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Botox Treatment: A Comprehensive Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

An exotoxin called botulinum toxin, or Botox, is isolated from *Clostridium botulinum*. It prevents the cholinergic nerve end plates from releasing acetylcholine, which causes the innervated muscles or glands to become inactive. Botox has proven to be effective for improving facial cosmetics, but new research has shown that it can also be used to treat a variety of surgical and medical disorders that aren't cosmetic. This article examines the available data regarding Botox use for non-cosmetic head and neck problems too. Because of its quick, distinct and long-lasting outcomes for the reduction of facial fine lines and wrinkles and cosmetic rejuvenation, injectable treatments like botulinum toxin are gaining popularity. Excessive sweating or hyperhidrosis can also be treated with Botox. Botox treatment can reduce the frequency of migraines, with chronic migraines requiring treatment in every three months. Additionally, Botox can help in reducing urinary incontinence caused by an overactive bladder. However, the usage of pure botulinum toxin dosages employed by certified healthcare professionals are strictly to be done under the stringent medical guidelines. When handled properly, botulinum toxins utilized in medicine are non-hazardous.

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1. INTRODUCTION

With breakthroughs in facial rejuvenation, beauty is a profession that is flourishing. Botulinum toxins are frequently utilized in aesthetic procedures aimed at enhancing the appearance of the face by smoothing out wrinkles and skin laxity [1]. The toxin generated by the bacteria *Clostridium botulinum* is the basis for the medication of botulinum toxin (Botox). It can be identified in an assortment of natural settings, like soil, lakes, woodlands and the guts of fish and mammals [2]. The botulinum toxin was first identified in 1897 after a group of Belgian musicians grew sick by eating the smoked ham when playing at a funeral. After such a dramatic incident, the ham was given to Emile van Ermengem, a professor of bacteriology at the University of Ghent, for analysis. The bacteria that were initially found by Dr van Ermengem, [3] was botulinum toxin type A (BTX-A), the strain of which was initially isolated in a rudimentary form in the 1920s. Also, Dr Herman Sommer of the University of California, San Francisco, made the first attempt to purify it. In 1946, pure BTX-A was isolated in crystalline form. Dr Vernon Brook's demonstration of BTX-A inhibition of acetylcholine release from motor nerve endings provided the first insights into the drug's mode of action in the 1950s.The bacteria was extensively investigated in the US throughout the mid-1900s. In the year 1960s & 1970s, Dr Scott predicted that BTX-A will eventually be proven helpful in a wide range of other disorders characterized by muscle spasms or hyperactivity because of the advantages he had demonstrated in the treatment of strabismus. Smith-Kettlewell Eye Research Foundation began testing BTX-A in monkeys as a possible therapy for strabismus. The landmark paper that first showed the safety and efficacy of BTX-A in the treatment of human disease came to the limelight in the year 1980.

Scott showed that selective weakening of specific extra ocular muscles with intramuscular injections of BTX-A could correct gaze misalignment in strabismus. The benefits he documented in the treatment of strabismus led Scott to predict that BTX-A would eventually be found useful in a wide range of other conditions characterized by muscle spasms or hyperactivity [4]. In an effort to treat the eye muscles following surgery for a retinal detachment, he administered the paralytic Botox to a patient for the first time in 1978. As a result of the success of this experiment, other patients, especially those with strabismus, or eye misalignment, could then be benefitted from it. As a result of his revolutionary studies, Dr Scott earned the title "Father of Botox" [3]. Since 1970s, Botox has been applied in the field of ophthalmology, and for the last 20 years it has been branched out to other disciplines in medicines, most notably dermatology [5].

2. TYPES OF BOTULINUM TOXIN AND THEIR USES

The bacteria *Clostridium botulinum* producing the neurotoxin known as botulinum toxin is one of the worst biological poisons ever discovered. Eight exotoxins (A, B, C1, C2, D, E, F, and G) produced by *C. botulinum* may be identified by their antigens. All serotypes disrupt brain communication by preventing the release of acetylcholine, the main neurotransmitter at the neuromuscular junction, which paralyzes muscles. The weakness brought on by a botulinum toxin injection often lasts for three months. In the management of a wide range of medical conditions, botulinum toxins now play a very important role, particularly in the management of strabismus, focal dystonia's, hemifacial spasm, and various spastic movement disorders, headaches, hyper salivation, hyperhidrosis and some chronic conditions that only partially respond to medical treatment. The number of potential brand-new indicators is continually growing [2].

