Review Article

BOTOX TREATMENT: ACOMPREHENSIVEREVIEW

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 professionalsare strictly to be done under the stringent medical guidelines.When handled properly, botulinum toxins utilized in medicine are non- hazardous.

Keywords: Botulinum toxin, treatment, microsurgical, neurotoxin, immuno-resistance.

Introduction:

Withbreakthroughsinfacialrejuvenation,beautyisaprofessionthatisflourishing.Botulinumtoxinsarefrequentlyutilizedinaestheticproceduresaimedatenhancingtheappearanceofthefacebysmoothingoutwrinklesandskinlaxity.(KroumpouzosG, 2021)Thetoxingeneratedbythebacteria*Clostridiumbotulinum*isthebasisforthemedicationofbotulinumtoxin(Botox).Itcanbeidentifiedinanassortmentofnaturalsettings,likesoil,lakes,woodlandsandthegutsoffishandmammals.(Nigam P, 2010). The botulinum toxin was first identified in 1897 afteragroupofBelgianmusiciansgrewsickbyeatingthesmokedhamwhenplayingatafuneral.Afterssuchadramaticincident,thehamwasgiventoEmilevanErmengem,aprofessorofbacteriologyattheU

niversityofGhent,foranalysis.Thebacteriathat wereinitiallyfoundbyDrvanErmengem,(JabbariB,2016)wasbotulinumtoxintypeA(BTX-A), the strain of which wasinitiallyisolatedinarudimentaryforminthe1920s.Also,DrHermanSommeroftheUniversityof California,SanFrancisco,madethefirstattempttopurifyit.In1946,pureBTX-Awasisolatedincrystallineform.DrVernonBrook'sdemonstrationofBTX-Ainhibition ofacetylcholinereleasefrommotornerveendingsprovidedthefirstinsightsintothedrug'smodeofaction inthe1950s.Thebacteriawasextensivelyinvestigatedintheusthroughoutthemid-1900s.Intheyear1960s&1970s,DrScottpredictedthatBTX-Awilleventuallybeprovenhelpfulinawiderangeofotherdisorderscharacterizedbymusclespasmsorhyperactivitybecauseoftheadvantageshehaddemonstratedinthetreatmentofstrabismus.Smith-KettlewellEyeResearchFoundationbegantestingBTX-Ainmonkeysasapossibletherapyforstrabismus.Thelandmarkpaperthatfirstshowedthesafetyandefficacyof BTX-Ainthetreatmentofhumandiseasecameto the limelight in the year 1980.

Scottshowedthatselectiveweakeningofspecificextra ocularmuscleswithintramuscularinjectionsofBTX-Acouldcorrectgazemisalignmentinstrabismus.Thebenefitshedocumentedinthetreatmentofstrabismusled Scotttopredictthat BTX-Awouldeventuallybefoundusefulinawiderangeofotherconditionscharacterizedbymusclespasmsorhyperactivity(CarruthersA,2001).Inanefforttotreattheeyemusclesfollowingsurgeryforaretinaldetachment,headministeredtheparalyticBotoxtoapatientforthefirsttimein1978.Asaresultofthesuccessofthisexperiment,otherpatients,especiallythosewithstrabismus,oreyemisalignment,could then bebenefittedfromit.Asaresultofhisrevolutionarystudies,DrScottearnedthetitle"FatherofBotox"(JabbariB,2016).Since1970s,Botoxhasbeenappliedinthefieldofophthalmology,and for thelast 20yearsithasbeen branchedouttootherdisciplinesinmedicines,mostnotablydermatology(SatriyasaBK,2019).

