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Article I: Clinical evaluation and treatment of patients. Study design, data collections and analysis. Writing the original text of the manuscript.

Article II: Clinical evaluation and treatment of patients. Study design, data collections and analysis. Writing the original text of the manuscript.

Article III: Clinical evaluation and treatment of patients. Study design, data collections and analysis. Writing the original text of the manuscript.

ABBREVATION

ACHE	acetylcholine
ALS	amyotrophic lateral sclerosis
BNT	Botulinum neurotoxin
BNT-A	Botulinum neurotoxin A
CFU	colony forming unit
CP	cerebral palsy
CT	computed tomography
EEG	electroencephalography
MAO-B	Monoamine oxydase B
MDS-UPDRS	Movement Disorders Society – Unified Parkinson's Disease Rating Scale
MRI	magnetic resonance imaging
ml	milliliter
PD	Parkinson's disease
PIGD	postural instability and gait disorder
SD	standard deviation
U	unit

1. INTRODUCTION

Intrasalivary gland injections of Botulinum neurotoxin type A (BNT-A) are effective in the treatment of drooling in patients with neurological disorders (Şen *et al*., 2015; Jost, 2016; Restivo *et al*., 2018). Drooling is a disruption of the co-ordinated control mechanism of orofacial and palatolingual musculature leading to excessive pooling of saliva in the anterior of the mouth resulting int the inadequate drainage of saliva from the mouth (Hussein *et al*., 1998; Basciani *et al*., 2011). Sialorrhea is a common symptom of different neurological disorders, such as cerebral palsy (CP) in children (Gonzalez-L *et al*., 2017) and amyotrophic lateral sclerosis (ALS) (Benson *et al*., 2007) and Parkinson's disease (PD) in adults (Odachi *et al*., 2017). In these neurological conditions, sialorrhea is usually associated with incomplete lip-closure, tongue protrusion, slow and stiff tongue movement, lack of voluntary control of jaw and tongue, and oral dyskinesia (Tahmassebi *et al*., 2003; McGeachan *et al*., 2017). Sialorrhea may also be caused by intraoral sensitivity disorder next to oral motor dysfunction and dysphagia (Dias *et al*., 2016). Furthermore, it may be a side-effect of treatments that increase the action of acetylcholine in the body (Layton *et al*., 2014).

Fiberoptic endoscopic evaluation of swallowing is suggested in the early stage of sialorrhea to evaluate dysphagia, the risk of aspiration and also to collect important data to help with therapy selection (Warnecke *et al*., 2010; Steffen *et al*., 2013).

BNT injections are a valuable treatment for many therapeutic indications: treatment of spasticity and dystonia, sphincter disturbances, paralysis of facial nerves, facial spasms, blefarospasm, strabismus, atypical headaches, bruxism, "gummy smile," Frey's syndrome, allergic rhinitis, excessive tear flow, etc. (Brin, 2009; Majid, 2010; do Nascimento Remigio *et al*., 2015; Rallmann *et al*., 2016; Zhang *et al*., 2017; Al-Fouzan *et al*., 2017). Treatments for sialorrhea include also salivary gland botulinum toxin next to anticholinergic drugs, surgery, salivary gland-targeted radiotherapy, and rehabilitation (McGeachan *et al*., 2017; Chaléat-Valayer *et al*., 2016). Although BNT-A can effectively treat sialorrhoea, its effect on salivary characteristics, including the amount of cariogenic bacteria, was not investigated.

2. REVIEW OF THE LITERATURE

2.1. Neurological disorders that cause sialorrhea

2.1.1. Parkinson's disease

PD is a slowly progressive neurodegenerative movement disorder, with tremor, rigidity, and bradykinesia as its main motor symptoms. The onset of PD usually occurs after the age of fifty, and its prevalence increases with age. Its pathophysiological mechanisms are related to the degeneration of dopaminergic neurons in the substantia nigra of the brain (Poewe *et al*., 2017). There are multiple pathways of pathogenesis in PD: α-synucleinproteostasis, mitochondrial function, oxidative stress, calcium homeostasis, axonal transport, and neuroinflammation (Poewe *et al*., 2017). It has been speculated that the cause of PD could be external, such as microbial, which could trigger the pathologic process in the corresponding organs, subsequently spreading to the central nervous system (Pereira *et al*., 2017).

The most common manifestations of PD are: resting tremor, slow reactions and responses, gait instability and short scuffy steps, lack of facial expression, a reduced blinking rate, a quiet monotone voice, dysartria, dysphagia, and sialorrhea (Kadastik-Eerme *et al*., 2016; Poewe *et al*., 2017). Hyposmia, rigidity, and involuntary jaw movements with orofacial pain may occur in PD patients. (Bakke *et al*., 2011; Adler *et al*., 2016). Among the non-motor symptoms of PD, sialorrhea is one of the most disabling and embarrassing for patients (Marras *et al*., 2016; Schirinzi *et al*., 2017).

According to the Hoehn and Yahr scale, PD is divided into five stages (Hoehn and Yahr, 1967). Stage 1 means mild disease and only one side of the body is affected, usually with minimal or no functional impairment. Stage 2 affects both sides of the body but posture and balance remain normal. In stage 3 both sides of the body are affected and there is some imbalance when standing or walking; it is also called moderate disease. Stage 4 is classified as advanced disease and affects both sides of the body with standing or walking problems requiring constant help and caregiving at home. Stage 5 is fully developed severe disease with restriction to a bed or wheelchair.

The pharmacotherapy of Parkinson's disease includes levodopa, dopamine agonists, inhibitors of monoamine oxidase (MAO-B) and catechol-o-methyltransferase (COMT), and amantadine (Oertel *et al*., 2016). Later in the course of the disease, motor complications may appear. The standard medication of PD is levodopa, which stimulates the remaining neurons in the basal ganglia and modifies into dopamine, thereby promoting synaptic transmission and improving function. Depending on the stage, different oral formulations of levodopa or dopamine agonists with a long half-life, administered through transdermal application or parenteral pumps for continuous drug supply, are determined (Taba *et al*., 2008). Levodopa's effect decreases with time, and after 5–10 years

of treatment at least half of the patients become partially unresponsive to the medication. There is still a degree of clinical uncertainty about when to initiate levodopa (Giladi *et al*., 2016). A combination of different drugs is commonly used. Dopamine receptor agonists are commonly used as first-line monotherapy for symptomatic control in the early stages of PD to delay the initiation of levodopa therapy. Also, it is used as an adjunctive therapy to levodopa when the disease progresses (Giladi *et al*., 2016). Treatment can lead to severe side effects, including dyskinesia of the head, face, and tongue, xerostomia, and dental attrition (Poewe *et al*., 2017).

There is evidence that drooling relates to PD pharmacotherapy and the stage of the PD (Bagheri *et al*., 1999; Tumilasci *et al*., 2006; Fereshtehnejad *et al*., 2017). Usually, there is a bigger problem with hyposalivation and xerostomia instead of excessive saliva flow in elderly patients and in PD patients. Approximately 30% of those aged sixty-five years and older are affected with hyposalivation, which is a consequence of systemic diseases, medications, and head and neck radiotherapy (Gupta *et al*., 2006). Usually, hyposalivation poses a higher risk for normal oral function and health than hypersalivation (Gupta *et al*., 2006). In PD patients, drooling is a delayed complaint and generally affects men twice as often as women (Postuma *et al*., 2015). Diurnal and nocturnal drooling are differentiated. Diurnal drooling is a complaint in about 28% of PD patients. It typically appears later in the disease's course and is associated with involuntary mouth opening and swallowing dysfunction due to weak musculature (Nutt, 2016). Nocturnal drooling appears with or without diurnal drooling in 58% of PD patients (Zlotnik *et al*., 2015). Drooling is treated with pharmacotherapy, including intraglandular BNT-A injections (Bruno *et al*., 2016).

2.1.2. Amyotrophic lateral sclerosis

ALS is a progressive neurodegenerative motor neuron disease with a terminal outcome with an incidence of ~2.16 per 100,000 person-years (Riva *et al*., 2016). Its prevalence is lowest in younger people (0.5 per 100,000 in those between 18 and 39 years old), and highest (20.0) among persons aged 70–79 years. The ratio of cases in males to females was 1.7:1 (Mehta *et al*., 2018). The mechanism leading to neurodegeneration of motor neurons is not fully understood. Loss of different protein function in genes (like C9orf72) and RNA toxicity with RNA binding protein sequestration are potential reasons for the disease's generation. The disease causes the death of neurons which control voluntary muscles. It causes progressive muscular weakness, leading to death by respiratory insufficiency within three to five years (Riva *et al*., 2016). Malnutrition is also a risk factor for death in ALS. No effective curative therapies are available. Only one drug (riluzole) was approved in recent years to treat ALS; in 2017 a second drug, edaravone was also approved (Mehta *et al*.,

2018). However, these can only prolong life by a matter of months. Thus, symptomatic and palliative care are usually implemented.

ALS phenotypes vary depending on the involvement of upper or lower motor neurons, the site of onset, and the rate of progression. ALS is characterized by rigid muscles, muscle twitching, and gradual weakening due to muscle atrophy. This results in impaired speech, dysphagia, and eventually breathing problems (Riva *et al*., 2016). Drooling is one of the autonomic symptoms in ALS. About 50% of patients with ALS have significant disorders in the control of saliva (Jackson *et al*., 2015). ALS may cause dysphagia, as well as limb weakness, dysarthria, respiratory failure, and emotional lability (Young *et al*., 2011). There is an increased risk of aspiration pneumonia due to increased mucous secretions in the throat and lungs, the inability to swallow saliva, and an impaired coughing reflex as a result of weakness and diaphragm and respiratory muscle fatigue (Banfi *et al*., 2015). Patients with ALS often have problems with thick and thin secretions coexistence (McGeachan *et al*., 2016). Sialorrhea is often treated with pharmacotherapy, including intraglandular BNT-A injections (Manrique, 2005; Jackson *et al*., 2015).

2.1.3. Infantile cerebral palsy

Cerebral palsy (CP) is a leading cause of physical disability in children with motor disorder of variable manifestations. The prevalence of CP is approximately 1.5 to 2.5 per 1,000 live births (Odding *et al*., 2006; Reid *et al*., 2012). Children may also have sensory dysfunction (seeing and hearing), cognitive and behavioral deficits, and dysfunctional oral motor control. CP leads to abnormal movements of the tongue and facial muscles (Liu *et al*., 2014). While drooling is usually considered abnormal in children over four years of age, it has been estimated to occur in approximately 10% to 37% of children with CP (Reid *et al*., 2012). Finding a solution to this problem is needed in order to simplify caregivers' work, because in many CP cases children are bedridden and it is necessary to periodically wipe up leaking saliva or aspirate excess saliva from the pharynx. Sialorrhea is generally caused by difficulty keeping saliva in the mouth, by swallowing difficulty, or by overproduction of saliva. Patients with drooling are at risk of aspirating saliva, food, or fluids into their lungs, which may result in aspiration pneumonia and breathing difficulties if there is a problem with gagging and coughing (Jongerius *et al*., 2004; Chaléat-Valayer *et al*., 2016; van Hulst *et al*., 2018). Inadequate swallowing and lip closure are the main causes of drooling in cases of mental retardation and CP (van Hulst *et al*., 2018). If the saliva is not swallowed, a large amount of saliva can be drooled from the mouth.

Oral health is more compromised in mentally disabled children (Radha *et al*., 2016; Jan *et al*., 2016). The oral health status of children with CP was not significantly different from that of normally developing children but the oral health-related quality of life of children with CP was significantly lower than

that of normally developing children (El Ashiry *et al*., 2016). Children with neurological disorders are at increased risk of induced pain. Bourseul *et al*. (2016) conducted a study on pain during daily care activities in children with motor disabilities and found that one of the most frequent painful activities was mouth care. Children with CP usually need dental treatment under general anesthesia or sedation (conscious or deep) carried out in a hospital depending on the severity of the main disease (Corcuera-Flores *et al*., 2014). CP children require extra help and rely on their parents or guardians to achieve and maintain good dental and oral hygiene (Liu *et al*., 2014).

2.2. Saliva

Saliva is a secretory fluid responsible for the lubrication of the alimentary tract with a mucin-rich film, making chewing and swallowing possible by moistening and helping with taste and digestion. It also protects the teeth and the oral and esophagus mucosa against viruses, bacteria, and fungi (Proctor *et al*., 2018). It cleans teeth regularly, reduces bacteria, acts as buffer medium, and protects hard tissue against acid damage after sugar intake (Llena-Puy, 2006; Proctor *et al*., 2018). The buffer medium in saliva remineralizes the enamel of teeth and stimulates wound healing. Saliva also helps in the regulation of oesophageal acidity and protects the upper respiratory tract from regurgitated gastric acid-induced infections. It is also important for speech articulation (Llena-Puy, 2006).

Saliva is produced by three major paired glands (parotid, submandibular, and sublingual glands) and by minor glands located all over the oral and pharyngeal mucosa. 90% of the total saliva is produced by the major glands and 10% by the minor glands. The parotid gland is the largest in size but the submandibular gland produces the most saliva (Bradley *et al*., 2011). About 70% of high-viscosity resting saliva is produced by the submandibular glands; the parotid and sublingual glands produce about 25% and 5% of secretions, respectively. The parotid glands are responsible for producing great amounts of watery enzyme (amylase) containing saliva while eating and drinking (Fejerskov *et al*., 2008). The minor glands prevent the feeling of dry mouth, and secrete mucin-rich saliva (Bradley *et al*., 2011).

