Osteopenic consequences of botulinum toxin injections in the masticatory muscles: a pilot study

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SUMMARY Patients with temporomandibular muscle and joint disorder (TMJD) increasingly seek and receive treatment for their pain with botulinum toxin (BoNTA; botulinum toxin A). Used intramuscularly in therapeutic doses, it produces localised paresis. Such paresis creates risk of reduced bone mineral density, or ‘disuse osteopenia’. Animal studies have frequently used BoNTA as a model of paralysis to induce bone changes within short periods. Osteopenic effects can be enduring in animals but have yet to be studied in humans. This is the first study in humans to examine bone-related consequences of BoNTA injections in the masticatory muscles, comparing oral and maxillofacial radiologists’ ratings of trabecular bone patterns in the condyles of patients with TMJD exposed to multiple masticatory muscle injection sessions with BoNTA to a sample of patients with TMJD not exposed to masticatory muscle injections with BoNTA. Cone-beam computed tomography (CBCT)-derived images of bilateral condyles were evaluated in seven patients with TMJD receiving 2+ recent BoNTA treatment sessions for facial pain and nine demographically matched patients with TMJD not receiving BoNTA treatment. Two oral and maxillofacial radiologists evaluated CBCT images for evidence of trabecular changes consistent with osteopenia. Both evaluators noted decreased density in all participants exposed to BoNTA and in none of the unexposed participants (P < 0.001). No other abnormalities associated with reduced loading were detected. These findings need replication in a larger sample and over a longer time period, to ensure safety of patients with TMJD receiving multiple BoNTA injections for their pain.

KEYWORDS: temporomandibular disorders, temporomandibular joint osteopenia, bone remodelling, botulinum toxin, bone density

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Introduction

Temporomandibular muscle and joint disorder (TMJD), involving pain and dysfunction in the masticatory muscles and joints, is a common condition. Prevalence studies suggest that 10–12% of the general female population experience a TMJD at any time point (1). The most common subtype involves masticatory muscle pain, a ‘myofascial’ TMJD condition (2–4). Women are affected approximately twice as often as men, with even higher gender ratios among careseekers (5, 6). Conservative and reversible treatment modalities (e.g. medications, stabilisation splints, jaw exercises, diet and habit modification) provide symptom management for many. Nevertheless, some patients fail to experience satisfactory symptom management from conservative treatments. One prospective study (7) found that
nearly one-third had persistent pain and more than one-third had recurrent pain at the end of 5 years of observation.

Temporomandibular muscle and joint disorder patients with refractory symptoms are inquiring about the safety and efficacy of botulinum toxin (BoNTA; botulinum toxin A; Botox®) treatment for their pain. Botulinum toxin A, considered ‘the most potent biological toxin known to man’ (8), acts as a pre-synaptic neurotoxin that blocks neuromuscular transmission by inhibiting acetylcholine release from motor and sympathetic nerve terminals. Used intramuscularly in therapeutic doses, it produces localised paresis. Injected extramuscularly, it also inhibits reactivity of non-cholinergic, nociceptive fibres (9–11).

Approved by the U.S. Food and Drug Administration (FDA) in 1989 for focal dystonia, BoNTA has subsequently been approved to treat migraine and a variety of specific neuromuscular spasms. In the United States, BoNTA is being aggressively promoted for off-label use for TMJD pain as ‘Dentox’ (http://dentox.com), with multiple nationally promoted continuing education (CE) courses training dentists and other health care providers in masticatory muscle injections for TMJD pain. Disturbingly, increasing use of BoNTA for myofascial TMJD is taking place with little evidence of its safety or efficacy for that indication. As such, BoNTA administration for myofascial TMJD presents a potential public health issue.

**Efficacy**

In a recent general review (12) of BoNTA for myofascial pain, Gerwin et al. concluded that ‘evidence is insufficient to guide clinical practice’. A Cochrane review of BoNTA for masseter hypertrophy (13) noted the lack of high-level (RCT) evidence to evaluate its effectiveness. More recently, a 21-patient multicentre RCT (14) concluded that BoNTA was not effective for myofascial TMJD, but we have noted (15) that the study was too small to detect clinically significant effects. Thus, the benefit of BoNTA treatment for myofascial TMJD has yet to be clearly established in an adequately powered, randomised and controlled clinical trial.

**Safety**

Botulinum Toxin A has demonstrated acceptable safety levels for a variety of indications (16) although rare reports associate its use with respiratory compromise, inability to swallow and even death (17, 18) [also see (19)]. A new and disturbing concern is that although BoNTA injections in current FDA-approved indications (e.g. aesthetics, blepharospasm, axillary hyperhidrosis) do not clearly reduce mechanical load on bones, BoNTA injections in masticatory muscles reduce usual loading of the mandibular condyle and alveolar bone, introducing risk of ‘disuse osteopenia’ (20, 21) in those structures.