Botulinum toxins commercially available in the market are onabotulinumtoxin A (Botox), abbotulinumtoxin A (Dysport), incobotulinumtoxin A (Xeomin) and prabotulinumtoxin A (Jeuveau) and rimabotulinumtoxin B (Myobloc).

3. BOTOXIN USED IN COSMETIC

Reducing the appearance of facial wrinkles is the main purpose of Botox. Botox injections are the most widely used cosmetic surgery in the US, according to the American Board of Cosmetic Surgery. The number of Botox procedures performed in 2016 was above 7 million. Depending on the type of therapy, the effects persist between three and twelve months. The following facial regions are often targeted for injection requests:

- Frown lines, glabellar lines, or elevens wrinkles between the brows
- Lines at the corners of the lips
- "Cobblestone" skin on the chin lines at the forehead's
- Horizontal creases lines around the eyes, known as crow's feet

But only the forehead and the area surrounding the eyes were given FDA approval for the injections. It has not been demonstrated through research that Botox. Courtesy:(www.medicalnewstoday.com)

4. BOTOXIN USED AS MEDICINES

Botulinum toxin injections are not only used to treat wrinkles, but this remarkable matter is also successful for treating quite a few other conditions, such as spasticity, migraines, overactive bladder and neck contracture (cervical dystonia). Botox is also used to treat hyperhidrosis, or profuse underarm sweating [6]. Botulinum toxin is helpful in the short-term management of face rhytids due to the fact that it promotes flaccid muscle paralysis by suppressing acetylcholine release at the neuromuscular junction [7]. Onabotulinum toxin A was the first variety of Botox to hit the market. The Food and Drug Administration (FDA) suggested using it as a cosmetic remedy for glabellar frown lines in 2002. Onabotulinum toxin A's second formulation, created in France, received FDA approval in 2009 and European Union authorization to be used for aesthetic purposes in 2006. The phrase "Botox type A" is now used by society to refer to all substances utilized in cosmetic procedures. Botox A has been used as a cosmetic procedure since a 1994 study shown its efficacy in reducing the look of facial wrinkles [5] BT injection is the treatment of first preference in the majority of patients, especially in the elderly and the physically sedentary [8]. When conservative therapies, including the spray and stretch technique developed by Travell and Simons, [9], physical therapy and its modalities, heat/cold treatments, transcutaneous nerve stimulation, electrical muscle stimulation, ultrasound, iontophoresis, myofascial release, massage, hydrotherapy, stretching and strengthening exercises, pharmacotherapy and standard TP injections, fail to result in a long-term symptomatic response, BTs have gained more acceptance as a treatment option. The use of BT is strongly supported by the potential for complete remission of symptoms in a considerable number of refractory cases and a significant reduction in drug use [10].