Saliva contains many components such as calcium, phosphorus, proteins, enzymes, and bicarbonates. Saliva's most important function is to bathe the teeth in a supersaturated solution of calcium and phosphorus so that tooth enamel is constantly exposed, leading to the replacement of any loss of tooth structure due to demineralization. Salivary secretion is regulated and controlled by both the parasympathetic and sympathetic autonomic nervous systems; both nerve stimulations lead to increased saliva production, but the salivary composition is different. During the mastication process, parasympathetic stimulation produces a large volume of low-protein saliva, but in the case of stress and high activity, the sympathetic stimulation produces a smaller volume of

protein-rich saliva. Higher salivary calcium may act as a risk factor for periodontal disease through the threat of raised dental plaque mineralisation (Gupta *et al*., 2016). Compared to age-matched control groups, PD patients have a higher concentration of salivary sodium, chloride, and amylase (Tumilasci *et al*., 2006).

Salivary flow rate is an indicator that shows the amount of saliva produced by salivary glands in a span of time (mL/min). This is divided into stimulated salivary flow rate and unstimulated salivary flow rate. Stimulated saliva is secreted due to gustatory and masticatory sensory stimulation. Unstimulated saliva does not depend on the presence of such stimuli as food or chewing. Saliva is also divided into "duct" and "whole" saliva (Proctor *et al*., 2018). Whole saliva consists of duct saliva and as well as secretions of oral, nasal and pharynx mucosa. It also contains food derbis, microorganisms, desquamated epithelial, and blood cells. The daily production of whole saliva is normally 0.5–1 liters (Fejerskov *et al*., 2008; van Hulst *et al*., 2018). Individuals with deviations in salivary flow rate, consistency, and composition are predisposed to a higher risk of oral diseases (Radha *et al*., 2016). Saliva flushes plaque and bacteria from oral mucosal and dental surfaces.

Saliva amount, flow rate, pH, and buffering capacity are significant aspects of oral health (Gopinath *et al*., 2006). Salivary flow pH and buffering capacity are factors that influence the risk of dental caries (Liu *et al*., 2014).

Saliva pH depends on the secretion rate. Normal salivary pH in a healthy individual is about 6.3, varying between 6.0 and 7.5. Most alkaline values are obtained under stimulated flow conditions (Fejerskov *et al*., 2008). When the pH of saliva decreases below 5.5, demineralization usually follows. Saliva functions to buffer the pH of the oral environment to prevent it from becoming too acidic. Buffer capacity is the ability of saliva to protect and repair oral mucosa and dental remineralization. This depends on the saliva's acids and bases. Lower levels of salivary pH lead to caries and higher levels lead to dental calculus (Sharma *et al*., 2012). Buffering capacity indicates how quickly oral pH can reach its normal value after a meal. Saliva buffering capacity neutralizes acids present in the mouth and maintains the mouth's usual pH after a meal. Salivary buffering capacity protects the teeth from dental caries (El-kwatehy *et al*., 2016). Individuals with a high salivary buffer capacity are often caries-resistant (El-kwatehy *et al*., 2016).

Salivary buffering capacity can be attributed to several systems such as the phosphate system and the carbonic acid/bicarbonate system. The main bicarbonate buffer is effective only at high salivary flow rates. When the acid content of saliva increases, the concentration of hydrogen ions also increases and thereby lowers the pH. The enzyme carbonic anhydrase found in saliva catalyzes the reaction between the free hydrogen ions from the acid and the bicarbonate ions. Water and carbon dioxide gas are the end products of this reaction which is released from the oral cavity. As more free hydrogen ions interact with bicarbonate ions, the pH begins to rise and the saliva begins to return to normal pH levels. The buffering capacity of saliva varies from person

to person. Salivary flow designates the capacity of saliva to buffer against acid. The greater the salivary flow is, the more bicarbonate ions are available to combine with free hydrogen ions. When acids get into the oral cavity, salivary flow is stimulated and increases within minutes.

Normal salivary flow rates are generally 0.1–0.6 mL per minute. A salivary flow rate of less than 0.1 mL per minute is considered low. Without the protective buffering capacity of saliva, enamel would be demineralized and lost (Perkins *et al*., 2001). However, this buffering capacity of saliva is limited and can be overloaded by frequent or long-term exposure to acids. Reduction in salivary flow and increase in calcium and phosphate concentration in composition promotes calculus formation and periodontal disease (Gupta *et al*., 2016). Salivary flow rate and pH are significantly lower in patients with oral lesions compared to those without oral lesions (Foglio-Bonda *et al*., 2017). Basal salivary flow rate is measured by collecting unstimulated "whole saliva" (Proctor *et al*., 2018). Whole saliva stays in the mouth fourteen hours per day and protects oral tissues. Stimulated salivary flow rate shows the secretion during eating and also two hours after food intake. Unstimulated whole saliva salivary flow rate is considered to be a more clinically reliable parameter than stimulated salivary flow rate. Many diseases can alter oral homeostasis. From earlier studies "risk patients" for saliva change are detected. Lichen and leukoplakia in oral cavity reduces the unstimulated whole saliva salivary flow rate values. It is also known that antihypertensive drugs decrease oral pH. Furthermore, cancer or liver disease decrease the unstimulated whole saliva salivary flow rate (Foglio-Bonda *et al*., 2017).

Low salivary flow (resting time saliva formation < 60 sec. and amount of saliva collected in 5 min. < 3.5 mL), highly acidic pH (5.0–5.8), and lower values of buffering capacity (0–5) increase the risk of caries.

2.3. Sialorrhea

Sialorrhea is defined as the inability to control oral secretions, resulting in excessive saliva accumulation in the oropharynx. Sialorrhea, also called "drooling," "dribbling," and "drivelling" is the involuntary escape of saliva from the mouth. In the literature, sialorrhea, ptyalism and hypersalivation are synonymous. To avoid confusion, it is important to distinguish drooling from hypersalivation. Hypersalivation is overproduction of saliva and it is swallowed without problems. Drooling is normal in growing children up to the age of eighteen months (Erasmus *et al*., 2011; van Hulst *et al*., 2018). Pathological drooling can be a primary symptom but this condition is rare and usually not associated with neurological disorders (Hussein *et al*., 1998; van Hulst *et al*., 2018). Cardona *et al*., (2015) reported that in the pediatric population the parotid and submandibular salivary glands do not differ in size between children with or without drooling. Drooling as a secondary symptom is caused by different neurological disorders like ALS, CP, and PD.

Dysregulation of swallowing leads to dysphagia and drooling. Sialorrhea is divided into “anterior” and “posterior drooling.” Anterior drooling is the unintentional loss of saliva from the mouth leading to negative psychosocial and physical consequences. It can produce perioral infections and impaired dentition. Posterior drooling is a pharyngeal problem, wherein saliva flows over the tongue through the oropharyngeal isthmus and increases the risk of saliva aspiration to the lungs. Aspiration in children with CP usually occurs silently (without clear coughing or choking) and chronic aspiration of saliva might lead to significant lung injury.

2.4. Different options for sialorrhea treatment

Sialorrhea treatment includes anticholinergic drugs, salivary gland botulinum toxin, surgery, salivary gland-targeted radiotherapy, and rehabilitation (McGeachan *et al*., 2017; Chaléat-Valayer *et al*., 2016).

2.4.1. Pharmacotherapy

Anticholinergic medications reduce secretions in the mouth, throat, airway, and stomach. They work by blocking the activity of acetylcholine in the body, which decreases secretions (Banfi *et al*., 2015; Harms *et al*., 2009). (For example glycopyrrolate is used in 1–2mg doses 3 times/day; atropine eye drops: 1–2 drops 4–6 times/day; amitriptyline: 25–50mg at bedtime; scopalamine transdermal patch: 0.5 mg every 3 days; hyoscyamine sulfate: 0.125–0.25 mg tablet or elixir 4 or 6 times/day; and diphenhydramine: 25–50 mg 3 times/day). Oral medication like glycopyrrolate and topical agents are also used, but the effect is quite variable (Layton *et al*., 2014). Intraoral tropicamide films offer short-term relief from sialorrhea (Lakraj *et al*., 2013). Anticholinergic drugs cause a thickening of mucous secretion in the throat and lungs, which is a dangerous complication. Scopolamine skin patches may result in pupillary dilatation, skin reactions, and urinary retention as adverse side effects. Anticholinergic drugs are contraindicated in the presence of heart diseases, glaucoma, pyloric stenosis, prostatic hypertrophy, and hepatic or renal insufficiency (Banfi *et al*., 2015; Lakraj *et al*., 2013).

Antireflux medications also are suggested for use in the case of drooling (Lakraj *et al*., 2013).

A novel option in the treatment of advanced PD drooling is rotigotine patches, a non-ergolinic dopamine agonist with continuous transdermal delivery. Schirinzi *et al*. (2017) observed that rotigotine 4mg/24h dosage significantly improved drooling.

2.4.2. Botulinum neurotoxin A treatment

BNT-A injections are suggested as a first-choice treatment for adult sialorrhea (Shetty *et al*., 2006). BNT-A injections are less invasive than surgical treatment (i.e. duct ligation of the salivary gland). Different studies have used several BNT serotypes (A and B), treatment regimens, and routes of administration (direct or transductal approach) with varying outcomes (Banfi *et al*., 2015). In the treatment of sialorrhea, the administration of BNT into salivary glands is the most effective way to control saliva flow (Meece *et al*., 2010; Lakraj *et al*., 2013). BNT-A injections are mostly needed in cases of neurological disorders (Manrique, *et al*., 2005). The most frequently treated diseases are infant CP (30%), PD (20%), and ALS (15%) (Møller *et al*., 2011; Fuster-Torres *et al*., 2007; Møller *et al*., 2015). BNT-A injections are effective in about 50% of ALS-conditioned sialorrhea cases. However, for many patients, the treatment is not enough to decrease salivary flow (Verma *et al*., 2006). BNT-A injections have also demonstrated good results in the treatment of recurrent parotitis, i.e. in Sjörgen syndrome (O'Neil LM *et al*., 2016).

Researchers have different priorities in their choice of which salivary glands should be targeted for treatment. Over half of them injected BNT-A into the parotid glands, 9.5% into the submandibular glands, and 38% into both (Fuster-Torres *et al*., 2007). The BNT-A injection procedure can be done blindly or under ultrasound guidance. As a complication, BNT-A injection may damage muscles, arteries, or nerves around the salivary glands, or muscle weakness may result in an improper BNT-A injection. The injection can also cause weakness in adjacent muscles, resulting in swallowing or mastication difficulties. There is a higher failure rate when injecting the submandibular glands blindly compared with the parotid glands. To reduce the complication rate, ultrasound guidance is suggested (So *et al*., 2017). The safest way to inject BNT-A blindly, depending on the anatomical landmarks, is to inject at the point that is 20%–35% from the mandible angle on the inferior view and 1.5 cm below the inferior line of the mandible on the lateral view. The needle should be inserted to a depth of 2.0 cm from the skin surface (Lee *et al*., 2010).

2.4.2.1. Botulinum neurotoxin

BNT is produced by the anaerobic bacterium *Clostridium botulinum*. The mechanism of the BNT is based on targeting the release of acetylcholine from cholinergic nerve endings, thereby causing paralysis of muscles and/or glands (Awan, 2017). There are seven different serotypes of BNT (BNT-A, -B, -C, -D, -E, -F, and -G) (Orsini *et al*., 2015). Only the A and B serotypes are available as drugs and used for sialorrhea treatment (Bentivoglio *et al*., 2015). The toxin is isolated, purified, and stabilized in order to be used as a drug. Acetylcholine is a neural transmitter that stimulates muscles and the secretion of glands. The toxin's action leads to the blockade of the cholinergic nerves. BNT diffuses into

human tissue and binds irreversibly to the presynaptic terminal of the neuromuscular or neuroglandular junction and, by cleaving specific membrane proteins, causes acetylcholine excretion. BNT action does not occur immediately. The maximum effect can be seen after a few weeks, and the duration of the effect varies from 2–6 months (de Maio *et al*., 2007). The effect of BNT slowly decreases over time as the affected axons grow new nerve terminals and restore the impaired transmission leading to damaged synapse regeneration (de Maio *et al*., 2007).

There are three main BNT-A products available: onabotulinum toxin-A (Botox®), abobotulinum toxin-A (Dysport®), and incobotulinum toxin-A (Xeomin®) (Jost *et al*., 2015; Scaglione, 2016). These toxin brands vary in their specific activity, packaging, constituents, excipient, and storage. Dysport® has the best cost-efficacy profile (Scaglione, 2016). The concept of calculating the dosage units for the different products is not clearly understood. The dosage units of different products of Botox® and Dysport® do not relate to each other. The ratio fluctuates from 1:1 to as high as 1:11 (Scaglione, 2016). For Botox® and Dysport®, the most commonly used ratio is close to 1:2.5–1:3, based on the available data from placebo-controlled clinical trials. Xeomin® has a 1:1 ratio to Botox®, according to the manufacturer (Scaglione, 2016). Different products also have a distinct molecular weight, which is thought to affect the toxin's spread, but this has not been proven. The toxin's spread to areas away from the injection site could be undesirable because this may increase the risk of adverse effects. Botox® with the highest complex size of 900 kD is the least diffusible, whereas Xeomin® with the 150-kD neurotoxin is the most diffusible. The standard dilution for Botox® and Dysport® is 2.5 ml (de Maio *et al*., 2007).

