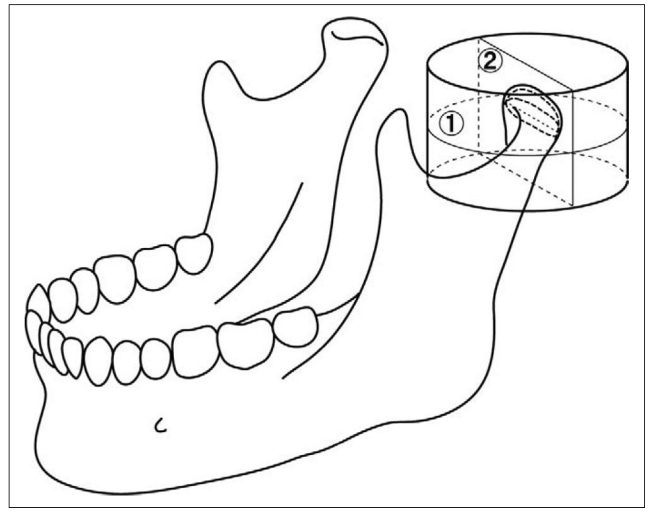

Fig. 3. The standard measurement planes. (1) Axial plane: the plane parallel to the Frankfort horizontal (FH) plane and running along the midpoint of the medial and lateral poles of the mandibular condyle. (2) Frontal plane: the plane running along the long axis of the mandibular condyle vertical to the FH plane.

1/2-inch-long needle. Injections were performed at two points that were 1 cm apart at the center of the lower one-third of the masseter muscle (Fig. 2).

3. CBCT

CBCT scans of both temporomandibular joints (TMJs) were performed before and at 6 months after the first BoNT injection into the masseter muscles in both groups. The CBCT images were obtained using an Alphard 3030 instrument (Asahi Roentgen Industries) at 80 kV and 5 mA, with a scan time of 17 seconds at a field of view of 20.0×17.9 cm. Participants were instructed to sit in an upright position with maximum intercuspation. The CBCT data were converted into Digital Imaging and Communication in Medicine (DICOM) files. Reconstructing and sectioning were guided by a previously reported protocol used in radiological condyle bone research and implemented using standard software (OnDemand 3D; Cybermed Inc.) [7].

4. Measurements

The images were interpreted and compared by one highly experienced radiologist (a professor of oral and maxillofacial radiology with 20 years of experience in this field). Bony changes in the cortical layers of the condyle heads were evaluated based on the presence of flattening, erosion (i.e., irregularities), and thickening. The axial plane was parallel to the Frankfort horizontal (FH) plane and ran along the midpoint of the medial and lateral poles of the mandibular condyle. The frontal plane ran along the long axis of the mandibular condyle and vertical to the FH plane. The long axis was the distance between the medial and lateral ends of the mandibular condyle, and the short axis was the distance between two lines drawn parallel to the long axis and tangential to the outer margin of the condyle. The long and short axes were measured on both mandibular condyles (Fig. 3) [7].

The thickness at the thinnest point of the roof of the glenoid fossa was quantitatively measured as described previously [18] by one highly experienced radiologist (a professor of oral and maxillofacial radiology and 20 years of experience in this field) on the imported DICOM data sets using the OnDemand 3D software. The equivalent region of the thinnest point of the roof of the glenoid fossa was identified among the coronal sections on the monitor [19]. The thickness was measured at this point for both condyles.

5. Statistical Analysis

All of the quantitative values were compared. A paired t-test was used to compare the values measured on the right and left sides of each of the 20 participants and the values between the two groups measured before and after the BoNT injection were also compared. A probability value of

$p<0.05$ was considered to be indicative of a significant difference. All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Co.).

RESULTS

There was no significant difference between the pre-injection and post-injection CBCT images obtained for any of the subjects (Table 1). The left and right TMJs were both normal in 60% of the subjects and were unchanged at 6 months following the initial BoNT injection (Fig. 4). Furthermore, no changes in the cortical layers of the condyle heads were observed at 6 months after the BoNT injection among those participants who exhibited mild degenerative TMJ changes before the injection (Fig. 5). The participants experienced no clinical complications such as allergic reactions, local transient neurological palsy, or permanent muscle weakness.

The quantitative measurements (long axis, short axis, and the thickness of thinness part of glenoid fossa roof) did not differ significantly between pre- and post-injection, except for the long axis in Group I ($p<0.05$; Table 2). There were also no significant differences between the condylar measurements for the left and right sides according to t-test results (Table 3).

