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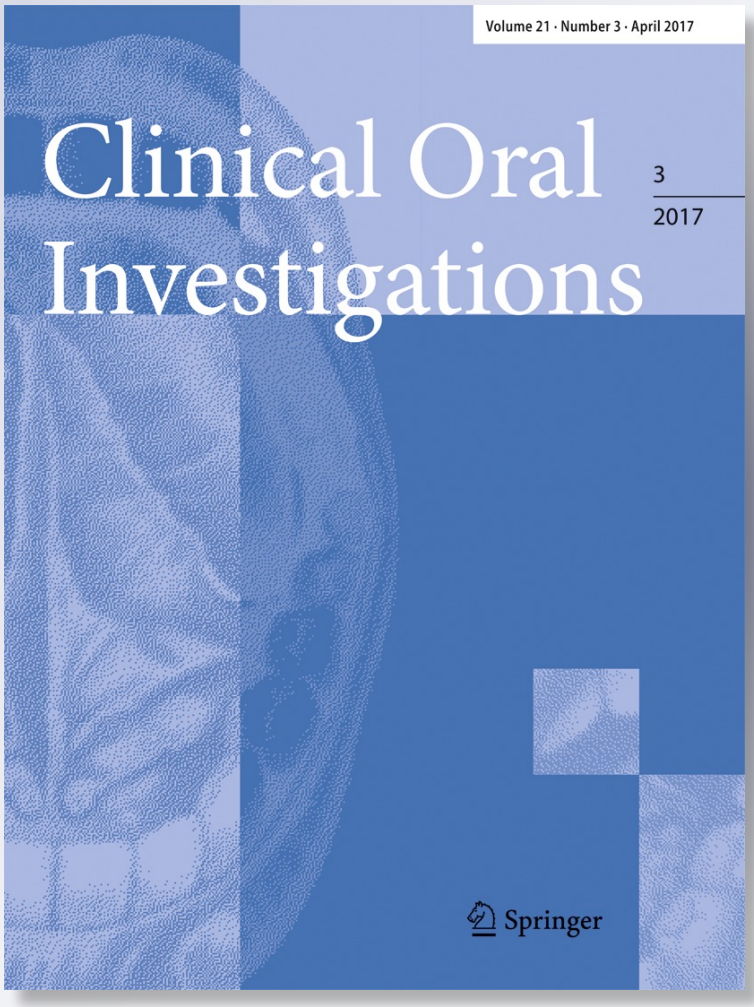

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ORIGINAL ARTICLE

Does Botulinum neurotoxin type A treatment for sialorrhea change oral health?

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Abstract
Objectives Botulinum neurotoxin type A (BNT-A) intrasalivary gland injections in patients with neurological disorders have been known to effectively treat hypersalivation. However, oral health can be compromised with increasing the dose. The aim of this study was to find out the therapeutic effect of low-dose, ultrasonography-controlled BNT-A injections into the bilateral parotid and submandibular glands on oral health in the treatment of sialorrhea.
Material and methods Twenty patients diagnosed with Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and other neurological disorders, including stroke or birth trauma, received BNT-A injections with salivary tests before and 1 month after the injections. Drooling was evaluated using subjective scales and objective assessment of salivary flow rate and oral health (salivary composition and cariogenic bacterial counts).
Results A significant decrease was found in salivary flow rate at 1- and 3-month follow-up in the BNT-A treated group. There was no significant change in salivary composition or cariogenic bacterial counts.
Conclusion BNT-A injections according to the current protocol can effectively manage sialorrhea while maintaining oral health.
Clinical relevance Oral health can be considered the mirror of general human health, as the cause of many diseases. Saliva plays a crucial role in protecting the oral cavity. The present study is of high clinical relevance because, although earlier research has proved the effect of Botulinum neurotoxin type A injections on reduction in saliva flow, data about the risks of the treatment method to the oral condition through affecting saliva composition has so far been missing.

Keywords Sialorrhea · Botulinum neurotoxin type A · Parkinson's disease · Amyotrophic lateral sclerosis · *Streptococcus mutans* · *Lactobacilli*

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Introduction

In the case of neurological diseases, children with e.g., cerebral palsy and adults with e.g., amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) often complain of drooling [1]. The prevalence of idiopathic PD in Estonia is 152 per 100,000 population which is the same when comparing the prevalence rates with other studies of Caucasian populations in Europe [2].