2.4.2.2. Dosages of BNT-A in the treatment of drooling

The recommended total dosage of BNT-A injections varies from 10–100 U of Botox® (onabotulinum toxin-A) or 30–450 U of Dysport® (abobotulinum toxin-A), according to different authors (Fuster Torres *et al*., 2007). Reduced production of saliva was observed following these injections, and the duration of the therapeutic effect was 1.5–6 months (Fuster Torres *et al*., 2007). The total dose of onabotulinum toxin-A (Botox®) recommended for salivary gland injections varies from 60 to 250 U. 20 to 100 U is diluted in 0.4 mL of saline solution and injected into two sites in each parotid gland and 20 to 25 U is diluted in 0.1 mL of saline solution and injected into a single site in each submandibular gland (Porta *et al*., 2001; Breheret *et al*., 2011; Banfi *et al*., 2015). Treatment with lower doses of the onabotulinum toxin-A, with 20 U into each parotid gland and 10 U into each submandibular gland, have a proven 90% success rate (Porta *et al*., 2001). It is suggested to start with lower doses, for instance 20 U per submandibular and parotid gland bilaterally (total dosage of 80 U), and raise the dose to 30 U per gland in cases of reinjection (Moller *et al*., 2015). There is also a scheme in use with a BNT-A dosage of 0.5 U/kg of

onabotulinum toxin-A (Botox®) injected into each submandibular and parotid gland under ultrasonographic guidance in children with sialorrhea, which is effective in 70.5% of cases (Jeung *et al*., 2012). Some authors prefer to inject only submandibular glands and use a total dosage of 30 U of BNT-A (Shetty *et al*., 2006). Suggested abobotulinum toxin-A (Dysport®, Ipsen Ltd., Slough, UK) doses in cases of child sialorrhea to submandibular glands are plotted according to the scheme of 1 U/kg/gland (Sidebottom *et al*., 2013). The study with the total dosage of 100 U incobotulinum toxin-A did not give the desired result of reducing salivation (Narayanaswami *et al*., 2016).

2.4.2.3. Changes in gland tissue parenchyma after BNT-A reinjections

Salivary gland atrophy or apoptosis have to be taken into consideration after recurrent injections. The anteroposterior distance of a gland is reduced significantly after BNT-A injections compared to the control group (Cardona *et al*., 2015). The first human study on salivary gland histological findings after BNT-A treatment showed no significant difference in the number of acinar cells by surface area (Mosseri *et al*., 2017).

2.4.2.4. Complications of BNT-A injections

Overall, BNT-A injections are considered to be a safe treatment option with only minor side effects. However, there are studies describing dysphagia, choking, and breathing difficulties with nocturnal coughing due to thickened saliva as a complication after BNT-A injection (Layton *et al*., 2014). Some patients complain about reduction in saliva viscosity (12.2%), swallowing difficulties (3.1%), temporarily deteriorated feeding behavior (6.1%), and xerostomia (1.7%) (Scheffer *et al*., 2010). There are some suggested measures for treating swallowing difficulties due to thickened saliva: pineapple juice as a lytic agent, cough assistance, saline nebulisers, and suctioning or mucolytic drugs like carbocisteine (McGeachan, 2017). The most frequent complications are weakness of adjacent muscles, leading to difficulty swallowing due to an incorrect injection of the toxin into the masseter or pharyngeal muscles. Other risks include hematoma and injury to the carotid arteries or damage to the facial nerve (So, 2017). A high number of postinjection complications (in about 23 cases out of 69) is also described, including aspiration pneumonia, dysphagia, and a decrease in head mobility control in children after BNT-A treatment. Eight of them where seriously manifested, requiring hospitalization in five cases and insertion of a nasogastric tube in two cases (Chan *et al*., 2013).

BNT-B is used for the treatment of drooling as well but the body's formation of neutralizing antibodies against BNT-B appears to be an important obstacle. BNT-B gives rise to non-neutralizing antibodies that also cross-react with BNT-A (Berweck *et al*., 2007). BNT reinjections in less than three months is a

contraindication as the neutralizing antibodies become evident in such cases (Orsini *et al*., 2015).

BNT treatment is contraindicated in cases of some relevant neuromuscular disorders (like neuromuscular junction diseases, including autoimmune acquired myasthenia gravis and Eaton-Lambert syndrome). It is not recommended when social and psychological support is missing (Orsini *et al*., 2015).

According to the study of Erasmus *et al*. (2011) 93 children were full responders to BNT-A injections while 33 children were unresponsive.

Nevertheless, despite the positive results with BNT-A injections, there remains a threat of compromised oral health through the changes in saliva.

2.4.2.5. Sialorrhea treatment in childhood

Approximately 13% cases of drooling are successfully treated in normally developed children while in children with slower development, the success rate is 18%. In early childhood, hypersalivation is considered to be a normal condition, but in older children (after 18 months) it may cause social problems (Montgomery *et al*., 2016).

Treatment with BNT-A is an invasive procedure and usually requires a general anaesthetic or sedation in children, depending on their anticipated ability to tolerate the procedure. Generally, the injections are performed by oral surgeons who locate the glands by palpation or under ultrasound guidance. With BNT-A intrasalivary gland injections to treat sialorrhea in neurologically dysfunctional children, around 60.4% of cases showed good results, while approximately 20.8% had moderate results, and 18.8% showed no improvement (Mahadevan *et al*., 2016). The effect of BNT-A is not related to the severity of the disability. Children with severe neurological dysfunction respond to BNT-A injections as effectively as their less impaired peers (Mahadevan *et al*., 2016).

Improved oral hygiene, including reduction in halitosis, reduced perioral dermatitis and improved speech and feeding, is a secondary benefit of the BNT-A injection. These effects were gone after eight months when BNT-A's impact wore off (Scheffer *et al*., 2010). A contraverse study showed that in the span of ten years, up to 30 percent of the CP children's drooling severity and frequency did not change significantly after the submandibular BNT-A injection (Erasmus *et al*., 2011).

2.4.3. Surgery

Submandibular gland excisions have been the treatment of choice for sialorrhea. One of the most relevant complications is marginal facial nerve paralysis. Despite this complication, this type of surgery is safe (Hernando *et al*., 2012). Transcervical procedure approach leads to external scars and is one of the reason to decline this surgical. Caregivers' satisfaction is correlated with the

treatment outcome and generally the appearance of scars do not influence satisfaction (Delsing *et al*., 2016).

Endoscopic transoral neurectomy of the submandibular and sublingual glands in the management of drooling in conventional treatment-resistant patients has good results in saliva production reduction (Ozturk *et al*., 2017).

Wilkie's original operation (1967) consists of the retropositioning of the parotid ducts into the tonsillar fossa region along with bilateral submandibular gland resection. These procedures have several variations: transposition, ligation, deviation of both submandibular and parotid ducts behind the anterior pillar of the soft palate (4-duct diversion); bilateral submandibular duct relocation with or without sublingual gland excision; a combination of ipsilateral parotid duct ligation and contralateral parotid duct repositioning; and ligation of both parotid and submandibular ducts (4-duct ligation). These procedures cannot be performed in patients with a history of recurrent aspiration pneumonia, as it may increase aspiration risk because it directs saliva posteriorly (Bafiv *et al*., 2015; Scheffer *et al*., 2010). Chronic sialadenitis and transient swelling are common complications with surgery (Hussein *et al*., 1998).

Surgical relocation of the submandibular gland ducts with removal of sublingual glands is effective in 75% of cases. The only complication noted was ranula formation (Hornibrook *et al*., 2012).

Sectioning of the parasympathetic neural pathway: Chorda tympani resection is considered a poor method for reducing stimulated salivary flow (Banfi *et al*., 2015).

2.4.4. Radiotherapy

Radiotherapy of the salivary gland has also been an effective method to reduce excessive drooling. There are different techniques like electron-based therapy and photon-based therapy. Electron-based therapy appears to be better tolerated than photon-based therapy. Photon-based therapy may result in acute toxicity symptoms (oral pain and mucositis during or immediately after irradiation) or delayed reactions (edema or xerostomia one month after irradiation or oral pain three months after irradiation). A total dose of 20 Gy is suggested to be administered in five intervals, encompassing the whole of the submandibular gland and excluding the upper part of the parotid gland. The benefits of radiation treatment last for 4–6 months. Single-dose radiotherapy with a single fraction of 7.5 Gy has also been proven to be beneficial in reducing excessive drooling (Banfi *et al*., 2015).

2.4.5. Rehabilitation

Physiotherapy and myogymnastics improve patients with swallowing problems due to excess saliva and food. For example, the postural chin-down maneuver is effective (Ayres *et al*., 2017).

Behavioral modifying therapy may be an option for controlling sialorrhea (van der Burg *et al*., 2009).

These methods are most appropriate for neurologically impaired children with poor oral skills or hypotonic perioral musculature (Zlotnik *et al*., 2015).

Chewing gum may reduce drooling in PD patients (Srivanitchapoom *et al*., 2014).

Oral stimulation plates are effective in neuropediadric patients (Steffen *et al*., 2011).

2.5. Oral diseases

There are two main problems dentists have to deal with: caries and periodontal disease. The pathogenesis of these diseases is different. Infection enters the organism through the tooth in cases of caries but in periodontal disease infection enters the organism through the periodontal tissues. Caries is related to low oral pH. In periodontal disease dental calculus formation is caused by high oral pH.

Saliva possesses a buffering capacity for neutralizing acids present in the mouth. This can be attributed to several systems such as the phosphate system and the carbonic acid or bicarbonate system. Through these systems, saliva can maintain its usual pH after a meal. Oral fluid supersaturation with calcium phosphates leads to remineralization and maturation of enamel and dentinal lesions (Newman *et al.*, 2015). By the same mechanism it may cause calcification of dental plaque resulting in calculus formation on tooth surfaces. Calculus is divided into sub- and supragingival and the aetiology of these two is different. Supragingival calculus is usually formed at the sites close to salivary gland ducts where caries rarely occurs. Exudate from periodontally inflamed tissue contains alkaline substances that increase the precipitation of minetal salts. This forms subgingival calculus, which is not related to salivary gland ducts and may appear around any tooth (Fejerskov *et al*., 2008).

2.5.1. Caries

Dental caries is also known as a tooth decay. It is a biofilm-dependent infectious oral disease (Ahn *et al*., 2018). Dental caries is a localized chemical dissolution of the tooth surface caused by metabolic events taking place in the biofilm (dental plaque) (Newman *et al*., 2012). Caries is closely related to diet, salivation, and the presence of a bacterial biofilm on dental surfaces (Radha *et al*., 2016).

The most cariogenic bacterium of all oral *streptococci* in dental biofilms is considered to be the *S. mutans*. The study of *Lactobacilli* is a new field in oral health scientific research. Decrease of plaque pH creates an environment that helps the growth of acidophilic microorganisms, such as *S. mutans* and *Lacto-*

bacilli, which promote caries and, with further pH drop, create areas of demineralization in the dental enamel. *S. mutans* is considered a primary cause of bacteriological caries. This microorganism plays a major role in the onset and progression of dental caries. *S. mutans* occurs in plaque forming on the surface of teeth and produces acids that can dissolve tooth enamel, eventually causing cavities. The presence of *S. mutans* is considered a strong indicator of high susceptibility to caries (Fejerskov *et al.,* 2008). The relation of *Lactobacilli* with caries is the opposite. It is considered, that some *Lactobacilli* species play a role in the progression of caries. Its appearance only in saliva and plaque outside of carious lesions is not significant. Probiotics can allow beneficial *Lactobacilli* to populate sites on teeth, preventing streptococcal pathogens from taking hold and inducing dental decay (Twetman *et al*., 2008). *Lactobacilli* presence becomes important when it is abundant not only in saliva and plaque but in carious lesions too (Jiang *et al*., 2016). It produces lactic acid, which can corrode teeth and cause existing carious lesions to progress, especially in cases of coronal caries. Another important factor in the emergence of caries lesions is the nutritional conditions. Fermentable carbohydrate intake causes pH fluctuations. Shifts in pH changes the chemical composition of biofilm fluid. When the pH in the biofilm drops, the dissolution (demineralization) of enamel surface appears. On the other hand, when pH goes up, the remineralization process of enamel surface redeposition takes place. These processes occur numerous times per day. The composition and thickness of the biofilm, salivary secretion rate and composition, and the diet and the floride ion concentration in the oral fluids are factors that influence the metabolic processes between biofilm and tooth surfaces (Fejerskov *et al*., 2008).

2.5.2. Dental calculus as a result of alkaline salivary pH and a causative factor for periodontitis

Dental calculus as a result of alkaline salivary pH is a form of hardened dental plaque and is caused by precipitation of minerals from saliva and gingival crevicular fluid onto the surface of teeth (Varghese *et al*., 2015). The rough and hardened surface that is formed provides an ideal surface for further plaque formation. This leads to calculus buildup, which compromises the health of the gingiva. Dental calculus as a result of alkaline salivary pH is divided into supragingival and subgingival calculus. The accumulation of dental plaque is not only due to the mineralization of non-viable microorganisms but is also a complex process supported by enzymes (Doğan *et al*., 2016). Higher salivary calcium content accelerates plaque hardening and thereby indirectly influences the level of oral hygiene (Varghese *et al*., 2015). The calcium concentration of submandibular gland-produced saliva is about 45% higher than parotid gland-produced saliva (Varghese *et al*., 2015). This is an important factor in oral health when choosing target glands for BNT-A injections. Periodontal health is disturbed at first by the enzymatic effect of dental plaque bacteria (like alkaline

phosphatase, lactate dehydrogenase, and acid phosphatase) and it worsens due to the retentive effect of dental calculus formation resulting in periodontal tissue inflammation (periodontitis).

