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Mandibular bone effects of botulinum toxin injections in masticatory muscles in adult

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Objective. Botulinum toxin (BTX) is injected into masticatory muscles to treat various conditions. Animal studies have demonstrated bone loss at the condylar and alveolar regions of the mandible after BTX injection into masticatory muscles. The aim of the present study was to investigate mandibular bone changes in patients who received BTX injections in masticatory muscles.

Study Design. Twelve adult patients who received BTX injections into masticatory muscles were included in this study. Cone beam computed tomography (CBCT) was performed before and 12 months after the injection. The condylar and alveolar regions of the mandible were analyzed by using texture analysis of the CBCT images with the run length method. Condylar cortical thickness was measured, and 3-dimensional analysis of the mandible was also performed. Six patients who did not receive BTX injections were used as controls.

Results. A run length parameter (gray level nonuniformity) was found to be increased in condylar and alveolar bones. A significant cortical thinning was found at the anterior portion of the right condyle. Three-dimensional analysis showed significant changes in the condylar bone and at the digastric fossa. No changes in mandibular angles were found.

Conclusions. This study identified mandibular bone changes in adult patients who received BTX injection into masticatory muscles. (Oral Surg Oral Med Oral Pathol Oral Radiol 2019;000:1−9)

Botulinum toxin (BTX) is a bacterial metalloprotease produced by *Clostridium botulinum*. This neurotoxin specifically blocks the release of acetylcholine at the presynaptic membrane of neuromuscular junctions.[1] It leads to a transient muscle paralysis through "functional denervation."[2] In laboratory animals, a single BTX injection in the quadriceps induces disuse bone loss at the tibia and the femur, with considerable muscle wasting.[3,4] In humans, the effects of BTX disappear after 2 to 4 months, and muscle strength starts to reappear 3 to 4 months later.[5] BTX A is the most widely used type in clinical medicine, and many parts of the human body are now being targeted for its use for various therapeutic purposes.[6]

In maxillofacial surgery, BTX is used for multiple indications: subcutaneous injections for aesthetic indications, blepharospasm, and hemifacial spasm and intraglandular injections for drooling.[7,8] BTX is also injected into masticatory muscles (mainly *Musculus masseter* and *Musculus temporalis*) for several indications, such as trismus, bruxism, masticatory myalgia, and temporomandibular joint disorders (TMDs) or *M. masseter* hypertrophy.[5] Repeated injections are needed in many cases to obtain a long-lasting effect.[9] Side effects of BTX are rare and reversible. The most common adverse effects are bruising and local pain at the injection site.[10] Systemic side effects (shock) and unwanted palsy of the nearby muscles are rarely reported.[1]

The mandible is a non−weight-bearing bone that is stimulated by masticatory muscles, mainly during the eating process. It is composed of trabecular and cortical bones; the roots of teeth are anchored into alveolar bone by the periodontal ligament. Alveolar bone has high plasticity and is remodeled at a high rate.[11] Its mechanical stimulation during mastication is essential to keeping teeth and underlying bone healthy. Loss of teeth leads to irreversible alveolar bone resorption.[12] Trabecular bone may be present in other parts of the mandible, such as the condylar process and the mandibular angle. Mandibular bone mineral density and cortical bone thickness are correlated with masticatory function and occlusal forces.[13] Muscles exert stresses

Statement of Clinical Relevance

Injection of botulinum toxin into masticatory muscles leads to mandibular bone changes at the condylar and alveolar regions and at the digastric fossa in adult humans.

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at the periosteum and control bone microarchitecture, according to Wolff's law.[14]

Injections of BTX decrease the force of *M. masseter* and/or *M. temporalis* contractions and reduce stress at the periosteum. Several animal studies have demonstrated profound bone loss at the mandibular condyle and at the alveolar region after BTX injections into masticatory muscles.[15-17] These bone alterations could constitute a risk factor for fractures, especially in patients receiving repeated BTX injections into masticatory muscles, and questions regarding this have arisen recently, as reported in the literature.[18] A few studies have investigated mandibular bone changes in humans and have shown the impact of BTX injections on condylar bone and bone volume.[19-21] Osteopenia and reduced bone volume may also increase the risk for periodontal disease, alveolar bone loss, and tooth loss.[22]

The aim of the present study was to investigate mandibular bone changes in patients who received BTX injections in masticatory muscles. We used texture analysis and comparison of 3-dimensional (3-D) mandibular models reconstructed from cone beam computed tomography (CBCT) to identify bone changes in human patients.