Sialorrhea is a frequent symptom in PD, occurring in almost 75 % of the patients [3]. Different authors report the proportion of drooling patients ranging between 10–58 % in cerebral palsy children [4]. About 50 % of ALS patients suffer from excess saliva [5].

Patients with hypersalivation are encouraged to periodically wipe up leaking saliva. Drooling is generally caused by difficulty with keeping saliva in the mouth, or with swallowing difficulty [1, 3–5], or with overproduction of saliva [6]. Drooling is more common in men than in women [6, 7], and it also appears to be more embarrassing for men. Drooling persons are at risk for aspiration of saliva, food, or

fluids into their lungs, which may cause further harm in case there is a problem with gagging and coughing [8].

According to clinical studies, Botulinum neurotoxin type A (BNT-A) injections into the salivary glands can effectively reduce drooling [9]. A change in the amount of saliva frequently leads to changes in salivary pH, oral flora, as well as in saliva characteristics. Salivary flow rate is related to oral pH, and a decrease in the amount of saliva could be linked with higher incidence of dental caries. Salivary pH and cariogenic bacterial counts are known to be associated with caries, while salivary *Streptococcus mutans* (*S. mutans*) and *Lactobacilli* play a key role [10]. Consequently, monitoring of the oral environment along with the saliva flow is particularly important in the management of patients with drooling.

S. mutans plays a major role in onset and progression of dental caries and is considered a primary cause of bacteriological caries. This bacterium occurs in plaque forming on the surface of teeth and produces acids that can dissolve tooth enamel which will eventually become cavitated. Presence of *S. mutans* is a strong indicator of high susceptibility to caries.

Although BNT-A can effectively treat sialorrhea, its effect on oral health characteristics, including the amount of cariogenic bacteria, is still unclear [4]. The aim of the present study was to establish if this treatment method is safe without compromising oral health.

Patients and methods

Participants

Twenty patients, 12 male and 8 female, with an average age of 63.15 years (ranging from 3 to 79 years), with profuse sialorrhea, were enrolled in the study. The patients were screened from October 2012 to October 2013 at Tartu University Hospital. The etiology of sialorrhea was caused by chronic neurodegenerative diseases, in 75 % of the cases and by other reasons in 15 % of the cases. In 12 cases, the patients had PD, and in three cases, they had ALS; in two cases, sialorrhea was caused by birth hypoxia; two patients had atypical headache, and one patient had experienced stroke with abnormal glutition. Basic medication for the disease was continued.

Treatment efficacy and safety were assessed at baseline, and at follow-up 1, 2, and 3 months after the BNT-A injections, using Item 2.2 from Part I (Non-Motor Aspects of Experiences of Daily Living) of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS, Appendix) [11] and side effects surveillance [3].

Medications known to influence severity of drooling were not allowed throughout the study. The patients, their carers, and their families were aware of the possible adverse effects and risks related to the study interventions.

Procedures of BNT-A injections into the salivary glands

All BNT-A injections into the submandibular and parotid glands were made by the same maxillofacial surgeon over a 1-year period from 2012 to 2013. The injections of BNT-A were performed with the application of local anesthesia with the ointment of lidocaine hydrochloride 5 % (Ung. Emla®). Placement of 27-gauge needles in the anteroposterior direction into each submandibular and parotid gland was done under ultrasound guidance. The 7.5 mHz linear transducer was positioned so that it was possible to perform an injection with the needle directed along the longitudinal axis of the transducer. This allowed quick visualization of the needle in the gland. The solution of 100 units of BNT-A (Dysport®) was prepared in a 2.5-mL volume of saline. The recommended dose for Botox® (Allergan) is weight-dependent: 1.4 U per 1 kg of body weight for injection to each parotid gland and 0.6 U/kg in each submandibular gland. The recommended Botox-A dose was tripled to obtain a relevant dose of Dysport®. The patients were followed up during 3 months after the injections.

Saliva collection

To measure the amount of stimulated saliva production, the patients chewed a piece of wax for 5 min before the collection of saliva, which accumulated in the oral cavity. Saliva was collected into a marked cup held close to the mouth.

Analysis of salivary composition

Testing of resting saliva consisted in a visual inspection of hydration level and saliva consistency, and pH measurements.

