GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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Evaluation of botulinum toxin type(A) as a treatment modality for migraine

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GSC Biological and Pharmaceutical Sciences, 2024, 29(03), 213-223

Publication history: Received on 12 November 2024; revised on 18 December 2024; accepted on 20 December 2024

Article DOI: https://doi.org/10.30574/gscbps.2024.29.3.0487

Abstract

Botulinum toxin, recognized as one of the most potent biological toxins identified, is a neurotoxin synthesized by the bacterium *Clostridium botulinum*. *C. botulinum* produces eight antigenically distinct exotoxins (A, B, C 1 , C 2 , D, E, F and G). The aim of this study was to Understand how botulinum toxin works to prevent or alleviate migraine symptoms, Evaluate the possible complications associated with this modality and investigate the efficacy of botulinum in those with chronic or episodic migraines.

Material and Methods: A total of 15 patients (10 males and 5 females) were examined, all of them had a chronic headache and any type of migraine, at the Department of Oral and Maxillofacial surgery/Collage of Dentistry, Al-Farahidi university between February to June 2024. The study received approval from the Faculty of Dentistry administration. Each patient signed Informed Consent, also The patient will be provided with detailed information about Botox injections, including potential side effects, expected outcomes, and the importance of compliance with follow-up visits. Botulinum toxin injections ought to be administered in close proximity to the designated markings in order to prevent the inadvertent tattooing of the skin at the injection site, while simultaneously instructing patients to execute specific facial expressions. locating the superior orbital rim, and avoid giving the toxin below or near the supraorbital foramina pinching the glabellar region to fully catch the muscle, penetrating the whole muscle thickness in order to get fine results providing the toxin into the procerus muscle, frontalis, orbicularis oculi, Temporalis Muscle.

Results: It shows that 15 subjects participated in this study with 10 males (66.7%) and 5 females (33.3%) aged 25-44 years old with mean ±Standard deviation 33.67±5.97, and it shows that 5 subjects (33.33%) with cluster headache while 10 subjects (66.67%) with Migraine, it shows that 10 cases (66.67%) take 100 unit of botulin while 5 cases (33.33%) take 50 units.

Conclusion: BoNT/A was found to be effective and safe in the prophylactic treatment of chronic migraine. BoNT/A is an effective treatment that could be administered by experienced neurologists to eligible CM patients regardless of previous prophylactic treatment.

Keywords: Botulinum toxin; *Clostridium botulinum*; Migraine; Modality

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1. Introduction

Migraine constitutes a multifaceted disorder with a genetic predisposition, distinguished by recurrences of moderate to severe cephalalgia experienced as a pulsating discomfort localized to one hemisphere of the cranium, typically concomitant with nausea and heightened sensitivity to photic and acoustic stimuli (1) Migraine attacks represent a multifaceted neurological phenomenon that manifests over a duration spanning hours to days, occurring in a recurrent fashion. The prevalence of migraine is notably high, impacting approximately 12% of the global population, with annual incidences reaching up to 17% among females and 6% among males. A familial predisposition to migraine is frequently observed. It consistently ranks as the fourth or fifth most prevalent cause for emergency department consultations, accounting for approximately 3% of all such visits on an annual basis. The occurrence of migraine tends to escalate during puberty and continues to rise until the ages of 35 to 39, subsequently diminishing in frequency later in life, particularly following menopause (2).

The etiology of migraine remains somewhat ambiguous; however, both genetic predispositions and environmental factors are acknowledged to contribute significantly. A majority of individuals afflicted with migraine experience spontaneous episodes, signifying that there are no specific actions or inactions that precipitate these occurrences. Conversely, certain individuals may experience episodes that can be traced to a specific causative factor. It is important to note that triggers vary considerably among individuals (3). Symptoms of migraine encompass intense throbbing or dull aching pain on one side of the head or both, as well as discomfort that exacerbates with physical exertion. Nausea or emesis, visual disturbances or scotomas, photophobia, hyperacusis, or olfactory sensitivity, fatigue and/or disorientation. Obstructed nasal passages, chills or perspiration, rigid or sensitive neck, pressure in the head, sensitive scalp (4) Migraine management include acute (abortive) and preventive prophylactic therapies. Patients experiencing frequent episodes typically necessitate both interventions. Strategies aimed at mitigating migraine triggers are typically recommended. Acute therapy seeks to counteract or halt the progression of an initiated headache. Preventive medication, administered even in the absence of a headache, seeks to diminish the frequency and intensity of migraine attacks, enhance the efficacy of abortive medicine for acute attacks, and potentially improve the patient's quality of life (5).

