Protocol

1. Project Title:

The Effect of Zinc Supplementation prior to Botulinum Neurotoxin Type A Injection in the Treatment of Spasmodic Dysphonia

2. Investigator(s):

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3. Abstract:

Botulinum toxin (BT) injections are commonly used to treat various cosmetic, muscular, anesthetic, and neurologic pathologies. In 2012, one study suggested that a combination of zinc and phytase supplementation could increase the duration and degree of effects of botulinum neurotoxin type A (BoNTA).[1] However, this study was met with scientific skepticism due to concerns of financial conflicts of interest, unmasking the study too early, and ambiguity of the dosage of zinc used.[2] In 2021, a new study with 25 patients receiving BoNTA injections for the treatment of excessive gingival display (EGD), showed that zinc supplementation significantly prolonged the effect of BoNTA injections.[3] There has been no further studies or commentary since these latest results were published. The purpose of this study is to evaluate if zinc supplementation can increase the positive effects of BoNTA for treatment of spasmodic dysphonia (SD). If positive effects are observed, this would strengthen the preliminary results of the 2021 study, encourage future larger studies on the same hypothesis, and, ultimately, reducing the burden of disease for patients with SD and any other condition that is treated with BT injections.

4. Background:

Botulinum toxin (BT) is produced by the anaerobic, gram-positive rod *Clostridium Botunilum,* which creates seven different serotypes of toxins—the most commonly used is type A.[4] Botulinum neurotoxin type A (BoNTA) is widely distributed in North America as the product Botox™. All serotypes of BT act by impeding transmission of nerve impulses through inhibiting the pre-synaptic release of acetylcholine neurotransmitter.[4] BT injections are commonly used to treat cosmetic concerns, such as dynamic rhytids in the aging face and excessive gingival display (EGD). BT injections also are effective, albeit temporary, treatments of various chronic neurologic, myogenic, and pain conditions. Thus, the use of BT injectables such as BoNTA continues to rapidly increase.[5]

Because the average effect of BT is 2-4 months, much research has been done to find ways to potentiate the duration of this effect. If a simple way was found to make the effect of BT last longer, this would have massive implications in the cosmetic, neurologic, and anesthetic fields of medicine. In 2012, one study suggested that a

combination of zinc and phytase supplementation could increase the duration and degree of effects of BoNTA.[1] However this study was met with scientific skepticism due to concerns of financial conflicts of interest, unmasking the study too early, and ambiguity of the dosage of zinc used.[2] The creators of that original pilot study make a product, Zytaze, which is a combination of zinc citrate and phytase and is marketed as a "Botox booster," based alone on the results of the 2012 study. Low amounts of zinc are often included in daily multivitamins, but specific zinc supplements are also sold in 30 mg or 50 mg tablets. In 2021, a study with 25 patients receiving BoNTA injection for the treatment of EGD, showed that a short course of 50 mg daily zinc supplementation prolonged the effect of BoNTA with statistical significance.[3] There have been no further studies or commentary since these latest results were published.

The use of Botox to treat spasmodic dysphonia is the accepted gold standard treatment worldwide for this disease.[6–9] Botox® injections are marketed worldwide for the safe, efficacious treatment of many cosmetic and therapeutic indications.[10] The use of Botox for the treatment of SD (or laryngeal dystonia) has been the accepted gold standard treatment globally for decades and has been found to be safe and effective.[11–14] The first injection of BoNTA to treat SD was performed in 1984, and since then dozens of publications have confirmed the safety and efficacy of this off-label treatment.[7] Risks of Botox® treatment for adductor SD include most commonly breathiness of voice, neck pain, and aspiration of fluids.[9,10,12,15–17] Other much more rare side effects include shortness of breath, cough, or odynophagia. There is one reported case of the Botox® injection causing an airway obstruction and requiring temporary intubation.[9]

The purpose of this study is to evaluate if zinc supplementation can increase the positive effects of BoNTA for treatment of spasmodic dysphonia (SD). If zinc supplementation prolongs the effect of BoNTA, this would strengthen the preliminary results of the 2021 Shemais study, encourage future larger studies on the same hypothesis, and, ultimately, could lead to reducing the burden of disease for patients with SD and any other conditions that are treated with BT injections.

5. Specific Aims:

To determine if zinc supplementation prior to injection of botulinum neurotoxin type A will affect the duration of the drug's effect in treating spasmodic dysphonia.