The level of hydration was visually assessed by guideline of Saliva-Check BUFFER in Vitro Test (GC EUROPE N.V. B-3001 Leuven, Belgium) by observing lower lip gland secretion under good light conditions [12]. The lower lip was everted, and the labial mucosa was gently blotted with a small piece of gauze. The time during which visible saliva droplets were forming at the orifices of the minor gland was assessed.

Testing of stimulated saliva consisted in assessment of the quantity and buffering capacity of saliva. For composition analysis, the Saliva-Check BUFFER in Vitro Test (GC EUROPE N.V. B-3001 Leuven, Belgium) was used.

Salivary cariogenic bacterial analysis

Salivary levels of the cariogenic bacteria *S. mutans* were measured using Dentocult *SM*, and *Lactobacilli* were measured by using Dentocult *LB* (Orion Diagnostica Co Ltd, Epsom, Finland).

For detection of *S. mutans* from a saliva sample and plaque, Orion Diagnostica's Dentocult SM Strip mutans was applied. The method makes use of a selective culture broth and is based

on the adherence and growth of *S. mutans* on the test strip [13]. *S. mutans* bacteria adhere to the rough area of the strip in proportion to their density in saliva/plaque. After incubation, the bacteria are visible as light to dark blue, raised colonies on the test strip.

Dentocult LB is a dipslide culture method for detecting aerobic aciduric bacteria (i.e., *Lactobacilli*) in stimulated saliva. The concentration of *Lactobacilli* in saliva represents a risk factor of caries [14]. Aciduric bacteria are seen as white to transparent colonies on modified Rogosa agar surface. Any negative test result should be confirmed. The dipslide should be examined sideways under bright light to detect colonies protruding from agar surface. Bacterial growth may consist of both large and small colonies. Colony density should be compared to a reference, using a model chart, irrespective of colony size. The medium also allows the growth of other oral aciduric organisms, including yeasts and *Streptococci* [15].

To avoid the effect of daily variation in *Lactobacilli* counts on the treatment result, it is recommended to take samples before noon. If this recommendation cannot be followed, samples from the same patient should be taken at the same time of the day during follow-up [16].

Prior to sampling, the patients did not eat, drink, smoke, or brush their teeth for 1–3 h. The incubation period for Dentocult *LB* and Dentocult *SM* was 96 and 48 h, respectively [17]. Dentocult LB test was placed in an incubator (36 ± 2 °C) for 4 days, and Dentocult SM test, for 2 days. To obtain colony count (CFU/ml), the slide was removed from the tube and colony density on agar surface was compared to the reference chart provided in the kit [16].

Statistical analysis

Data was analyzed with IBM SPSS Statistics V20 and expressed as estimates of mean, standard deviation, and median values. Differences between the study groups were compared with Paired samples *t* test, Wilcoxon matched pairs signed ranks test, and Fisher's exact test. *p* values <0.05 were considered statistically significant.

Approval of ethics

The study was approved by the Research Ethics Committee of the University of Tartu (Protocol No. 221/T-15). A signed informed consent was obtained from all patients.

Results

Almost all patients (19 out of 20, 95 %) reported a decrease in drooling after the injections. There was subjective improvement in sialorrhea at the first month, and improvements according to MDS-UPDRS [11] were recorded. A statistically significant decrease in the amount of saliva according to subjective assessment by the MDS-UPDRS scale Item 2.2 score from 3.7 (severe/moderate) to 2 (mild) was recorded in response to the question on the amount of saliva (Table 1; Appendix). The average numerical value of the amount of saliva according to the MDS-UPDRS scale was 3.7 before BNT-A treatment, 1.95 1 month after the injection; 1.93 2 months after the injection, and 1.5 3 months after the injection (Table 1; Appendix).

The amount of stimulated saliva also decreased in objective measurement after the BNT-A injections, and the length of resting saliva formation increased significantly when the baseline values were compared to drooling at 1 and 2 months after the injections. Buffering capacity increased, indicating improvement in salivary defense ability. There was no significant change in oral pH (Table 1), which is an evidence of preserved normal pH physiology.

All patients had saliva thickening around 2 months after the BNT-A injections. Drooling was very intensive at baseline; however, 1 month after the treatment the patients reported a decrease in drooling to a moderate level. One patient reported a decrease in drooling later, starting at 2 months after the injection. The change in saliva consistency is shown in Table 2: Before the BNT-A injections, the rate of watery clear saliva was the highest (90 % of the patients) and there was no case with sticky, frothy saliva. After the injections, the rate of frothy, bubbly saliva was the highest (65 % of the patients) and sticky, frothy saliva occurred in one case.