PATIENTS AND METHODS

Participants

This prospective study included patients who received BTX injections into the right and left *M. masseter* and *M. temporalis* in the Department of Maxillofacial Surgery, Nantes University Hospital (France), between January 2015 and December 2016. The indications for these injections were TMDs, *M. masseter* hypertrophy or spasm, bruxism, or masticatory myalgia. All participants were adults *i.e.,* over 18 years old.

All potential causes of mandibular bone disorder were set as exclusion criteria, which included diabetes, osteoporosis, neoplasia, previous mandibular surgery, long-course corticosteroid therapy, radiotherapy, a previous BTX injection, antiresorptive drug treatment, premolar/molar loss, and orthodontic treatment. All of the patients receiving BTX injections were screened (n = 90), and after applying the exclusion criteria, only 12 patients were finally included in the study.

All participants gave their informed consent before participating to the study. This experimental protocol was approved by the local ethical committee of the Angers University Hospital, and the study was performed in accordance with the institutional guidelines of the French Ethical Committee (protocol No. 2016-41) and according to the tenets of the 1964 Helsinki Declaration and its later amendments. All patients answered a series of questions about their status, including side of the mouth with symptoms, previous treatment (drugs, anterior repositioning splint [ARS]), and underwent a clinical examination.

Some patients who fulfilled the same exclusion criteria but had not received BTX injections were also enrolled and constituted the control group. They had had the same follow-up with similar clinical and CBCT examinations for preimplantation evaluation.

Experimental protocol

BTX type A (Botox, Allergan Inc., Irvine, CA) was used. Injections were performed in the 4 main masticatory muscles, that is, the 2 *M. masseter* and the 2 *M. temporalis* muscles, regardless of the symptoms being unilateral or bilateral, as is the current practice for patients with TMDs; pterygoideus muscles were not injected.[23-25] All injections were administered percutaneously and intramuscularly. Each injection was performed by using a 1-mL syringe and a 26-gauge needle, at a dilution of 100 U/mL of injectable saline.[26] Each patient received a total dose of 100 U—30 U for each *M. masseter* and 20 U for each *M. temporalis*. Ten points of injection were performed for each patient—3 per *M. masseter* and 2 per *M. temporalis* (Figure 1). The injection sites corresponded to areas of

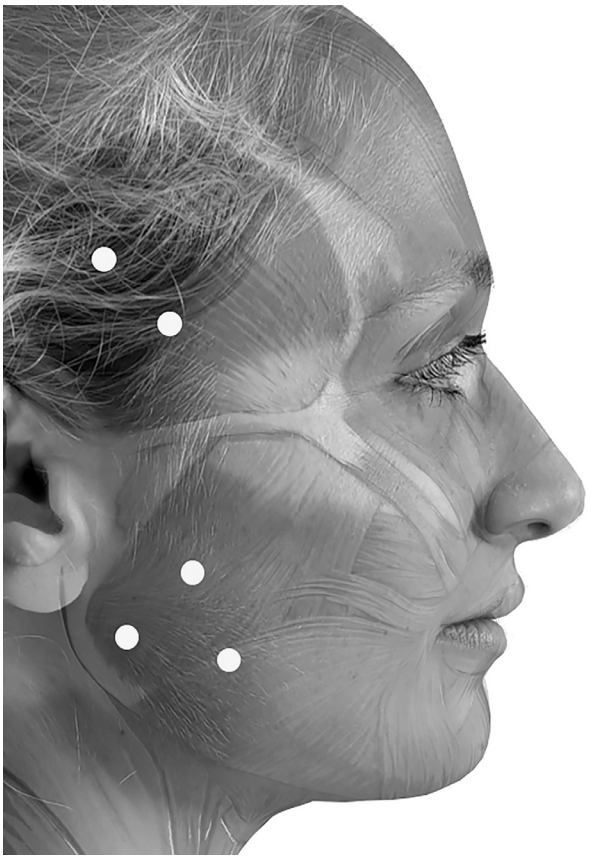

Fig. 1. Injection sites of botulinum toxin in masticatory muscles: 3 sites for *M. masseter* and 2 for *M. temporalis.*