6. Research Plan:

A prospective, controlled crossover study will be completed on patients seen at the University of Florida Department of Otolaryngology—Head and Neck Surgery. The UF Health Institutional Review Board will be consulted to approve this study.

Patients and Methods:

Inclusion Criteria:

-Adults patients
-18 years old and above
-Current diagnosis of Adductor spasmodic dysphonia
-Has had Botox injection treatments for SD > 6 months

Exclusion Criteria:
-Pregnant
-Diagnosis of Abductor spasmodic dysphonia
-Patients taking > 30 mg zinc supplementation daily prior to study beginning

Upon arrival to the previously scheduled BoNTA injection clinic visit, eligible patients will be invited to participate in the study. Those that agree will undergo informed consent. The consenting process will be done in a clinic room in the Shands ENT clinic. Paper copies of the consent form will be provided to the subject to read and review, and the study investigator will verbally explain the consent form in detail as well. The subject will have ample opportunity to ask questions. If a subject consents to participate in the study, they will sign a digital copy of the consent form. At this point, basic patient parameters that will be recorded for analysis including: age, sex, race, weight, body mass index, past medical history, otolaryngologic history, comorbidities, medications, and current vitamin supplement use.

The standard of care for treating patients with adductor spasmodic dysphonia is to have BoNTA injections into bilateral vocal cords every 2-4 months. That time frame is determined by how long it takes for the Botox to wear off and the patient's symptoms to return. This procedure is performed three to four times per year, and is often chronically performed for years and decades. This research study looks to see if simply taking five days of zinc supplementation prior to a BoNTA injection will affect the duration or degree of effect of that treatment. Thus, the intervention will consist of taking a 50 mg zinc gluconate supplement at breakfast for the five days leading up to their injection appointment. The subject will also answer questionnaires and provide audio recordings at various time points of the injection cycles.

The treatment procedure is as follows: the subject will come to outpatient clinic. For all subjects in this study, patients will have already had two cycles of BoNTA injections for SD. The attending physician will ask how the previous cycle went and if the patient feels like the dose was too small or too large and make adjustments accordingly with the next dose given. When the dose is established, the patient will be sitting upright with the head resting on a head rest. The anterior neck is cleaned with an alcohol swab and allowed to dry. At this time, a 27-gauge needle that is attached to the syringe containing the BoNTA and also wires that lead to an electromyography machine. The needle is then inserted through the anterior neck skin at a 30-degree superior and 30-degree lateral vector on the ipsilateral side. The patient then vocalizes, the position is confirmed with the electromyography readings, and the BoNTA is then injected. The exact procedure is then repeated on the contralateral side.

Data Collection

We will enroll patients in this study as each patient comes to a previously scheduled procedure for laryngeal BT injections for treatment of SD. The patient list of enrolled subjects will then be used to guide accessing the patients' existing electronic medical records (UF Epic). The electronic medical record (EMR) will be used to collect all information/data needed for this study. Information will be captured from existing clinic evaluations, laboratory findings and other diagnostic results. Medical records will be reviewed for patient demographic data (age, gender, co-morbidities).

After consenting to participate in the study, each subject will fill out the Voice-Related Quality of Life (V-RQOL) questionnaire, and we will obtain the initial audio recording of the subject's voice while reciting a scripted passage. The patient will provide demographic, past medical history and contact information. A study packet will be provided that includes a copy of the consent form, copies of the V-RQOL survey, zinc supplement tablets, and the script for the audio recordings. The patient will then be contacted via text, email, and telephone on post-injection day 2, 7, 14, 42, 70, and 98 to obtain audio recordings of the subject while reading the scripted passage and to fill out the V-RQOL questionnaire. Four days before the next scheduled injection procedure, each subject will begin daily taking one 50-mg zinc tablet after breakfast. A fifth tablet will be included for the patient to take the morning of the injection procedure.

At the patient's second BT injection of the study, they will then officially start cycle 2 and will crossover to the intervention arm of the study. Prior to receiving their injection, they will be asked the V-RQOL survey and an audio recording will be obtained reciting the scripted passage. They will specifically be asked if they feel their voice has improved since starting the zinc supplements. Data collection will then proceed exactly as it did in cycle 1. The patient will be contacted on post-injection day 2, 7, 14, 42, 70, and 98 to obtain audio recordings of the subject while reading the scripted passage and to fill out the V-RQOL questionnaire. The study will conclude when the subject returns for the third BT injection and a final V-RQOL and audio recording will be obtained.