DISCUSSION

BoNT injected into muscle rapidly and irreversibly binds to presynaptic neurons at the neuromuscular junction, and then becomes internalized. It interacts with a zinc-dependent endoprotease to break down part of the peptides required for acetylcholine release [20]. Blocking of acetylcholine release in neuromuscular junctions causes muscle paralysis and atrophy and this process could take up to two weeks. After muscle paralysis occurs due to a BoNT injection, even if exposure to the toxins increases, muscle function starts to return within a few weeks and is typically fully restored by six months, and there is still continuous turnover at the neuromuscular junction [21].

In both short-term (4 weeks) and long-term studies, Rafferty et al. [9] examined the impact of BoNT injection on underlying bone alterations in adult rabbit masseter muscles (12 weeks). After 4 weeks, bone volume and

Table 1. Interpretation and comparison of the TMJs in CBCT images obtained from 20 subjects in Group I (subjects 1-10) and Group II (subjects 11-20)

Subject #	Age (y)	Sex	Pre-injection	Post-injection	Pre–post difference
1	27	M	Normal	Normal	No
2	32	M	Normal	Normal	No
3	24	F	Left flattening	Left flattening	No
4	25	F	Left and right flattening	Left and right flattening	No
5	25	F	Normal	Normal	No
6	28	F	Normal	Normal	No
7	30	F	Normal	Normal	No
8	42	F	Right irregularities and left flattening	Right irregularities and left flattening	No
9	50	F	Right flattening	Right flattening	No
10	26	F	Normal	Normal	No
11	29	F	Normal	Normal	No
12	29	M	Left flattening	Left flattening	No
13	26	F	Normal	Normal	No
14	28	F	Right flattening and left irregularities	Right flattening and left irregularities	No
15	27	M	Right flattening	Right flattening	No
16	29	M	Left and right flattening	Left and right flattening	No
17	26	M	Normal	Normal	No
18	39	F	Normal	Normal	No
19	36	F	Normal	Normal	No
20	42	F	Normal	Normal	No

TMJ, temporomandibular joint; CBCT, cone-beam computed tomography; Pre–post, pre- to post-injection; M, male; F, female.