greatest muscle mass on palpation. The depth of the injection was determined as follows: When the needle came into contact with bone, it was removed for a distance of about 5 mm to inject BTX directly into the muscle.[23-25] All injections were performed by trained maxillofacial surgeons. No electromyographic examination was performed. No sedation or local anesthesia was used.

CBCT was performed with a NewTom VGI (NewTom, Verona, Italy) with the following acquisition parameters: high-frequency generator, 0.3 mm focal spot, 15 × 15 cm full field of view, 110 kV, 20 mA. A first acquisition was performed before the BTX injection for etiologic and morphologic assessment (T0). The second CBCT acquisition was performed in the same conditions 1 year later (T2). All patients had a regular follow-up (3 months, 6 months, and 12 months after the injection for the BTX recipients) and after 12 months for the control group.

Bone texture analysis

CBCT images in the Digital Imaging and Communications in Medicine format were converted to 256-gray-level images in the BMP (Bitmap) format by using ImageJ 1.5 (US National Institutes of Health, Bethesda, MD).[27] The DataViewer software (release 1.5; Brucker microCT, Kontich, Belgium) was used to re-slice the stacks of 2-dimensional images: (1) At the mandibular condyle, the area of interest was selected on a sagittal plane at the mid-condyle with the largest trabecular bone surface and including the mandibular notch; and (2) for alveolar bone, the area of interest was selected on a plane comprising the second premolar, molars, mandibular canal, and the retromolar area. Areas of interest were selected manually by 1 investigator (Figure 2).

Texture analysis was performed by using MaZda 3-D Editor 4.6 software (Polytechnika Institute of Electronics, Lodz, Poland). A region of interest (ROI) was drawn manually on the selected images and served as a mask for the texture analysis, using the run-length distribution described by Galloway, as previously reported.[28,29] Analysis was done in triplicate by the same investigator with determination of the intraoperator variation coefficient. The following parameters were determined in the horizontal and vertical directions of the image:

- Run length nonuniformity measures the similarities of the length of the runs throughout the image. Run length nonuniformity is expected to be large if the number of runs of same length increases throughout the image.
- Gray level nonuniformity (GLN) measures the similarity of gray level values throughout the image. GLN is expected to be large if the number of runs of same gray level increases throughout the image.
- Short run emphasis is highly dependent on the occurrence of short runs and is expected to be large for fine textures.
- Long run emphasis is highly dependent on the occurrence of long runs and is expected to be large for coarse structural textures.

Cortical thickness of condylar bone

Cortical bone thickness at the condyle was measured by using ImageJ on the same images at 3 different locations. Anterior and posterior thicknesses were obtained parallel to the Frankfurt plane through the lowest point of the articular eminence. Superior thickness was obtained on an orthogonal line intersecting the anteroposterior plane and the midpoint of the condyle.