Botulinum neurotoxins (BoNTs) are produced as multimolecular complexes by anaerobic bacteria of the genus *Clostridium* spp (6). Seven different serotypes of BoNTs have been characterized (A–G), and these serotypes are active on many different types of vertebrates (7). Botulinum neurotoxins (BoNTs) are proteins of approximately 1300 amino acids and are organized into three domains of comparable size, each around 50 kDa. The NH2-terminal domain, referred to as the L-chain domain, is a Zn2+-endopeptidase that constitutes the catalytic domain exhibiting protease activity. The remaining two domains, covalently linked to constitute the H-chain, are the central domain, which facilitates the membrane translocation of the L-chain into the neuronal cytoplasm, and the COOH-terminal domain, including two equally sized subdomains responsible for neurospecificity binding (8).

The cellular mechanism of BoNTs involves four steps: (i) the binding of BoNTs to the neuronal presynaptic membrane through interactions with gangliosides, synaptic vesicle protein 2 (SV2), and/or synaptotagmin, contingent on the serotype; (ii) the internalisation of BoNTs via endocytosis of the BoNTs-receptor complex within the neurones; (iii) the translocation of the L-chain of BoNTs from the endocytosed vesicle to the neuronal cytosol; and, finally, (iv) the cleavage of specific proteins involved in neuroexocytosis by Zn2+-endopeptidase activity (9,10). The proteins include: SNAP-25, cleaved by BoNT/A, /E, and /C; VAMP/synaptobrevin, cleaved by BoNT/B, /D, /F, and /G; and syntaxin, cleaved by BoNT/C. All of these proteins participate in the formation of the core complex of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptors), which is essential for the proper docking and fusion of neurotransmitter vesicles with neuronal membranes. The cleavage of a single protein is adequate to obstruct the proper assembly of the SNARE core complex, hence hindering the fusion of synaptic vesicles with the presynaptic neuronal membrane and preventing neurotransmitter release. This impact is reversible, with the duration of the action contingent upon the serotype. The specifics of the binding mechanism, internalisation, and action modes of various BoNT serotypes are beyond the purview of this review and can be located in other sources (11). The medical use of BoNTs as therapeutic drugs began after purification of BoNT/A, and the subsequent discovery that the injection of small amounts of BoNT/A into a hyperactive muscle blocked the release of acetylcholine (ACh) from motor nerve endings, causing temporary “muscle relaxation”. This finding led to the first use of BoNT/A as a therapeutic drug to treat human strabismus as an alternative to the conventional surgery (12).

Nowadays, the clinical indications for BoNT/A are rapidly growing, ranging from treatment of overactive skeletal and smooth muscles, to management of hypersecretory (hyperhidrosis, sialorrhea) and painful disorders, such as myofascial pain syndrome, trigeminal neuralgia and chronic migraine. The potential for BoNTs as a treatment for headaches was discovered casually during clinical trials to determine the efficacy of BoNT/A as a treatment of cranio-

facial dystonia: patients reported a reduction of headache attacks together with beneficial effects of BoNT/A on dystonia. A retrospective review of headache patients who were receiving BoNT/A injections for neurology, otolaryngology or cosmetic indications also suggested a reduced headache frequency (13).

Botulinum toxin is FDA-approved for the treatment of migraines, which is currently the sole chronic ailment sanctioned for its application. The therapeutic impact stems from the capacity to reduce muscle tension and promote muscle relaxation (14). Migraine typically manifests prior to middle age, however it may occur later in life; it impacts approximately 20% of females and 6% of males at some stage in their lives. Migraine is typically easily recognizable from the medical history; however atypical forms may induce ambiguity (15).