With significantly decreasing amounts of salivation and lengthening of salivation time, there was no statistically

Table 1 Comparison of salivary characteristics before and 1 month after the injections

Characteristic	Before injections	1 month after injections	*p*
Subjective evaluation of salivation (MDS-UPDRS Item 2.2 score)	3.7 (±0.6)	2.0 (±1.2)	0.001*
Resting saliva time (s)	20.7 (±15.1)	34.3 (±19.7)	0.002*
Stimulated saliva amount (ml)	7.1 (±5.6)	4.5 (±3.4)	0.006*
Oral pH	6.7 (±0.7)	6.8 (±0.7)	0.494
Buffering capacity (points)	6.5 (±3.2)	8.2 (±3.1)	0.082

* *p* is statistically significant

Table 2 Saliva consistency before and 1 month after the injection according to assessment of patients, $p = 0.004$

Saliva consistency	Before injection No. of patients (%)	1 month after the injection No. of patients (%)
Sticky frothy (increased viscosity)	0 (0 %)	1 (5 %)
Frothy bubbly (increased viscosity)	2 (10 %)	13 (65 %)
Watery clear (normal viscosity)	18 (90 %)	6 (30 %)

significant change in oral health characteristics (oral pH and buffering capacity) (Table 1). Comparison of different *Lactobacilli* and *S. mutans* colony-forming unit (CFU) count groups before and 1 month after BNT-A injections revealed a tendency of increase in the number of patients in the groups with higher microbe levels; however, the change was not statistically significant (Table 3).

The patients and their carers had to report adverse events at each evaluation. All participants reported no adverse events and tolerated the treatment well. There were no complaints of swelling or pain in the study group. One patient showed no response to the BNT-A injections, did not participate in the follow-up examination, and was excluded from the study.

Discussion

Although BNT-A can effectively treat sialorrhea, its effect on oral health characteristics, including the amount of cariogenic bacteria, is still unclear [4]. The present study demonstrated an effective control of sialorrhea with BNT-A without compromising oral health.

Intraglandular application of BNT-A controlled by high-resolution ultrasonography can provide reliable treatment of sialorrhea with favorable results [18, 19]. In our study, the bilateral parotid and submandibular glands served as treatment targets to optimize the therapeutic outcome of BNT-A intrasalivary gland injections. Suskind et al. [20] have reported favorable treatment outcome in 30 % of patients when injecting the submandibular gland alone, and favorable treatment outcome in 80 % of patients when injecting the bilateral submandibular and parotid glands. In several studies, the toxin was injected into the parotid gland, possibly owing to its more superficial location, easier access, and marked contribution to total saliva output [21]. However, in other studies, injections were performed solely into the submandibular glands, to avoid reduction in parotid output at the time of eating and drinking [8].

The flow rate, amount, pH, and buffering capacity of saliva are significant characteristics of oral health [22]. Saliva has a self-cleaning ability for teeth. Patients with neurological disorders are at a higher risk of dental decay [23]. The amount of saliva and its flow rate are highly important salivary characteristics associated with dental caries formation, especially in the elderly and in children, whose manual function is weaker in comparison with the healthy young adult population. Salivary pH can be considered an important factor of oral health as its lower level leads to caries, and higher level leads to dental calculus [24]. Buffering shows the capacity of saliva to respond to changes, in the oral cavity and to correct them. Buffering capacity and pH have direct impact on oral health.

Factors associated with caries have been addressed in several studies. When lower salivary flow rate and lower pH play

