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Case Report

Neutralizing Antibodies to Botulinum Toxin Type A as a Secondary Treatment Failure: A Case Report from Mumbai, India

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ABSTRACT

Botulinum toxin use in aesthetic medicine for treatment of facial rhytids is common and has become more and more popular in India in recent times. Botulinum toxin (BoNT) is produced by clostridium botulinum which is an anaerobic, spore forming, rod shaped bacteria. The botulinum toxin complex contains a 150 KD neurotoxin together with a complex additional protein. The neurotoxin is cleaved by the clostridial proteases into heavy and light chain. The heavy chain (100 KD) after cleavage binds to gangliosides and a protein receptor on the presynaptic nerve ending, whereas the light chain (50 KD) blocks the release of acetylcholine, leading to a dose dependent weakening of the target muscle.

Keywords
Botulinum neurotoxin type A; Neutralizing antibodies; Secondary treatment failure; Aesthetic treatment; Facial rhytides.

INTRODUCTION

There are the seven distinct serotypes of botulinum toxin A-G, of which A and B are used clinically. Various commercially available botulinum toxin type A (BoNT-A) commonly used in India are Incobotulinum-Xeomin®, Onabotulinum-Botox®, and Abobotulinum-Dysport®; respectively. Botulinum toxin was first used clinically in 1970s in ophthalmology to treat strabismus,[1] and over last two decades has gained widespread use in conditions requiring inhibition of excessive muscle spasm. Medical indication of BoNT include movement disorders like spasticity, cervical dystonia, urological disorders like overactive bladder, dermatology conditions like hyperhydrosis and cosmetic indications of facial rhytids. The indicated clinical conditions are chronic in nature, and BoNT effects do not last more than few months; patients need to be treated repeatedly. Over the last 20-years botulinum toxin has gained popularity especially for cosmetic and aesthetic indications. However, there some patients who stop responding to the BoNT-A injections due to a variety of reasons, including emergence of immunoresistance as a result of neutralizing antibodies (NAb).

Botulinum Toxin Structure and Function

BoNT is composed of core neurotoxin and associated non-toxic accessory proteins called NAP. The core neurotoxin consists of a 150 kD precursor protein that contains a 100 kD heavy chain and a 50 kD light chain, linked with a disulphide bond.[2] BoNT binds to the glycoprotein receptors on the cholinergic nerve terminal membrane, here the native vesicle recycling mechanisms are used to facilitate endocytosis of the 50 kD light chain into the cytoplasm.[3,4] The light chain cleaves the soluble N-etheylmaleimide sensitive factor attachment protein receptor (SNARE) proteins involved in eventual transport of acetylcholine (Ach) vesicles and their docking with the presynaptic membrane before releasing Ach into the synaptic cleft. Different serotypes of BoNT affect different SNARE proteins, synaptosomal-associated protein-25 (SNAP-25) is cleaved by BoNT-A, BoNT-C, and BoNT-E, and VAMP (synaptobrevin) is cleaved by BoNT-B, BoNT-D, and BoNT-F.[3,5] The impaired Ach exocytosis interferes with synaptic neural transmission in striate muscles as well as cholinergically innervated structures e.i. smooth muscles or exocrine glands.[5] The core neurotoxin is accompanied by NAPs, which is comprised of hemagglutinin (50 kD) and non-hemagglutinin proteins (130 kD), this associates with the core neurotoxin to help prevent degradation.[6,7] Different BoNT types have different NAP compositions. The only product where all NAPs have been removed is Incobotulinumtoxin A.[8] The core neurotoxin and NAP are mixed with excipients which vary

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among the different brands, include albumin, sucrose, lactose, sodium chloride, and disodium succinate.[5,9-13] The active neurotoxin dissociates completely from the complexing proteins on reconstitution. Therefore, complexing proteins do not influence the therapeutics effect of the core neurotoxin (Table 1).[1,14]

Immunogenicity

The relevance of immunogenicity in aesthetics is debated, however many reports suggest it should be considered seriously.[6-8,16-24] The immunogenic potential of BoNT-A products depends on multiple factors including their formulations, quantity of antigenic protein, accessory proteins, and treatment related like toxin dosage, frequency and earlier exposure to toxin.[25] Immunogenicity is described as proteins ability to induce an immune response, as a consequence, stimulate antibody formation.[26] Like any other non-human, foreign protein, commercial BoNT-A preparations can initiate immune reaction on injection, particularly when these injections have been repeated several times.[27,28] It is important to define the terms primary non-responders and secondary non-responders.