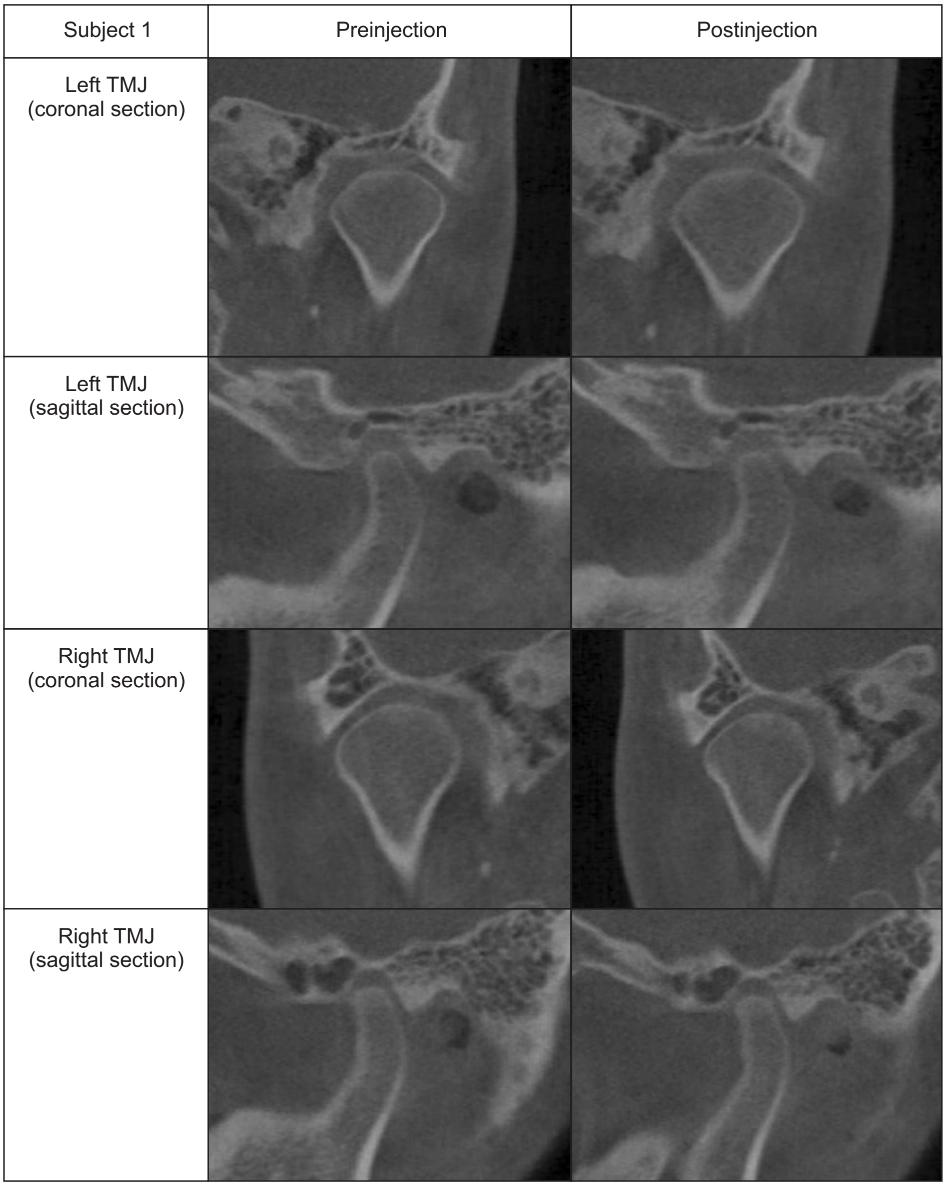

Fig. 4. CBCT images obtained before and 6 months after bilateral BoNT injections into the masseter muscles in subject 1. CBCT, cone-beam computed tomography, BoNT, botulinum toxin; TMJ, temporomandibular joint.

functional parameters were reduced and, after 12 weeks, there was partial recovery. Taking into account the disparity in metabolic rates between people and rabbits, the condylar bone changes were expected to gradually recover after 6 months [22]. Kim et al. [23] reported that the repeated injection of BoNT increased the treatment effect and its duration period. Lee et al. [22] reported that BoNT-A injections that were given repeatedly may cause changes in the bone volume around the mandibular angle. Thus, in this study, CT scans were performed at 6 months after the first BoNT injection to investigate mandibular condylar bone changes and the second injection was performed at 4 months after the first injection to investigate whether repeated injections of BoNT caused more mandibular condyle changes than a single injection.

After a BoNT injection, neither group's mandibular condylar morphology showed any significant changes, according to our observations. This result suggested that injecting BoNT bilaterally into the masseter muscles may not alter the morphology of the mandibular condyle in healthy adults. This finding is in agreement with the report by Raphael et al. [17] who showed that no clinically significant difference was found in TMJ bone-related changes with injections into the masticatory muscle.

Our study results were different from those of animal experiments [8-12] in which changes in the condyle were