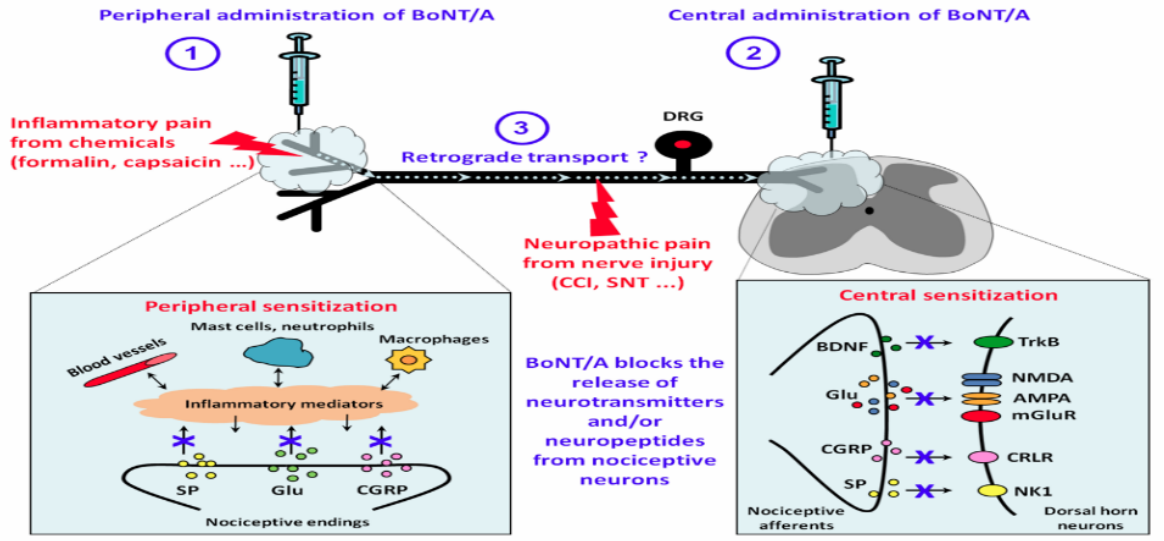
5. PHARMACOLOGY AND IMMUNOLOGY OF BOTOX

Few therapeutic medicines were better understood in terms of mechanism of action prior to clinical application or had a stronger favourable influence on patients' functioning than botulinum toxin. [11] The usefulness of this medicinal agent stems from its ability to prevent the release of acetylcholine from presynaptic nerve terminals, resulting in local chemo denervation. There are seven immunologically unique toxins; but types A and B have received the most attention and have been utilized extensively, but the basic pharmacology and therapeutic uses of other types of toxins, particularly C, D and F are also being investigated [12].

Despite their antigenic differences, the seven neurotoxins share structurally homologous subunits. Botulinum toxin is a single chain polypeptide with a molecular weight of 150 kDa that is broken by trypsin or bacterial enzymes into a heavy chain (100 kDa) and a light chain (50 kDa). The neurotoxins have a binding domain (heavy chain), a catalytic domain (light chain) and a translocation domain, according to their three-dimensional structure. Botulinum toxin activity is comprised of four steps: a) The heavy chains attach to acceptors on the presynaptic membrane of cholinergic nerve terminals with high affinity and serotype specificity, b) Internalization of the complex by acceptors that requires energy (endocytosis), c) Moving from cytosol to the acidic endosome and d) the light chain, a zinc-dependent protease, blocking the release of acetylcholine into the synapses.

A group of proteins known as SNARE or SNAP receptor are involved in the controlled fusion of the plasma membrane, synaptic vesicle and in regulating exocytosis. SNAP stands for soluble NSF Botulinum toxin A and E both cleave SNAP-25, although at distinct sites: botulinum toxin B, D and F cleave synaptobrevin (VAMP), while type C cleaves both SNAP-25 and syntaxin. attachment protein, while NSF stands for N-ethylmaleimide sensitive factor. The complex consists of two target (t-SNARE) proteins, syntaxin and plasma membrane synaptosome associated with protein (SNAP-25) and VAMP (vesicle-associated membrane protein), sometimes referred to as v-SNARE or synaptobrevin [13].

a)



b)

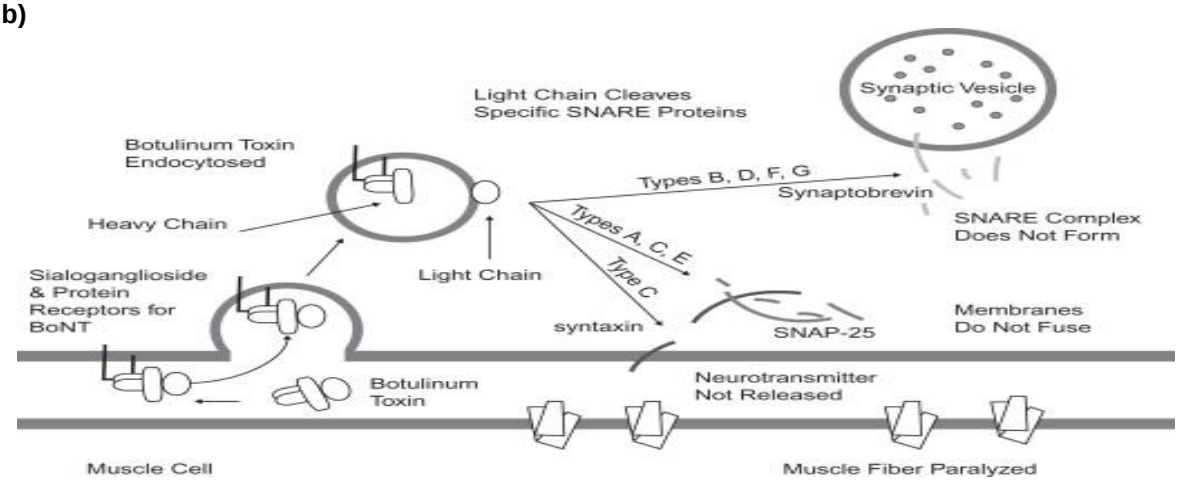


Fig. 1 (a, b). Mechanism of action of botulinum toxin, Courtesy
https://www.mdpi.com/2072-6651/2/12/2890,
https://www.sciencedirect.com/topics/medicine-and-dentistry/botulinum-toxin-f