Surgeons

The procedures will be performed by one experienced laryngologist at the University of Florida (Dr. Neil N. Chheda). If a resident otolaryngologist is involved in the procedure, the training level and degree of involvement will be recorded.

Data analysis

Since this is a preliminary study, we were not able to run a power analysis. Based off of the related studies by Paniello (n=9) and by Shemais (n=25), and as the attending surgeon has a wide range of experience, we have a goal to have a sample size of 30 patients. With the assumption that about 10% of patients may not complete surveys or audio recording follow up and about 10% that may be excluded for various reasons, we request an additional 6 patients. Hence, targeted total enrollment will be 36 patients. Patients will be enrolled in the study in three consecutive months at Dr. Chheda’s monthly SD Botox clinic. After three months, almost all of the patients who are treated by Dr. Chheda for SD will be seen in one of those clinics and have the opportunity to enroll the study.

All information/data needed for this study will be obtained prospectively from patient surveys and audio recordings or retrospectively from medical records. Each patient included in the study will be assigned a subject ID. All identifiers (MRN, patient names) will be removed, and dates of birth converted to ages after completion of data collection. All data collected will be stored in a separate password-protected file with the study ID using the REDCap’s secure web platform. The file containing the MRN, patient names, and the study IDs will be destroyed after data analysis. Only designated study staff will have access to the data collected. Those with access will be compliant with the institutional requirement established by the University of Florida. After all subjects have

completed both cycles, all of the accrued audio recordings will be analyzed. After this analysis is completed, all audio recordings will be deleted.

7. Possible Discomforts and Risks:

BoTNA injections as a standard of care for treating SD have risks involved with the procedure. Transcervical BT injections into the bilateral thyroarytenoid muscles usually cause minimal discomfort, and the patient is able to walk out of the office immediately afterwards. There is no appreciable blood loss. Patients with adductor SD typically notice an improvement in vocal symptoms within seven days, and typically enjoy two to three months of relief. Risks of Botox treatment for SD include most commonly breathiness of voice, neck pain, and aspiration of fluids.[9] Other more rare side effects include shortness of breath, cough, or odynophagia. There is one reported case of the Botox injection causing an airway obstruction and requiring temporary intubation.[9]

The specific risks of this study involve the zinc supplementation and collecting of patient information. Zinc is considered to be relatively nontoxic.[18] There are no consensus recommendations on the dosage of zinc supplementation that is too high, but there are known cases of zinc toxicity after chronic supplementation over many months and years.[19] Excess zinc can be associated with nausea, vomiting, fatigue, anemia, weakened immune system and copper deficiency. The intervention of this study will be only a short five-day course of zinc supplementation, so the risk of zinc toxicity is low. The exact dosage and course of zinc supplementation will be identical to Shemais' study with 25 patients which had no reported toxicities.[3] There does remain a possibility that zinc reacts to the BT injection in unpredictable ways, thus there is potential that the effects of the BT could be shortened, weakened, prolonged, or augmented compared to prior procedures. All patient health information and identifiers will be kept in secure, password-protected files on REDCap's web platform, however there still remains a low risk of a breach in confidentiality of subject's health information if that database were to be subjected to a cyber attack and compromised. These risks will be discussed with patients at length upon enrollment into the study.

Every subject will be assessed at every clinic visit for complications and adverse events related to both the standard of care BoTNA injections and the zinc supplementation. Additionally, the ENT clinic has a 24/7 answering service that patients can call if they experience any concerning side effects of the treatment. Stopping criteria would include any signs of life-threatening respiratory distress. Other unwanted side effects could be reasonably avoided with lowering subsequent Botox dosages.

Data Safety Monitoring Board:

The PI and lead co-investigator (Drs. Chheda and Gebhard) will meet at least once a month to review for any adverse events. A written report be generated.

8. Possible Benefits:

There is a possibility that the intervention will increase the duration of BT injections, and the patient will have improved vocal symptoms from SD for a longer duration. There is also a chance that the study intervention may restore zinc homeostasis if the patient was previously zinc-deficient.[19]

9. Conflict of Interest:

There are no conflicts of interest.

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