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MANDIBULAR BONE EFFECTS OF BOTULINUM TOXIN INJECTIONS IN MASTICATORY MUSCLES IN ADULT HUMAN

CONSEQUENCES OSSEUSES MANDIBULAIRES D'INJECTIONS DE TOXINE BOTULIQUE DANS LES MUSCLES MASTICATEURS CHEZ L'HOMME

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Liste des abréviations

3D	Three-dimensional
ARS	Anterior repositioning splint
BTX	Botulinium toxin type A
CBCT	Cone-beam computed tomography
GLN	Gray Level Non-uniformity
LRE	Long Run Emphasis
M	Musclus
OA	Osteoarthritis
RLN	Run Length Non-uniformity
ROI	Region of interest
SRE	Short Run Emphasis
TMJD	Temporomandibular joint disorders

Plan

MANDIBULAR BONE EFFECTS OF BOTULINUM TOXIN INJECTIONS IN MASTICATORY MUSCLES IN ADULT HUMAN

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ABSTRACT

Introduction: Botulinum toxin (BTX) type A is injected in masticatory muscles to treat various clinical conditions. Animal studies have evidenced a bone loss at the condyle and alveolar regions of the mandible following BTX injection in masticatory muscles. The aim of the present study was to seek for mandibular bone changes in patients following BTX injections in masticatory muscles.

Methods: Twelve patients in which a BTX injection in masticatory muscles (*musculus masseter* and *musculus temporalis)* were included. Cone-beam computed tomography (CBCT) was performed before and twelve months after the BTX injection in each patient. The condylar and alveolar regions of the mandible were analyzed using texture analysis of the CBCT images with the run length method. Condylar cortical thickness was measured and 3D analysis of the whole mandible was also performed.

Results: A run length parameter (gray level non-uniformity) was found significantly increased in condylar bone (right side) and alveolar bone (both sides). A significant cortical thinning was found at the anterior portion of the right condyle. 3D analysis showed significant bone changes of condylar bone and at the digastric fossa. No change was found on mandibular angles.

Conclusion: This study identified mandibular bone changes in adult patients after BTX injection into masticatory muscles.

INTRODUCTION

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 by a "functional denervation" [2]. In laboratory animals, a single BTX injection in the quadriceps induces a disuse bone loss at the tibia and femur with a considerable muscle wasting [3, 4]. In humans, the effect of BTX disappears after two to four months and muscle strength starts to reappear three to four months later [5, 6]. BTX type A is the most widely used in clinical medicine and many parts of the human body are now being targeted for various therapeutic purposes [7]. In maxillofacial surgery, BTX is used for multiple indications: subcutaneous injections for aesthetic indications, blepharospasm and hemifacial spasm or intraglandular injections for drooling [8-10]. BTX is also injected in masticatory muscles (mainly *musculus (M) masseter* and *M. temporalis*) for several indications such as trismus, bruxism, masticatory myalgia, temporomandibular joint disorders (TMJD) or *M. masseter* hypertrophy [11, 12]. Repeated injections are needed in many indications to obtain a long-lasting effect [6]. Side effects of BTX are rare and reversible. The most common adverse effects are bruising and local pain at the injection site [13]. Systemic side effects (shock) or unwanted palsy of the nearby muscles are rarely reported [1].

The mandible is a non-weight bearing bone stimulated by masticatory muscles mainly during the eating process. It is composed of trabecular and cortical bone; the teeth roots being anchored into the alveolar bone by the periodontal ligament. Alveolar bone has a high plasticity and is remodeled at a high rate [14]. Its mechanical stimulation during mastication is essential in keeping the teeth and underlying bone healthy. Loss of teeth leads to an irreversible alveolar bone resorption [15]. Trabecular bone may also occur in other parts of the mandible such as the condylar process and mandibular angle.

Mandibular bone mineral density and cortical bone thickness are correlated with masticatory function and occlusal forces [5]. Muscles exert stresses at the periosteum and control bone microarchitecture according to the Wolff's law [16].

Injections of BTX decrease the force of *M. masseter* and/or *M. temporalis* contractions and reduce stress at the periosteum. Several animal studies have shown a profound bone loss at the mandibular condyle and at the alveolar region after BTX injections in masticatory muscles [5, 17-20]. These bone alterations could constitute a risk factor for fractures, especially in patients receiving repeated BTX injections in masticatory muscles. Few articles have studied the mandibular bone changes in humans. None have shown marked impact on condylar bone after BTX injection [21-23]. Osteopenia and reduced bone volume may also increase the risk for periodontal disease, alveolar bone loss and tooth loss [24].