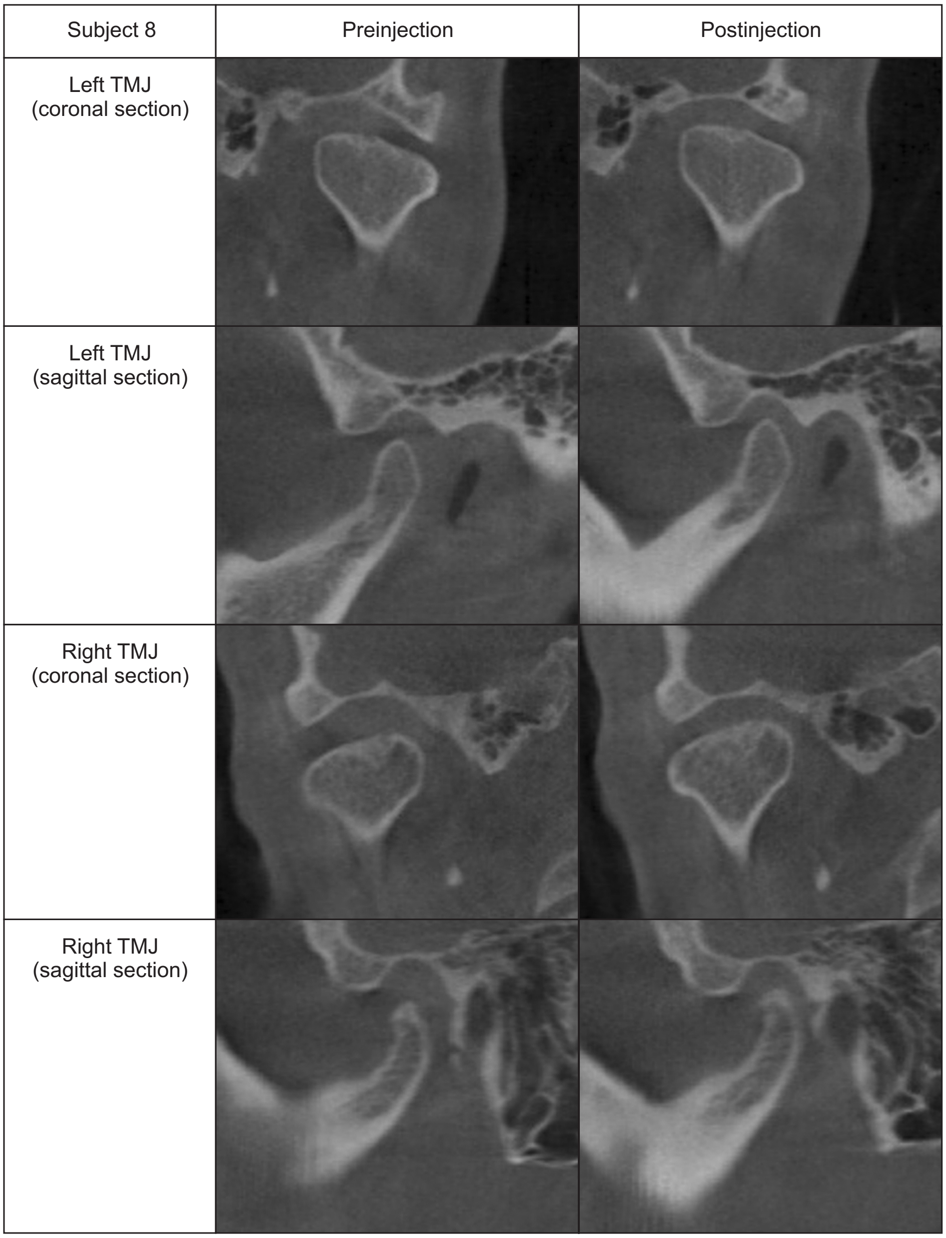

Fig. 5. CBCT images obtained before and 6 months after bilateral BoNT injections into the masseter muscles in subject 8. CBCT, cone-beam computed tomography, BoNT, botulinum toxin; TMJ, temporomandibular joint.

observed after BoNT was injected into the masseter muscle. This difference could be attributed to the dose of BoNT injected in this study not reducing the muscle strength of the masseter muscle enough to cause a condylar change, unlike in animal experiments [22,24]. Similarly, a case report suggested that degeneration was observed in the left mandibular condyle in patients who injected 140 U of BoNT into the left masseter muscle more than four times at three-month intervals in patients with a TMJ disorder history [13], however, no condylar change was observed in this study. This suggests that the usual BoNT treatment dosage may not reduce the muscle strength of the masseter muscle to cause a condylar change in healthy adults.

In addition, our results were inconsistent with the results of a study that reported a decrease in condylar cortical bone quality after BoNT injection into the temporalis and masseter muscle in TMJ patients that was accompanied by splint treatment [15,16]. This difference may be due to the differences among participants. In this study, healthy adults were involved, while two other studies included TMD patients. It is possible that orofacial pain influenced the masticatory performance and condyle [15]. In addition, while this study injected BoNT only into the masseter muscle, in the two previous studies, the injection of BoNT into the masseter and temporalis muscles may have made a difference. Chewing actions involve various muscles, including the masseter, temporalis, pterygoid, and infrasuprahyoid muscles. Since only several muscle fibers of the masseter muscle are temporally paralyzed when BoNT is injected, if voluntary movement of the jaw is still possible, the effect of this paralysis may be minimal on the condyle in terms of bony change.

Morphological changes of the condyle can be identified by comparing quantitative data. The long and short axes are only two of several dimensions related to the condylar size, and measuring these axes is not sufficient for detecting quantitatively morphological changes of the condyle. Therefore, measuring the thickness at the thinnest part of the roof of the glenoid fossa was adopted in this study to analyze any such changes. This parameter reflects changes of the condyle caused by osteoarthritis, which is characterized by degenerative bony changes such as erosion, irregularity, and flattening [25].