Types of botulinum toxin and their uses:
Thebacteria*Clostridiumbotulinum*producestheneurotoxinknownasbotulinumtoxin,oneoftheworstbiologicalpoisonseverdiscovered.Eightexotoxins(A,B,C1,C2,D,E,F,andG)producedby*C.botulinum*maybeidentifiedbytheirantigens.Allserotypesdisruptbraincommunicationbypreventingthereleaseofacetylcholine,themainneurotransmitteratttheneuromuscularjunction,whichparalyzes

muscles.Theweaknessbroughtonbyabotulinumtoxininjectionoftenlastsforthreemonths.Inthemanagementofawiderangeofmedicalconditions,botulinumtoxinsnowplayaveryimportantrole,particularlyinthemanagementofstrabismus,focaldystonia’s,hemifacialspasm,andvariousspasticmovementdisorders,headaches,hyper salivation,hyperhidrosisandsomechronicconditionsthatonlypartiallyrespondtomedicaltreatment.Thenumberofpotentialbrand-newindicatorsiscontinuallygrowing.(NigamP,2010)

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

Botoxin used in Cosmetic: Reducingtheappearanceoffacialwrinklesisthemainpurposeof Botox.Botoxinjectionsarethemostwidelyusedcosmeticsurgeryintheus,accordingtotheAmericanBoardofCosmeticSurgery.Thenumberof Botoxproceduresperformedin2016wasabove7million.Dependingonthetypeoftherapy,theeffectspersistbetweenthreeandtwelvemonths.Thefollowingfacialregionsareoftentargetedforinjectionrequests:

- Frownlines,glabellarlines,orelevenswrinklesbetweenthebrows
- Linesatthecornersofthelips
- "Cobblestone"skinonthechinlinesattheforehead's
- Horizontalcreaseslinesaroundtheeyes,knownascrow'sfeet

Butonlytheforeheadandtheareasurroundingtheeyesweregiven FDAapprovalfortheinjections.Ithasnotbeendemonstratedthroughresearchthat Botox.Courtesy:(www.medicalnewstoday.com)

Botoxin used asMedicines: 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 (Frank, 2022). 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. (Freeman M, 2021). 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

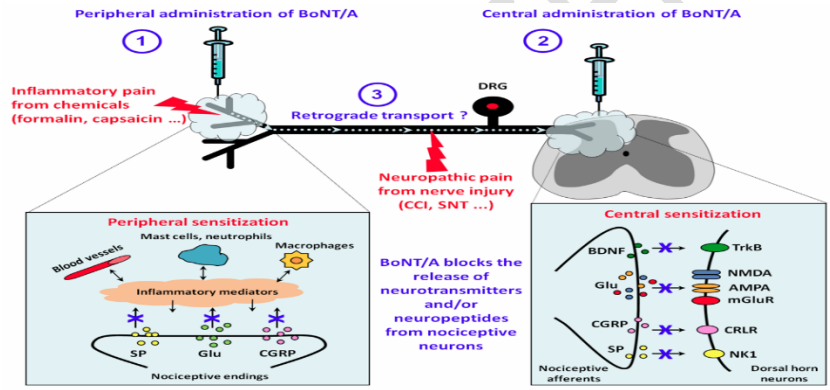
purposes in 2006. The phrase "Botox type A" is now used by society to refer to all substances utilized in cosmetic procedures. Botox A has been used as a cosmetic procedure since a 1994 study shown its efficacy in reducing the look of facial wrinkles. (Satriyasa BK, 2019) BT injection is the treatment of first preference in the majority of patients, especially in the elderly and the physically sedentary. (Yoshimura DM, 1992).When conservative therapies, including the spray and stretch technique developed by Travell and Simons,(Travell JG,1983),physical therapy and its modalities, heat/cold treatments, transcutaneous nerve stimulation, electrical muscle stimulation, ultrasound, iontophoresis, myofascial release, massage, hydrotherapy, stretching and strengthening exercises, pharmacotherapyand standard TP injections, fail to result in a long-term symptomatic response, BTs have gained more acceptance as a treatment option. BT use 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.(Lalli F,1999).