Migraine generally manifests as an intermittent debilitating headache that disrupts routine tasks. Migraine headaches are often associated with nausea, vomiting, photophobia, phonophobia, and osmophobia. It may be preceded by an aura of neurological dysfunction, including visual abnormalities, vertigo, numbness, or weakness. The discomfort may be moderate or debilitating. The frequency of migraines varies significantly. In many patients, migraine is triggered by specific factors, such as menses, weather changes, irregular sleep, alcohol, or certain foods. Migraine is also often relieved by sleep. The lifetime prevalence of migraine is estimated to be near 35%, and it affects greater than 17% of women and 6% of men (16).

2. Material and methods

2.1. Patients

In this prospective study, a total of 15 patients (10 males and 5 females) were examined, all of them had a chronic headache and any type of migraine, at the Department of Oral and Maxillofacial surgery/Collage of Dentistry, Al-Farahidi university between February to June 2024. The study received approval from the Faculty of Dentistry administration. Each patient signed Informed Consent, also The patient will be provided with detailed information about Botox injections, including potential side effects, expected outcomes, and the importance of compliance with follow-up visits. Informed consent is obtained before proceeding with the treatment.

2.1.1. Inclusion and Exclusion Criteria.

- **Age:** Generally, patients should be at least 20 years old. However, Botox injections may be considered for patients younger than 18 in specific cases, such as for the treatment of certain medical conditions.
- **Good Health:** Patients should generally be in good overall health without any underlying medical conditions, especially those that may increase the risk of complications.
- **No Known Allergies or Sensitivities:** Patients should not have a known allergic reaction or sensitivity to Botox or any of its components.
- **Realistic Expectations:** Patients should have realistic expectations about the outcomes, risks, and limitations of Botox injections. They should understand that the effects of Botox are temporary and that repeat treatments may be necessary to maintain the desired results.

2.2. Methods

2.2.1. Preparation of Type A Toxin

The significance of standardising manufacturing and purifying settings is evident due to the impact of proteolytic 'nicking' on poisonous activity and the increased vulnerability of the protein toxin to inactivation by proteolytic enzymes. Culture. Cultures of C. botulinum from a validated seed stock are cultivated and injected into a 30-liter fermenter operated under anaerobic conditions, while toxin production and other cultural parameters are monitored. Upon reaching the maximum toxin yield (2x10^6 mouse LD50/ml), often after 72 hours, the toxin is extracted using centrifugation following the acidification of the culture. The poison can be preserved in this form before purification. Cultures are rigorously examined for toxin activity, identification, and contamination absence.

- **Purification**: The precipitated crude toxin is re-dissolved and purified using a sequence of processes that include ammonium sulphate precipitation and ion-exchange chromatography. The processes involve monitoring for contamination by extraneous microorganisms, as well as assessing the toxicity and protein composition of the extracts.

- **Formulation and Freeze Drying:** The efficacy of the purified toxin is evaluated, and a suitable volume of the purified toxin solution is incorporated into a diluent comprising lactose and human serum albumin. The diluent is intended to safeguard the toxin during freeze-drying and serve as a bulking agent for the freeze-dried product. Before freeze-drying, the diluted toxin is allocated into vials, and upon completion of the drying process, the vials are assessed for integrity, sterility, moisture content, and potency.

2.2.2. Equipment's

1-1mL syringes with 20-gauge needles (for drawing up) and 30 gauge 0.5-inch needles (for injecting). Alternatively, insulin syringes with attached 31-gauge needles can be used.

- 2- 0,9 % sodium chloride (preferably bacteriostatic/preserved)
- 3_Alcohol pads
- 4_Gauze
- 5_ botulinum toxin (Botox).
- 6_Topical anesthetic (optional)
- 7_ surgical Marker and ruler

2.2.3. Treatment technique

Before the treatment, an informed consent was obtained from all patients. The protocol and procedure for migraine injection with Botox involve the following steps

