MEDICAL APPLICATIONS OF BOTULINUM NEUROTOXIN

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Certificate

This is to certify that the work titled "**Medical Applications of Botulinum Neurotoxin**" submitted by **Ridhima Verma** in partial fulfilment for the award of degree of bachelors of technology in of Jaypee institute of information technology, Noida, has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

Signature of supervisor

Name of supervisor: Dr. Rachana

Designation: Associate Professor

Date: 30th April 2018

Acknowledgement

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Signature of student

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Date: 30th April 2018

Abstract

Botulinum toxin or Botox is a neurotoxic protein produced by bacterium *Clostridium botulinum*. It prevents the release of neurotransmitter acetylcholine from axon endings. There are eight types of botulinum toxin, named type A–H. Botulinum toxin is used to treat a number of disorders characterized by overactive muscle movement. In this report we are discussing 4 diseases that are Migraine, Facial Rhytides, Overactive Bladder and Eye twitching. This medication is given by injection by an experienced health care professional. It is injected into the affected muscles (intramuscularly) when treating eye disorders, muscle stiffness/spasms, and wrinkles. When used to prevent migraines, it is injected into the muscles of the head and neck. It is injected into the skin (intradermally) for the treatment of excessive sweating. When treating overactive bladder, it is injected into the bladder. The number of injections, the site of injections, and how often you receive the medication will be determined by your condition and your response to therapy. Most people start to see an effect within a few days to 2 weeks, and the effect usually lasts 3 to 6 months. Botox is generally considered safe if used in small amounts and administered in small amounts and by a licensed professional, but the rug is not without risks. In 2009 the U.S Food and Drug Administration (FDA) required Botox carry a black box warning – the strongest type of warning label on any drug, cautioning the drug has been linked to serious side effects.

Signature of student	**Signature of supervisor**
Name: Ridhima Verma	Name: Dr. Rachana
Date: 30th April 2018	Date: 30th April 2018

1. Introduction

1.1 Botulinum Neurotoxin

Botulinum toxin, one of the most poisonous biological substances known, is a neurotoxin produced by the bacterium *Clostridium botulinum*. *C. botulinum* elaborates eight antigenically distinguishable exotoxins (A, B, C_1, C_2, D, E, F and G). All serotypes interfere with neural transmission by blocking the release of acetylcholine, the principal neurotransmitter at the neuromuscular junction, causing muscle paralysis. The weakness induced by injection with botulinum toxin A usually lasts about three months.[2]

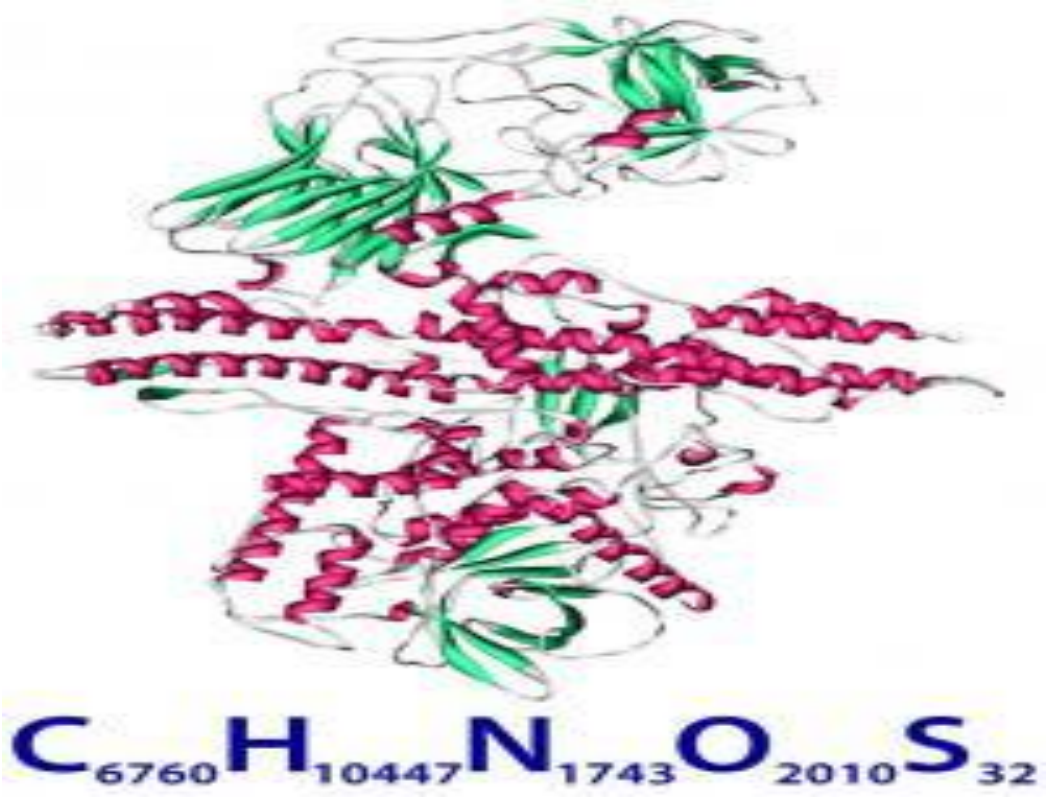


Figure 1: Botulinum toxin (Adapted from Ref no. [1])