Calculus blocks the effect of oral hygiene procedures and is also a reservoir for endotoxins and bacterial antibodies. Calculus formation is influenced by age, gender, eating habits, oral care, bacterial composition, host response, systemic diseases, and prescribed medications. Not all the plaque becomes calcified and some patients with deficient oral hygiene otherwise do not have dental calculus and the reason for this situation is still unknown.

There is some evidence that fetuin-A inhibits apatite formation (Schafer *et al*., 2003). Higher gingival crevicular fluid and saliva fetuin-A levels were detected in patients with dental calculus than in patients without dental calculus, which may be the result of an adaptive mechanism to inhibit mineral precipitation and eventually calculus formation (Doğan *et al*., 2016). Fetuin-A is an effective inhibitor protein that is synthesized in the liver and secreted into the circulatory system in the case of ectopic calcification. It prevents calcium-phosphate precipitation in the calcification processes inside the organism, such as in the kidneys (Aksoy *et al*., 2010), coronary arteries (Lehtinen *et al*., 2007), bones (Yang *et al*., 2007), and brain (Geroldi *et al*., 2005).

2.5.3. Periodontal disease

Chronic periodontal disease is an inflammatory condition in tooth-supporting structures with multifactorial aetiology, caused by anaerobic Gram-negative microorganisms. The predominant periodontal pathogens are *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, Tannerella forsythensis, Eikenella corrodens* and *Treponema denticola* (Gurav *et al*., 2014). Anaerobic species adhere to teeth forming bacterial plaque. The target points of inflammation are the gums, periodontium and alveolar bone (Puşcu *et al*., 2016). Numerous studies have shown an association between periodontitis and various systemic conditions, such as diabetes mellitus, atherosclerosis, cardiovascular diseases rheumatoid arthritis, Alzheimer's disease, chronic nephropathies, dyslipidemia, obesity, etc. (Suvan *et al*., 2011; Newman *et al*., 2012; Ruospo *et al*., 2014; Gurav *et al*., 2014; Sanchez *et al*., 2017; Dietrich *et al*., 2017; Kitagawa *et al*., 2017; Äyräväinen *et al*., 2017). Periodontitis is associated with lower intakes of niacin, vitamin C, and iron, especially in women and non-smokers (Park *et al*., 2016).

Periodontal tissue inflammation is responsible for various cardiovascular diseases, including atherosclerosis (Newman *et al*., 2012). Earlier studies showed that systemic illnesses, especially metabolic disorders, affect oral health (Löe *et al*., 1993; Liu *et al*., 2017), but recent studies prove that the association between oral health and systemic health is bidirectional and it appears that oral health may affect systemic health likewise (Puşcu *et al*., 2016). It is hypothesised that *Treponema* from the oral cavity must have gained access to the

cerebral cortex via the trigeminal nerve (Gurav *et al*., 2014), leading to brain infection and damage. A significant association has been demostrated between spirochetes and Alzheimer's disease. *Spirochetes* were detected in the brain in 93.7% of Alzheimer's disease cases and in only 33.3% of the control group. Periodontal disease and diabetes mellitus are conditions which usually begin after the age of fifty and negatively influence one another. Usually the inflammatory reaction in patients with periodontal disease and diabetes is more intense than in the patients with periodontal disease without diabetes (Puşcu *et al*., 2016). Äyräväinen *et al*., (2017) reported in their study that nearly 80% of rheumatoid arthritis patients suffer under periodontitis versus 40% of the control group. Smoking is known to be one of the reasons for exacerbation of periodontitis (Roshan *et al.*, 2016).

2.6. Prevention of oral diseases

Maintaining adequate dental and oral hygiene is a challenge especially for elderly people and young children due to their limited motor skills (Chand *et al*., 2014; Sjögreen *et al*., 2015). Patients with PD typically have poor oral health (Pradeep *et al*., 2015) and smoking also increases the morbidity of periodontitis (Kitagawa *et al*., 2017). To combat periodontal disease, PD patients need health guidance about tooth brushing and information about the importance of quitting smoking and controlling obesity. Oral hygiene in disabled patients can be maintained with increased mouth rinsing after meals to remove accumulated food from the mouth. Powered toothbrushes, topical administration of chlorhexidine, and fluoridated products and calcium phosphate are suggested to reduce the risk of caries. (DeBowes *et al*., 2007; Chibinski *et al*., 2011; Rath *et al*., 2013).

Despite the achieved therapeutic effect of the BNT-A treatment, the risk for poorer oral condition and decreased self-cleaning ability remains. Caregivers and patients should cooperate with dentists to improve oral hygiene and maintain good oral health. They need instructions on how to take care of oral hygiene, especially in the first month after BNT-A injections when the effect of the treatment on sialorrhea is maximal.

2.7. Summary of literature review

Our study was designed to assess the effect and safeness of BNT-injections in sialorrhea treatment because there was earlier worldwide effective off-label use reports (Brin, 2009; Laskawi, 2008; Majid, 2010), but in Estonia that treatment was not implemented previously. BNT-A was not in indication list of Estonian State Agency of Medicines for sialorrhea also.

Earlier studies describe oral health changes only in patients with central nervous system diseases due to their bad manual coordination (Pradeep *et al*.,

2015) and insufficient brushing, but studies including BNT-A treatment role on saliva composition change and through that to dental and periodontal health are missing. This study direction is important because BNT-A injections are novel approach in sialorrhea treatment and they are always more frequently used method. Because of the lack of information, patients receiving BNT-A injections have to be under the careful supervision of a dentist or a dental hygienist.

3. AIMS OF THE STUDY

General aim of our study was to evaluate the BNT-A safety and efficacy in treatment of sialorrhea in neurologically disabled patients. All three of the studies are focused to evaluate the efficiency of the BNT-A treatment.

1. To elucidate clinical factors that play a role in sialorrhea with CP in Study I.
2. To evaluate the safety to oral health after the ultrasonography-controlled bilateral BNT-A injections into the parotid and submandibular glands in the management of sialorrhea in patients with neurodegenerative diseases. Study II and III evaluate the risks of BNT-A injections to oral health.
3. To evaluate and compare salivary compositions and microbiota change after BNT-A injections. Study II and III are oriented to evaluate saliva changes after the BNT-A injections.

4. SUBJECTS AND METHODS

The study has been approved by the Ethics Review Committee on Human Research of the University of Tartu (protocol Nr 192/T-3; 26.04.2010) and the State Agency of Medicines (Protocol no. 01-09.02.15). The research has been registered to the European Clinical Trials Database (EUDRA 2015-000682-30 EE 20150417 CTA). Informed consent has been obtained from all study participants.

Study I was an open label, descriptive, non-blinded prospective study investigating sialorrhea in children. Study II and III were prospective clinical trials in the adult population.

4.1. Subjects of the studies

4.1.1. Study I

Twelve children with CP from the Department of Neurology and Neurorehabilitation of the Children's Clinic of the Tartu University Hospital were admitted to the Study I (The use of Botulinum neurotoxin A in uncontrolled salivation in children with cerebral palsy: A Pilot Study). Three participants were excluded due to the parents' refusal to give permission to participate in the study, bringing the total number of paediatric patients who completed the study down to nine. Patients aged 1.6 to 11 years old (four male and five female) with CP, were screened from January 2011 to August 2011. All subjects had neurological manifestations of CP: spastic hemiparesis, tetraparesis, or dystonic movement disorder. The patients were selected according to the Gross Motor Function Classification System (Palisano *et al*., 1997). This is a five-level classification that differentiates children with cerebral palsy based on the child's current gross motor abilities, limitations in gross motor function, and need for assistive technology and wheeled mobility. All selected patients had moderate to severe intellectual disability.

4.1.2. Study II

Study II (Effect in salivary parameters after BNT-A treatment in sialorrhea patients) includes twenty patients, among them twelve males and eight females, with an average age of 63.2 years, all exhibiting remarkable hypersalivation. The patients were screened from October 2012 to October 2013 at the Tartu University Hospital. In 75% of the cases, the aetiology of sialorrhea was caused by chronic neurodegenerative diseases. In twelve cases the patients had PD, and three patients 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.

4.1.3. Study III

The participants in Study III (Saliva changes in PD patients after injection of Botulinum neurotoxin type A) included patients diagnosed with PD who had sialorrhea. Thirty-eight subjects (sixteen female and twenty-two male; age range of 58–88 years; mean age of 71.1 years) were screened at the Tartu University Hospital from April 2015 to January 2016 and enrolled in the study. The participants were selected from the cohort of patients included in the PD epidemiology study at the Department of Neurology and Neurosurgery of Tartu University. The healthy control group was recruited from the department of Dentistry. The patient assessment was based on screening by the Movement Disorders Society Unified Parkinson's Disease Rating Scale (MDS-UPDRS) questionnaire, Item 2.2 from Part II (Non-Motor Aspects of Experiences of Daily Living) (Goetz *et al*., 2008). All participants were divided into three groups: Group 1 of twelve PD patients (nine male and three female, with an average age of 71.3 years) with sialorrhea, was treated with salivary gland BNT-A injections. Group 2 included thirteen PD patients without hypersalivation (seven male and six female, average age 71.5 years) who did not receive BNT-A injections. Group 3 comprised thirteen age- and sex-matched healthy volunteers (six male and seven female, average age 70.6 years).

4.2. Methods

Intervention with BNT-A injection procedures was performed in all three studies. In Studies II and III, salivary analysis was performed alongside the BNT-A procedure. For the control group, salivary tests were performed without BNT-A injections.

4.2.1. BNT-A injection techniques

The study groups received BNT-A injections into the salivary glands. The solution of 100 U of BNT-A (Botox®) was prepared in a 2.5 mL volume of saline solution. The dose was calculated according to body weight. The recommended dose for Botox® (Allergan) was weight-dependent: 1.4 U per kg of body weight for injection to each parotid gland, and 0.6 U per kg in each submandibular gland (total dose of 2 U per kg) (Reddihough *et al*., 2010). The recommended Botox® dose was tripled to get the relevant dose of Dysport® (Reddihough *et al*., 2010). In the children of Study I, BNT-A injections were done under general anesthesia. The injection procedures were done under local anesthesia for adult patients in Study II and III. Ultrasound guidance was used in all cases of BNT-A injections. The 7.5 mHz linear transducer was positioned so that it was possible to perform an injection with a needle directed along the

longitudinal axis of the transducer. 27-gauge needles were placed in the antero-posterior direction into each submandibular and parotid gland.

4.2.2. Assessing BNT-A treatment effectiveness

To determine children's responses to the treatment in Study I, a structured telephone interview with one parent or caregiver was used. The interview schedule for Study I was designed based on the literature review and past experience. Drooling severity at baseline and reduction of sialorrhea during treatment were assessed using a parent's questionnaire containing previously known scales. Drooling intensity and frequency were evaluated with the Drooling Severity and Frequency Scale (Table 1) (Thomas-Stonell *et al*., 1988) and Teacher Drooling Scale (a 5-point scale: 1- no drooling, 2- infrequent drooling, small amount 3- occasional drooling, on and off all day, 4- frequent drooling, but not profusely 5- constant drooling, always wet) (Reid *et al*., 2010). The baseline rate of salivation was assessed before the BNT-A injection. After BNT-A treatment the rates of salivations were assigned with biweekly evaluations over a six-month span with self-assessed rating scales for drooling intensity, discomfort, and treatment efficacy (Benson *et al*., 2007). The patient interview included an evaluation of their medical and psychological history, and a consideration of their sialorrhea aetiology. Reporting on drool reduction, bib changes, need for suctioning, respiratory distress, self estimated quality of life, and complications such as facial swelling and swallowing dysfunction was included in the interview. Treatment efficacy and safety were assessed at baseline, and in a one-month follow-up after the BNT-A injections. Side effect prevalence was also measured (Mancini *et al*., 2003).

Participants of Study II and III were evaluated by questionnaire twice, before injections, and one month after the BNT-A treatment, to investigate the effect and changes in self estimated quality of life. Teacher Drooling Scale and MDS-UPDRS Item 2.2 from Part II (Non-Motor Aspects of Experiences of Daily Living) and was used in Study II and III (Reid *et al*., 2010; Goetz *et al*., 2008).

Table 1. Drooling Severity and Frequency Scale

Drooling severity scale
1=Never drools, dry
2=Mild – drooling, only lops wet
3=Moderate – drool reaches the lips and chin
4=Severe – drool drips off chin and onto clothing
5=Profuse – drooling off the body and onto objects (furniture, books)

Drooling Frequency Scale
1=No drooling
2=Occasionally drools
3=Frequently drools
4=Constant drooling

4.2.3. Salivary tests

Salivary tests were used in the subjects of Study II and III. In Study III (Group 1), patients took salivary tests before the injections and one month afterwards and control group subjects (Group 2 and 3) took salivary tests once. Throughout the study, medications known to influence the severity of drooling were not used. The patients, their caregivers, and their families were made aware of the possible adverse effects and risks related to the study interventions.

The Saliva-Check BUFFER in Vitro test (GC EUROPE N.V. B-3001 Leuven, Belgium) was used to determine the quality, pH, and buffering capacity of saliva.

In order to test resting saliva, three different tests were used:

1. Resting saliva formation was tested by visual inspectation of level of hydration. The lower lip has to be everted and labial mucosa gently blotted with a small piece of gauze. Then, under good light, observe how quickly the droplets of saliva express from the minor glands (in seconds).
2. Saliva consistency was also assessed by visual evaluation. Saliva was characterised as (1) sticky-frothy, (2) frothy-bubbly, or (3) watery-clear. Watery-clear saliva is referred as of normal viscosity. Sticky-frothy and frothy-bubbly saliva is characterised by increased viscosity.
3. For pH measurement, subjects had to expectorate pooled saliva into the collection cup. pH test strips were held in samples for ten seconds and compared with the chart in the kit.