- **Diagnosis and Evaluation:** A neurologist or headache specialist will conduct a thorough examination and medical history review to diagnose migraines and assess their severity and frequency.
- **Pre-Treatment Preparation:** The healthcare professional will explain the treatment process, potential benefits, risks, and any alternative options. They will also review the patient's medical history, current medications, and any allergies to ensure the safety of the procedure.
- **Treatment Plan:** Based on the patient's diagnosis and evaluation, a customized treatment plan will be developed.
- **Injection Procedure:** The Botox injections are usually administered in an outpatient setting. using a small needle or a specialized injection technique, Botox delivered into specific muscles associated with migraines. The injections typically take about **10-30** minutes to complete

Beginners may utilise an erasable marker or pencil to indicate injection sites; however, injections should be administered immediately adjacent to the markings to prevent tattooing of the skin, as illustrated in Fig (2.1).

Figure 1 Marking the injecting point while asking the patients to perform certain expressions

Injection techniques exhibit considerable variety and must be customized to meet the specific demands of each patient. Male patients generally possess larger and more robust facial muscles, necessitating increased dosages. The patient should move the brows medially and pinch the medial brow to palpate the tightened muscle, as depicted in figure (2.2). In this area, the practitioner should administer the injection deep (supraperiosteal) to circumvent the more superficially located frontalis muscle. Laterally, during muscle contraction, the insertion can be identified by the puckering of the overlying forehead skin. A shallow injection should be utilized in this area to circumvent the frontalis muscle. To prevent the diffusion of toxin into the levator palpebrae superioris (LPS) resulting in ptosis, the practitioner should provide the injection to the corrugator 1 cm above the superior eyebrow and instruct the patient to maintain an upright position for 2-3 hours following the injection. Extra care must be taken to ensure that the lateralmost corrugator injections are administered superficially and gradually to prevent diffusion.

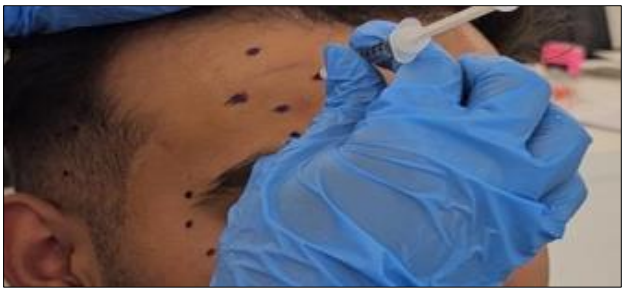

Figure 2 Locating the superior orbital rim, and avoid giving the toxin below or near the supraorbital foramina

The procerus is situated in the glabellar area, as illustrated in figure (2.3). In the absence of a horizontal rhytid when the patient medially elevates the eyebrows, chemical denervation of the procerus may be unnecessary. Injections are administered superficially, at a depth not exceeding 4 mm, in the area between the eyebrows. Dosing for the glabellar complex varies from 8 to 40 units, administered over 3 to 7 injection sites between the corrugator supercilia and procerus muscles.

Figure 3 Penetrating the whole muscle thickness in order to get fine results

- **The frontalis** is administered either intramuscularly or superficially, immediately beneath the subcutaneous tissues. Typically, injection is conducted no less than 1.5 cm above the superior orbital margin to avert ptosis. Dosing varies from 8 to 25 units, administered between 4 to 8 injection sites.
- **Injection of the orbicularis oculi** must be performed superficially, at a depth of roughly 1 to 2 mm, creating small wheals of toxin injected directly beneath the skin 1 cm lateral to the lateral orbital rim or 1 cm lateral to the lateral canthus.
- The clinician may instruct the patient to tightly close their eyes to facilitate the identification of muscle fibres. Furthermore, this will enable the provider to assess if the contraction of the orbicularis oculi induces depression of the lateral brow. Consequently, lateral and superior injection of this muscle may lead to an elevation of the lateral brow position during rest. Dosing varies from 4 to 15 units each side, distributed between 1 to 5 injection sites.
- **Temporalis Muscle injection:** The temporalis muscle is located on the sides of the head, above the ears. The provider will palpate the muscle to locate the optimal injection sites, usually around 4-6 injection points per side.
- **Occipitalis Muscle injection:** The occipitalis muscle is located at the back of the head, near the base of the skull. Injection points are typically along the hairline at the back of the head, with several injections distributed on both sides.
- **Trapezius Muscle:** The trapezius muscle runs from the back of the neck to the shoulder and upper back region. Multiple injection sites are typically used along the length of both sides of the trapezius muscle.
- **Post-Treatment Care:** After the injections, patients may experience some mild discomfort or redness at the injection sites, but this usually subsides quickly. It should be recommend avoiding strenuous exercise or lying down flat for a few hours following the procedure.
- **Follow-Up and Monitoring:** Regular follow-up appointments will be scheduled to assess the treatment's effectiveness and make any necessary adjustments to the treatment plan. This allows to ensure the patient's response is being optimized and address any concerns.