Bone remodelling in response to mechanical loading is a continuous process. Disuse of limbs due to immobilisation or lack of gravity during a spaceflight leads to a rapid loss in bone strength (22–24). Botulinum Toxin A has been used in an animal model of hind limb muscle paralysis to induce dramatic bone loss in short periods (20, 21). Osteopenic effects of BoNTA can be durable. Studies of BoNTA-induced calf muscle paralysis in mice observed continuation of bone loss after 12 weeks post-injection (25) and even 16 weeks post-injection (26, 27). Mice studies have also observed persistently low bone density even after muscle activity recovery (25, 28). A rabbit study found that unilateral masseter muscle BoNTA injection produced ‘profound’ morphological changes and bone loss in the mandibular condyle, as well as bone loss in the alveolar region (29) similar to that found in an earlier study (30). Parallel findings in rats demonstrate alterations in bony structures attached to the masseter muscle following BoNTA injection (31, 32).

Osteopenia and reduced bone volume may increase the risk for periodontal disease (33–35), alveolar bone loss (36, 37) and tooth loss (37–43) and show increased risk of jaw fracture following impact (44–46). Reduction in bone volume and density may also adversely influence future therapeutic options, such as regenerative bone augmentation and dental endosseous implants.

Translation of research findings from animal models to humans is not without problems. Recent meta-analytic research (47) demonstrates that factors related to trabecular bone patterns differ for humans and small mammals, raising caution about extrapolating small animal bone biomechanical results to humans. Given the absence of data in humans on

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bone-related consequences of masticatory muscle BoNTA injections concerns raised by animal studies, this pilot study aimed to compare trabecular bone patterns in the condyles of patients with TMJD exposed to multiple masticatory muscle injections with BoNTA to a similar patient sample unexposed to such injections. Only female patients with TMJD were studied because (a) women are disproportionately affected (5, 6) and (b) women have a higher risk of osteoporosis and osteopenia (48).

Material and methods

This study compared a small sample of women with oro-facial pain who either did or did not receive multiple BoNTA injections into the masticatory muscles. Two oral and maxillofacial radiologists rated bilateral cone-beam computed tomography (CBCT) images of the TMJ region for mandibular condyle morphological characteristics and trabecular bone density patterns associated with osteoporotic and osteoarthritic diseases.

Participants

Participants ‘exposed’ to BoNTA injections for oro-facial pain treatment. Patients self-reporting facial pain consistent with myofascial TMJD were recruited from women responding to an NYU School of Medicine IRB-approved survey posted on the website of the TMJ Association (www.tmj.org), an advocacy and self-help organisation for individuals suffering from TMJD. Women viewing the website were asked to complete a short survey regarding their oro-facial pain, history of Botox treatment and major risk factors for bone health (i.e. age, menopausal status and osteoporosis/osteopenia status). Individuals willing to learn more about participating in a further study of Botox were asked to supply contact information.

Based on survey results, a group of 22 women (i) reported receiving at least one treatment session of BoNTA injections for muscle-based facial pain, (ii) met eligibility criteria (age 18+, no history of TMJ surgery, history of facial pain treatment and history of severe facial pain as indicated by lifetime worst pain of at least 5 on a 0–10 Characteristic Pain Intensity Scale) and (iii) agreed to receive contact from NYU investigators. A research coordinator then determined whether these women were located near a facility where they could receive suitable cone-beam computed tomography (CBCT) imaging of their TMJ region. Facilities needed to use imaging units with reasonably comparable fields of view, producing three-dimensional images that could be compared in terms of morphological features, if not condylar bone density and volume. If within an acceptable commuting distance, the coordinator attempted to contact respondents to describe basic study elements and confirm eligibility (study candidate spoke adequate English, history of muscle-based facial pain and treatment within the prior 3 months). Women expressing interest in further participation were consented in a follow-up phone call.

Participants ‘unexposed’ to BoNTA treatments for oro-facial pain treatment. From the survey, individuals who indicated that they had never received BoNTA injections, for any reason, were recruited if eligible (at least age 18, no history of TMJ surgery and lifetime worst pain at least 5 on the Characteristic Pain Intensity Scale). Unexposed participants were recruited to match exposed participants on several self-reported risk factors for osteoporosis or osteopenia: age (±5 years), menstrual status, historical worst pain severity and TMJD treatment within the prior 3 months.

Botulinum Toxin A exposure status was confirmed by review of medical records from treatment providers identified by participants and released to us, after clinicians received a medical release form signed by participants. Participants were interviewed regarding self-reported treatment parameters, demographic factors, potentially confounding comorbid medical factors (e.g. rheumatoid arthritis, current menstrual status, etc.) and personal and familial history of osteoporosis or osteopenia. Participants were also asked about characteristic pain intensity (49) during their worst-ever 2 weeks of facial pain, to ensure that we were potentially accruing individuals with a history of similarly severe pain, regardless of exposure to BoNTA. Given the possibility that BoNTA produced marked pain reduction, it was inappropriate to ask about recent pain severity.