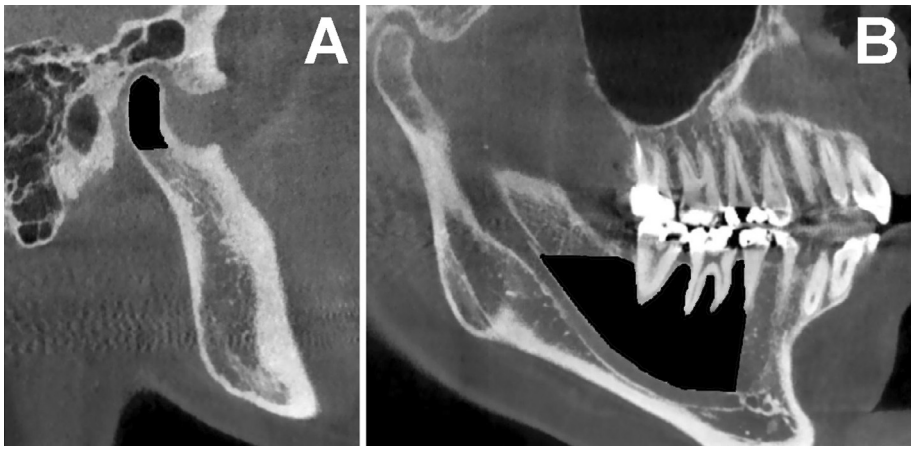

Fig. 2. Areas of interest *(in black)* used to measure bone texture of condylar bone **(A)** and alveolar bone **(B)** on 2-dimensional sections of the mandible obtained by cone beam computed tomography.

Measurements were made in triplicate and expressed in millimeters.

3-D analysis of the mandible

After CBCT acquisitions, semiautomatic segmentations of mandible volumes were performed with ITK-Snap v. 3.6 open-source software (http://www.itksnap.org).[30] A global threshold was used to segment bone in all of the patients.[31] 3-D models were generated and analyzed with 3-D Slicer v. 4.8 software (National Alliance for Medical Image Computing; http://www.slicer.org).[32] The *Surface Registration* tool of the CMF registration module (Cranio Maxillo Facial) was used to superimpose 3-D models of the mandible before (T0) and after BTX injection (T1). This tool is based on a superimposition process using the entire surface mesh of both 3-D models. Then, the Model to Model Distance module of MeshMetric 3-D plugin (https://www.nitrc.org/projects/meshmetric3 d) was used to compute the point-by-point distance between 2 triangular meshes of the superimposed 3-D models. Finally, the combined image was analyzed with the Shape Population Viewer v. 1.4 software (https://www.nitrc.org/projects/shapepopviewer) to create color maps showing mandibular surface differences expressed in millimeters. The lookup table provided a color bar and was set with 3 colors: Blue indicated a negative difference corresponding to a bone loss; red corresponded to a positive difference (bone apposition); and green represented a steady state. 3-D superimposed mandibles were analyzed on 7 ROIs: condylar processes, coronoid processes, mandibular angle region, and the lingual side of the symphysis. Condylar processes were analyzed in 3 areas: anterior, upper, and posterior. Because Chang et al., reported that differences of less than 1 mm were observed after 3 months with the same technique, in this study, a difference greater than 1 mm was considered significant.[33]

Statistical analysis

Statistical analysis was done with Systat statistical software v. 13 (Systat Software, Inc., San José, CA). All data were expressed as mean ± standard deviation. Differences between before and after results on each side were determined; and the average of the right−left values before and after treatment for each parameter was determined by using the Student paired *t* test with the Bonferroni adjustment. Differences were considered significant when $P < .050$.

RESULTS

Clinical characteristics

From the 90 patients enrolled in this study, only 12 patients met the selection criteria used here; mean age was 31.5 ± 13.3 years (range 18−58 years). Clinical details of the patients are given in Table I. Female-to-male ratio was 5:1. Ten patients presented with a TMD, 1 had *M. masseter* hypertrophy, and 1 had *M. masseter* spasm. Of the 10 patients with TMDs, 5 patients presented with left unilateral symptoms, 4 patients had bilateral symptoms, and 1 patient presented with a right unilateral form. Nine patients with TMDs were also treated with an ARS during the study. Nine patients showed subjective improvement of symptoms after the BTX injection. No side effect was observed in this series of patients. Of the 6 participants enrolled in the control group, none had worsening of symptoms.