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

PATIENTS AND METHODS

1. Participants

All patients receiving BTX injections into the right and left *M. masseter* and *M. temporalis* at the maxillo-facial surgery department of Nantes University Hospital (France) between January 2015 and December 2016 were included. The indications for injections were: TMJD, *M. masseter* hypertrophy or spasm, bruxism or masticatory myalgia.

Any potential cause of mandibular bone disorder constituted exclusion criteria: diabetes, osteoporosis, neoplasia, previous mandibular surgery, long-course corticosteroid therapy, radiotherapy, anti-resorptive drug treatment, premolar/molar loss and orthodontic treatment. All participants gave their informed consent before participating to the study. This experimental protocol was approved by the local ethical committee of Angers University Hospital and was done in accordance with the institutional guidelines of the French Ethical Committee (protocol number 2016-41) and with the 1964 Helsinki declaration and its later amendments. All patients answered a series of questions about their status: the side of the symptoms, previous treatment (drugs, anterior repositioning splint (ARS)) and had a clinical examination.

2. Experimental Protocol

Botulinum toxin of type A (Botox®, Allergan Inc., Irvine, CA, USA) was used. The main four masticatory muscles, i.e. the two *M. masseter* and the two *M. temporalis* were injected, regardless of whether the symptoms were unilateral or bilateral. Each injection was performed using a 1 mL syringe and a 26G needle, at a dilution of 100 U/mL of injectable saline [25]. 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* (Fig. 1). All injections were performed by trained maxillo-facial surgeons.

CBCT was performed using a NewTom VGI (NewTom, Verona, Italy) with the following acquisition parameters: high frequency generator, field of view size: 15x15cm, 110 kV, 20 mA. A first acquisition was performed before BTX injection for etiological and morphological assessment. The second CBCT acquisition was performed one year after. All patients had a regular follow-up (3 months, 6 months and 12 months after the injection).