A mouse protection assay (MPA) provides the standard unit for assessing the efficacy of various preparations: 1 unit of botulinum toxin is the amount of toxin administered intraperitoneally that was determined to kill 50% (LD_{50}) of a group of mice. Although mice are employed in these tests, there are significant variances between species in their sensitivity to particular neurotoxins. As a result, it is vital to understand that different preparations require different dosages, expressed in mouse units, to elicit a similar therapeutic impact.

While the majority of patients continue to respond to successive botulinum toxin treatments, some become unresponsive as a result of acquiring neutralizing or blocking antibodies. The reasons of this immuno-resistance are unknown; however, studies have demonstrated that the botulinum toxin protein's heavy chain (HC) component contains epitopes recognized by anti-HC Abs and HC primed T cells. Only antibodies directed against the 150 kDa neurotoxic complex inhibit toxin function, not antibodies directed against the complex's light chain or non-toxin protein components. There are various ways for detecting botulinum toxin antibodies, but the mouse protection assay (MPA) is thought to be the most clinically relevant because it detects blocking antibodies Cross reactivity may result in immune-resistance to the other serotype due to epitope homology

between the multiple serotypes. Immuno-resistance prevention is critical in maintaining the beneficial response to botulinum toxin injections. This is accomplished by using botulinum toxin preparations with the lowest possible antigenicity, keeping the dose per treatment session as low as possible, and keeping the inter-dose interval as long as possible (at least 2.5 months) [14].

6. COMPOSITION OF BT DRUGS

Botulinum neurotoxin (BNT), complexing proteins (CP, also known as auxiliary proteins), and excipients are components of BT medicines. Excipients may differ amongst BT medications on the market. They include human serum albumin and buffer systems in onabotulinumtoxinA, abobotulinumtoxinA, incobotulinumtoxinA and rimabotulinumtoxinB, NaCl in onabotulinumtoxinA, lactose in abobotulinumtoxinA, sucrose in incobotulinumtoxinA and H_20 and disodium succinate in rimabotulinumtoxinB. The BT component includes all protein elements and is made up of BNT and CP. CP are protein aggregates made up of nontoxic nonhemagglutinin (NTNHA) proteins of roughly 130kD and hemagglutinin (HA) proteins of around 50kD. BNT and CP create distinct protein aggregates depending on the BT type. In BT type A, BNT and CP form three protein aggregates: small 280kD aggregates, medium 600kD aggregates and giant 900 kD aggregates. The CP content of the various BT type A medicines varies. During the production procedure, all CP was removed from incobotulinumtoxinA. Each of these proteins may result in the formation of BTAB. When they are directed towards therapeutically important BNT epitopes, they are referred to as neutralising BTAB and have the potential to diminish the effects of BT. When they are directed against therapeutically irrelevant BNT epitopes or CP, they are referred to as non-neutralizing BTAB and do not interfere with the actions of BT.

Although several conversion ratios have been proposed, the motor actions of onabotulinumtoxin A and rimabotulinumtoxin B appear to be comparable on a 1:40 ratio. The conversion ratio for autonomic diseases may differ from the conversion ratio for motor signs. Fig. 2 is showing the dosage form of Botox and route of administration. Overall, BT-B exhibits slightly higher autonomic effects and slightly lesser motor effects than BT-A [15].