Pharmacology and Immunology of Botox: Fewtherapeuticmedicineswerebetterunderstoodintermsofmechanismofactionpriortoclinicalapplicationorhadastrongerfavourableinfluenceonpatients'functioningthanbotulinumtoxin.(WhelchelDD,2004)Theusefulness ofthis medicinal agentstemsfromitsabilitytopreventthereleaseofacetylcholinefrompresynapticnerveterminals,resultinginlocalchemodenervation.Therearesevenimmunologicallyuniquetoxins;but typesAandBharevereceivedthemostattentionandhave been utilizedextensively,butthebasicpharmacologyandtherapeuticusesofothertypesoftoxins,particularlyC,DandF arealsobeinginvestigated.(EleopraR,2004)

Despitetheirantigenicdifferences,thesevenneurotoxinssharestructurallyhomologoussubunits.Botulinumtoxinisasinglechainpolypeptidewithamolecularweightof150kDathatisbrokenbytrypsinorbacterialenzymesintoaheavychain(100kDa)andalightchain(50kDa).Theneurotoxinshaveabindingdomain(heavychain),acatalyticdomain(lightchain)andatranslocationdomain,accordingtotheirthree-dimensionalstructure.Botulinumtoxinactivityiscomprisedoffoursteps: 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.(AdlerM,2001)

a)



b)

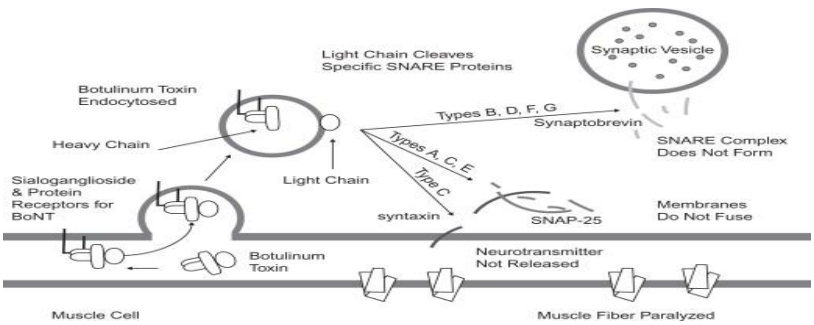


Figure1 (a, b):Mechanismofactionofbotulinumtoxin,

Courtesy:https://www.mdpi.com/2072-6651/2/12/2890,

https://www.sciencedirect.com/topics/medicine-and-dentistry/botulinum-toxin-f

Amouseprotectionassay(MPA)providesthestandardunitforassessingtheefficacyofvariouspreparations:1unitofbotulinumtoxinistheamountoftoxinadministeredintraperitoneallythatwasdeterminedtokill50%(LD_{50})ofagroupofmice.

Althoughmiceareemployedinthesetests,therearesignificantvariancesbetweenspeciesintheirsensitivitytoparticularneurotoxins.Asaresult,itisvitaltounderstandthatdifferentpreparationsrequiredifferentdosages,expressedinmouseunits,toelicitasimilartherapeuticimpact.

Whilethemajorityofpatientscontinuetorespondtosuccessivebotulinumtoxintreatments,somebecomeunresponsiveasaresultofacquiringneutralizingorblockingantibodies.Thereasonsofthisimmuno-resistanceareunknown;however,studieshavedemonstratedthatthebotulinumtoxinprotein'sheavychain(HC)componentcontainsepitopesrecognizedbyanti-HCAbsandHCprimedTcells.Onlyantibodiesdirectedagainstthe150kDaneurotoxiccomplexinhibittoxinfunction,notantibodiesdirectedagainstthecomplex'slightchainornon-toxinproteincomponents.Therearevariouswaysfordetectingbotulinumtoxinantibodies,butthemouseprotectionassay(MPA)isthoughttobethemostclinicallyrelevantbecauseitdetectsblockingantibodiesCrossreactivitymayresultinimmune-resistancetotheotherserotypeduetoepitopehomologybetweenthemultipleserotypes. Immuno-resistancepreventioniscriticalinmaintainingthebeneficialresponsetobotulinumtoxininjections.Thisisaccomplishedbyusingbotulinumtoxinpreparationswiththelowestpossibleantigenicity,keepingthedosepertreatmentsessionaslowaspossible,andkeepingtheinter-doseintervalaslongaspossible(atleast2.5months).(HughesAJ,1994)