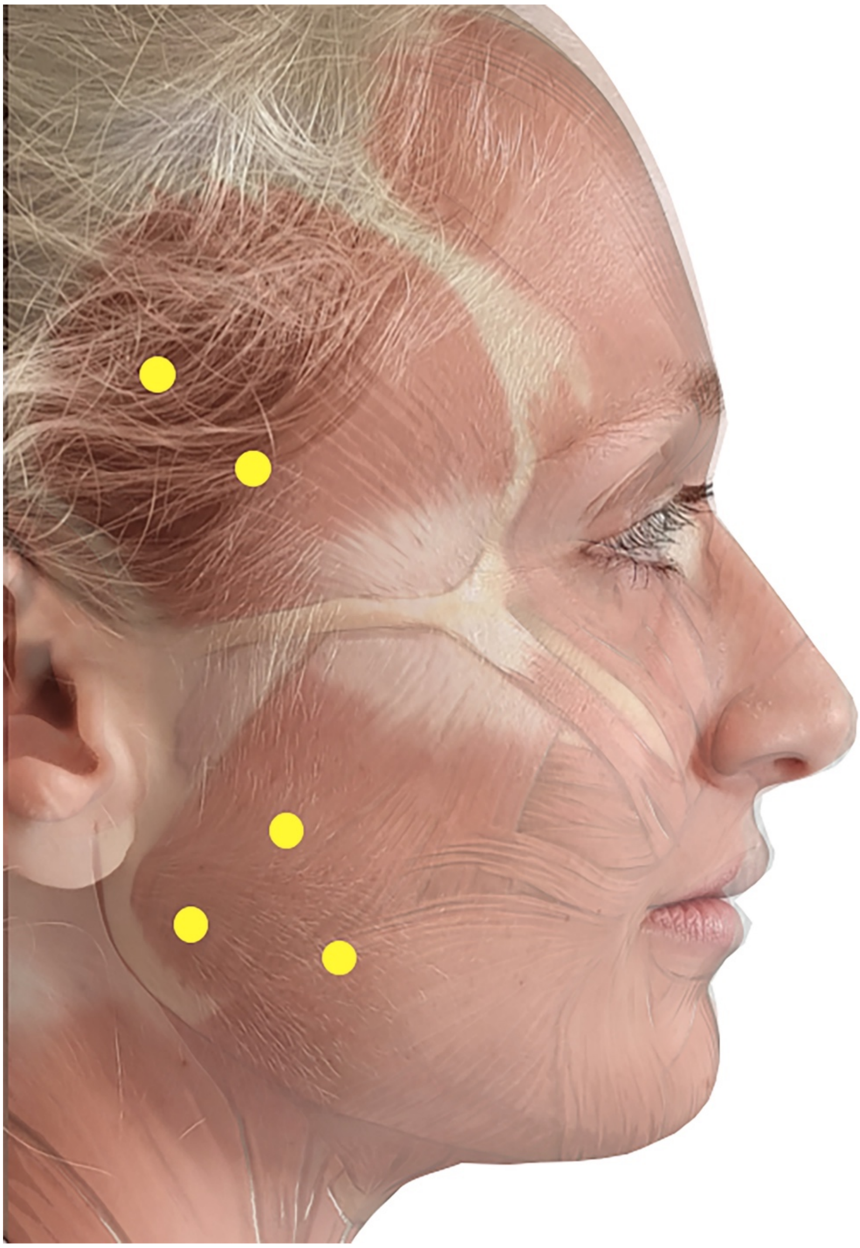

Figure 1: Injection sites of botulinum toxin in masticatory muscles: three sites for *M. masseter* and two for *M. temporalis*.

3. Bone texture analysis

DICOM images obtained by CBCT were converted in 256-gray-level images in the BMP format using ImageJ 1.5 (US National Institutes of Health, Bethesda, MD, USA) [26]. The DataViewer software (release 1.5; Brucker microCT, Kontich, Belgium) was used to reslice the stacks of 2D images: i) 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; ii) for alveolar bone, the area of interest was selected on a plane comprising the second premolar, molars, mandibular canal and the retromolar area (Fig. 2).

Texture analysis was performed using MaZda 3D 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 [27, 28]. Analysis was done in triplicate by the same investigator with determination of the intra-operator variation coefficient. The following parameters were determined in the horizontal and vertical direction of the image:

- Run Length Non-uniformity (RLN) measures the similarities of the length of the runs throughout the image. RLN is expected large if the number of runs of same length increases throughout the image.
- Gray Level Non-uniformity (GLN) measures the similarity of gray level values throughout the image. GLN is expected large if the number of runs of same gray level increases throughout the image.
- Short Run Emphasis (SRE) is highly dependent on the occurrence of short runs and is expected large for fine textures.
- Long Run Emphasis (LRE) is highly dependent on the occurrence of long runs and is expected large for coarse structural textures.