Table I. Clinical presentations of the patients included in the study

Patient No.	*Diagnosis*	*Treatment (other than BTX)*
#1	TMD/bilateral pain	ARS nightly
#2	TMD/bilateral pain	ARS nightly
#3	TMD/bilateral pain	ARS nightly
#4	TMD/pain on the right side	None
#5	TMD/bilateral pain	ARS nightly
#6	TMD/pain on the left side	ARS nightly
#7	TMD/pain on the left side	ARS nightly
#8	Left masseter hypertrophy	None
#9	TMD/pain on the left side	ARS nightly
#10	TMD/pain on the left side	ARS nightly
#11	TMD/pain on the left side	ARS nightly
#12	Spasm	None
Control #1	Preimplantation evaluation	None
Control #2	Bruxism without TMD	ARS nightly
Control #3	Preimplantation evaluation	None
Control #4	Preimplantation evaluation	None
Control #5	Rhinoplasty	None
Control #6	Facial traumatology	None

ARS, anterior repositioning splint; *BTX,* Botox; *TMD,* temporomandibular joint disorder.

Bone texture analysis

Intraoperator variation coefficients for the ROI and the texture parameters were less than 5%. On the CBCT performed 12 months after BTX injection, GLN was found to be significantly different at the right condylar bone, in the horizontal ($P = .003$) and vertical directions ($P = .014$) (Figure 3). Similarly, GLN also increased significantly ($P = .048$) at the right alveolar bone in the horizontal direction but not in the vertical direction ($P = .059$). At the left alveolar bone, GLN was significantly different in both the horizontal ($P = .003$) and vertical directions ($P = .017$). GLN was also significantly increased in both the horizontal ($P = .003$) and vertical ($P = 0.006$) directions for averages of the right and left sides of alveolar bone. No difference was found for GLN at the left condylar bone. No difference