1.2 Biochemical aspects

C. botulinum elaborates eight antigenically distinguishable exotoxins (A, B, C_1, C_2, D, E, F and G). Type A is the most potent toxin, followed by types B and F toxin. Types A, B and E are commonly associated with systemic botulism in humans.[1] All botulinum neurotoxins are produced as relatively inactive, single polypeptide chains with a molecular mass of about 150 kDa with a high degree of amino acid sequence homology among the toxin types. The polypeptide chain consists of a heavy (H) chain and a light (L) chain of roughly 100 and 50 kDa respectively, linked by a disulfide bond. The botulinum toxin neurotoxin complex is also associated with various other nontoxic proteins, which may also have hemagglutinating properties.[2]

1.3 How Botulinum Toxin Works

All the serotypes interfere with neural transmission by blocking the release of acetylcholine, which is the principal neurotransmitter at the neuromuscular junction. Intramuscular administration of botulinum toxin acts at the neuromuscular junction to cause muscle paralysis by inhibiting the release of acetylcholine from presynaptic motor neurons. Botulinum toxins act at four different sites in the body: The neuromuscular junction, autonomic ganglia, postganglionic parasympathetic nerve endings and postganglionic sympathetic nerve endings that release acetylcholine. The heavy (H) chain of the toxin binds selectively and irreversibly to high affinity receptors at the presynaptic surface of cholinergic neurones, and the toxin-receptor complex is taken up into the cell by endocytosis. The disulphide bond between the two chains is cleaved and the toxin escapes into the cytoplasm. The light (L) chain interact with different proteins (synaptosomal associated protein (SNAP) 25, vesicle associated membrane protein and syntaxin) in the nerve terminals to prevent fusion of acetylcholine vesicles with the cell membrane.

1.4 Applications of Botox

2. Cervical dystonia- severe neck muscle spasms
3. Blepharospasm – an uncontrollable eye twitch or muscle spasm
4. Severe and excessive underarm sweating
5. Eye alignment or fixing ‘cross eyes’
6. Bruxism or teeth grinding
7. Overactive bladder
8. Chronic migraine
9. Facial Rhytides

IN THIS WE WILL DISCUSS ABOUT ONLY 4 APPLICATIONS AND THAT ARE: EYE TWITCH, OVERACTIVE BLADDER, CHRONIC MIGRAINE AND FACIAL RHYTIDES.

2. Botox for Migraine

2.1 What is Migraine?

A **migraine** is a primary headache disorder characterized by recurrent headaches that are moderate to severe. Associated symptoms may include nausea, vomiting, and sensitivity to light, sound, or smell. The pain is generally made worse by physical activity. There are four possible phases to a migraine, although not all the phases are necessarily experienced.[5]

- The prodrome, which occurs hours or days before the headache
- The aura, which immediately precedes the headache
- The pain phase, also known as headache phase
- The postdrome, the effects experienced following the end of a migraine attack

Migraines are associated with major depression, bipolar disorder, anxiety disorders, and obsessive compulsive disorder.[6]
Specifically, activation of the trigeminovascular system, cortical spreading depression, and neuronal sensitization are seen as playing important roles in migraine pathophysiology.

2.1.1 Activation of trigeminovascular system

The major structures involved in activation of the trigeminovascular system are shown in **Figure 2**. Sensory neurons from the trigeminal ganglion and upper cervical dorsal roots innervate dural-vascular structures (e.g., pial vessels, dura mater, large cerebral vessels). Input from dural-vascular structures and from cervical structures through the upper cervical dorsal root ganglia project to second order neurons in the trigeminocervical complex (TCC).

2.1.2 Cortical spreading depression

Cortical spreading depression, a self-propagating wave of cellular depolarization that slowly spreads across the cerebral cortex and is associated with depressed neuronal bioelectrical activity and altered brain function, has been linked to migraine aura and headache. Cortical spreading depression is thought to activate neurons in the trigeminal nucleus caudalis, leading to inflammatory changes in pain-sensitive meningeal vascular structures, which produces headache via central and peripheral reflex mechanisms.[6]

2.1.3 Neuronal sensitization

Neuronal sensitization, the process by which neurons become increasingly responsive to nociceptive and non-nociceptive stimulation, is thought to play a role in migraine attacks. Sensitization results in decreased response thresholds, increased response magnitude, expansion of receptive fields, and development of spontaneous neuronal activity. Peripheral sensitization in the primary afferent neuron and central sensitization of higher-order neurons of the spinal cord and brain have been shown to play an important role in somatic pain.[6]