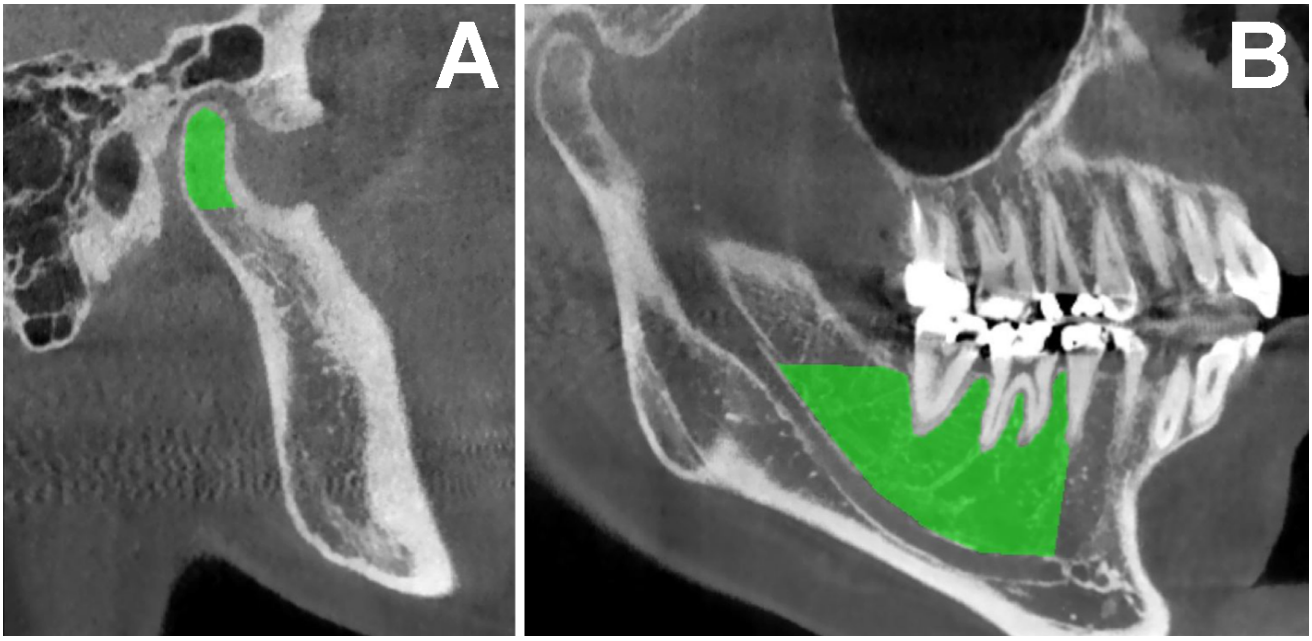

Figure 2: Areas of interest (in green) used to measure bone texture of condylar bone (A) and alveolar bone (B) on 2D sections of the mandible obtained by cone-beam computed tomography.

4. Cortical thickness of condylar bone

Cortical bone thickness at the condyle was measured using ImageJ on the same images at three different locations. Anterior and posterior thicknesses were obtained on a parallel to the Franckfurt plane through the lowest point of the articular eminence. Superior thickness was obtained on an orthogonal line intersecting the antero-posterior plane and the mid-point of the condyle. Measurements were made in triplicate and expressed in mm.

5. 3D analysis of the mandible

After CBCT acquisitions, semi-automatic segmentations of mandible volumes were performed with ITK-Snap v3.6 open-source software (http://www.itksnap.org) [29]. 3D models were generated and analyzed with 3DSlicer v4.8 software (National Alliance for Medical Image Computing NA-MIC; http://www.slicer.org) [30].

The *Surface Registration* tool of the CMFregistration module was used to superimpose 3D 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 3D models. Then, the *Model to model distance* module of MeshMetric3D plugin (https://www.nitrc.org/projects/meshmetric3d) was used to compute point-by-point distance between two triangular meshes of the superimposed 3D models. Finally, the combined image was analyzed with the *Shape population viewer* v1.4 software (https://www.nitrc.org/projects/shapepopviewer) to create color maps showing mandibular surface differences expressed in mm. The lookup table provided a colorbar and was set with three colors: blue indicated a negative difference corresponding to a bone loss, red for a positive difference (bone apposition) and green for steady state. 3D superimposed mandibles were analyzed on three ROI: condylar processes, coronoid processes, mandibular angle region and the lingual side of the symphysis. Condylar processes were analyzed on three areas: anterior, upper and posterior. A difference greater than one millimeter was considered as significant.

6. Statistical analysis

Statistical analysis was done with Systat statistical software v13 (Systat Software, Inc., San José, CA, USA). All data were expressed as mean ± standard deviation. Differences between the left and right side was search for each parameter and compared using a Student paired t test. Differences were considered significant when $p < 0.05$.

RESULTS

1. Clinical characteristics

Twelve patients were included in the study; the mean age was 31.5 ± 13.3 years. Sex ratio was 5 women for 1 man. Ten patients presented a TMJD, one had a *M. masseter* hypertrophy and one a *M. masseter* spasm. In patients with TMJD, five patients presented left unilateral symptoms, four patients presented bilateral symptoms and one patient presented a right unilateral form. Nine patients presenting a TMJD were also treated with the use of an ARS during the study. Nine patients presented subjective improvement of symptoms after BTX injection. No side effect has been observed.

2. Bone texture analysis

On the CBCT performed twelve months after BTX injection, the GLN parameter was found significantly different at the right condylar bone, in the horizontal (p=0.003) and vertical directions (p=0.014) (Fig. 3). Similarly, GLN also increased significantly (p=0.048) at the right alveolar bone in the horizontal direction but not in the vertical direction (p=0.059). At the left alveolar bone, GLN was significantly different both in the horizontal (p=0.003) and vertical directions (p=0.017). GLN was also significantly increased in both horizontal (p=0.003) and vertical (p=0.006) directions for averages of right and left sides of alveolar bone. No difference was found for GLN at the left condylar bone. No difference could also be evidenced for other texture parameters. Intra-operator variation coefficients were found less than 5%.