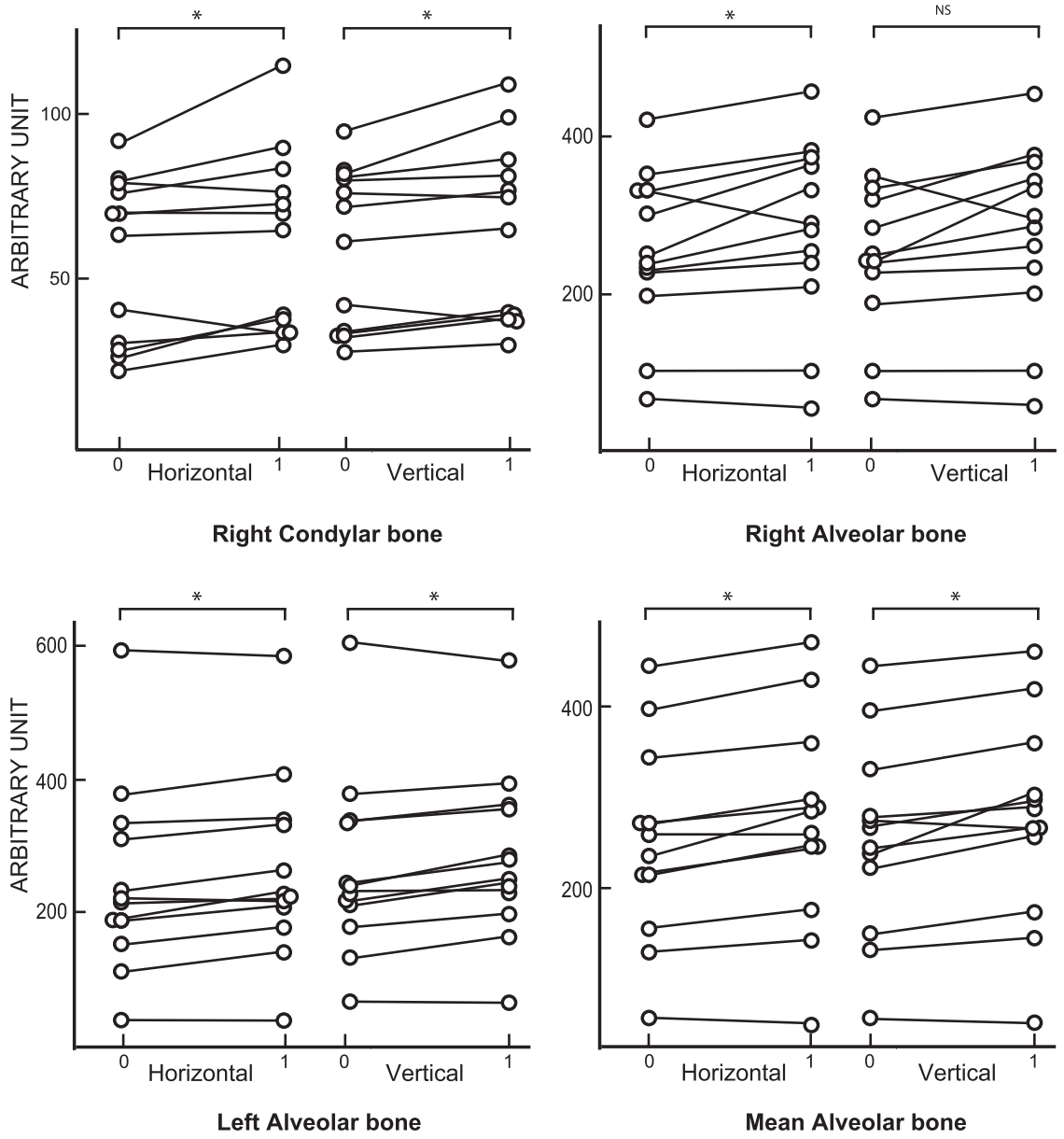

Fig. 3. Gray-level nonuniformity (GLN) measurements for alveolar bone and condylar bone in horizontal and vertical directions. Measurements are expressed for right side, left side, and average of right and left sides (mean). *Significant difference ($P < .05$) between T0 and T1 (1 year after BTX injections.

could also be seen for other texture parameters. No difference was observed for any texture parameter in the control group as well.

Cortical thickness at condylar bone

Intraoperator variation coefficients were less than 5% for cortical thickness determination. Significant cortical thinning was found at the anterior portion of the right condyle 1 year after BTX injections: 1.71 ± 0.50 mm at T0 vs 1.51 ± 0.57 mm at T1 ($P = .008$). Similar differences were seen when both sides were averaged: 1.74 ± 0.53 mm vs 1.52 ± 0.48 mm ($P = .008$) (Figure 4). Average condylar cortical thinning was −0.22 ± 0.24 mm. No significant differences could be seen for the other directions.

No differences were observed for cortical thickness in the control group.

3-D analysis of the mandible

3-D condylar analysis showed significant bone changes in most of the patients in the study (Table II). Bone thickening at the lingual side of the mandibular symphysis was present in the digastric fossa in 3 cases (Figure 5A). Six patients presented bone changes in the anterior area of the condyle on the combined image: bone formation (in 3 patients) and bone loss (in 3

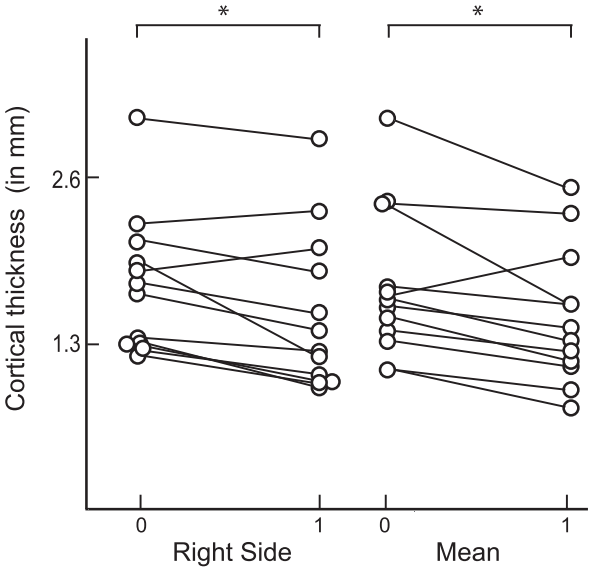

Fig. 4. Cortical thickness measurements (mm) of anterior condylar bone showing a significant cortical thinning at the anterior portion of the right condyle 1 year after BTX injection (right side) and averaged sides (mean). *Significant difference ($P < .05$) between T0 and T1 (1 year after BTX injections).