Exclusion criteria

Exposed and unexposed survey completers were excluded from further participation if they indicated a history of TMJ surgery, had no treatment for facial
pain within the prior 3 months, had lifetime worst pain of <5, were under 18 or were not fluent in English. Due to the risk of radiation exposure, women who were pregnant or at risk of becoming pregnant were excluded.

After providing written consent, participants were sent an order for bilateral CBCT imaging of their temporomandibular joint region, with results forwarded to NYU. Before upload to a double-password-protected server at NYU, personal identifiers were stripped from DICOM files using Trialwire. Two oral and maxillofacial radiologists viewed and rated the deidentified images.

Cone-beam computed tomography

Cone-beam computed tomography is increasingly used to evaluate osseous changes in the TM joint (50–53). Its ability to detect subtle osseous changes in the TMJ is equal or similar to that of multislice medical grade CT (54–58), and its superior spatial resolution and lower radiation dose makes it a preferable imaging modality. It was not possible to use the same CBCT imaging instrument in this national sample. The Accuitomo 170 and the Newtom VGI Flex units were used most often. Exposed participants were imaged within 6–10 weeks following their last BoNTA treatment to capture periods when effects of bone unloading due to BoNTA might be detectable. Unexposed participants were asked to undergo bilateral imaging of their TMJ regions at their earliest convenience.

Evaluation of trabecular density changes

As animal research (29) suggests that bone density alteration in the trabecular area is the most profound change produced by BoNTA-induced unloading, two oral and maxillofacial radiologists (blind to BoNTA exposure status) rated bone density patterns in the trabecular region of the condyle. They could rate each side for ‘no change’, ‘patterns consistent with increased density’ or ‘patterns consistent with decreased density’. Specific morphological abnormalities associated with osteoarthritis, such as osteophytes or erosion, were identified. Any other morphological abnormalities were noted. Multiple serial sections were viewed to make a judgment. See Fig. 1 for an example of data from one exposed and one unexposed participant.

Statistical analysis

Inter-rater reliability was assessed by the kappa statistic. Group differences in rates were evaluated with the Fisher exact test and mean differences with the Mann–Whitney test. All analyses used IBM SPSS, v21.

Results

In total, 157 women completed the website survey. Of these, 143 indicated that a health care professional told them that they had a muscle-related TMJ or TMD. One hundred and twenty-nine (129) women indicated whether they had ever received BoNTA injections for facial pain. Forty-three reported such treatment: 26 reported receiving three or more injection sessions, 4 reported two sessions, 6 reported one session, and 7 did not indicate frequency of sessions. Of the 30 receiving two or more injections, 25 (83.3%) agreed to additional contact. Of 86 women reporting a muscle-based TMJ/TMD diagnosis and no history of any BoNTA use, 63 (73.3%) agreed to additional contact.

Initial telephone or email contact was made with 17 exposed women who appeared to be eligible on the basis of the online survey and who lived near a CBCT imaging facility; eight of these women provided verbal and written consent. One exposed participant withdrew after the telephone interview, leaving seven BoNTA-exposed participants with radiological data. Eleven (11) unexposed women (i) met eligibility criteria according to the online survey, (ii) lived within a moderate distance of a CBCT imaging facility (iii) and matched an exposed participate on menstrual status, age (±10 years) and self-reported osteoporosis/osteopenia status. The research coordinator made telephone or email contact with all 11 women; nine gave verbal and written consent to participate.

Interview results were similar in exposed and unexposed women. Average worst-ever 2 weeks of facial pain (rated 0–10, where 10 = worst pain imaginable)
was 9.11 (SD 1.27) for unexposed and 9.29 (SD 1.11) for BoNTA-exposed women ($P > 0.10$). Mean age for both groups was 45 (SD of 10.80 for exposed and SD of 9.48 for unexposed). Personal history of osteoporosis or osteopenia was reported by two unexposed and zero exposed women. One additional unexposed woman reported a first-degree relative history of osteoporosis or osteopenia (Fisher’s exact, $P > 0.10$). Three unexposed and two exposed women indicated that they were post-menopausal, with one additional exposed woman reporting irregular periods suggestive of perimenopause ($P > 0.10$). One exposed and one unexposed woman endorsed Hispanic ethnicity and race; all others reported their race as white. Thus, exposed and unexposed groups appeared well matched on self-reported characteristics that could confound bone comparisons.

Of the 16 subjects with radiological data, six of the seven exposed and six of the nine unexposed participants also provided treatment charts. Chart reviews indicate that the total number of masticatory muscle BoNTA injection sessions ranged from 2 to 7 among exposed participants. Only one received two BoNTA sessions, and only one received seven such treatment sessions, with a median of 4. Time between sessions ranged from 6 to 17 weeks, with a median (and mean) between-treatment interval of approximately 3 months.