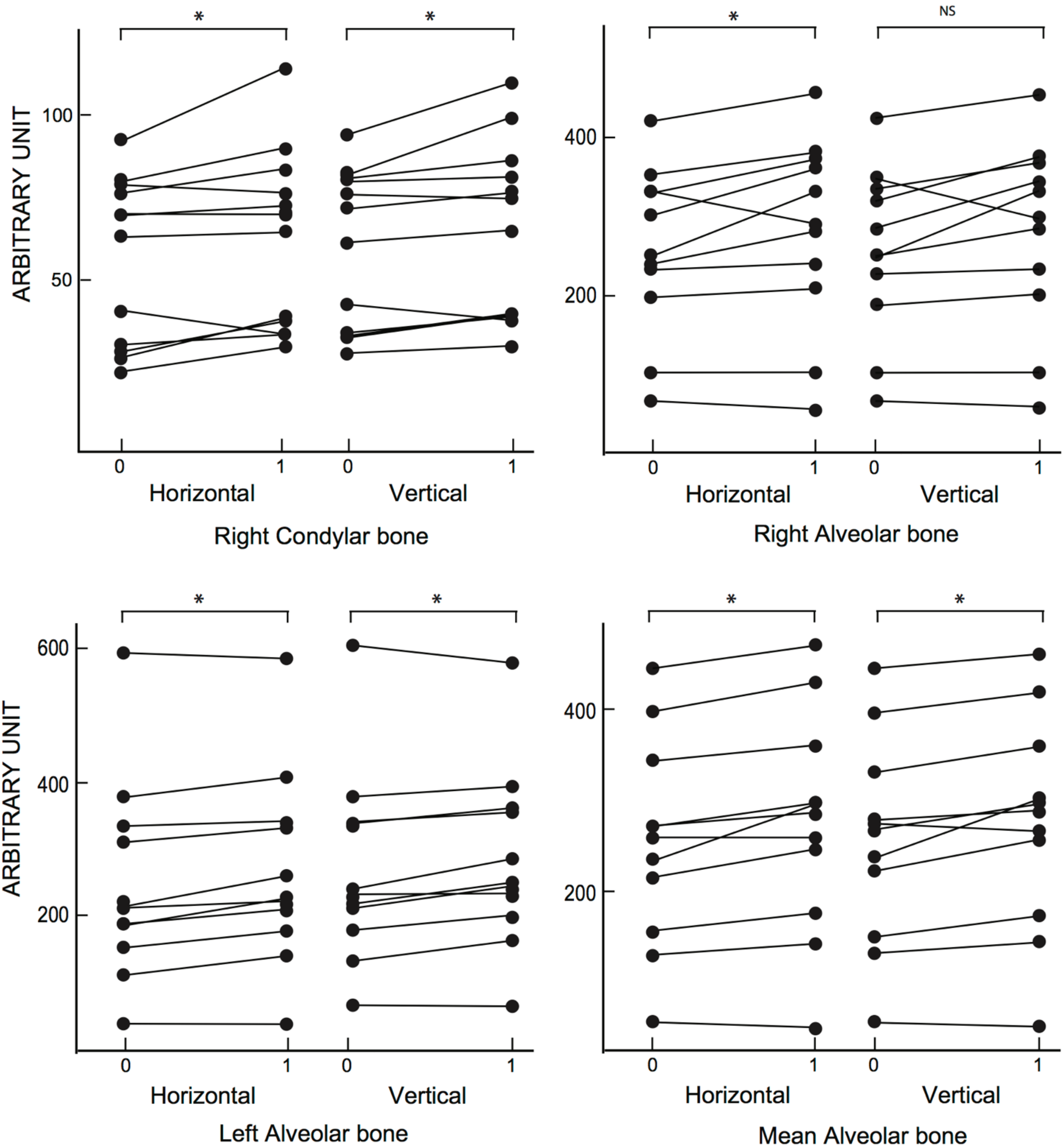

Figure 3: GLN (Gray Level Non-uniformity) measurements for alveolar bone and condylar bone in horizontal and vertical directions. Measurements are expressed for right side, left side and average of right and left sides (Mean). * Significant difference ($p < 0.05$) between T0 and T1 (one year after BTX injections).

3. Cortical thickness of condylar bone

A significant cortical thinning was found at the anterior portion of the right condyle one year after BTX injections: 1.71 ± 0.50 mm at T0 vs. 1.51 ± 0.57 mm at T1 (p=0.008). Similar differences were shown when both sides were averaged: 1.74 ± 0.53 mm vs. 1.52 ± 0.48 mm (p=0.008) (Fig. 4). Average condylar cortical thinning was – 0.22 ± 0.24 mm. No significant difference could be evidenced for the other directions. Intra-operator variation coefficients were found less than 5%.