In order to test stimulated saliva, two different tests were used:

1. The amount of saliva was determined by the quantity of saliva secretion over the course of five minutes. Subjects had to chew a piece of wax to stimulate salivary flow. Every thirty seconds patients had to expectorate the accumulated saliva into the marked collection cup. The amount was checked with the ml markings on the side of the cup. It is noted that a normally stimulated flow rate varies between 1 ml/min–1.6 ml/min.
2. To assess buffering capacity, Buffer test strips were used. The strips were placed onto absorbent tissue and the saliva drop was then dispensed onto each of the three test pads with the pipette. Excess saliva was soaked up with absorbent tissue. After two minutes the test pads changed their colour and the final result was calculated from 0 to 4 points, according to the final colour of each pad. The combined total scores were described as follows: 0–5 points show very low buffering ability of saliva; 6–9 points show a low buffering ability; and 10–12 points show normal or high buffering ability.

4.2.4. Analysis of salivary cariogenic bacteria

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). To detect *S. mutans* in a saliva sample, Dentocult SM Strip mutans was applied. The method was based on the adherence and growth of *S. mutans* on the test strip (Thorhild *et al*., 2002). *S. mutans* bacteria adhere to the rough area of the strip in proportion to their density in saliva. After incubation, the bacteria are visible as light to dark blue. Dentocult LB is a dipslide culture method for detecting aerobic aciduric bacteria (i.e. *Lactobacilli*) in stimulated saliva. Aciduric bacteria are seen as white to transparent colonies on modified Rogosa 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.

To avoid the effects 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 sampling (Birkhed *et al*., 1981). Prior to sampling, the patients did not eat, drink, smoke, or brush their teeth for two hours. The incubation periods for Dentocult LB and Dentocult SM were 96 and 48 hours, respectively (Schlagenhauf *et al*., 1995). Dentocult LB sample was placed in the incubator (36 ± 2°C) for four days, and Dentocult SM sample, for two days. To assess 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 (Birkhed *et al*., 1981).

4.3. Statistical analysis

The data was analysed using IBM SPSS Statistics V20 (IBM Corporation, Armonk, NY, USA). All three studies used descriptive analytical methods: the calculation of means, standard deviations (SDs), and median values were used for continous variables, depending on the distribution. Differences between the study groups in Study II were compared with the Paired Samples t-test, Wilcoxon Matched Pairs Signed Ranks Test, and Ficher's Exact Test. Differences between the study groups in Study III were compared with Kruskal-Wallis or ANOVA test. The data without normal distribution was analysed with the Wilcoxon Signed Ranks test. Pearson's correlation was used to analyse relationships between the different parameters in Study III.

The two-sample t-test, the two-proportion z-test, and the Mann–Whitney test were used to assess the differences of the variables of interest between the two independent groups. The Wilcoxon signed-rank test was used to analize non-parametric data, and paired t-test for parametric data analysis. Pearson's correlation analysis was used to evaluate the associations between variables. Differences with p values < 0.05 were considered statistically significant.

5. RESULTS

5.1. Use of Botulinum neurotoxin A in uncontrolled salivation in children with cerebral palsy (Study I)

The results of the first part of our study (Study I) showed an increased frequency of malocclusion (mostly open bite) and insufficient lip closure in children with CP that occurs due to weak head and neck musculature and dysphagia, which causes breathing through the mouth while at rest, instead of normal breathing through the nasal cavity. When the mouth is held open, saliva spills out. Anterior drooling was found in five cases, and posterior drooling in three cases out of nine; one patient had both types of drooling. All patients had oral spasticity, with slow uncoordinated tongue movements noted in eight patients. The clinical characteristics of patients are shown in Table 2.

Children with sialorrhea had a maximum response to the BNT-A injection between two weeks and two months post-injection. A direct decrease in drooling according to the Drooling Severity and Frequency Scale was reported at the first week, subjectively. The number of bibs used per day (Figure 1) and the need for suctioning (Figure 2) also dropped by almost half. About two weeks after the BNT-A injection, patients' saliva flow decreased for the liquid component. Saliva thickened. Drooling decreased from a score of 5 (profuse) at baseline to 3 (moderate) by two weeks after treatment (Figure 1).

One child out of nine had no response to the BNT-A injection, and one child had difficulties swallowing for three weeks as a complication of BNT. There were no complaints of pain or swelling in the research group.

The main effect on self estimated quality of life was seen in the first two months but later decreased. Three caregivers reported a remarkable increase in quality of life, another three described a good increase, two reported a moderate increase, and one did not see any change in quality of life (Figure 3).

Parents of CP patients preferred to undergo this treatment option in winter during the season of respiratory infections, which may cause saliva-soaked clothes.

Table 2. Clinical characteristics of cerebral palsy patients with sialorrhoea.

Patient's No	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Sex	Female	Female	Female	Male	Male	Female	Female	Male	Male
Age (Years)	6	10	3	4	7	1.6	11	3	10
Aetiological factor of CP	Herpes-encephalitis	Cytomegalo virus	Birth-trauma	Birth-trauma	Birth-trauma	Unknown	Encephalitis	Birth-trauma	Unknown
Motor disorder	Spastic	Dyskinetic	Dyskinetic	Dyskinetic	Spastic	Spastic	Dys-kinetic	Dys-kinetic	Spastic
Aspiration	no	yes	yes	no	yes	yes	no	no	yes
Feeding	Oral	Oral	Gastro-stomy	Oral	Gastro-stomy	Oral and Gastro-stomy	Oral	Oral	Gastro-stomy
Reflux	no	no	yes	yes	yes	yes	no	no	yes
Breathing	Self	Self	Self	Self	Tracheo-stomy	Tracheo-stomy	Self	Self	Self
Lip seal	No	No	No	No	No	Yes	No	No	No
Open-bite	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes
Drooling	Anterior	Anterior	Posterior	Anterior	Both	Posterior	Anterior	Anterior	Posterior

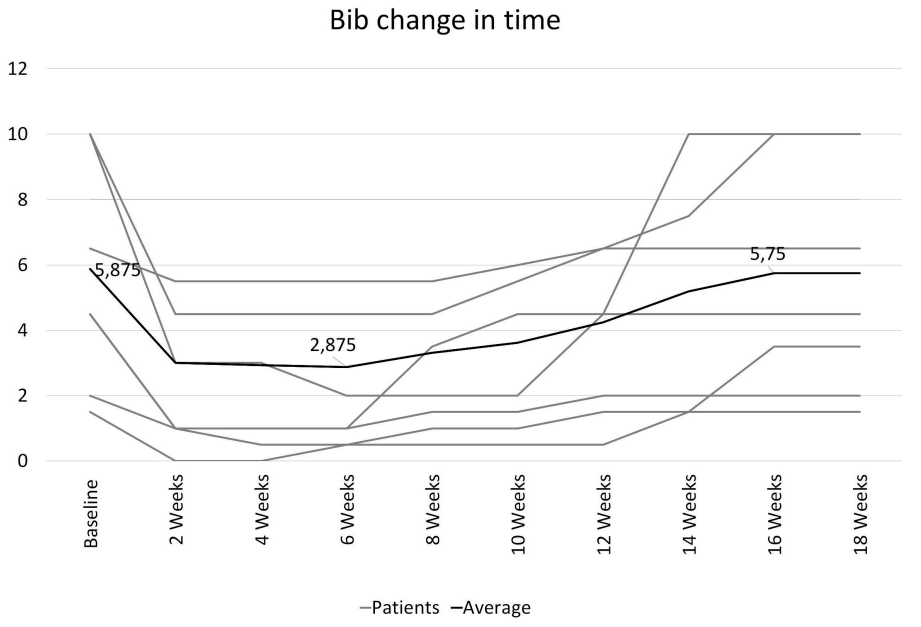

Figure 1. The number of bibs used per day

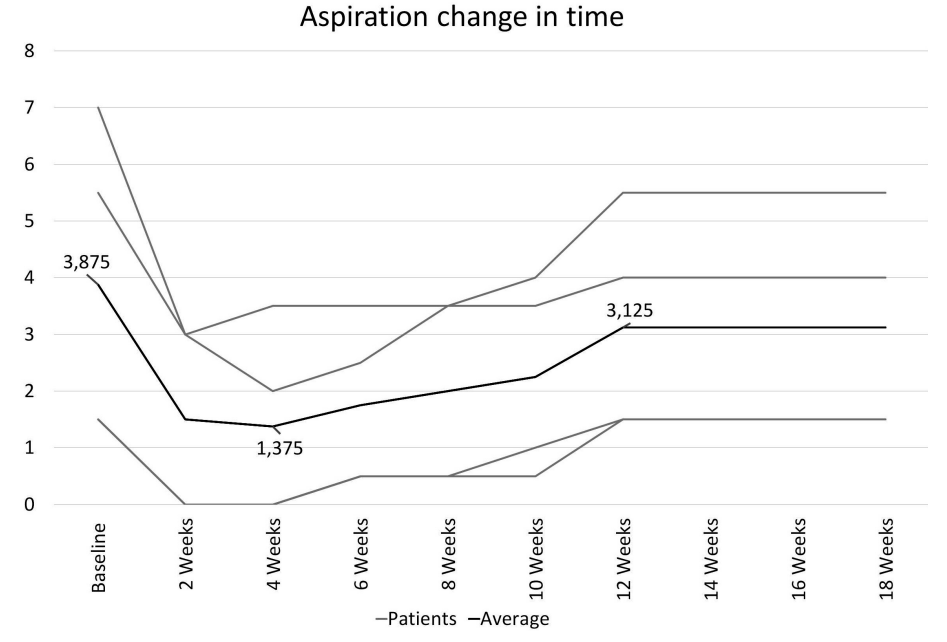

Figure 2. Need for saliva aspiration in time

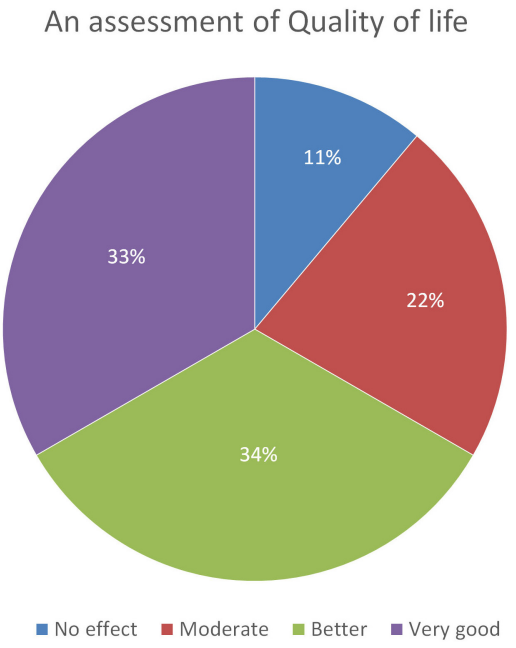

Figure 3. Patients self estimated quality of life

5.2. Effect in salivary parameters after BNT- A treatment in sialorrhea patients (Study II)

Treatment efficacy and safety were assessed in twenty patients with profuse sialorrhea (twelve male and eight female) at baseline, and at a follow-up after the BNT-A injections, using Item 2.2 from Part II, MDS-UPDRS (Goetz *et al*., 2008) and salivary tests**.** The average age of participants was 63.15 years (ranging from 3 to 79 years). The aetiology of sialorrhea was caused by chronic neurodegenerative diseases in 75% of the cases, and by other reasons in 15% of the cases. In twelve cases the patients had PD; in three cases ALS was diagnosed; in two cases birth hypoxia was a cause of sialorrhea; two patients complained atypical headache; and one patient had experienced stroke with dysphagia.

Subjective severity was recorded according to the MDS-UPDRS scale Item 2.2 at baseline and one month after the injection procedure. Of all the study patients, 95% reported a significant decrease in drooling one month after the injections. A statistically significant decrease in the amount of saliva was found according to the Teacher Drooling Scale, dropping from the severe/moderate level to mild. The amount of stimulated saliva decreased, and the duration of at-rest saliva formation increased significantly. Buffering capacity went up, which showed that salivary defence ability had improved. Oral pH had no significant changes. In the follow-up visit, there was no statistically significant change in oral pH and buffering capacity compared to the initial assessment. The results are shown in Table 3

Like the results of the first study, all patients in Study II had saliva thickening for two months after the BNT-A injections. Saliva consistency changed significantly according to Ficher's exact test (p = 0.004). Before the BNT-A injections, watery-clear saliva was the most common consistency (90% of the patients), and none had sticky-frothy saliva. After the injections, the most common consistency by the visual assessment was frothy-bubbly saliva (65% of the patients), and sticky-frothy saliva occurred in one case.

A comparison of different *Lactobacilli* and *S. mutans* colony forming unit (CFU) count groups before and one month after the BNT-A injections demonstrated a tendency for increased microbial levels, but the change was not statistically significant (Table 3).

All participants tolerated the treatment well. There were no complaints of swelling or pain in the study group.