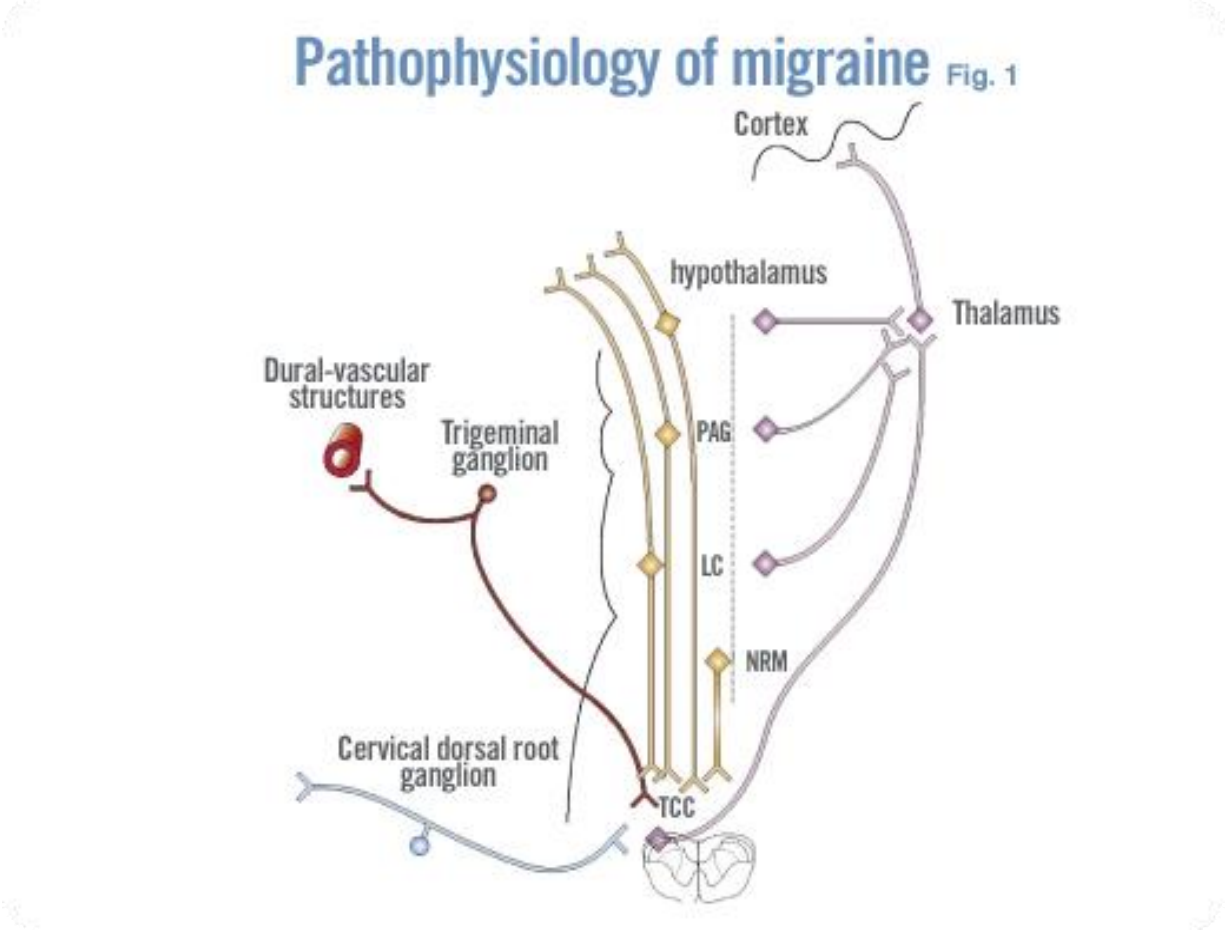


Figure 2. ***Pathophysiology of migraine. Trigeminocervical complex=TCC; periaqueductal gray=PAG; pontine locus coeruleus=LC; nucleus raphe magnus=NRM.(Adapted from Ref no. 6)***

2.2 How Botox Helps In Treatment of Migraine

You'll get several shots of Botox around your head and neck once every 12 weeks to dull or prevent migraine headaches on the sites shown in figure 3.