2.3. Statistical Analysis

Data description, analysis and presentation were performed using Statistical Package for social Science (SPSS version - 22, Chicago, Illionis, USA), Pie chart bar, frequency, percentage, minimum, maximum, mean, standard deviation, Fisher exact, level of significance is when p value less than 0.05.

3. Results

3.1. Distribution

Results show that 15 subjects participated in this study with 10 males (66.7%) and 5 females (33.3%) aged 25-44 years old with mean ±Standard deviation 33.67±5.97

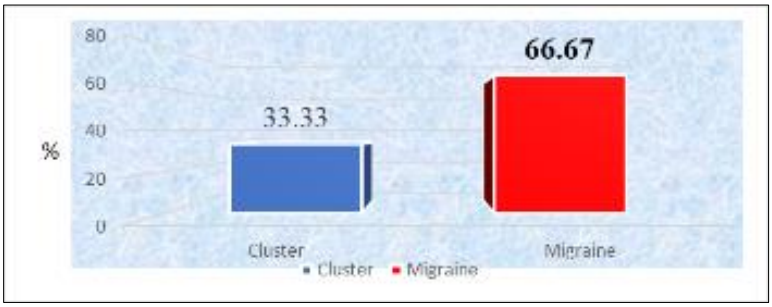

Figure 4 Distribution of subjects by headache type

Results above show that 5 subjects (33.33%) with cluster headache while 10 subjects (66.67%) with Migraine.

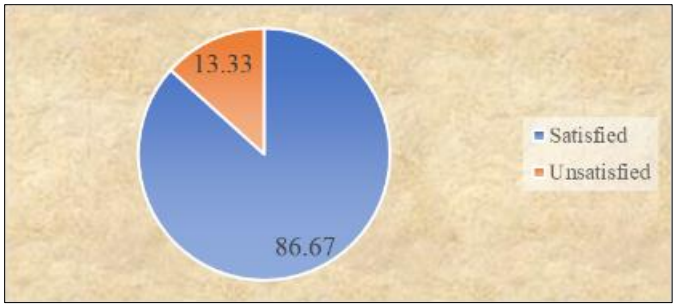

Figure 5 Distribution of subjects by satisfaction.

Results above show that 13 subjects satisfied (86.67%) while 2 subjects unsatisfied (13.33%).

Results above show that 13 cases success (86.67%) while 2 cases failed (13.33).

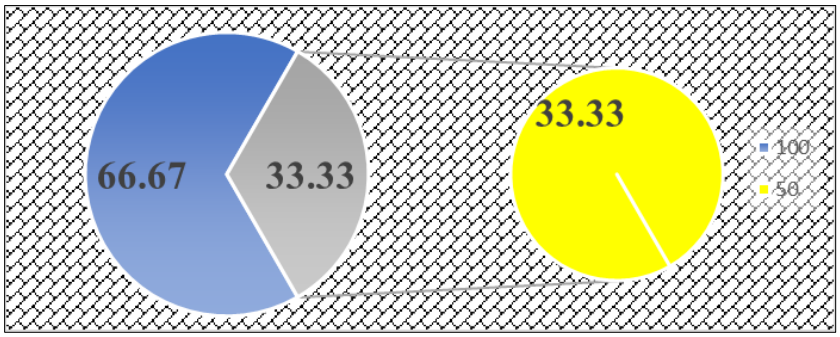

Figure 6 Distribution of subjects by botulin dose.