Table 3. Comparison of salivary characteristics before and one month after the injections

Characteristic	**Before injections** (SD)	**1 month after injections** (SD)	**p**
Subjective evaluation of salivation (MDS-UPDRS Item 2.2 score)	3.7 (± 0.6)	2.0 (±1.2)	0.001*
Resting saliva time (sec)	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
Lactobacilli (CFU/ml)	0.9×10^6	3.1×10^6	0.052
S. mutans (CFU/ml)	10^5–10^6	10^5–10^6	0.707

p* statistically significant
Wilcoxon Signed Ranks Test, n=20

5.3. Saliva changes in Parkinson's disease patients after BNT-A injections (Study III)

This research was performed on elderly individuals with PD. Differences in salivary tests were compared across three groups: Group 1 consisted of twelve PD patients (nine male and three female) who suffered from drooling and received BNT-A injections in their salivary glands, Group 2 consisted of thirteen PD patients without excess saliva (seven male and six female) who were not injected with BNT-A, and Group 3 consisted of thirteen age-matched healthy controls (six male and seven female). The characteristics of the study PD participants according to the groups are described in Table 4 An association was found between long-term levodopa use and drooling (Pearson's correlation, p=0.034). Group 1, which received BNT-A treatment for drooling, had a

significantly longer levodopa treatment duration. The amount of saliva was greater in patients who were treated with levodopa (Pearson's correlation, p = 0.016) and less in patients on MAO-B inhibitors (Pearson's correlation, p = 0.020).

Table 4. Characteristics of the study participants according to the group.

Characteristic	Group 1 (n=12)	Group 2 (n=13)	p-value
PD onset age, yr (mean ± SD)	57.7 ± 9.6	63.7 ± 8.1	0.102
PD duration, yr (mean ± SD)	13.4 ± 6.6	7.8 ± 4.6	0.019*
Clinical subtype of PD, n (%)			
Tremor-dominant	1 (8%)	9 (69%)	0.0018*
Akinetic-rigid	8 (67%)	4 (31%)	0.005*
PIGD	3 (25%)	0 (0%)	0.055
HY, median (ranges)	3 (2–4)	2.5 (1.5–3)	0.005*
MDS-UPDRS part II (mean ± SD)	17.7 ± 7.7	9.9 ± 4.1	0.004*
Item 2.2. Saliva and drooling (mean ± SD)	2.9 ± 1.1	0.2 ± 0.4	<0.001*
MDS-UPDRS part III (mean ± SD)	50.1 ± 19.8	27.2 ± 9.6	0.001*
Antiparkinsonian treatment, n (%)			
Amantadine	7 (58%)	3 (23%)	0.074
MAO-B inhibitors	3 (25%)	3 (23%)	0.907
Dopamine agonists	7 (58%)	8 (62%)	0.838
Levodopa	11 (92%)	9 (69%)	0.151
LEDD, mg (mean ± SD)	1024.8 ± 576.5	405.4 ± 288.3	0.002*
Levodopa daily dose, mg (mean ± SD)	568.2 ± 234.8	355.6 ± 142.4	0.029*
Duration of levodopa treatment, yr (mean ± SD)	9.2 ± 5.2	4.5 ± 3.7	0.034*

Mann-Whitney U test for statistical significance
[a] number (proportion) of patients; two proportion z-test for statistical significance
* Statistically significant
Abbrevations: PD= Parkinson's disease; SD = standard deviation; yr = years; PIGD = postural instability and gait disorder; HY = Hoehn and Yahr stage; MDS-UPDRS = Movement Disorders Society Unified Parkinson's Disease Rating Scale; LEDD = levodopa equivalent daily dose.

Resting time saliva formation shows how quickly a drop of saliva appears from the minor salivary glands of the lower lip. It was slower in patients with later disease onset of PD.

An association was found between the initial amount of 5-minute saliva and the leading symptom (Pearson's correlation, p = 0.016). The patients with akinesia-rigidity as a leading clinical syndrome had more sialorrhea than patients with tremor-dominant PD (Pearson's correlation, p = 0.0019).

All patients in Group 1 reported thickening of saliva for one month after BNT-A injections. Subjective assessment by the MDS-UPDRS Item 2.2 demonstrated the change in drooling from very intensive at the baseline, to the moderate level (mean 3.55, SD 0.688, median = 4.00) and one month after the BNT-A injections (mean 1.55, SD 1.214, median = 1.00) (p = 0.01), and the amount of saliva collected in the 5-minute test showed a significant decrease (Table 5). The consistency of saliva and the pH values did not change after the injections. The higher buffering capacity values after the injection demonstrated the ability of saliva to maintain a normal oral pH (Table 5). There were no significant changes in the count of *S. mutans*, but the *Lactobacilli* counts were statistically significantly increased (Table 5).

The treatment was generally well-tolerated and there were no complaints of swelling or pain by the patients.

Table 5. Change in saliva parameters across Groups.

Saliva parameters	Group 1			Group 2	Group 3
	Before BNT-A injection	1 month after BNT-A injection	p		
Resting saliva formation time (sec) (range)	15 (5–60)	25 (16–75)	0.05*	20 (7–78)	26 (10–60)
Amount of 5 min collected saliva (ml± SD)	8.6 ± 6.79	4.8 ± 4.09	0.018*	6.0±3.5	6,3±4.0
Buffering capacity (range)	5 (2–12)	9 (3–12)	0.037*	9 (3–12)	8 (2–12)
Consistency (range)	3 (2–3)	2.5 (2–3)	0.059	3 (3)	3 (2–3)
pH (±SD)	7.0 ± 0.74	7.0 ± 0.95	1.00	7.3±0.86	7.2±0.8
Dentocult LB counts (*Lactobacilli*) (range)	1 (0–3)	2 (0–3)	0.047*	2 (0–3)	1 (0–2)
Dentocult SM counts (*S.mutans*) (range)	2 (0–3)	2 (2–3)	0.206	2 (0–3)	2 (0–3)

p* statistically significant
Abbrevations: BNT-A, Botulinum neurotoxin type A
Median ± SD (range)
Wilcoxon Signed Ranks Test, n=38

6. DISCUSSION

6.1. Sialorrhea etiology

Excessive drooling can be a serious physical and social disability, affecting communication, and greatly reducing quality of life. In neurological disorders (CP, PD, ALS) usually the main problem with saliva excess is related to dysfunctional oral motor control when the swallowing process is abnormal due to muscle weakness (Hussein *et al*., 1998; van Hulst *et al*., 2018).

Clinical factors like head position, lip seal, voluntary control of tongue movements, and mental stage, influence drooling (Dias *et al*., 2016), which is in concordance with the results of our study. Tongue mobility is associated with drooling control. The results of the first part of our study (Study I) showed an increased malocclusion in children with CP sialorrhea that is explained with weak head and neck musculature and disturbed swallowing. While at rest, nasal breathing is changed to mouth breathing. When the mouth is held open, saliva spills out.

The results of Study III showed remarkable sialorrhoea in PD patients with a prominent clinical syndrome of akinesia and rigidity than in patients with tremor as a leading symptom, which demonstrates a connection between a clinical subtype of the disease and swallowing dysfunction. Another study has shown an association between hypokinesia and rigidity and swallowing function, which may be worsened by malnutrition with decreased liquid intake and eating soft sticky food (Müller *et al*., 2011).

Furthermore, PD medications, including levodopa, dopamine agonists, MAO-B inhibitors, amantadine, and anticolinergics may have oral implications like xerostomia, bruxism, dry throat, gingivitis, tongue edema, abnormal taste, and glossitis (DeBowes *et al*., 2013; Zlotnik *et al*., 2015). Our study showed an association between long-term levodopa use and drooling. Sialorrhea was more prevalent in patients who were treated with levodopa and less so in patients treated with MAO-B inhibitiors. Most PD medications including levodopa are responsible for xerostomia emergence in the early stage of PD. Levodopa might stimulate the salivary flow rate and lead to an excessive amount of saliva in a later stage of PD (DeBowes *et al*., 2013). This could also be explained by the fact that levodopa's effect decreases with time: after 5–10 years of treatment, at least half of the patients become partially unresponsive to the medication (Giladi *et al*., 2016).

6.2. Sialorrhea treatment effects with BNT-A

Intraglandular application of BNT-A guided by a high-resolution ultrasound can provide reliable treatment of sialorrhea with a favourable outcome (Wilken *et al*., 2008; Sriskandan *et al*., 2010; Petracca *et al*., 2015). Our results are in line

with previous studies showing that BNT-A reduces drooling effectively (Ou *et al*., 2015; Gómez-Caravaca *et al*., 2015).

Lee *et al*. (2010) recommend blind injections with BNT-A based on anatomical landmarks, but we chose to use ultrasonic guidance for safety reasons. The parotid glands are easy to locate blindly, but there is a higher failure rate when injecting the submandibular glands blindly due to their more difficult detection (So *et al*., 2017).

There are different options for the technique used to administer BNT-A into the parotid gland regarding the number of injection points (Manrique *et al*., 2005). We used the single-point technique to target glands. Many authors, including us, prefer to inject both the parotid and submandibular glands but some authors consider this less effective than submandibular injections alone. Scheffer *et al*. (2010) prefer the submandibular glands as these are responsible for 60% to 70% of unstimulated saliva production, but the parotids mainly secrete saliva during mastication. In some studies, injections were performed solely into the submandibular glands, to avoid reduction in parotid output at the time of eating and drinking (Jongerius *et al*., 2004). On the contrary, Gómez-Caravaca *et al*. (2015) showed a favourable result in 65% of cases, injecting only the parotid glands blindly, which are accessible due to their superficial location and make a large contribution to total saliva output (Fuster-Torres *et al*., 2007). Suskind *et al*. (2002) showed a favorable outcome in 30% of patients when injecting only their submandibular gland, and 80% in patients injected in both their submandibular and parotid glands. The first part of our study (Study I) showed that the BNT-A injection into both the submandibular and parotid glands was effective in 89% of cases using that technique. We selected both bilateral parotid and submandibular glands as the treatment targets to optimize the therapeutic outcome of BNT-A intrasalivary gland injections.

The wide range of dosages of BNT used for treatment of hypersalivation is related to the diagnosis of the patient, the severity of the sialorrhea, the injection technique, and the experience of the physician (Laskawi *et al*., 2008). Several studies have shown that the effect of BNT is dosage-related: the higher the dose of BNT-A, the higher the efficacy of injections (Restivo *et al*., 2018). Patients in our study received a weight-dependent dosage total of 6 U/kg abobotulini (Dysport). As the total dosage of BNT-A was calculated according to bodyweight, the cause of unresponsiveness in one child might be related to an insufficient dose.

The return of hypersalivation four to seven months after BNT-A treatment was noted in 21% of patients who requested a second injection (Ellies *et al*., 2004). Salivary flow rates in our CP study dropped remarkably within one week after the injections but increased again after twelve weeks. Our results showed that high salivation rates returned after three to four months. The duration of the toxin's effect varies widely among individuals. Generally, the effect of 50 to 65 U of BNT-A (Botox®; Allergan, Irvine, CA) in both the submandibular and parotid glands under sonography application lasted for about three months (Ellies *et al*., 2004). We speculate that the reason for the wide range in the

duration of BNT-A's effect could be related to differences in total dosages between studies. Moreover, the injection technique might have an impact on the duration of effect, as overly superficial injections might lead to some extra-glandular absorption.

Earliest study showed the appearance of many complications such as dysphagia, xerostomia, and chewing difficulties (Alvarenga *et al*., 2017), but there is a study describing no side effects of BNT-A treatment (Mahadevan *et al*., 2017). In our CP study group, one patient had swallowing difficulties for three weeks, and six patients had extensive thickening of saliva lasting eight weeks. Thickened saliva after the BNT-A injections affects the mouth's natural self-cleaning ability as the thick saliva cannot wash the dental plaque off teeth. El-kwatehy *et al*. (2016) showed that salivary flow rate does not correlate with dental caries.

BNT-A injections into salivary glands do not need general anesthesia but can be performed under intravenous sedation (Çiftçi *et al*., 2013). We used general anesthesia for children with infantile CP sialorrhea (Study I and II). Injections for adult patients were done with local anesthesia without any problems (Study II and III). Some parents of children in Study I did not want reinjections because of short duration of its effect and the need for general anesthesia. Crysdale *et al.* (2006) and Scheffer *et al.* (2010) compared surgical treatment with BNT injections in children with sialorrhea, and found that both interventions were effective, but surgery had a larger and longer-lasting effect. In their protocol, the surgical treatment included submandibular duct relocation to the posterior area with sublingual gland removal, which was noted as un-suitable for children with a high risk of aspiration (posterior drooling). Removal of the submandibular gland may reduce the salivary flow by more than 80% (Hernández-Palestina *et al*., 2016) and is suggested to CP patients who do not respond to BNT-A treatment. Out of nine CP children, only one was non-responsive.

6.3. Salivary parameters as a risk factor for caries and periodontitis

BNT-A injections decrease the amount of saliva and may lead to changes in the oral environment and health. Study II demonstrated an effective control of sialorrhea with BNT-A without compromising saliva's protective effect on the oral environment. Earlier studies showed that BNT-A can effectively treat sialorrhea but its effect on saliva characteristics, including the amount of cariogenic bacteria, was unclear (Pei-Hsuan *et al*., 2011).

It has been proven that children with different disabilities had more problems with oral health than the healthy control group (Gaçe *et al*., 2014; Bartolomé-Villar *et al*., 2016). Children with intellectual disablity have less plaque and a lower salivary pH (Radha *al*., 2016). Proper mastication is an

important factor in oral health (Sanjay *et al*., 2014). Several studies have reported that children with various disabilities have higher levels of untreated caries and periodontal disease than children without disabilities (Gace *et al*., 2014; Lewis *et al*., 2009). A higher incidence of periodontal disease is associated with a lack of manual dexterity (Radha *et al*., 2016). Our study showed a statistically significant decrease in the amount of stimulated saliva collected during the 5-minute test, mostly produced by the parotid glands. A decrease of this parameter may be explained by our injection scheme with higher doses in the parotid glands.