Table 1 summarises judgments of trabecular bone patterns by the two oral and maxillofacial radiologists. As shown, raters had perfect agreement (kappa = 1.0) when rating reduced trabecular density. When rating normal or increased density, agreement was still ‘good’ (kappa = 0.62).

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**Fig. 1.** Condylar changes associated with exposure to BoNTA. Note decreased density (more dark areas) of the central, trabecular bone, as well as reduced thickness of the cortical bone in the exposed participant.
Decreased density was noted in all BoNTA-exposed and no unexposed participants (Fisher’s exact test, \( P < 0.001 \)). No abnormalities associated with reduced loading, other than reduced trabecular density, were noted by either rater. Not shown in Table 1, unilateral or bilateral changes associated with mild to severe osteoarthritis were detected in all but one unexposed participant and all of the exposed.

### Discussion

This pilot study is the first to document differences in mandibular condylar trabecular bone patterns, suggesting a bone density change associated with BoNTA injections in the masticatory muscles. It shows some fidelity between rabbit (29) and human studies. All women receiving BoNTA masticatory muscle treatment sessions 6–10 weeks before imaging showed abnormally low trabecular density patterns on evaluation of CBCT images by two oral and maxillofacial radiologists. None of the demographically similar unexposed women with a TMJD complaint showed low-density patterns; in fact, depending on rater, 3 or 4 of the 9 unexposed women showed increased trabecular density patterns.

The clinical significance of these findings is unclear. Does reduced trabecular bone density normalise over time after BoNTA treatment cessation? Does it have important consequences for overall bone quality and strength? Of note, no other abnormalities associated with reduced density, such as fractures, were detected. Given the small sample and cross-sectional nature of these findings, longitudinal research with a larger sample is needed. Future studies would benefit from use of a single CBCT imaging platform and quantification of bone density in the cancellous and trabecular regions. Use of quantitative CBCT to evaluate bone density is gaining acceptance (52, 53, 59–63). Although values produced are not equivalent to Hounsfield units (52, 53), they can be used to compare groups and individuals to one another and within an individual over time, evaluating magnitude of difference or change.

A larger study can evaluate dose–response effects related to the number of injection sessions, varying repeat dosing protocols and overall time duration after injection initiation. These data suggest the possibility of significant morphological changes to mandibular condyles with longer-term BoNTA treatment, as condylar bone loses density before it has time to fully remodel.

Based on strategic sampling of unexposed participants to maximise similarity to exposed participants, the two groups appeared well matched on characteristics that might affect condylar trabecular density.

**Table 1.** Oral radiologists’ ratings of trabecular bone changes in the condyle of women with oro-facial pain who were exposed or unexposed to BoNTA injections for their masticatory muscle pain

<table>
<thead>
<tr>
<th>BoNTA exposure status</th>
<th>Rater 1 condylar trabecular change</th>
<th>Rater 2 condylar trabecular change</th>
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<tbody>
<tr>
<td></td>
<td>Right</td>
<td>Left</td>
</tr>
<tr>
<td>Unexposed S1</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Unexposed S2</td>
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</tr>
<tr>
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<td>Normal</td>
</tr>
<tr>
<td>Unexposed S4</td>
<td>Increase</td>
<td>Increase</td>
</tr>
<tr>
<td>Unexposed S5</td>
<td>Increase</td>
<td>Increase</td>
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<tr>
<td>Unexposed S6</td>
<td>Normal</td>
<td>Increase</td>
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<td>Normal</td>
</tr>
<tr>
<td>Exposed S1</td>
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<td>Decrease</td>
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<tr>
<td>Exposed S2</td>
<td>Decrease</td>
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<tr>
<td>Exposed S3</td>
<td>Decrease</td>
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<tr>
<td>Exposed S4</td>
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<tr>
<td>Exposed S5</td>
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<td>Exposed S6</td>
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<tr>
<td>Exposed S7</td>
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</tbody>
</table>
However, power to detect differences in a small sample is limited. Ideally, a measure of systemic bone density, such as standard bone density dual-energy X-ray absorptiometry (DXA), would have ensured that overall bone density did not differ by chance between exposed and unexposed participants.

In sum, this first study of trabecular bone patterns in the mandibular condyles of women exposed or unexposed to BoNTA for masticatory muscle pain suggests that BoNTA does cause a consistently detectable decrease in trabecular bone density. The magnitude of risk, generalisability of findings to male patients and long-term clinical consequences of this effect should be evaluated in future larger studies.

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References

55. Sirin Y, Guven K, Horasan S, Sencan S. Diagnostic accuracy of cone beam computed tomography and conventional


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