Results above show that 10 cases (66.67%) take 100 unit of botulin while 5 cases (33.33%) take 50 units

Results above show that 13 cases with no complication (86.67%) while one case (6.67%) for each asymmetrical brow and eyelid ptosis.

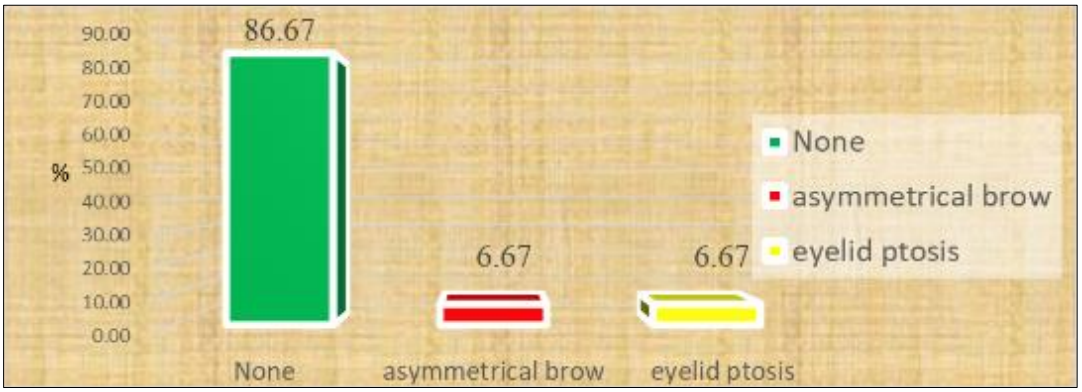

Figure 7 Distribution of subjects by complication.

3.2. Cross Tabulation

Table 1 Association between studied variables and gender

Vars.		**Gender**					
		Males		**Females**			
		N.	**%**	**N.**	**%**	**Fisher exact**	**P value**
Headache	cluster headache	0	.00	5	100.00	15.00	0.000
	migraine	10	100.00	0	.00		
Dose	50	0	.00	5	100.00	15.00	0.000
	100	10	100.00	0	.00		
Satisfaction	satisfied	10	76.92	3	23.08	4.615	0.095
	not satisfied	0	.00	2	100.00		
Success	success	10	76.92	3	23.08	4.615	0.095
	Failure	0	.00	2	100.00		
Complication	None	10	76.92	3	23.08	4.615	0.095
	asymmetrical brow	0	.00	1	100.00		
	eyelid ptosis	0	.00	1	100.00		
Modality	non	10	76.92	3	23.08	4.615	0.095
	referral to neurologist	0	.00	2	100.00		

Results above show that there is only significant association between gender with dose of botulin and headache type while success, satisfaction, complication and modality not associated with gender Table (3.1).

Table 2 Association between studied variables and headache type

Vars.		Headache				Fisher exact	P value
		Cluster headache		Migraine			
		N.	%	N.	%		
Dose	50	5	100.00	0	.00	15.00	0.000
	100	0	.00	10	100.00		
Satisfaction	Satisfied	3	23.08	10	76.92	4.615	0.095
	not satisfied	2	100.00	0	.00		
Success	Success	3	23.08	10	76.92	4.615	0.095
	Failure	2	100.00	0	.00		
Complication	None	3	23.08	10	76.92	4.615	0.095
	asymmetrical brow	1	100.00	0	.00		
	eyelid ptosis	1	100.00	0	.00		
Modality	Non	3	23.08	10	76.92	4.615	0.095
	referral to neurologist	2	100.00	0	.00		

Results above show that only dose of botulin is significant association with headache type while satisfaction, success, complication and modality is not significant associated with dose Table (3.2).

Table 3 Association between dose, complication and satisfaction**.**

Vars.		Satisfaction				Fisher exact	P value
		satisfied		un satisfied			
		N.	%	N.	%		
Dose	50	3	60.00	2	40.00	4.615	0.095
	100	10	100.00	0	.00		
Complication	None	13	100.00	0	.00	15.00	0.011
	asymmetrical brow	0	.00	1	100.00		
	eyelid ptosis	0	.00	1	100.00		

Results above show that only complication is associated with patient satisfaction while dose is not significant associated Table (3.3).