Table 3 Amount of bacteria before and 1 month after BNT-A injections

Amount of *S. mutans*	Before injections		1 month after injections		
Microbe count groups (CFU/ml)	Number of patients	Percentage	Number of patients	Percentage	*p*
0 (10^3)	3	15.0	2	10.5	0.8635 NS
1 (10^4)	3	15.0	1	5.3	0.7072 NS
2 (10^5)	10	50.0	13	68.4	0.6195 NS
3 (10^6)	4	20.0	3	15.8	0.8866 NS
Amount of Lactobacilli	Before injections		1 month after injections		
Microbe count groups (CFU/ml)	Number of patients	Percentage	Number of patients	Percentage	*p*
0 (10^3)	9	45.0	6	33.3	0.4612 NS
1 (10^4)	4	20.0	2	11.1	0.4524 NS
2 (10^5)	6	30.0	5	27.8	0.8813 NS
3 (10^6)	1	5.0	5	27.8	0.0544 NS

p is considered significant when <0.05

CFU colony-forming unit, *NS* not significant

the key role then *S. mutans* and *Lactobacilli* colony counts have also been associated with caries development [25]. Although presence of high numbers of *Lactobacilli* (10^5 CFU/ml or higher) indicates a caries-inducing environment, the role of *Lactobacilli* in caries initiation is limited [26]. According to literature data, colony counts of >10, 000 CFU/ml are considered high and counts of <1000 CFU/ml are considered low [15]. Carbohydrate intake [27] has been shown to correlate with salivary *Lactobacilli* count in the mouth but without sites for microbial retention [28]. High *Lactobacilli* counts can be linked with low salivary secretion rate, low salivary buffering capacity [29], and presence of glucose in saliva [30].

In the current study, the salivary flow rate decreased while salivary pH and the levels of *S. mutans* and *Lactobacilli* did not change significantly after the injections. The salivary flow rate, which is related to caries development, decreased but favorably. The other characteristics (including bacterial count) did not demonstrate changes.

Factors related to development of dental caries have been debated. In the present study, we failed to demonstrate any influence of decreased salivary flow rate either on salivary pH level or on the amount of cariogenic bacteria. Normally, saliva has a self-cleaning ability for teeth and a 2-month period of thickened saliva after the BNT-A injections might affect the oral health condition as thick saliva cannot clean dental plaque. Our findings showed a slight increase in the amount of the cariogenic bacteria after the injections. Although the change was not statistically significant, the possible risk for worsening of the microbiological status of the oral cavity should be considered and special attention should be paid to oral hygiene after injections.

Further studies should be made to inspect the saliva characteristics (e.g., bacterial count) during longer period of time after the BNT-A treatment.

As a secondary finding, the dental status of neurological patients was often poor already before the BNT-A injections, which is in concordance with other studies [31]. All elderly patients had partial *adentia*, and the main problem in all patients was dental calculus and chronic marginal periodontitis. Although the oral health of neurologically disabled people has not been investigated, it is an important aspect of general health.

Patients receiving BNT-A injections must remain under a careful supervision of a dentist or a dental hygienist. These patients need instructions on how to take care of their oral hygiene. As elderly and neurological patients also have problems with manual coordination and movements, brushing may be insufficient. They should be encouraged to use electrical toothbrushes [32, 33] combined with mouth rinsing with chlorhexidine [34].

Our findings suggest that BNT-A is an effective method to treat sialorrhea in patients with neurological diseases, which provides a favorable therapeutic effect without compromising normal salivary composition and microflora. We demonstrated that sonography-controlled percutaneous intrasalivary BNT-A bilateral injections into the parotid and submandibular glands can effectively reduce drooling while maintaining oral health. Patients and their caregivers need instructions on how to take care of oral hygiene, especially in the first month after BNT-A injections when the effect of treatment on sialorrhea is maximal.

Authors roles The study was conceived and designed by Janne Tiigimäe-Saar, Pille Taba, and Tiia Tamme. Acquisition, analysis, and interpretation of the data were performed by Janne Tiigimäe-Saar. Critical revision of the manuscript was made by Pille Taba and Tiia Tamme.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. (State Agency of Medicines reference number is 15-024. Research Ethics Committee of the University of Tartu Protocol nr is 247/M-1.)

Informed consent Informed consent was obtained from all individual participants included in the study.

Appendix

Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) (Goetz et al., 2008).

Part I: non-motor aspects of experiences of daily living Item 2.2: SALIVA & DROOLING: Over the past week, have you usually had too much saliva during when you are awake or when you sleep?

0: Normal: Not at all (no problems).

1: Slight: I have too much saliva, but do not drool.

2: Mild: I have some drooling during sleep, but none when I am awake.

3: Moderate: I have some drooling when I am awake, but I usually do not need tissues or a handkerchief.

4: Severe: I have so much drooling that I regularly need to use tissues or a handkerchief to protect my clothes.

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