7. CURRENT THERAPEUTIC USES OF BOTOX

i) As of right now, BTX-A is the go-to medication for treating the majority of focal dystonia types and is used for over a dozen goals. Instead of only treating the lines' appearance, the use of BTX-A to treat hyper functional lines in the face is attractive since it enables the clinician to relax the muscles that create the lines, without the need for surgery. Botulinum type B is marketed as a 5,000 U/mL solution that can be diluted more if preferred. In the past, injectors have calculated BT doses for various disorders based on muscle size (patient) and spasm severity, either by using muscle-specific dosage or by beginning with BT dystonia and spasticity data. This extrapolation to MPS and headache appears to be supported by clinical experience; however, when using BTB, caution is advised. Even in patients who have previously experienced toxins, start at a maximum dose of 2500 to 5000 U and increase based on clinical response [16]. Using the same injectate volume for BTA and BTB may not be recommended until more research is done since variations in complex size, pH, and other parameters may induce diffusion variances that increase the possibility for remote dissemination. Although not recommended by the manufacturers, using preservative-free or saline-containing local anaesthetic as a diluent does not appear to break down the protein and reduces the discomfort of local injections, particularly when employing BTB [17].

ii) Hypophonia and breathy voice (abductor type) are among the symptoms or stifled speech pauses and hoarseness (adductor sort) [18]. An improvement of almost one standard deviation was seen in all dependent voice-related Quality of Life variables that were assessed in a meta-analysis of 30 randomized controlled trials (RCTs) incorporating Botox therapy for adductor laryngeal dystonia [19,20]. A further RCT confirmed the positive benefits of Botox in laryngeal dystonia and revealed that patients with the greatest degree of disability showed the greatest recovery [21]. Additionally, laryngeal Botox treatment improved patients with laryngeal

dystonia's mean Voice Handicap Index by 9.6%, according to a recent prospective study (n = 133) [22].

iii) Numerous multicentre, double-blind, placebo-controlled studies have shown that Botox is an effective preventative treatment for migraines. Injecting Botox into muscles innervated by the trigeminal or facial nerves, particular pain distribution sites, or a combination of both is part of the procedure [23,24,25]. Patients in the Botox trial arm showed a significant reduction from baseline in terms of headache and migraine days, total hours of headache, and recurrence of moderate/severe headache days [26]. These beneficial effects of Botox were confirmed by a recent meta-analysis, but only for the treatment of chronic daily headaches and chronic migraines (>15 episodes per month) [27].

iv) Cervical Dystonia is described as a chronic neurological movement disorder that results in significant cervical pain and abnormal cervical postures due to the neck's involuntary turning to the left, right, upward, or downward [28]. It may be the main cause of another neurological disorder or a secondary one. Two Cochrane systematic reviews of thirteen (677 members for Botox A) and thirty-eight (308 members for Botox B) high-quality RCTs provide evidence in favour of using Botox to treat cervical dystonia [29,30]. These meta-analyses showed that a single Botox injection can be safely redone if necessary and is effective, as indicated by both objective and subjective rating scales. Subsequent RCTs have confirmed the safety and effectiveness of Botox in treating cervical dystonia in patients who have had previous treatments as well as those who have never had Botox [31]. According to studies, Botox not only reduces contractures and irregular movements but also prevents secondary degenerative changes of the cervical spine and associated radiculopathy [32,33].

v) The abnormal, involuntary bilateral contraction or twitching of the muscles controlling the eyelids is its defining feature. The condition manifests as excessive blinking and spasms of the eyes, uncontrollably twitching or contracting the muscles surrounding the eyes and the face, dry eyes, and sensitivity to bright light and the sun. First documented in 1985, Botox has since established itself as the go-to treatment for blepharospasm. [34,35,36] The authors of a recent Cochrane systematic review stated that, given the obvious benefits and high efficacy of Botox in treating blepharospasm, it would be unethical to conduct additional RCTs to prove its value over a placebo (saline) [37].

vi) Several years ago, it was demonstrated that BT injections into the forehead might reduce depression. Four subsequent randomized controlled trials confirmed these anti-depressive effects whilst the fifth one intended for eventual drug registration failed to produce significant therapeutic effects. Even more so than with pain indications, mechanisms underlying BT effects on depression are unknown. Another potentially interesting indication area for BT therapy could be the modification of inflammatory reactions as originally suggested several years ago. Intradermal BT injections are usually ineffective for articular pain reduction.

Fig. 2 a) Dosage form of Botox **Fig. b) Route of administation**

8. COST AND PERTINENT FACTS OF BOTOX TREATMENT

Respecting a patient's budget is critical, as it is developing a treatment plan that accommodates both benefit and budget. Botox costs between $100 and $400 to treat a single region. An outline of global market value of Botox has been depicted in Fig. 3.