You may need 30 to 40 shots in all, and you'll get an equal number on each side of your head. If you have migraine pain in one particular spot, you may need more shots there. You could see results 2 to 3 weeks after your first treatment.[7]

Recommended injection sites for chronic migraine:

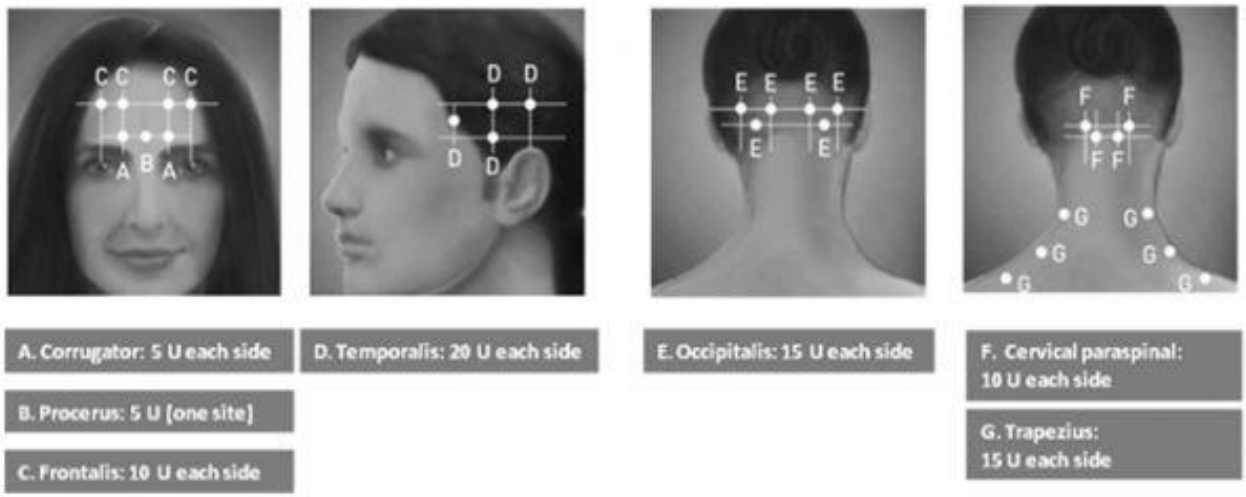


Table 1: BOTOX Dosing by Muscle for Chronic Migraine

Head/Neck Area	Recommended Dose (Number of Sites[a])
Frontalis[b]	20 Units divided in 4 sites
Corrugator[b]	10 Units divided in 2 sites
Procerus	5 Units in 1 site
Occipitalis[b]	30 Units divided in 6 sites
Temporalis[b]	40 Units divided in 8 sites
Trapezius[b]	30 Units divided in 6 sites
Cervical Paraspinal Muscle Group[b]	20 Units divided in 4 sites
Total Dose:	**155 Units** divided **in 31 sites**

[a] **Each IM injection site = 0.1 mL = 5 Units BOTOX**
[b] **Dose distributed bilaterally**

Figure 3 : Injection Points for Migraine of Botox (Adapted from Ref no. [7])

2.3 Case Study

- In 2012 an experiment was performed where a 45-year-old female presented to a chiropractic office with ongoing neck pain and headaches. Her headaches were on a daily basis and varied in intensity from 6–9/10.

- She associated the headaches with neck stiffness, numbness and tingling into her hands.

2.3.1FOR TREATMENT

- During the first session she was given Botox therapy through 31 injection sites on seven key areas around his head and neck muscles as shown in Figure 3.
- The second session was scheduled for 8 weeks later to make sure that she was not presenting with any side-effects from the first application.
- Three more sessions were scheduled to take place over the following 9 months to complete the treatment.

2.3.2RESULTS

- The patient reported that the number of days with strong headaches and migraines had nearly halved since the application of Botox.
- She also noted that the intensity and length of his migraines had lessened, making his migraine days much more manageable.[6]

3. Facial Rhytides

3.1 What are facial Rhytides?

A rhytid, is a wrinkle in the skin. The skin is composed of three layers: the epidermis, the dermis, and subcutaneous fat. The dermis, the middle layer of skin, has many functions. It is the source of sweat glands and sensation. It also grows hair and produces oil to keep skin smooth. It is the location of blood vessels that feed skin and remove toxins.[8]

The subcutaneous fat layer is the bottom layer of skin. With its special connecting tissue, it attaches the dermis to muscles and bones. It supports the function of nerve cells and blood vessels. It is also responsible for controlling the temperature of the body and padding muscles and bones to protect them from bumps and falls.

Each layer of skin is affected by aging, genetics, and environmental factors. The layers tend to become thinner when tissue production slows, making the skin susceptible to damage and change. Thinner skin that is no longer filled in with collagen and fat cells begins to show lines and wrinkles.

3.1.1Causes of Wrinkles

Wrinkles are caused by a combination of different conditions.