Composition of BT drugs:

Botulinumneurotoxin(BNT),complexingproteins(CP,alsoknownasauxiliaryproteins),andexcipientsarecomponentsofBTmedicines.Excipientsmaydifferamongstmedicationsonthemarket.Theyincludehumanserumalbuminandbuffersystemsinonabotulinumtoxin A,abobotulinumtoxin A,incobotulinumtoxin Aandrimabotulinumtoxin B,NaClinonabotulinumtoxin A,lactoseinabobotulinumtoxin A,sucroseinincobotulinumtoxin AandH_20anddisodiumsuccinateinrimabotulinumtoxin B.TheBTcomponentincludesallproteinelementsandismadeupofBNTandCP.CPareproteinaggregatesmadeupofnontoxicnonhemagglutinin(NTNHA)proteinsofroughly130kDandhemaggl

utinin(HA)proteinsofaround50kD.BNTandCPcreatedistinctproteinaggregatesdependingonthe BTtype.InBTtypeA,BNTandCPformthreeproteinaggregates:small280kDaggregates,medium6 00kDaggregatesandgiant900kDaggregates.TheCPcontentofthevariousBTtypeAmedicinesvari es.Duringtheproductionprocedure,allCPwasremovedfromincobotulinumtoxinA.Eachofthesepr oteinsmayresultintheformationofBTAB.Whentheyaredirectedtowardstherapeuticallyimportant BNTepitopes,theyarereferredtoasneutralisingBTABandhavethepotentialtodiminishtheeffectso fBT.Whentheyaredirectedagainsttherapeuticallyirrelevant BNTepitopesorCP,theyarereferredto asnon-neutralizingBTABanddonotinterferewiththeactionsofBT.

Figure 2:a)DosageformofBotoxb)Routeofadministation

Althoughseveralconversionratioshavebeenproposed,themotoractionsofonabotulinumtoxinAan drimabotulinumtoxinBappeartobecomparableona1:40ratio.Theconversionratioforautonomicdi seasesmaydifferfromtheconversionratioformotorsigns. Figure 2 is showing the dosage form of Botox and route of administration. Overall,BT-BexhibitsslightlyhigherautonomiceffectsandslightlylessermotoreffectsthanBT-A.(DresslerD,2017)

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.BotulinumtypeBismarketedasa5,000U/mLsolutionthatcanbedilutedmoreifp referred.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 (O'Brien C, 2002). 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. (Setler P,2002)

ii) Hypophonia and breathy voice (abductor type) are among the symptomsor stifled speech pauses and hoarseness (adductor sort) (Rosenfield, 1990). 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 (Boutsen, 2002; Brazeau, 2010). 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. (Cannito, 2004)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) (Novakovic, 2011).

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(Aurora, 2010; Diener ,2010; Dodick, 2010). 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 (Durham and Cady, 2011). 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).(Jackson, 2012)

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 (Velickovic ,2001). 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(Costa, 2005a, 2005b). 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(Comella, 2011). According to studies, Botox not only reduces contractures and irregular movements but also prevents secondary degenerative changes of the cervical spine and associated radiculopathy (Jankovic, 2011; Ruiz, 2011).

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.(Jankovic, 1988a, 1988b; Scott, 1985) 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) (Costa, 2005c).

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.