Our study showed statistically significant retardation in at-rest saliva formation, which is measured from the lower lip's minor glands. This decrease is an unexpected finding with injections of BNT-A only into the major glands. We speculate, that could be related to the systemic effect of BNT-A injection and not only to the local effect.

Saliva amount, flow rate, pH, and buffering capacity are significant aspects of oral health (Gopinath *et al*., 2006). Salivary flow pH and buffering capacity are factors that influence the risk of dental caries (Liu *et al*., 2014). Low salivary flow (resting time saliva formation < 60 sec. and amount of saliva collected in 5 min. < 3.5 mL), highly acidic pH (5.0–5.8), and lower values of buffering capacity (0–5) increase the risk of caries.

Buffering capacity and pH can impact oral health directly. Buffering is saliva's capability to respond to changes and to correct them in the oral cavity. Low buffering capacity is associated with caries development through its impaired neutralization of plaque acids and reduced remineralization of early enamel lesions (El-kwatehy *et al*., 2016). Salivary pH can be referred to as an indicator for assessment of caries risk and general oral health. Caries-active individuals who have a higher risk for caries show larger quantities and faster rates of acid production compared to caries-free individuals. Salivary pH was significantly lower in caries-affected children than in caries-free children (El-kwatehy *et al*., 2016). The results of Study II and III showed no statistically significant change in pH before and one month after BNT-A injection showing that pH change is not a risk factor for caries in sialorrhea treatment. In Study II buffering capacity had no statistically significant change but in Study III statistical significance was found in oral environment improvement after BNT-A treatment. This could be related to main diagnosis of participants. Study II consist of patients with different genesis of sialorrhea (CP, ALS and PD) but Study III involved only PD patients. ALS patients often complain about sticky secretions but this is not only due to salivary glands but may also originate from the nasal and oral mucous glands (Jackson *et al*., 2015). Sticky secretions as a result of a BNT-A injections may be an issue for patients with ALS too. That could be a reason for the worsening of dysphagia and the refusal of reinjections.

6.4. Salivary *S.mutans* and *Lactobacilli* counts after BNT-A treatment

Individuals with intellectual disabilities tend to have inferior oral hygiene and higher rates of dental diseases, including caries and periodontitis, in comparison with the general population. Changes in the oral environment's microbial ecosystems increase the potential for pathogenicity within a microbial ecosystem and subsequently initiate and promote oral diseases (Baliga *et al*., 2013). Individuals with intellectual disabilities have a significantly higher proportion of *S. mutans* and *S. sobrinus* among their oral bacteria and have a higher risk of dental caries (Oda *et al*., 2016). No significant difference was found in the microbiome diversity in caries-affected children compared to caries-free children, while the level of *Rothia dentocariosa, Actinomyces graevenitzii, Veillonella sp*. oral taxon 780, *Prevotella salivae, Lactobacillus Scardovia* and *S. mutans* was higher in the caries-affected group (Jiang *et al*., 2016). *Lactobacilli* and *Streptococcus mutans* are the two main cariogenic microorganisms affecting oral health and caries formation (Petersson *et al*., 2002). Because of that, we focused on these microbes in our study. The concentration of salivary *Lactobacilli* represents a risk factor of caries (Pienihäkkinen *et al*., 1995; Peterssen *et al*., 2002; Kutsch, V.K., 2014). Colony counts of > 10,000 CFU/ml are considered high, and counts of < 1,000 CFU/ml are considered low, according to literature data (Crossner *et al*., 1977). Carbohydrate intake has been shown to correlate with salivary *Lactobacilli* counts in the mouth (Gabre *et al*., 1999). In our study the patients with PD tended to have increased *Lactobacilli* counts of > 10^5 CFU/ml, showing higher caries active oral environment, compared to healthy controls, who had counts of < 10^5 CFU/ml showing lower caries active oral environment, though the difference was not statistically significant. However, the presence of high numbers of *Lactobacilli* (10^5 CFU/ml or higher) indicates a caries-inducing oral environment.

S. mutans counts in our study did not differ statistically significantly before sialorrhea treatment between PD patients and healthy controls, being in the range of 10^5–10^6. Still, we should consider that the controls were elderly as age-matched to the PD group, and also that they might have a more caries-active oral environment.

High *Lactobacilli* counts are related to low salivary secretion rate, low salivary buffering capacity, and the presence of glucose in saliva (Larmas, 1992). Our second study of sialorrhea patients with different neurological diagnoses (Study II) did not find statistically significant changes in salivary *Lactobacilli* and *S. mutans* counts after BNT-A treatment though there was a tendency for there to be increased *Lactobacilli* counts, but in our third study on PD patients (Study III) we found an increase in *Lactobacilli* counts after BNT-A treatment, which is important to note as a risk factor for caries (Pienihäkkinen *et al*., 1995). Our results in Study II showed that the salivary flow rate decreased while salivary pH and the levels of *S. mutans* and *Lactobacilli* did not

change significantly after the injections. We had a favorable decrease in the salivary flow rate, which still might be related to the development of caries.

Lactobacilli is considered to play a role in caries formation but in cases of periodontal disease it could produce the opposite effect. The rise in our study's *Lactobacilli* counts does not mean an absolute worsening of the oral environment. Innovative researches have expressed a hypothesis on the possible beneficial effects of some *Lactobacilli* on oral health (Kutsch 2014; Ahn *et al*., 2018). *Lactobacillus acidophilus* culture relieves gingivitis and periodontitis. Some *Lactobacillus* strains can inhibit biofilm formation of oral pathogenic bacteria (Ahn *et al*., 2018). They produce antimicrobial molecules, improve the epithelial barrier function, and inhibit the adherence of pathogens to epithelial cells (Ahn *et al*., 2018). *Lactobacillus plantarum, Lactobacillus reuteri, and Lactobacillus rhamnosus GG* inhibit *S. mutans* biofilm formation (Ahn *et al*., 2018). There is also a study showing that oral administration of *Lactobacilli* changes the bacterial population in subgingival plaque in chronic generalized periodontitis in a positive way (Imran *et al*., 2015; Kõll *et al*., 2007). Probiotic drink intake containing *Lactobacillus casei* leads to a significant reduction in the three main pathogenic bacteria of periodontitis (*Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Prevotella intermedia*) and has a beneficial effect on periodontal conditions (Imran *et al*., 2015). Probiotics like lactobacilli are considered to be useful in reducing gingival inflammation as well as levels of pathogenic microorganisms in the saliva and subgingival plaque. They are nonpathogenic bacteria that change or replace the intestinal microbiota and have a beneficial effect on the host (Imran *et al*., 2015). There is some evidence that products containing probiotic *Lactobacilli* also reduce the recurrence of apthous ulcers, decrease caries risk, and the number of *Streptococcus mutans* in the oral cavity (Imran *et al*., 2015). The results of Study III demonstrated a statistically significant increase in *Lactobacilli* counts but still does not clarify its negative or positive consequences on oral health as the strains of *Lactobacilli* were not specified.

6.5. Treatment of sialorrhoea with BNT-A in Parkinson's disease patients patients

Study III focused on the oral condition of PD patients who had been described as disturbed compared to controls due to their resting tremor, bradykinesia, akinesia, and limited mobility (Bakke *et al*., 2011; Müller *et al.,* 2011); these patients have more caries, dental plaque and food derbis, and missing teeth. Orofacial dysfunction with lower mastication and jaw opening lead to dysphagia and food retention in PD patients. However, Fukayo *et al*. (2003) did not find poorer oral health in PD patients than in the control group. Drooling is more likely associated with oropharyngeal bradykinesia, hypomimia, dysphagia, and more severe involuntary mouth opening in PD (Kalf *et al*., 2012; Karakoc *et al*.,

2016). Several studies have reported that patients with PD have lower salivary flow, but increased excretion velocity to stimulus compared to healthy controls (Tumilasci *et al*., 2006; Nicaretta *et al*., 2008; Fedorova *et al*., 2015). Our results showed that salivary parameters (resting saliva formation, consistency, amount of saliva collected in the span of 5 minutes, pH, and buffering capacity) did not differ before BNT-A injections between PD patients and controls showing that our patients have sialorrhea due to impaired swallowing ability and not overproduction of saliva, as other studies have shown.

Xerostomia is an early manifestation of PD due to significantly lower saliva production than in the control group (Proulx *et al*., 2005). Droolers are usually older, have a more severe stage of PD, and have a longer disease duration (Zlotnik *et al*., 2015). Problems with drooling can be a result of levodopa treatment (Llena-Puy, 2006; Tumilasci *et al*., 2006; Fereshtehnejad *et al*., 2017). Dopamine has been shown to modulate salivary secretion (Bagheri *et al*., 1999; Tumilasci *et al*., 2006; Fereshtehnejad *et al*., 2017). Levodopa stimulates salivary flow and leads to an excessive amount of saliva. Saliva production correlates significantly with levodopa dosage and xerostomia or drooling symptoms (Proulx *et al*., 2005). The prevalence of sialorrhea in PD patients ranges widely, from 10% to 84% (Srivanitchapoom *et al*., 2014; Bruno *et al*., 2016). Our study showed that PD patients with drooling were statistically more often on levodopa treatment, supporting the findings by Ou *et al*. (2015).

Few studies have shown a relation between drooling and motor subtypes of the disease (Karakoc *et al*., 2016). Our study revealed that PD patients with a tremor-dominant subtype of the disease reported drooling less frequently than those with an akinetic-rigid and PIGD-dominant subtype of PD, which may be explained by the more prominent hypokinesia of swallowing musculature in patients with these clinical subtypes.

There are no data from earlier studies on BNT-A treatment and saliva composition or microbial changes. Our results on at-rest saliva formation time shortened, but it did not overcome statistically a critical value of > 60 sec, showing that the oral cavity remained hydrated and healthy during the treatment with BNT-A. Saliva's normal pH is considered 6.7–7.4 (Hand *et al*., 2014). There is a higher risk for dental caries in an acidic oral pH state, and a higher risk for the dental calculus in an alkaline pH condition (Dawes, 2007). We did not find significant alterations in salivary pH after the BNT-A injections but revealed an increase in buffering capacity during the study period, indicating an improvement in salivary defence ability that could be related to saliva flow decrease and mouth closure.

6.6. Strengths and limitations of the study

The strengths of this research were its broad approach and comprehensive examination of sialorrhea patients, including neurological testing and charac-

terization of salivary parameters. Patients with different neurological disorders with childhood, adult and geriatric sialorrhea were included.

Study III also contains the comparison of salivary parameters between patients with neurological diseases, and controls. Saliva characteristics were measured both in patients with neurological diseases and controls, and also compared between PD patients treated with BNT-A injections or those who were not. Dynamic changes after the treatment were followed as well.

The main limitation of the study is the relatively small sample size of the study groups, which might be a source of low statistical reliability. Another limitation is the focus only on *S. mutans* and *Lactobacilli* of cariogenic microflora and not on other periodontal pathogens that may also influence the oral environment.

6.7. Practical implications and future perspectives

According to this study, BNT-A injections are an effective, well-tolerated, and low-risk procedure in the treatment of sialorrhea. Most of the more frequent complications of this treatment are related to swallowing disturbances, which ware off in a matter of weeks. During the study, a pattern for dosage and specified target point selection were performed and the reinjection schedule was arranged, in addition to treating the clinical aspects of sialorrhoea in neurological diseases that have been described. With these findings we can confirm that BNT-A intrasalivary gland injections are beneficial and this treatment improves the self estimated quality of life of CP, PD and ALS patients with sialorrhea.

Also, possible complications of BNT-A injections were studied, including their impact on microflora and possible role for the oral health, specially to development of caries, which is of high practical importance. Our study was focused on *Lactobacilli* and *S. mutans* but periodontal pathogens need further study.

Also cost-effectiveness studies are of importance as BNT-A injections are expensive, and in some cases, e.g. for children, general anesthesia is needed. Compared to BNT-A injections, surgery has higher risk of complications, but despite of that it has been the treatment of choice for sialorrhea because of permanent effect (Delsing *et al*., 2016; Ozturk *et al*., 2017). Anticholinergic drugs are not so practicable because they may cause a thickening of mucous secretion also in lungs as a dangerous complication and are contraindicated in the presence of heart diseases, glaucoma, pyloric stenosis, prostatic hypertrophy, and hepatic or renal insufficiency (Banfi *et al*., 2015; Lakraj *et al*., 2013).

Generally, this study has provided the practical implications of the clinical management of sialorrhoea by developing the procedure for BNT-A injections in salivary glands in order to treat sialorrhoea in patients with chronic neurological diseases.

7. CONCLUSIONS

BNT-A injections into the salivary glands to treat drooling is an effective and safe method if done under sonographic guidance. It is a good therapeutic option for treatment of sialorrhea in different neurological diseases like CP, PD and ALS, to improve patients' quality of life.

1. Sialorrhea is influenced by different clinical factors like lip seal, head position, control of voluntary movement functions, above all tongue, and psychological maturity.
2. Our findings in Study II showed that BNT-A provides a favorable therapeutic effect with improvement in saliva's buffering capacity without compromising salivary pH and microflora. Bilateral percutaneous sonography-guided intrasalivary BNT-A injections into the parotid and submandibular glands effectively reduced drooling while maintaining oral health.
3. The results of Study II did not demonstrate changes in oral health but the results of Study III showed a statistically significant increase in levels of *Lactobacilli*, which is a possible risk factor for caries. BNT-A injections can effectively treat sialorrhea, but the change of oral microflora has to be taken into consideration and patients should be under dentists' care more frequently.