Some people are genetically predisposed to develop wrinkles. Some of the causes of wrinkles include:

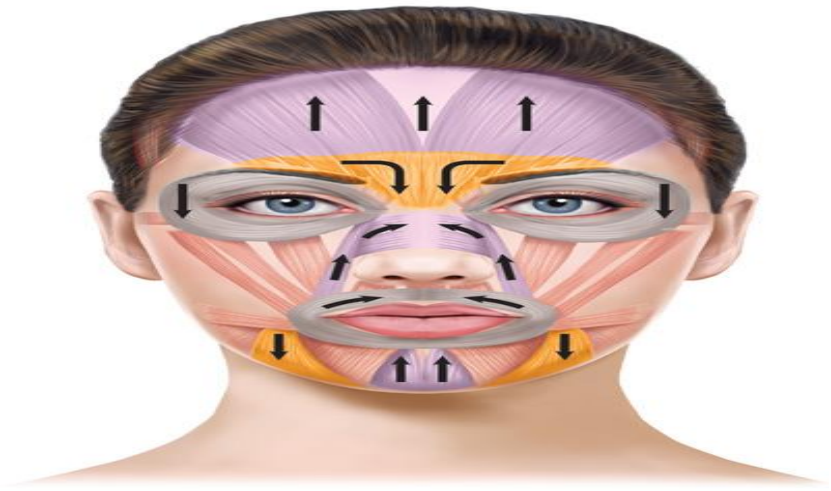
- Age
- Exposure to harmful substances for a prolonged period of time
- Artificial tanning
- Ultraviolet light
- Smoking

The constant contraction of facial muscles, either by squinting or smiling can also lead to the development of fine lines and wrinkles.

With thinner skin, the muscles are no longer able to maintain its flexibility and retain its shape.

2.2 Treatment of Facial Rhytides with Botox

- Botulinum toxin is supplied as a powder and is reconstituted at the time of treatment into a solution using sterile normal saline.
- Dilution volumes range from 1 to 4 mL per 100-unit vial. The botulinum toxin dose injected into the targeted muscles
- Wrinkles are formed by dermal atrophy and repetitive contraction of underlying facial musculature. Injection of small quantities of botulinum toxin into specific overactive muscles causes localized muscle relaxation that smooths the overlying skin and reduces wrinkles.[6]
- It exerts its effect at the neuromuscular junction by inhibiting the release of acetylcholine, which causes temporary chemical denervation. At the cellular level, botulinum toxin functions by cleaving a docking protein (synaptosomal-associated protein of 25 kDA [SNAP-25]) on the internal surface of neuronal membranes, thereby inhibiting vesicle fusion and release of acetylcholine.[7] Botulinum toxin effects in the targeted muscles diminish over time as SNAP-25 regenerates, and neuromuscular signaling and muscle contractility are restored.
- Figure 4 shows the various muscles that need to be targeted for treatment of wrinkles.[8]



Expression lines	Muscle	Action
Frown lines	Corrugator supercilii	Eyebrows drawn medially
	Procerus and depressor supercilii	Eyebrow depressors
Horizontal forehead lines	Frontalis	Eyebrow levator
Crow's feet	Lateral orbicularis oculi	Lateral eyebrow depressor
Eyebrow lift	Superior lateral orbicularis oculi	Superior lateral eyebrow depressor
Bunny lines	Nasalis	Nose drawn up and medially
Radial lip lines	Orbicularis oris	Lip puckering
Marionette lines and downturned smile	Depressor anguli oris	Corner of mouth depressor
Gummy smile and nasolabial fold	Levator labii superioris alaeque nasi	Central lip levator
Chin line and pebbly chin	Mentalis	Chin texture and lower lip levator

Key: orange = depressor muscles; purple = levator muscles; gray = sphincteric muscles.

Figure 4: ***Muscles To Be Injected with Botox For Treatment Of Facial Rhytides(Adapted from Ref no. [8])***

3.3 Case Study

A 41-year-old woman with moderate forehead wrinkling was treated by botulinum toxin. Decrease of upper forehead wrinkling with the appearance of lower frontalis and glabellar protrusion was shown one week after treatment. By four weeks, this protrusion had diminished and remained absent through 16 weeks follow-up.[9]

Figure 5 : (A) Before and (B) After Botox Treatment for Forehead Wrinkles(Adapted from Ref no. [9])

4. Overactive Bladder

4.1 What is Overactive Bladder?

Overactive bladder causes a sudden urge to urinate. The urge may be difficult to stop, and overactive bladder may lead to the involuntary loss of urine (urge incontinence)[10]

4.1.1 Symptoms

With an overactive bladder, you may:

- Feel a sudden urge to urinate that's difficult to control
- Experience urge incontinence — the involuntary loss of urine immediately following an urgent need to urinate
- Urinate frequently, usually eight or more times in 24 hours
- Awaken two or more times in the night to urinate (nocturia)

Although you may be able to get to the toilet in time when you sense an urge to urinate, unexpected frequent urination and nighttime urination can disrupt your life.[10]