This study involved manual visual comparisons of condyle images, which is more subjective and less reliable than comparing quantitative data. However, quantitative comparisons are hampered by the condyle shape, which varies markedly among individuals [25]. Even if various condyle shapes had been categorized, any bony changes might still not have been detectable due to the research period being too short for the bony changes to have resulted in a category change. Therefore, qualitative visual examinations were considered to be an effective method for comparing the shapes of the condyles. However, the reliability of the findings is questionable and these measurements should be confirmed in future studies.

In this study, statistical significance of differences before and after BoNT injection was observed only for the long axis of Group I, when BoNT was injected once. Data from measuring the size of the condyle and the thickness of the mandibular fossa before and after BoNT injection were significantly different. This is thought to have occurred due to an error of about 0.1 mm to 0.2 mm when measuring the length on the screen due to the small number of samples. Moreover, it was observed that there was no statistically

Table 2. Results of applying paired t-tests to quantitative condylar data to compare pre- and post-injection measurements

Measurement	Group I				Group II				Group I and II
	Pre-injection	6 months post-injection	Δ		Pre-injection	6 months post-injection	Δ		p-value[b]
			Mean±SD	p-value[a]			Mean±SD	p-value[a]	
Long axis (mm)	20.139	20.018	0.122±0.100	0.006	19.220	19.175	0.045±0.107	0.215	0.514
Short axis (mm)	9.222	9.283	−0.061±0.147	0.249	8.920	8.875	0.045±0.138	0.331	0.408
Thinnest part of fossa (mm)	1.056	1.067	−0.011±0.049	0.512	0.970	0.965	0.005±0.037	0.678	0.717

Δ, change between pre-injection and 6 months post-injection; SD, standard deviation.
[a]Comparison of the changes between pre-injection and 6 months post-injection(s) in each group. [b]Intergroup comparison of the changes between pre-injection and 6 months post-injection.

Table 3. Results of applying paired t-tests to quantitative condylar data to compare pre- and post-injection measurements on both condyle sides (p=0.050)

Condylar variables	Paired differences					t	df	Significance (two-tailed)
	Mean	SD	SEM	95% CI of the difference				
				Lower	Upper			
Pre injection								
Rt.–Lt. at long axis	0.595	1.709	0.392	−0.229	1.418	1.517	18	0.147
Rt.–Lt. at short axis	0.031	0.780	0.179	−0.344	0.407	0.177	18	0.862
Rt.–Lt. at thinnest point	0.105	0.465	0.107	−0.119	0.330	0.987	18	0.337
Post injection								
Rt.–Lt. at long axis	0.653	1.614	0.370	−0.125	1.430	1.763	18	0.095
Rt.–Lt. at short axis	0.021	0.836	0.192	−0.382	0.424	0.110	18	0.914
Rt.–Lt. at thinnest point	0.111	0.401	0.092	−0.083	0.304	1.201	18	0.245

SD, standard deviation; SEM, standard error of the mean; CI, confidence interval; Pre–post, pre- to post-injection; Rt.–Lt., right minus left.

significant difference in the long axis of Group II, which was injected twice, and no significance was observed between Group I and II. The statistically significant difference in the long axis of Group I should be further investigated through follow-up studies (Table 2).

The findings of this study should be interpreted in the light of several limitations. First, the number of subjects studied herein was insufficient to allow definitive conclusions to be drawn. Moreover, changes in trabecular bone density could not be detected due to limitations in the image quality and histological examinations. Further studies with larger numbers, a longer assessment period, three-dimensionally reconstructed CT images, measurement of the occlusal force, and additional BoNT-A injections will provide more data to determine the effects of BoNT injections into the masseter muscle on the morphology of mandibular condyle bone.

In conclusion, within the limitations of this study, it appears that injecting BoNT bilaterally into the masseter muscles does not alter the morphology of the mandibular condyle in healthy adults. However, considering previous studies, further studies are needed in animals, TMD patients, or adolescents who are growing to validate the injection of BoNT into the masticatory muscle affecting the mandibular condyle.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

FUNDING

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AUTHOR CONTRIBUTIONS

Conceptualization: NK, STK. Data curation: HP. Formal analysis: JH, HP. Funding acquisition: None. Methodology: NK, STK. Project administration: JH. Visualization: YP. Writing original draft: YP, JH. Writing review & editing: NK, STK.

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