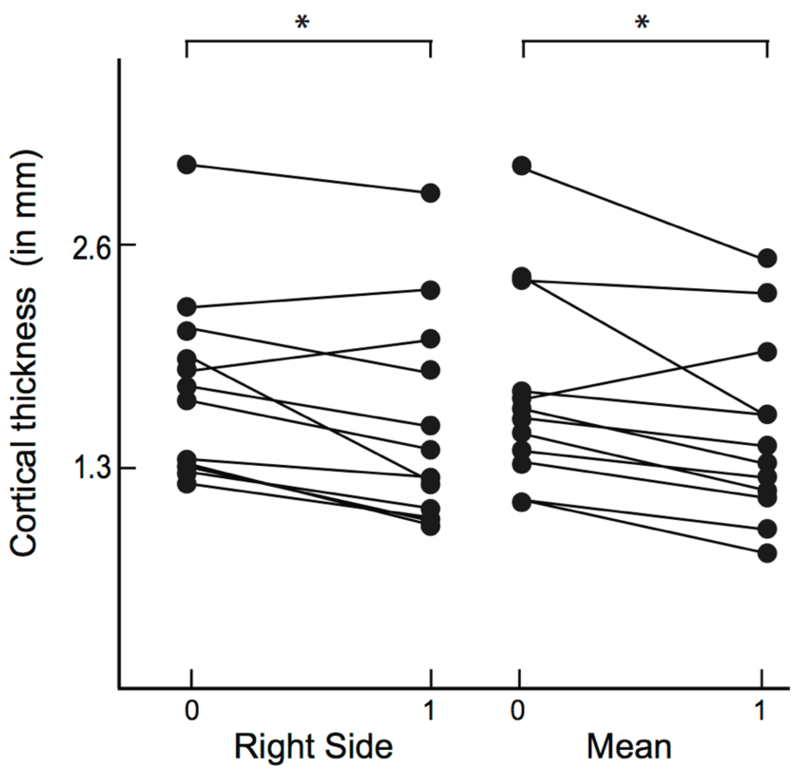

Figure 4: Cortical thickness measurements (mm) of anterior condylar bone showing a significant cortical thinning at the anterior portion of the right condyle one year after BTX injection (Right side) and averaged sides (Mean). * Significant difference (p <0.05) between T0 and T1 (one year after BTX injections).

4. 3D analysis of the mandible

3D condylar analysis showed significant bone changes in most of the patients of the study (Table 1). Bone thickening at the lingual side of the mandibular symphysis was present in three cases in the digastric fossa (Fig. 5A). At the anterior area of the condyle, six patients presented bone changes on the combined image: bone formation (in three patients) and bone loss (in three patients). Two patients had a significant bone loss at the anterior portion of the right condyle (Fig. 5B). At the upper condylar portion, seven patients had bone changes: five with new bone formation and two with bone loss. At the posterior area, seven patients presented bone changes: three with new bone formation and four with bone loss. One patient presented new bone formation of the coronoid process on both sides. No significant difference could be evidenced at the mandibular angle.

Table I : Table showing mandibular bone changes observed at the anterior, upper and posterior areas of the mandibular condyle and at the digastric fossa in 3D 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