Primary and Secondary Non-Responders

There are several studies which have reported that some patients have never responded to BoNT-A, they were categorized as primary non-responders. In series of 235 patients receiving BoNT-A for multiple indications 9.1% were thought to have primary resistance and 7.5% secondary resistance[29] Primary resistance was defined as a less than 25% response from the first injection despite two or three consecutive injections of increasing dosages. Primary resistance however is a rare phenomenon in clinical populations.[30]

Secondary non-response is characterized by initial benefit in symptoms after BoNT-A treatment followed by loss of response with subsequent injection at some point in time of series of injection treatment. These patients cannot be categorized as non-responders, they fall under the definition of secondary non-responders wherein there is an absence of clinical response and absence of any adverse effects after at least two consecutive treatment visits. Immunogenicity due to presence of Nabs is not the only or main cause of secondary non-response. Poor or no response to BoNT-A is more frequently due to an insufficient dosage, inappropriate muscle selection, or improper injection technique.[31] In a meta-analysis of 8525 patients reported in 61 studies, the prevalence of Nabs was 3.5% among clinically responding patients and 53.5% in patients with secondary non-response, but half of the patients with SNR did not have NAbs.[32] There are various studies which have used different assays for NAbs, which accounts for the marked variability in the reported frequency of the NAbs and poor correlation between the presence of NAbs and clinical response.

Botulinum Toxin Antibodies

BoNT antibodies can be divided into NAbs, targeting the core neurotoxin binding at the heavy chain, and non-neutralizing antibodies, targeting the accessory protein which may be clinically irrelevant. Some antibodies have been found to have bind to the regions of light chain of BoNT-A[33] Antibodies detected against BoNT are typically of IgG-type, are sero specific. Patients who do not respond to BoNT-A due to development of NAbs usually respond to BoNT-B. However, this switch of serotypes exposes them to a higher risk of developing resistance to alternate type of BoNT, which can be attributed to about 30% structural homology in the heavy chain of BoNT-A and BoNT-B.[34]

NAbs can decrease over prolonged duration, one of the studies showed that the average duration between the detection of NAb and subsequent reversal to a NAb-negative status was about 30-months.[35] However, the immunologic response to the same BoNT serotype can be reactivated by repeat treatments.[35,36] An increase in dosage up to four times can restore the therapeutic response in some patients with partial SNR associated with NAbs.[37] Alternative strategy to gain the clinical response in patients with

Table 1. *Pharmaceutical, Biological and Clinical Properties of BoNT/A Products*[15]

Botulinum Toxin type A	Onabotulinum	Abobotulinum	Incobotulinum
Brand name	Botox® ,Vistabel®	Dysport®,Azzalure®	Xeomin®
Aproved aesthetic indication	Galbellar rhytides, lateral canthal rhytides	Moderate to severe Galbellar rhytides	Moderate to severe Glabellar rhytides and lateral canthal rhytides
Presentation	Vacuum-dried powder for reconstitution	Freeze dried (Lyophilized) powder for reconstitution	Freeze dried (Lyophilized) powder for reconstitution
Isolation Process	Precipitation	Precipitation and chromatography	Precipitation and chromatography
Composition	Clostridium botulinum toxin type A: HA and non-HA proteins	Clostridium botulinum toxin type A: HA and non-HA proteins	Clostridium botulinum toxin type A
Excipients*	100 U vial HSA 0.5 mg; NaCl 0.9 mg	500 U vial HSA 125 µg; Lactose 2.5 mg	100 U vial; HSA 1mg; Sucrose 4.6 mg
Molecular weight	900(150)	Not published (150)	150
Total Protein content ng per 100l U	5.0	4.87	0.44
Neurotoxin protein load (ngneurotoxin per100 U*)	0.73	0.65	0.44
Neurotoxin potency U/ng	137	154	227
Shelf life	2-8 °C 2-3-years**	2-8 °C 2-years	Room Temp 3-4-years**
Storage after reconstitution	2-8 °C 24 hrs	2-8 °C 4 hrs	2-8 °C 24 hrs

*Units of measurements for three commercially available BoNT/A preparations are proprietary to each manufacturer and are not interchangeable.
**Depending on the number of units

SNR is to transition them to the formulation of BoNT that lacks the complexing proteins and hence have potentially lower immunogenicity.[36] Antibodies to NAPs do not result in loss of efficacy and hence are not clinically relevant as antibodies targeting the heavy or light chains,[38] Many factors concerning the structure of the BoNT, such as the ratio of active to inactive core neurotoxin, can affect its immunogenicity. Inactive protein refers to the 150 kD precursor protein formed prior to further protease cleaving into 100 kD heavy chain and 50 kD light chain. Although inactive, this 150 kD protein bears the same epitopes as the heavy and the light chain and remains immunogenic.[39] As a consequence, products with a higher ratio of inactive to active toxin are more prone to generating antibodies and have a lower specific biologic activity, which denotes the ratio of biologic activity to antigenic toxin.[40] Patients' characteristics may also affect the immunogenicity, as patients may already have antibodies related to prior botulism or vaccinations. Though there is no animal or human data support, some have theorized that tetanus vaccinations may contribute BoNT NAb formation, as tetanus toxin has a greater than 50% homology in amino acids to BoNT-A and BoNT-B.[27]