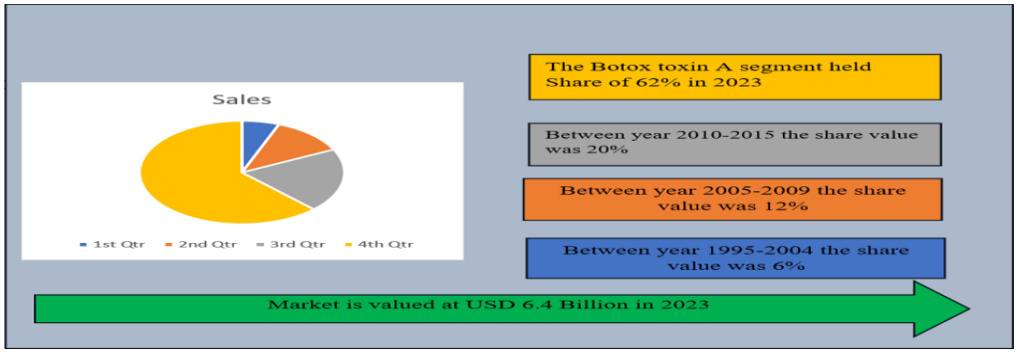


Figure 3: Global market value ofBotox

Cost and pertinent facts of Botox Treatment: Respectingapatient'sbudgetiscritical,asisdevelopingatreatmentplanthataccommodatesbothbenefitandbudget.Botoxcostsbetween$100and$400totreatasingleregion.An outline of global market value of Botox has been depicted in Figure 3. Bothintrinsicandextrinsicfactorscontributetoaging.OurDNA,ethnicity,andevencertainmedicaldisordersareinherentandbeyondourcontrol.Wehavegreaterinfluenceoverextrinsicfactorssuchasairpollution,stressandsmoking.Educatingthepatientaboutthemanytypesofagingandhavinganopendialogueabouttheirspecificbehaviours,environmentalexposures,nutritionandlifestylechoiceswillassistleadthestrategy,maximizethebenefits,andoptimizetheoutcome.Botoxrequiresmultipleinjections.A person mayneedtotreatdifferentsectionsofyourfacedependingonyourfacialmuscles. Botoxmaintenancemaynecessitatetwotosixsessionseveryyear.

InordertomakeBotoxhealthyandlong-lasting, aperson should avoid the direct sun's rays, temperaturesthatareextremelyloworhigh, to avoid tobacco (smoking)andalcoholconsumption, any kindofexcessive musclepressureand last but not the least rubbingtheinjectionsite.(https://www.lybrate.com/topic/botox)Someproductsthatmayinteractwiththisbotoxinclude certainantibiotics likeGentamicinandPolymyxin(aminoglycosides), Quinidine (anti-cancer drug), Galantamine, Rivastigmineand Tacrine (drugs used in Alzheimer's disease), Warfarin (Anticoagulants), Ambenonium and Pyridostigmine (drugs used in myasthenia gravis)

Side Effects and Contraindications of Botox Treatment: Botox,asatreatmentforlookingyounger,isstillinitsinfancy.BotoxwasapprovedforsomecosmeticpurposesbytheUnitedStatesFoodandDrugAdministration(FDA)in2002.Althoughspecialistshavedeemed Botoxtobelargelysafe,investigationsonlong-

termeffectsandotheraspectsarestillongoing.Forexample,in2016,researchersdiscoveredthatgreaterdosesofBotoxcanmigratealongnervecellsbeyondtheinjectionsite.TheFDAhasissuedawarningaboutBotox,butitisstillpermittedinreduceddosesforthetemporaryreductionofwrinklesonthefore head,aroundtheeyes,andaroundthelips.Botoxalsocarriestheriskofabotchedjobiftoomuchofthene urotoxinisutilizedorinjectedinthewrongarea.A"frozen"orexpressionlessface,asymmetricalissue s,ordroopingareallsignsofbadBotox.Thesameistrueforanylittlebruisingthatmayoccurafterreceiv inginjections,whichshouldresolvewithinafewdays.
Courtesy:(https://www.healthline.com/health/beauty-skin-care/guide-to-botox)

Botoxinjectionsaregenerallywelltolerated,andadverseeffectsareuncommon.However,dependin gonthereasonfortheinjectionsandtheindividual'sreaction,Botulinumtoxincanhavecertainunfavo urablesideeffects like pain, swelling or bruising at the injection site, ectropion, decreased strength of eye-closure, xerophthalmia and headache or influenza like symptoms.