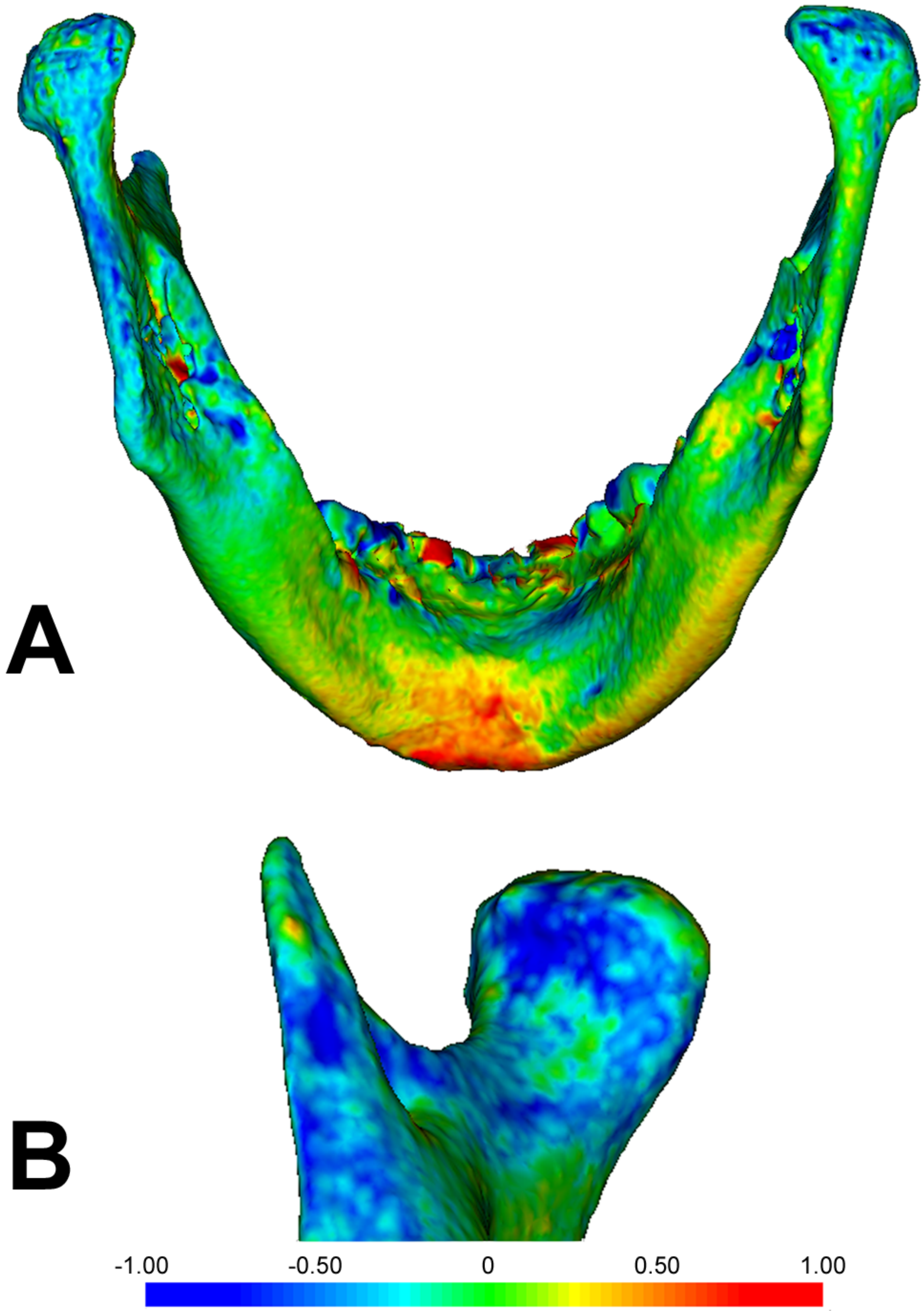

Figure 5: A) Color map of the 3D model of a mandible showing surface differences at one year expressed in millimeters on the colorbar. Note the significant bone thickening at the left digastric fossa. B) Color map of 3D model of a right condyle showing a significant thinning at the anterior part at one year.

DISCUSSION

In the present study, we found that one year after a BTX injection in the masticatory muscles, bone changes could be evidenced by texture analysis and 3D 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 microCT images and high resolution CBCT for GLN [21]. Significant differences were found in the present study at both sides for alveolar bone, but only on the right side of the condylar bone. Similarly, bone thickness at the anterior area was significantly reduced only at the right condyles. These unilateral changes occurring at the condylar bone could be explained by a mastication desequilibrium as toothed subjects are normally alternating the side of chewing. Our series was mainly composed of patients with TMJD in which mastication is often unilateral. They chewed often on the side of the injured TMJ which is therefore less stressed as previously reported by others [31, 32]. This specificity of chewing may explain the asymmetry of condylar bone loss in our series of patients.

The present study analyzes for the first time the 3D changes of condylar bone after BTX injections in humans. Condylar bone changes were observed in half of the patients, particularly at the anterior part of the right condyle where three of them had a significant bone loss. These results are consistent with measurements obtained by texture analysis and bone thickness evaluation. New bone formation of the condyle was also observed in three patients at the anterior and posterior areas and in five patients at the upper area.

Medical management of TMJD consists of preferring reversible and non-invasive treatments such as the use of ARS as recommended by the French Oral and MaxilloFacial society [33]. Several studies have shown that ARS directly affects bone remodeling at the condyle.

New bone formation was more often noted at the posterior part of the condyle, probably because ARS moved the condyles downwards and forwards thus increasing both posterior and medial joint spaces [34]. ARS is reported also to induce new bone formation and cortical thickening predominantly in the anterior portion of the condyle, but also posteromedial and posterior intermediate areas [35].

TMJD can be an age-related disease when osteoarthritic lesions (OA) occur [36]. The condylar morphology of untreated patients with OA of the TMJ was found significantly different from that of asymptomatic patients [37]. There are few articles in the literature on the natural history of condylar bone changes in patients with TMJD. Significant condylar bone changes were found in 68% of cases at the TMJ after radiological follow-up during 13.4 months [3-18] in patients presenting TMJD [38].