patients). Two patients had significant bone loss in the anterior portion of the right condyle (Figure 5B). Seven patients had bone changes in the upper condylar portion: new bone formation in 5 and bone loss in 2. Seven patients presented bone changes in the posterior area: new bone formation in 3 and bone loss in 4. One patient presented new bone formation of the coronoid process on both sides. No significant differences could be seen at the mandibular angle. No differences were observed in the control group. We have conducted an intraobserver variation study concerning image registration, and the difference (verified 3 times) was negligible (only 1 pixel difference).

DISCUSSION

In the present study, we found that 1 year after a BTX injection into masticatory muscles, bone changes were

Table II. Mandibular bone changes observed in the anterior, upper, and posterior areas of the mandibular condyle and at the digastric fossa in 3-dimensional analysis (expressed in number of patients)

Bone status	*Anterior area*	*Upper area*	*Posterior area*	*Digastric fossa*
Bone formation (n)	3	5	3	3
Bone loss (n)	3	2	4	2
No change (n)	6	5	5	7

See the online Supplementary Table containing all data from all patients and controls.

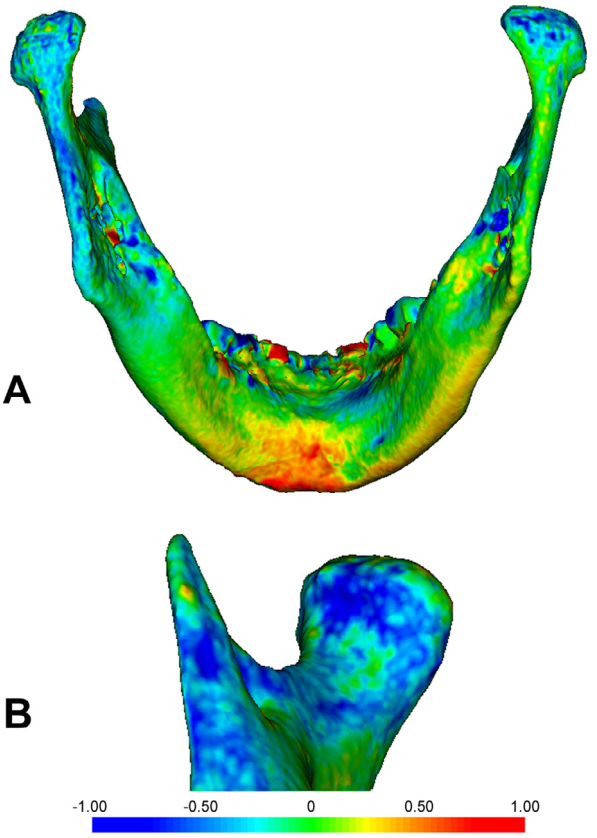

Fig. 5. **(A)** Color map of the 3-dimensional (3-D) model of a mandible showing surface differences at 1 year, expressed in millimeters on the color bar. Note the significant bone thickening at the left digastric fossa. **(B)** Color map of 3-D model of a right condyle showing a significant thinning at the anterior part at 1 year.

evidenced by texture analysis and 3-D reconstruction of the mandible. Texture analysis showed significant differences for GLN on images of the right condyle and alveolar bone in the horizontal and vertical directions. A significant correlation of the measurements was found between texture analysis of micro-CT images and high-resolution CBCT for GLN.[34] Significant differences were found in the present study on both sides for alveolar bone, but only on the right side for condylar bone. Similarly, bone thickness in the anterior area was significantly reduced only at the right condyles. It has been previously shown that significant correlations exist between bone volume and texture parameters (in particular GLN) measured on radiographic images.[35-39] In this clinical condition, GLN could reflect trabecular bone loss. These unilateral changes occurring in condylar bone could be explained by mastication disequilibrium as dentate patients normally alternate between sides while chewing. No change was observed in the series of control patients. Our series was mainly composed of patients with TMDs, in which mastication is often unilateral. They

often chewed on the side of the injured temporomandibular jaw (TMJ), which is, therefore, less stressed, as previously reported by others.[40] This specificity of chewing side may explain the asymmetry of condylar bone loss in our series of patients.