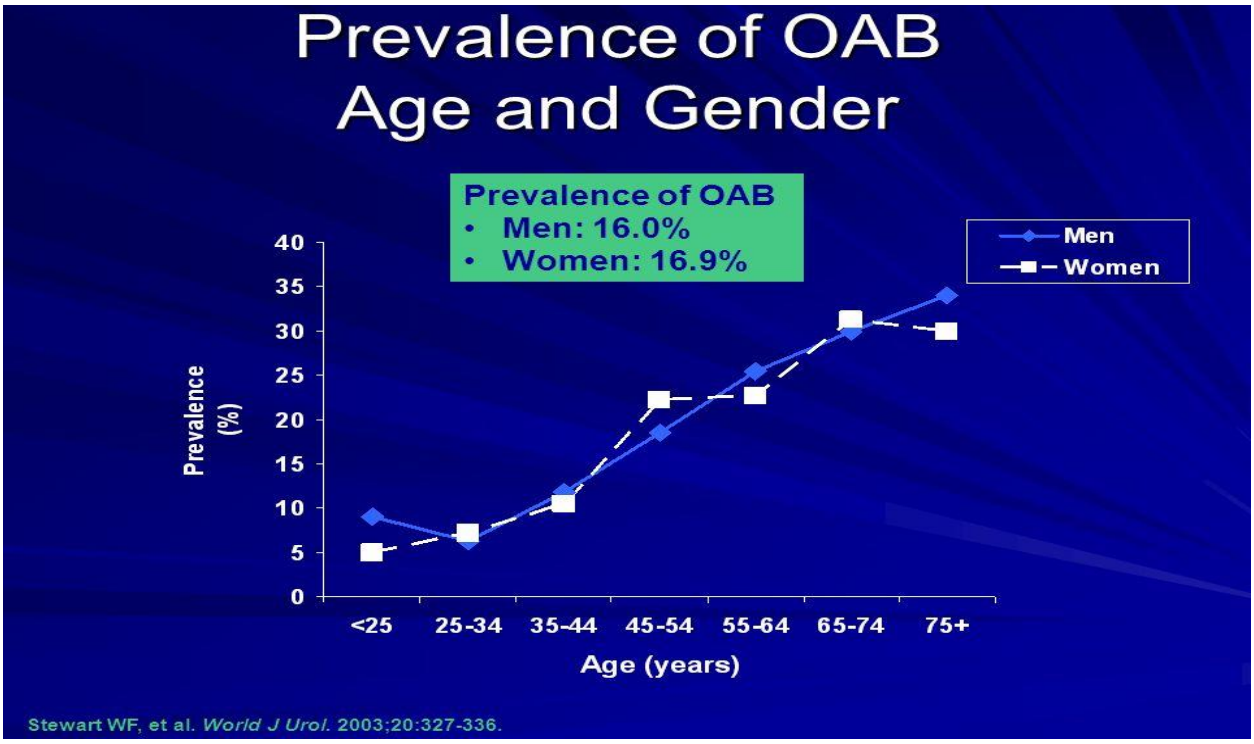


Figure 6: A graph showing Prevalence of OAB according to age and gender (Adapted from Ref no. [10])

4.2 Treatment with Botox

BoNT/A is composed of a 100 kDa heavy chain polypeptide and a 50 kDa light chain polypeptide, as is joined via a disulfide bond. The initially-proposed mechanism of BoNT/A was that by attachment of the heavy chain to the protein receptor SV2 on axon terminals, the toxin could enter the neuron by endocytosis [16]. Then the light chain cleaves synaptosomal-associated protein (25 kDa) (SNAP-25), a protein from the soluble *N*-ethylmaleimide-sensitive factor attachment receptor (SNARE) family. As a result, the fusion of neurosecretory vesicles and release of acetylcholine(Ach) from presynaptic nerve terminals are blocked [11]. With inhibition of Ach release, the effect on suburothelial afferent and detrusor parasympathetic nerve endings was abolished, which was similar to the action of anticholinergic agents.