To date three studies in the literature report human mandibular bone assessment following BTX injections in *M. masseter* [22, 23, 39]. Differences were described by only one author who found significant differences in total volume of mandibular angle area. These differences were found 6 months after BTX injections, only for patients who received two injections at a four months interval [39]. In the present study, no significant difference in the angular region of the mandible was found. This is consistent with other previous studies in patients having received a single BTX injection [22, 23, 39].

The 3D study of lingual side of mandibular symphysis showed that bone thickening in the digastric fossa was present in three cases. In a study conducted in the rat, a hypertrophic bone proliferation was noted on the paralyzed side at the mandibular the *M. Digastricus* enthesis in all animals with a BTX-induced paralysis of *M. masseter* and *M. temporalis* [17]. An increased activity of *M. Digastricus* on the paralyzed side probably leads to a local increase of mechanical strains at the mandible which compensates the loss of activity of the paralyzed muscles.

An increased muscle activity is known to stimulate bone remodeling leading to a higher bone mass and to induce bone proliferation at the entheses [40, 41].

This is the first study showing 3D changes at the condylar bone one year after BTX injection into human masticatory muscles. These changes were evidenced by texture analysis and 3D reconstruction of the mandible. Half of the patients presented bone changes with new bone formation and/or bone loss depending on the bony territories and redistribution of the muscle strains.

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LIST OF FIGURES

LIST OF TABLES

TABLE OF CONTENTS

KAHN Alexis

Conséquences osseuses mandibulaires d'injections de toxine botulique dans les muscles masticateurs chez l'homme

RÉSUMÉ

Introduction: La toxine botulique de type A (BTX) est utilisée en injection dans les muscles masticateurs pour diverses indications. Des études animales ont montré l'existence d'une perte osseuse mandibulaire condylienne et alvéolaire après injection de BTX dans les muscles masticateurs. L'objectif de cette étude était de rechercher les modifications osseuses mandibulaires après injections de BTX dans les muscles masticateurs chez l'homme.

Méthodes: Douze patients ayant reçu une injection de BTX dans les muscles masséters et temporaux ont été inclus. Une tomographie par faisceau conique (cone-beam computed tomography (CBCT)) a été réalisée avant et douze mois après l'injection de BTX. Les régions alvéolaires et condyliennes mandibulaires ont été analysées par analyse de texture sur les images CBCT avec la méthode des longueurs de plage. L'épaisseur corticale condylienne a été mesurée et une analyse 3D de la mandibule a été effectuée.

Résultats: Un paramètre de l'analyse de texture (la non-uniformité des niveaux de gris) a été retrouvé significativement différent dans l'os condylien droit et l'os alvéolaire droit et gauche. Un amincissement cortical significatif a été retrouvé à la partie antérieure du condyle droit. L'analyse 3D a montré des changements osseux significatifs de l'os condylien et de la fosse digastrique. Aucun changement n'a été trouvé sur les angles mandibulaires.

Conclusion: Cette étude montre l'apparition de changements osseux mandibulaires chez l'homme après injection de BTX dans les muscles masticateurs.

Mots-clés : Toxine botulique ; mandibule ; remodelage osseux; muscle masticateur

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

ABSTRACT

Introduction: Botulinum toxin (BTX) type A is injected in masticatory muscles to treat various clinical conditions. Animal studies have evidenced a bone loss at the condyle and alveolar regions of the mandible following BTX injection in masticatory muscles. The aim of the present study was to seek for mandibular bone changes in patients following BTX injections in masticatory muscles.

Methods: Twelve patients in which a BTX injection in masticatory muscles (*musculus masseter* and *musculus temporalis)* were included. Cone-beam computed tomography (CBCT) was performed before and twelve months after the BTX injection in each patient. The condylar and alveolar regions of the mandible were analyzed using texture analysis of the CBCT images with the run length method. Condylar cortical thickness was measured and 3D analysis of the whole mandible was also performed.

Results: A run length parameter (gray level non-uniformity) was found significantly increased in condylar bone (right side) and alveolar bone (both sides). A significant cortical thinning was found at the anterior portion of the right condyle. 3D analysis showed significant bone changes of condylar bone and at the digastric fossa. No change was found on mandibular angles.

Conclusion: This study identified mandibular bone changes in adult patients after BTX injection into masticatory muscles.

Keywords: Botulinum toxin; mandible; bone remodeling; masticatory muscle

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