Antibody Detection

Various laboratory assays have been used to detect antibodies in patients with possible immune resistance. Structural assays such as ELISA and immunoprecipitation assays (IPA)[41] are sensitive in the detection of BoNT antibodies however, they are unable to discriminate between neutralizing and non-neutralizing antibodies. Bioassays such as mouse protection assay or mouse hemidiaphragm assay utilize animals to identify neutralizing antibodies that impact clinical efficacy. There are other methods like the western blot assay, synaptosome inhibition assay, sternocleidomastoid test, electrical stimulation of the injected muscle and clinical test such as Unilateral brow injection (UBI) or Frontalis antibody test (FTAT). UBI test consists of injecting a standard amount of BoNT in the right (by convention) medial eyebrow.[19] FTAT involves similar BoNT injections used by some investigators in the past for injections in the frontalis muscle and subsequent assessment of asymmetry of forehead wrinkling on eyebrow elevation.[42]

The possibility of neutralizing antibodies in secondary treatment failure is real and one such case has been discussed when a patient stopped responding to the toxin after series of treatments over a period of 3-years.

CASE REPORT

A 52-year-old female patient who is a professional theatre artist, came in seeking treatment for facial rhytids, after a detailed facial analysis and an insight into her professional requirements of need for expressions on stage; a treatment plan was charted for her. She did not desire any treatment for glabellar rhytids but expressed the need for treatment for lateral canthal rhytids. She had been under treatment for more than three years. She had responded to multiple treatments done during the year 2017 and 2018. The patient complained of no response following treatment in the year 2019, prompting an investigation in August 2019, when a couple of my patients stopped taking the medication due to lack of results. The same month, a final treatment was offered to corroborate her complaint, and to my astonishment, there was no response to the BoNT-A treatment two weeks after injection. In August 2019 with patients consent I sent the blood sample for serology testing for neutralizing antibodies to Toxogen GmbH laboratory in Hannover Germany, we received the results after about one month which was positive (Figures 1 and 2).

Timeline	Preparation	Indication and Dosage	Dosage	Frequency	No of Injection Points	Duration of Effect
2017	Botulinum toxin type A	Lateral canthal lines	9 IU each side	Every 3/4-months	3	3-months
2018	Botulinum toxin type A	Lateral canthal lines	12 IU each side	Every 3/4-months	6	3-months
2019	Botulinum toxin type A	Lateral canthal lines	12 IU each side	Two times followed by serum test	6	None

Figure 1. *Before - 10th August 2019*

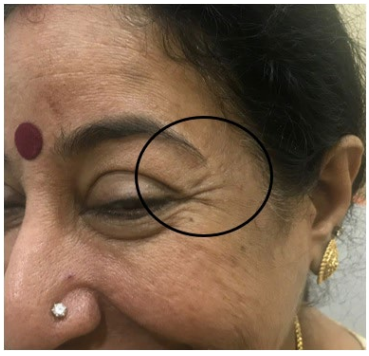
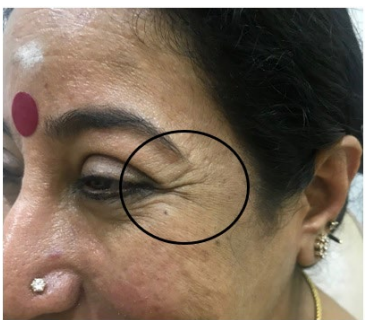


Figure 2. *After- 26th August 2019*



DISCUSSION

All botulinum toxins are capable of inducing the neutralizing antibodies following repeated injections.[26] Antibodies blocking the pharmacological effect of the botulism neurotoxin are termed as neutralizing antibodies[43] non-neutralizing antibodies do not influence the therapeutic effect, but increase the foreign protein load and hence increase the immunogenic risk of forming neutralizing antibodies.[14] Aesthetic indications require lower doses of botulinum toxin preparations however, repeated treatments are required for a sustained outcome, which puts the patients at risk for immunologic reactions with possible formation of neutralizing antibodies and secondary treatment failure. Several papers have reported neutralizing antibodies in this population.[44-47] Information concerning antibody formation relates to the therapeutic use mostly. There is no published data on the prevalence of non-response in the aesthetic field however, with the indications and duration of treatment increase; the reports of patients not responding after an initial response will also be increasingly reported. The prevalence of patients developing neutralizing antibodies after long-term treatment of botulinum toxin type A seem to depend on the condition to be treated and the dose given i.e., higher the dose higher the risk, the global incidence varies from 0.3-6% according to the literature.[16,17,19,20,48,49]

CONCLUSION

In aesthetics practice the treatments repetition with botulinum toxin type A, along with dose requirement is of importance to be able to observe the patient's response with time. This case report concludes that development of neutralizing antibodies is a possibility even with low dose use of botulinum toxin type A used in aesthetic practice and may result in secondary treatment failure This is probably the first such case reported from Mumbai, India.

CONSENT

The authors have received written informed consent from the patient.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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