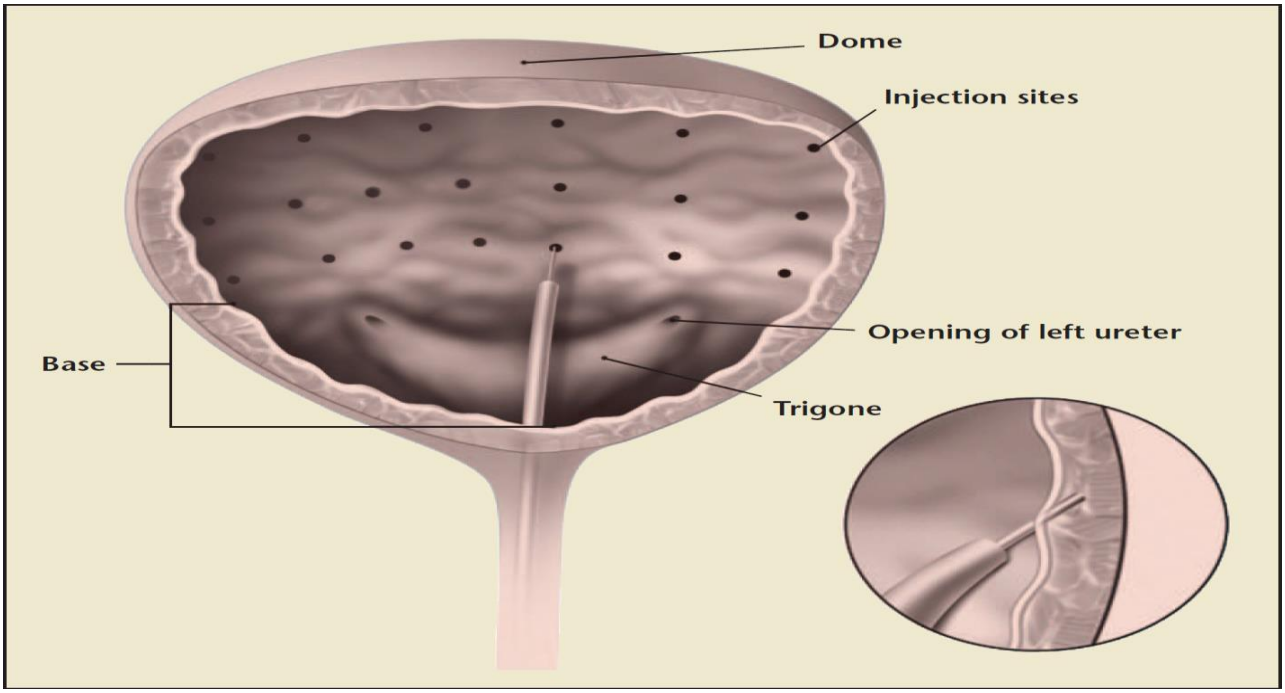


Figure 6. Example of an injection template showing 20 injections throughout the posterior and lateral walls of the bladder sparing the trigone and dome. The inset shows an injection into the detrusor. Image courtesy of Allergan Inc. (***Adapted from Ref no. [11]***

4.3 Case Study

In 2015 study a 50 year old women was dealing with the problem of overactive bladder.

She had to pee after every hour and when pills were not the effective solution of the problem, to manage OAB, her doctor may recommend different types of treatments that work by calming the nerves and muscles around your bladder. Botox is approved in people for whom anticholinergic drugs didn't work. Botox works by calming the nerves that usually overstimulate your bladder muscles and lead to an urgent feeling of needing to urinate.

Botox is approved in people for whom anticholinergic drugs didn't work. Botox works by calming the nerves that usually overstimulate your bladder muscles and lead to an urgent feeling of needing to urinate.

The effects of a Botox injection can last for up to eight months. After that, your doctor will let you know if you need another injection. There is no limit to how long you can use Botox therapy for OAB. However, the FDA recommends that treatments be at least 12 weeks apart.[11]

5. Eye Twitching

5.1 What is Eye Twitching?

An eyelid twitch, or myokymia, is a repetitive, involuntary spasm of the eyelid muscles. A twitch usually occurs in the upper lid, but it can occur in both the upper and lower lids. For most people, these spasms are very mild and feel like a gentle tug on the eyelid. Others may experience a spasm strong enough to force both eyelids to close completely; this is a different condition called blepharospasm.[11]

Spasms typically occur every few seconds for a minute or two. Episodes of eyelid twitching are unpredictable. The twitch may occur off and on for several days. Then, you may not experience any twitching for weeks or even months.

The twitches are painless and harmless, but they may bother you. Most spasms will resolve on their own without the need for treatment. In rare cases, eyelid spasms may be an early warning sign of a chronic movement disorder, especially if the spasms are accompanied by other facial twitches or uncontrollable movements.

5.1.1 What causes eyelid twitches?

- eye irritation
- eyelid strain
- fatigue
- lack of sleep
- physical exertion
- medication side effects
- stress

5.2 Treatment of Eye Twitching with Botox

- Botox blocks the chemical messenger in the muscle, freezing and weakening the muscle so stopping it moving
- The effects last only three months so patients must return regularly for further injections.
- However, many patients can miss out on the benefits of the jab, as the key to success is giving the right dose at the right points in the muscle.
- Figure 7 shows the injection sites for treatment of eye twitching