4. Discussion

4.1. Distributions

In this study, 86 % of patients are satisfied with the result because a number of them received relief and their symptoms disappeared 14% of patients were not satisfied with the treatment This is because they did not get the desired result, and some patients had complication such as:

4.1.1. lip ptosis

A further consequence is an asymmetric smile resulting from injection into the zygomaticus major muscle. The injection must not be positioned near the inferior edge of the zygoma to prevent lip ptosis. A multitude of muscles in the lower central face, particularly those utilised for facial expressions, also participate in the functions of the lips and cheeks (17).

4.1.2. asymmetry eye brows

A range of functional deleterious consequences is linked to various muscle responses to botulinum toxin or its improper administration. They are particular to botulinum toxin and result from the toxin's direct pharmacological activity. They occur less frequently than injection reactions and are mostly attributed to transient denervation of nearby muscles beyond the targeted treatment area. These issues are contingent upon technique, as they typically arise from improper toxin placement or varying muscle responsiveness (18).

4.1.3. eyelid ptosis

Eyelid ptosis poses a considerable risk when injections are administered on or underneath the central area between the eyebrows along the midpupillary line. This occurs due to the diffusion of the toxin through the orbital septum fascia to the levator palpebrae superioris, a muscle responsible for elevating the top eyelid. Injections into the orbicularis oculi, corrugator supercilia, and procerus muscles have the greatest probability of causing lid ptosis (19), and we referred the patients to a neurologist.

4.2. Distribution of subjects by headache type

Results in this study showed that 5 subjects (33.33%) with cluster headache while 10 subjects (66.67%) with Migraine.

The changes in brain activity affect blood in the brain and surrounding tissues, causing a range of symptoms. In addition to severe head pain, migraine sufferers may experience some or all of the following symptoms: Nausea. Increased sensitivity to light, sound or smells (20).

Hormonal changes, specifically fluctuations and estrogen that can occur during menstrual periods, pregnancy and perimenopause can trigger a migraine attack. Additional recognized triggers encompass specific pharmaceuticals and alcohol consumption. According to the Global Burden of Disease (GBD) 2019 study, the projected global prevalence of migraine escalated from 721.9 million (95% UI: 624.9–833.4) in 1990 to 1.1 billion (95% UI: 0.98–1.3) in 2019.

Migraine impacts about one billion individuals annually worldwide and is among the most prevalent neurological disorders, exhibiting significant prevalence and morbidity, particularly in young adults and females. Migraine is linked to various comorbidities, including stress, sleep difficulties, and suicide. The intricate and often ambiguous mechanisms underlying migraine formation have led to the identification of numerous social and biological risk factors, including hormone imbalances, genetic and epigenetic impacts, along with cardiovascular, neurological, and immunological disorders. This review provides a thorough examination of the latest literature regarding epidemiology, risk factors, and high prevalence (21).

A number of studies in different populations have made conflicting findings about the associations migraine has with obesity, diabetes, hypertension, and hypothyroidism (22-24). Individuals suffering from migraines may have a variety of problems, such as sickness absence and reduced productivity at work, school, and home. Hence, the disease imposes a heavy burden on society (25). Recently, The World Health Organization and The Lifting the Burden Campaign against Headache published the “Atlas of Headache Disorders and Resources in the World, 2011”.

Their worldwide investigation found that rates of migraine were as high as 15-20% of the population in European countries and as low as 4% of African countries with rates in Asian countries being similar to [Atlas of headache disorders and resources in the World] (26).

4.3. Distribution Regarding Gender

Results show that 15 subjects participated in this study with 10 males (66.7 %) and 5 females (33.3 %) aged 25-44 years old with mean + Standard deviation 33.67 * 5.97

So results show the migraine occur in male more than female because Stress, Physical exertion and production of lactate increased levels of a molecule called calcitonin gene-related peptide during exercise dysfunction of a molecule called hypocretin produced by your hypothalamus. The study that contradicted the research results written by Laura Kelley on June 16, 2023 Ten percent of the world’s population suffers from migraines, with women suffering from the painful headaches at significantly higher rates than men, according to the Journal of the American Medical Association (26).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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