The present study analyzed for the first time the 3-D changes of condylar bone after BTX injections in humans. Condylar bone changes were observed in half the study patients, particularly in the anterior part of the right condyle, where 3 of the patients had significant bone loss. These results are consistent with the measurements obtained through texture analysis and bone thickness evaluation. Formation of new bone at the condyle was also observed in 3 patients in the anterior and posterior areas and in 5 patients in the upper area.

Medical management of TMDs consists of the preferred reversible and noninvasive treatments, such as the use of ARS, as recommended by the French Oral and Maxillo-Facial Society.[41] Several studies have shown that ARS directly affects bone remodeling at the condyle. New bone formation was more often noted in the posterior part of the condyle, probably because ARS moved the condyles downward and forward thus increasing both posterior and medial joint spaces.[42] ARS is reported to induce cortical thickening predominantly in the anterior portion of the condyle but also in the posteromedial and intermediate posterior areas.[42,43]

TMDs can be age related when lesions of osteoarthritis (OA) occur.[44] Condylar morphology in untreated patients with OA of the TMJ is reported to be significantly different from that in asymptomatic patients.[45] There are only a few reports in the literature on the natural history of condylar bone changes in patients with TMDs. Significant condylar bone changes are found in 68% of cases at the TMJ after radiologic follow-up during 13.4 months (range 3−18 months) in patients presenting with TMDs.[46]

At the present time, there are 3 reports in the literature regarding human mandibular bone assessment after several BTX injections into only the *M. masseter* muscle.[19,20,33] Raphael et al.[20] described condylar bone changes 6 to 10 weeks after the last injection, and Lee et al.[19] found significant differences in the total volume of mandibular angle area 6 months after BTX injection, but only in patients who received 2 injections at a 4-months interval. In the present study, no significant differences were found in the angular region of the mandible. This is consistent with other previous studies in patients who had received a single BTX injection.[19,33] The mandibular angle contains a limited amount of trabecular bone in laboratory animals (mice, rat, rabbit), and similar findings are also noted in humans.[47] The masseter enthesis being larger than the digastric one, it is likely that muscle strains are less intense and distributed in a more diffuse way with less consequences on bone volume.

The 3-D study of the lingual side of the mandibular symphysis showed that bone thickening in the digastric fossa was present in 3 cases, whereas such changes were not observed in the control patients. Increased muscle activity is known to stimulate bone remodeling, leading to a higher bone mass, and to induce bone proliferation at the entheses.[48] In a study rats, hypertrophic bone proliferation was noted on the paralyzed side at the mandibular enthesis of *M. digastricus* in all animals with BTX-induced paralysis of *M. masseter* and *M. temporalis*.[15] In the present study, as well as in the study by Kün-Darbois et al., it seems that there is increased activity of *M. digastricus* on the paralyzed side, which leads to local increase of mechanical strain at the mandible. This is surprising because the compensation of the loss of muscle activity is usually provided by agonist muscles. In that case, compensation for the loss of *M. masseter* and *M. temporalis* activity should be provided by agonist muscles, such as *M. pterygoideus medialis*. This bone proliferation at the *M. digastricus* enthesis seems to be caused by a disequilibrium in muscle activity, which remains to be investigated more precisely in future studies.

CONCLUSIONS

This is the first study showing 3-D changes at condylar bone 1 year after BTX injection into human masticatory muscles. These changes were evidenced by texture analysis and 3-D reconstruction of the mandible. Half the study patients presented bone changes, with new bone formation and/or bone loss, depending on the bony territories and the redistribution of muscle strains. These bone changes can have clinical expression, and some recently published case reports tend to confirm this hypothesis.[18,21]

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.oooo.2019.03.007.

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