Botox is contraindicated in gestationperiod, lactation phase, neurologicaldisorders, allergytoinjectionsofBotox, Diabeticconditions, Psoriasis, patientstakinganti-HIVdrugs, amyotrophiclateralizingsclerosismyopathies, bodydysmorphicdisorder and keloidalscarring(PaddaIS,2021)

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 promising future in medicinal applications, but additional study is still required to investigate these applications. Testing the recombinant BoNT as a therapeutic agent is one of the most crucial areas since it might be the foundation for the engineering of novel BoNTs in the future. Botox has unquestionably been shown to be quite beneficial in the treatment of numerous non-cosmetic disorders related to head and neck surgery and otorhinolaryngology. The number of people getting Botox and the spectrum of clinical applications will undoubtedly grow as more research is conducted. It seems that Botox lives up to the tagline as "the poison that heals."

REFERENCES

1. 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 Feb;39(2-3):233–43.
2. Aurora, S.K., Dodick, D.W., Turkel, C.C., et al, 2010. Onabotulinum- toxina for treatment of chronic migraine: results from the double- blind, randomized, placebo-controlled phase of the preempt 1 trial. Cephalalgia 30, 793–803.
3. Boutsen, F., Cannito, M.P., Taylor, M., Bender, B., 2002. Botox treatment in adductor spasmodic dysphonia: a meta-analysis. J. Speech Lang. Hear. Res. 45, 469–481.
4. Brazeau, G.A., 2010. Is there time for student intellectual development and scholarly pursuits? Am. J. Pharm. Educ. 74, 18.
5. Cannito, M.P., Woodson, G.E., Murry, T., Bender, B., 2004. Perceptual analyses of spasmodic dysphonia before and after treatment. Arch. Otolaryngol. Head Neck Surg. 130, 1393–1399.
6. Carruthers A, Carruthers J. Botulinum toxin type A: History and current cosmetic use in the upper face. Seminars in Cutaneous Medicine and Surgery. 2001 Jun;20(2):71–84.
7. Comella, C.L., Jankovic, J., Truong, D.D., Hanschmann, A., Grafe, S., 2011. 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. 308, 103– 109.
8. Costa, J., Espirito-Santo, C., Borges, A., et al, 2005a. Botulinum toxin type a therapy for cervical dystonia. Cochrane Database Syst. Rev. 1, CD003633.
9. Costa, J., Espirito-Santo, C., Borges, A., et al, 2005b. Botulinum toxin type b for cervical dystonia. Cochrane Database Syst. Rev. 1, CD004315.
10. Costa, J., Espirito-Santo, C., Borges, A., et al, 2005c. Botulinum toxin type a therapy for blepharospasm. Cochrane Database Syst. Rev. 1, CD004900.
11. Diener, H.C., Dodick, D.W., Aurora, S.K., et al, 2010. Onabo- tulinumtoxina for treatment of chronic migraine: results from the double-blind, randomized, placebo-controlled phase of the preempt 2 trial. Cephalalgia 30, 804–814
12. Dodick, D.W., Turkel, C.C., DeGryse, R.E., et al, 2010. Onabo- tulinumtoxina for treatment of chronic migraine: pooled results from the double-blind, randomized, placebo-controlled phases of the preempt clinical program. Headache 50, 921–936.
13. Dressler D, Bigalke H. Immunological aspects of botulinum toxin therapy. 2017 May