(https://www.healthline.com/health/beauty-skin-care/guide-to-botox). Both intrinsic and extrinsic factors contribute to aging. Our DNA, ethnicity, and even certain medical disorders are inherent and beyond our control. We have greater influence over extrinsic factors such as air pollution, stress and smoking. Educating the patient about the many types of aging and having an open dialogue about their specific behaviours, environmental exposures, nutrition and lifestyle choices will assist lead the strategy, maximize the benefits, and optimize the outcome. Botox requires multiple injections. A person may need to treat different sections of your face depending on your facial muscles. Botox maintenance may necessitate two to six sessions every year. In order to make Botox healthy and long-lasting, a person should avoid the direct sun's rays, temperatures that are extremely low or high, to avoid tobacco (smoking) and alcohol consumption, any kind of excessive muscle pressure and last but not the least rubbing the injection site [38]. Some products that may interact with this botox include certain antibiotics like Gentamicin and Polymyxin (aminoglycosides), Quinidine (anti-cancer drug), Galantamine, Rivastigmine and Tacrine (drugs used in Alzheimer's disease), Warfarin (Anticoagulants), Ambenonium and Pyridostigmine (drugs used in myasthenia gravis).

9. SIDE EFFECTS AND CONTRAINDICATIONS OF BOTOX TREATMENT

Botox, as a treatment for looking younger, is still in its infancy. Botox was approved for some cosmetic purposes by the United States Food and Drug Administration (FDA) in 2002. Although specialists have deemed Botox to be largely safe, investigations on long-term effects and other aspects are still ongoing. For example, in 2016, researchers discovered that greater doses of Botox can migrate along nerve cells beyond the injection site. The FDA has issued a warning about Botox, but it is still permitted in reduced doses for the temporary reduction of wrinkles on the forehead, around the eyes, and around the lips. Botox also carries the risk of a botched job if too much of the neurotoxin is utilized or injected in the wrong area. A "frozen" or expressionless face, asymmetrical issues, or drooping are all signs of bad Botox. The same is true for any little bruising that may occur after receiving injections, which should resolve within a few days.

Courtesy:(https://www.healthline.com/health/beauty-skin-care/guide-to-botox) [39]

Botox injections are generally well tolerated, and adverse effects are uncommon. However, depending on the reason for the injections and the individual's reaction, Botulinum toxin can have certain unfavourable side effects like pain, swelling or bruising at the injection site, ectropion, decreased strength of eye-closure, xerophthalmia and headache or influenza like symptoms.

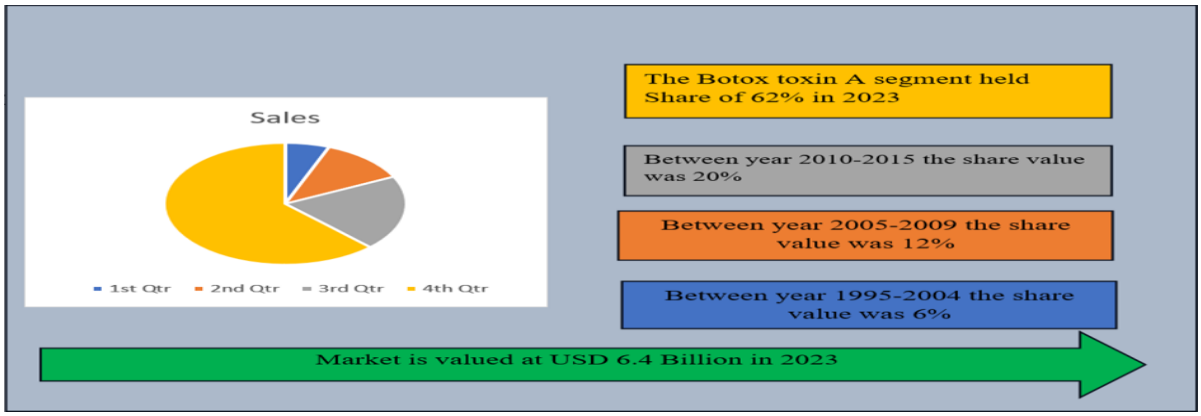


Fig. 3. Global market value of Botox

Botox is contraindicated in gestation period, lactation phase, neurological disorders, allergy to injections of Botox, Diabetic conditions, Psoriasis, patients taking anti-HIV drugs, amyotrophic lateralizing sclerosis myopathies, body dysmorphic disorder and keloidal scarring [40].