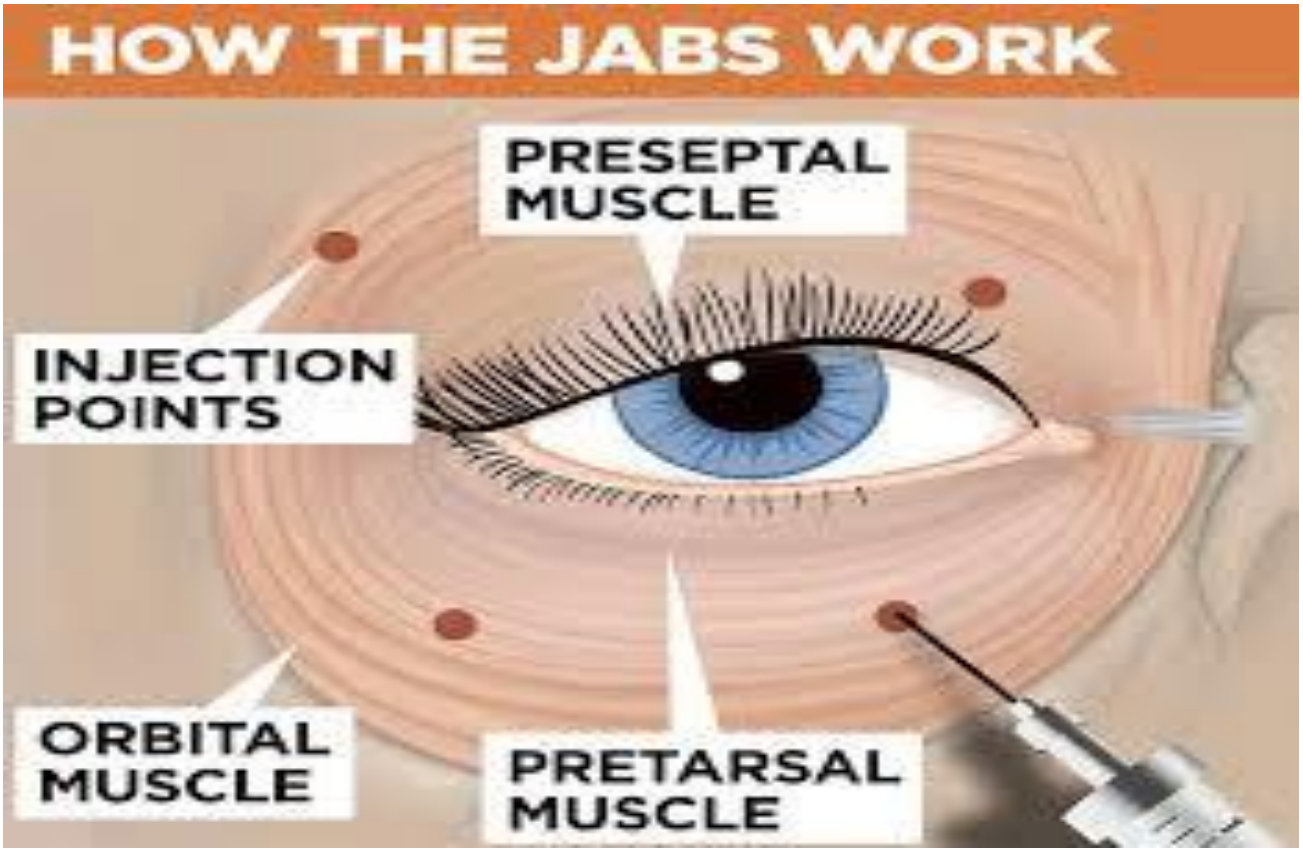


Figure 7 : Showing Injection Sites for Treatment of Eye Twitching (Adapted from Ref no. [11])

5.3 Case Study

- A 63 year old women named Sue was suffering from Eye Twitching for last 4 years.
- As the condition progressed, Sue's right eye would 'clamp shut' for long periods of time.
- Her face ached too just try holding a wink for a couple of minutes.
- Finally, when she started taking botox injections after four jabs in each eye the relief was almost instant.
- Sue now returns every three months for top up treatment.[11]

6. Conclusions

- *Clostridium botulinum*, the bacterium from which Botox is derived, is found in many natural settings, including soil, lakes, and forests.
- The bacterium can also be found in the intestinal tracts of mammals and fish and in the gills and organs of crabs and other shellfish.
- Despite botulinum toxin being so toxic, Botox is in huge demand.
- Despite this, botulinum toxin has proven to be a successful and valuable therapeutic protein.
- Injected botulinum toxin prevents the release of acetylcholine, preventing contraction of the muscle cells. Botulinum toxin causes a reduction in abnormal muscle contraction, allowing the muscles to become less stiff.
- Botulinum toxin is currently used to treat over 20 different medical conditions, with more applications under investigation.

6.2 Side-Effects involved with Botox

- Mild pain, local edema (fluid buildup) and/or erythema (reddening of the skin) at the injection site.
- Numbness.
- Headache.
- Malaise - feeling generally unwell.
- Mild nausea.
- Temporary unwanted weakness/paralysis of nearby muscles.
- Temporary upper lid or brow ptosis (drooping).
- Weakness of the lower eyelid or lateral rectus (a muscle controlling eye movement).
- Dysphagia - trouble swallowing.
- Neck weakness.
- Flu-like illness.
- Brachial plexopathy - a condition affecting the nerves either side of the neck and chest.
- Gallbladder dysfunction.
- Diplopia (double vision).
- Bleeding.
- Blurred vision.
- Decreased eyesight

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8.Plagiarism

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