14. Durham, P.L., Cady, R., 2011. Insights into the mechanism of onabotulinumtoxina in chronic migraine. Headache 51, 1573–1577.
15. 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 Mar 1 [cited 2021 Sep 10];19 Suppl 8: S53-59. Available from: https://pubmed.ncbi.nlm.nih.gov/15027055/
16. Frank. The Fascinating History of Botox [Internet]. Olansky Dermatology & Aesthetics. 2022. Available from: https://www.olanskydermatology.com/blog/the-fascinating-history-of-botox
17. 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 Jan 28;2(1):273250162098476.
18. Hughes AJ. Botulinum Toxin in Clinical Practice. Drugs. 1994 Dec;48(6):888–93.
19. Jabbari B. History of Botulinum Toxin Treatment in Movement Disorders. Tremor and Other Hyperkinetic Movements. 2016 Nov 28;6(0):394.
20. Jackson, J.L., Kuriyama, A., Hayashino, Y., 2012. Botulinum toxin a for prophylactic treatment of migraine and tension headaches in adults: a meta-analysis. JAMA 307, 1736–1745.
21. Jankovic, J., 1988a. Botulinum a toxin in the treatment of ble- pharospasm. Adv. Neurol. 49, 467–472.
22. Jankovic, J., 1988b. Blepharospasm and oromandibular-laryngeal- cervical dystonia: a controlled trial of botulinum a toxin therapy.Adv.Neurol.50,583-591.
23. Jankovic, J., Adler, C.H., Charles, P.D., Comella, C., Stacy, M., Schwartz, M., Sutch, S.M., Brin, M.F., Papapetropoulos, S., 2011. Rationale and design of a prospective study: cervical dystonia patient registry for observation of OnaBotulinumtoxinA efficacy (CD PROBE). BMC Neurol. 11, 140.
24. 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. https://doi.org/10.1111/jocd.14160
25. Lalli F, Gallai V, Tambasco N, et al. Botulinum A toxin versus lidocaine in the treatmentof myofascial pain: a double-blind randomized study. Presented at the InternationalConference 1999: Basic and Therapeutic Aspects of Botulinum and Tetanus Toxins.Orlando, November 16–8, 1999.
26. Nigam P, Nigam A. Botulinum toxin. Indian Journal of Dermatology, 2010;55(1):8.

27. Novakovic, D., Waters, H.H., D'Elia, J.B., Blitzer, A., 2011. Botulinu toxin treatment of adductor spasmodic dysphonia: longitudinal functional outcomes. Laryngoscope 121, 606–612.
28. 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. p. 64–76.
29. Padda IS, Tadi P. Botulinum Toxin [Internet]. PubMed. Treasure Island (FL): StatPearls Publishing; 2021.
30. Rosenfield, D.B., Donovan, D.T., Sulek, M., Viswanath, N.S., Inbody, G.P., Nudelman, H.B., 1990. Neurologic aspects of spasmodic dysphonia. J. Otolaryngol. 19, 231–236.
31. Ruiz, P.J., Castrillo, J.C., Burguera, J.A., et al, 2011. Evolution of dose and response to botulinum toxin a in cervical dystonia: a multicenter study. J. Neurol. 258, 1055–1057
32. 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 Apr;Volume 12(12):223–8.
33. Scott, A.B., Kennedy, R.A., Stubbs, H.A., 1985. Botulinum a toxin injection as a treatment for blepharospasm. Arch. Ophthalmol. 103, 347–350.
34. Setler P, Burke D, Callaway J, Wang C. Stability of Myobloc (botulinum toxin type B) inpreserved saline. Poster presentation at Toxins 2002. Hannover, Germany June 8–11, 2002.
35. Travell JG, Simons DG. Myofascial pain and dysfunction: the trigger point manual.Baltimore: Williams & Wilkins; 1983.
36. Velickovic, M., Benabou, R., Brin, M.F., 2001. Cervical dystonia pathophysiology and treatment options. Drugs 61, 1921–1943.
37. 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.
38. Yoshimura DM, Aminoff MJ, Tami TA, Scott AB. Treatment of hemifacial spasm with botulinum toxin. Muscle & Nerve. 1992 Sep 1;15(9):1045–9.