10. CONCLUSION

In recent years, a number of illnesses have been treated with the potent drug botulinum toxin. Unwanted adverse effects can be reduced by being aware of anatomical landmarks, muscle function, recognizing baseline asymmetries, accounting for possible toxin migration and taking site-specific measures. By taking advantage of their ability to interfere with a variety of physiological processes, from reducing muscle contraction to relieving pain, BoNTs, in particular BoNT/A and B, have been employed to treat a vast number of neurological illnesses. BoNTs are a flexible therapy option for an increasing range of ailments owing to their distinctive qualities and pharmacological capabilities. BoNTs have a bright future in medicine, but more research is needed to fully explore these possibilities. One of the most important areas is testing the recombinant BoNT as a therapeutic agent, as this could lay the groundwork for future engineering of novel BoNTs. Undoubtedly, Botox has demonstrated significant benefits in the management of several non-cosmetic conditions associated with head and neck surgery and otorhinolaryngology. With more research being done, the number of people receiving Botox and the range of its clinical applications will surely increase. Botox appears to live up to its marketing claim of being "the poison that heals."

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kroumpouzos G, Kassir M, Gupta M, Patil A, Goldust M. Complications of botulinum toxin a: an update review. Journal of Cosmetic Dermatology. 2021;20(6):1585-1590.
Available:https://doi.org/10.1111/jocd.14160
2. Nigam P, Nigam A. Botulinum toxin. Indian Journal of Dermatology. 2010;55(1):8.
3. Jabbari B. History of botulinum toxin treatment in movement disorders. Tremor and Other Hyperkinetic Movements. 2016; 6(0):394.
4. Carruthers A, Carruthers J. Botulinum toxin type A: History and current cosmetic use in the upper face. Seminars in Cutaneous Medicine and Surgery. 2001;20(2):71–84.
5. Satriyasa BK. Botulinum toxin (Botox) A for reducing the appearance of facial wrinkles: a literature review of clinical use and pharmacological aspect. Clinical, Cosmetic and Investigational Dermatology [Internet]. 2019;12(12):223–8.
6. Frank. The Fascinating History of Botox [Internet]. Olansky Dermatology & Aesthetics. 2022.
Available:https://www.olanskydermatology.com/blog/the-fascinating-history-of-botox
7. Freeman M, Sayegh F, Sarosi A, Chopra K, Codner M, Sanati-Mehrizy P, et al. Millennials Are Interested in Botulinum Toxin Injections for Prevention of Facial Rhytids. FACE. 2021;2(1): 273250162098476.
8. Yoshimura DM, Aminoff MJ, Tami TA, Scott AB. Treatment of hemifacial spasm with botulinum toxin. Muscle & Nerve. 1992;15(9):1045–9.
9. Travell JG, Simons DG. Myofascial pain and dysfunction: the trigger point manual.Baltimore: Williams & Wilkins; 1983.
10. Lalli F, Gallai V, Tambasco N, et al. Botulinum A toxin versus lidocaine in the treatment of myofascial pain: a double-blind randomized study. Presented at the InternationalConference 1999: Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins.Orlando. 1999:16–8.
11. Whelchel DD, Brehmer TM, Brooks PM, Darragh N, Coffield JA. Molecular targets of botulinum toxin at the mammalian neuromuscular junction. Movement Disorders. 2004;19(S8):S7–16.
12. Eleopra R, Tugnoli V, Quatrale R, Rossetto O, Montecucco C. Different types of botulinum toxin in humans. Movement Disorders: Official Journal of the

Movement Disorder Society [Internet]. 2004.19 Suppl 8: S53-59.
Available:https://pubmed.ncbi.nlm.nih.gov/15027055/
Accessed on: 2021 Sep 10