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SUMMARY IN ESTONIAN

Kesknärvisüsteemi haigusest tingitud sialorröa ravi botulismitoksiiniga

Liigset süljeeritust ehk sialorröad võib esineda mitmete kesknärvisüsteemi haiguste korral, sh laste tserebraalparalüüs, amüotroofne lateraalskleroos ja Parkinsoni tõbi (Gonzalez, L. *et al*., 2017; Odachi *et al*., 2017). Neuroloogiliste haigustega patsientide probleem ei ole seotud mitte niivõrd sülje rohkusega, kuivõrd neelamisfunktsiooni häirega. Pideva süljevooluse tõttu on häiritud artikulatsioon ja efektiivne kommunikatsioon. Sialorröa on tingitud neelu, suupõhja, keele ja suulae lihaste pareesist, hüpokineesiast või düskoordinatsioonist (Meece *et al*., 2010). Osadel patsientidel nõrgeneb köharefleks ja seetõttu võib neil esineda sisse hingates suurenenud risk süljeaspiratsiooniks, mistõttu võib tekkida aspiratsioonipneumoonia; samuti võib tekkida naha matseratsioon. Nende komplikatsioonide tõttu langeb nii patsientide kui ka nende hooldajate elukvaliteet (Gonzalez, L. *et al.*, 2017).

Alates 1980. aastatest on botulismitoksiini kasutatud mitmete kesknärvisüsteemi haiguste ravis: peamiselt fokaalsete düstooniate ja spastilisuse leevendamiseks. Uue näidustusena on lisandunud botulismitoksiin A (BNT-A) süstimine liigse süljevooluse vähendamiseks hüpersalivatsiooni korral (Rallmann *et al.*, 2016; Zhang *et al.*, 2017; Al-Fouzan *et al.*, 2017).

Botulismitoksiini toime

Botulismi tekitaja, *Clostridium botulinum*'i produtseeritud toksiin toimib perifeerses kolinergilises süsteemis, blokeerides atsetüülkoliini vabanemist presünaptilistelt närvilõpmetelt ning põhjustades neuromuskulaarset või neuroglandulaarset pöörduvat paralüüsi (Awan, 2017). Süljeerituse vähendamine ja suukuivus on üks botulismitoksiini mõjudest ja seda kasutatakse liigse süljeerituse ravis. Terapeutilistel eesmärkidel kasutatakse põhiliselt A-alatüübi toksiini (Bentivoglio *et al*., 2015), toime kestvuseks on märgitud kaks kuni kaheksa kuud (Meece *et al.,* 2010). Enamasti manustatakse BNT-A-d nii kõrvasüljenäärmetesse kui ka submandibulaarsetesse näärmetesse, kuid on kasutatud ka süstimist ainult submandibulaarnäärmetesse (Fuster-Torres *et al*., 2007).

Varasemad uuringud on kirjeldanud BNT-A ravi positiivset mõju süljeerituse pärssimisele, kuid suuõõne tervist käsitlevaid uuringuid on vähe (Pei-Hsuan *et al*., 2011). Sülje koguse vähenemine võib mõjutada suuõõne mikrofloorat ja sülje omadusi (nt pH ja puhvervõime), mistõttu süljevoolu langus võib olla seotud pH ja isepuhastumisvõime vähenemisega suuõõnes ning hambakaariese sagenemisega (Sharma *et al*., 2012). Sülje pH-l ja kariogeensete bakterite hulgal (*S. mutans* ja laktobatsillid) on suuõõnetervise seisukohalt võtmeroll (Pienihäkkinen *et al*., 1995). *S. mutans* on oluline hambakaariese tekkes ja progressioonis ning seda mikroobi peetakse bakteriaalse kaariese esmaseks põhjustajaks. Ta esineb hamba pinnale kinnitunud katus ja kasutab elutege-

vuseks suhkruid, mille ümbertöötlemise tulemusena tekivad hambaemaili lagundavad happed. *S. mutans*'i esinemine mikroflooras näitab suurenenud riski hambakaariese tekkeks (Fejerskov *et al.*, 2008).

Uuringu eesmärgid

Hinnata BNT-A efektiivsust ja ohutust sialorröa ravis kesknärvisüsteemi haigustega patsientidel.

1. Hinnata kliiniliste faktorite rolli tserebraalparalüüsiga laste sialorröa puhul.
2. Hinnata BNT-A süstide mõju suuõõnetervisele sialorröa patsientidel.
3. Hinnata ja võrrelda sülje koostist ja suuõõne mikrofloora muutumist pärast BNT-A süstimist süljenäärmetesse ultraheli kontrolli all sialorröa ravis.

Metoodika

Uuringu esimene osa hõlmas 12 tserebraalparalüüsiga last Tartu Ülikooli Kliinikumi lasteneuroloogia osakonnast, kellest 9 (4 poissi ja 5 tüdrukut vanuses 1,6–11 aastat) osalesid uuringus täies mahus. Uuringu teine osa hõlmas 20 sialorröaga täiskasvanut Tartu Ülikooli Kliinikumi näo-lõualuudekirurgia osakonnast (12 meest ja 8 naist), keskmise vanusega 63,2 aastat. Uuringu kolmandas osas uuriti 38 Parkinsoni tõvega patsienti Tartu Ülikooli Kliinikumi neuroloogia ja näo-lõualuudekirurgia osakonnast (16 naist ja 22 meest), kelle keskmine vanus oli 71,1 aastat. Patsientide skriininguks kasutati MDS-UPDRS küsimustiku igapäevaelu mittemotoorsete aspektide osa (Goetz *et al*., 2008). Uuritavad jagati kolme rühma: (1) 12 Parkinsoni tõvega patsienti (9 meest ja 3 naist keskmise vanusega 71,3 aastat), kes said liigse süljeerituse raviks BNT-A süste; (2) 13 Parkinoni tõvega patsienti (7 meest ja 6 naist keskmise vanusega 71,5 aastat) ilma BNT-A ravita; (3) 13 samaealist tervet vabatahtlikku kontrollrühmana (6 meest ja 7 naist keskmise vanusega 70,6 aastat).

Ohutuse eesmärgil süstiti BNT-A-d süljenäärmetesse ultraheli kontrolli all; süstiti mõlema poole lõuaaluseid- ja kõrvasüljenäärmeid. BNT-A (Abobotulinumi/ Dysport®) doos arvutati patsientide kehakaalu järgi, kogudoosiga 6 TÜ/kg.

BNT-A-ga tehtud ravi efektiivsust hinnati intervjuu ja küsimustiku abil enne BNT-A süstimist süljenäärmetesse ja pärast süstimist kahenädalaste vahedega kuue kuu jooksul. Intervjuu hõlmas haiguse kulgu, sialorröa põhjust, sülje hulka, süljelappide vahetuse hulka, aspiratsiooni sagedust, hingamisprobleeme ja teisi komplikatsioone ning elukvaliteeti. Teises ja kolmandas uuringu osas kasutati MDS-UPDRS küsimustikku (igapäevaelu mittemotoorsed aspektid) ja sülje parameetrite mõõtmist; patsiente hinnati enne BNT-A süstimist süljenäärmetesse ja üks kuu hiljem. Mõõdetavad põhiparameetrid olid suuõõnest kogutud sülje hulk, koostis ja mikrobioloogiline seisund. Uuriti nii puhkeaja kui ka stimuleeritud sülge: puhkeaja sülge hinnati märgumise aja, konsistentsi ja pH järgi; stimuleeritud sülge hinnati koguse ja puhvervõime järgi. Sülje koostist analüüsiti, kasutades Saliva-Check BUFFER *in vitro* testi (GC EUROPE N.V.

B-3001 Leuven, Belgium). Kariogeensete mikroobide uurimiseks kasutati Dentocult SM ja Dentocult LB teste (Orion Diagnostica Co Ltd, Epsom, Finland).

Uuringu tulemused ja arutelu

BNT-A süstimise järel vähenes süljevoolus tserebraalparalüüsiga lastel subjektiivselt juba esimesel nädalal. Kui süstimiseelses seisundis oli süljeeritus väga intensiivne (väga intensiivne võrdus skaalal viie punktiga), siis kahe nädala möödudes hinnati seda keskmiseks (keskmine võrdus kolme punktiga). Süljelappide arv ja aspiratsioonivajadus vähenes poole võrra. Maksimaalne raviefekt esines kahest nädalast kahe kuuni. Ühel lapsel üheksast positiivset efekti ei olnud, kuna komplikatsioonina esines neelamisraskus. Elukvaliteet paranes oluliselt kaheks kuuks.

Teise uuringu tulemused näitasid, et 95%-l sialorröa patsientidest oli kuu aega hiljem sülje hulk vähenenud, kui BNT-A-d oli süstitud süljenäärmetesse, seejuures sülje pH, puhvervõime ja sülje laktobatsillide ja *S. mutans*'i tase statistiliselt olulist muutust ei näidanud ($p>0,05$). Stimuleeritud sülje hulk vähenes oluliselt, olles enne süstimist 7,1 (±5,6) ml ja üks kuu pärast süstimist 4,5 (±3,4) ml. Puhkeaja sülje teke aeglustus, olles enne süstimist 20,7 (±15,1) sekundit ja üks kuu pärast süstimist 34,3 (±19,7) sekundit.

Kolmanda uuringu tulemused, mis hõlmasid ainult Parkinsoni tõvega patsiente, erinesid mõnevõrra teise uuringu tulemustest, kuhu olid kaasatud erineva geneesiga sialorröahaiged. Võrreldes süstimise-eelse tulemusega esinesid üks kuu pärast süstimist statistiliselt olulised muutused: viie minuti jooksul kogutud sülje hulk vähenes (enne 8,6 (±6,8)ml; 1 kuu pärast 4,8 (±4,1)ml; $p=0,018$) ja puhkeaja sülje teke aeglustus (enne 15 sek; 1 kuu pärast 25sek; $p=0,05$). Puhvervõime oli üks kuu pärast BNT-A süste paranenud võrreldes süstimiseelse väärtusega (enne 6,3 (±3,3); 1 kuu pärast 8,8 (±2,8); $p=0,037$), mis näitab sülje paremat võimet normaliseerida oma pH-d pärast sööki. Sülje konsistents ja pH ei muutunud oluliselt. Sialorröad esines rohkem hüpokineetilis-rigiidse alatüübiga Parkinsoni tõve patsientidel, samuti ilmnes seos pikaajalise levodopa kasutamise ja liigse süljeerituse vahel.

BNT-A ravi ei muutnud *S. mutans*'i taset süljes, kuid siiski esines statistiliselt oluline laktobatsillide tõus, mis näitab kaariese tekke riski. Samas, periodontaalhaiguste tekke seisukohalt on varasemate uuringutega tõestatud, et laktobatsillide osakaalu suurenemine vähendab igemehaigusi tekitavate patogeenide osakaalu suuõõnes (Imran *et al.*, 2015).

Järeldused

BNT-A süstid on efektiivsed krooniliste neuroloogiliste haigustega esineva sialorröa ravis, kuid arvesse tuleb võtta suuõõne mikrofloora muutumise riski ja sülje hulga vähenemisest tingitud sülje isepuhastumisvõime langust, mis võib kaasa tuua kaariese esinemissageduse tõusu.

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PUBLICATIONS

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2014–2018	PhD Student, Institute of Dentistry, University of Tartu, Tartu, Estonia

Professional empoyment

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Membership in professional societies

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Scientific work

The major areas of my research include the salivary changes after the intraglandular BNT-A treatment of the central nervous system diseases induced sialorrhea. I am interested in modern treatment method with BNT-A injections for sialorrhea. My main focuses of research include the BNT-A effect and its potential risks to oral health.

Pubications

1. Tiigimäe-Saar, J.; Leibur, E.; Kolk, A.; Talvik, I.; Tamme, T. (2012) Use of Botulinum neurotoxin A in uncontrolled salivation in children with cerebral palsy. A pilot study. International Journal of Oral and Maxillofacial Surgery. 41, 1540–1545.
2. Tiigimäe-Saar, J.; Taba, P., Tamme T. (2017) Does the Botulinum neurotoxin type A treatment for sialorrhea change oral health? Clin Oral Invest. 21, 795–800.

3. Tiigimäe-Saar, J.; Tamme, T.; Kadastik-Eerme, L.; Rosenthal, M.; Taba, P. (2018) Saliva changes in Parkinson's disease patients after injection of Botulinum neurotoxin type A. Neurological Sci. https://doi.org/10.1007/s10072-018-3279-4.
4. Tiigimäe-Saar, J,; Tamme T. (2017) Hüpersalivatsiooni ravi botulismitoksiiniga ja selle mõju suuõõne tervisele. Hambaarst. 1, 34–36.
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2002–	Eesti Näo-lõualuudekirurgia Selts

Teaduslik tegevus:
Peamiseks uurimisvaldkonnaks on muutused süljes pärast näärmesisest ravi BNT-A-ga kesknärvisüsteemi haigustega patsientidel. Olen huvitatud sialorröa modernsest ravimeetodist BNT-A-ga. Peamine uurimissuund on BNT-A mõjuaeg ja riskid suuõõnetervisele.

Publikatsioonid:

1. Tiigimäe-Saar, J.; Leibur, E.; Kolk, A.; Talvik, I.; Tamme, T. (2012) Use of Botulinum neurotoxin A in uncontrolled salivation in children with cerebral palsy. A pilot study. International Journal of Oral and Maxillofacial Surgery, 41, 1540–1545.
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