13. Adler M, Keller JE, Sheridan RE, Deshpande SS. Persistence of botulinum neurotoxin A demonstrated by sequential administration of serotypes A and E in rat EDL muscle. Toxicon. 2001;39(2-3):233–43.
14. Hughes AJ. Botulinum Toxin in Clinical Practice. Drugs. 1994;48(6):888–93.
15. Dressler D, Bigalke H. Immunological aspects of botulinum toxin therapy; 2017.
16. O'Brien C. Discussion: optimal doses for treatment with botulinum toxins. In: Lees A,editor. Optimal patient management with botulinum toxins: evidence and experience.Round Table Series 74. London: The Royal Society of Medicine Press.2002:64–76.
17. Setler P, Burke D, Callaway J, Wang C. Stability of Myobloc (botulinum toxin type B) in preserved saline. Poster presentation at Toxins 2002. Hannover, Germany; 2002.
18. Rosenfield DB, Donovan DT, Sulek M, Viswanath NS, Inbody GP, Nudelman HB. Neurologic aspects of spasmodic dysphonia. J. Otolaryngol. 1990;19:231–236.
19. Boutsen F, Cannito MP, Taylor M, Bender B. Botox treatment in adductor spasmodic dysphonia: a meta-analysis. J. Speech Lang. Hear. Res. 2002;45:469–481.
20. Brazeau GA. Is there time for student intellectual development and scholarly pursuits? Am. J. Pharm. Educ. 2010;74:18.
21. Cannito MP, Woodson GE, Murry T, Bender B. Perceptual analyses of spasmodic dysphonia before and after treatment. Arch. Otolaryngol. Head Neck Surg. 2004;130:1393–1399.
22. Novakovic D, Waters HH, D'Elia JB, Blitzer A. Botulinu toxin treatment of adductor spasmodic dysphonia: longitudinal functional outcomes. Laryngoscope. 2011; 121:606–612.
23. Aurora SK, Dodick DW, Turkel CC, et al. Onabotulinum- toxina for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the preempt 1 trial. Cephalalgia. 2010;30:793–803.
24. Diener HC, Dodick DW, Aurora SK, et al. Onabo- tulinumtoxina for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the preempt 2 trial. Cephalalgia. 2010;30:804–814
25. Dodick DW, Turkel CC, DeGryse RE, et al. Onabo- tulinumtoxina for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the preempt clinical program. Headache. 2010;50:921–936.
26. Durham PL, Cady R Insights into the mechanism of onabotulinumtoxina in chronic migraine. Headache. 2011;51: 1573–1577.
27. Jackson JL, Kuriyama A, Hayashino Y. Botulinum toxin a for prophylactic treatment of migraine and tension headaches in adults: a meta-analysis. JAMA. 2012;307:1736–1745.
28. Velickovic M, Benabou R, Brin MF. Cervical dystonia pathophysiology and treatment options. Drugs 2001;61:1921–1943.
29. Costa J, Espirito-Santo C, Borges A, et al. Botulinum toxin type a therapy for cervical dystonia. Cochrane Database Syst. Rev. 2005ª;1:CD003633.
30. Costa J, Espirito-Santo C, Borges A, et al. Botulinum toxin type b for cervical dystonia. Cochrane Database Syst. Rev. 2005b;1:CD004315.
31. Comella CL, Jankovic J, Truong DD, Hanschmann A, Grafe S. Efficacy and safety of incobotulinumtoxina (nt 201, xeomin(r), botulinum neurotoxin type a, without accessory pro- teins) in patients with cervical dystonia. J. Neurol. Sci. 2011; 308:103–109.
32. Jankovic J, Adler CH, Charles PD, Comella C, Stacy M, Schwartz M, Sutch SM, Brin MF, Papapetropoulos S. Rationale and design of a prospective study: cervical dystonia patient registry for observation of OnaBotulinumtoxinA efficacy (CD PROBE). BMC Neurol. 2011; 11:140.
33. Ruiz PJ, Castrillo JC, Burguera JA, et al. Evolution of dose and response to botulinum toxin a in cervical dystonia: a multicenter study. J. Neurol. 2011;258: 1055–1057
34. Jankovic J. Botulinum a toxin in the treatment of ble- pharospasm. Adv. Neurol. 1988a;49:467–472.
35. Jankovic J. Blepharospasm and oromandibular-laryngeal- cervical dystonia:

a controlled trial of botulinum a toxin therapy.Adv.Neurol. 1988b;50:583-591.
36. Scott AB, Kennedy RA, Stubbs HA. Botulinum a toxin injection as a treatment for blepharospasm. Arch. Ophthalmol. 1985;103:347–350.
37. Cost J, Espirito-Santo C, Borges A, et al. Botulinum toxin type a therapy for blepharospasm. Cochrane Database Syst. Rev. 2005c;1:CD004900.
38. Available:https://www.lybrate.com/topic/botox
39. Available:https://www.healthline.com/health/beauty-skin-care/guide-to-botox
40. Padda IS, Tadi P. Botulinum Toxin [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2021.

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