



Composition and Effect of Botox injections: a study Awareness of Female Medical Staff in Some Benghazi Clinics, Libya

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ABSTRACT

Background: Freshness despite age includes smooth and charming skin without skin folds, loss of volume and sagging skin, all this is possible only because of these cosmetic procedures that are mainly attributed to Botox treatment. **Objectives:** To study the awareness of towards Botox injections among women working in medical clinics. Also, to determine whether bacterial multiplication occurred in vials of reconstituted BTX-A. **Methods:** A self-administered questionnaire was developed to collect the necessary data to achieve the research objectives. Analyzing samples of Botox in a laboratory, they were distributed in Petri dishes to grow bacteria. **Results:** The results of the questionnaire showed that highest participants in the age group of women 31-40 years was 30% . Female respondents 90%, were doctors, 30% were pharmacists. The extent for cosmetology with

Botox 50% disagreed while 35% agreed. Knowledge of Botox extracted from the bacteria *Clostridium botulinum*, show only 27% of women in medical and paramedical staff are aware of this information. About 70% of women in the target group disagree that Botox-A injections used as a drug without a prescription. 19% only knew the side effects of Botox injections on human health and environment. In addition, the second part of the study when analyzing the Botox in the lab revealed that the Botox used, was free of bacteria *Clostridium botulinum*. **Conclusion:** This study concluded that there is a weaker level of awareness of Botox treatment among the medical staff. We also that BTX-A used in Benghazi was free of botulinum bacteria.



Keywords: Botox injection, Botulinum toxin, Cosmeceuticals, Botulinum; reconstituted; microbiological assay.

INTRODUCTION

Botulinum toxin:

Botulinum toxin is one of the most poisonous biological substances known. It is a neurotoxin produced by a gram-positive anaerobic bacteria known as *Clostridium botulinum* ^[1]. There are at least seven serotypes of neurotoxin discovered till now: botulinum toxin A, botulinum toxin B, botulinum toxin C, botulinum toxin D, botulinum toxin E, botulinum toxin F, botulinum toxin G, but only the first two are medically used. The neurotoxin produced by this bacteria causes a disease known as botulism, this can cause a life-threatening neuroparalysis ^[2].

Even after knowing its side effects, several people get attracted to it just in order to maintain their appearance ^[3,4]. Botulinum neurotoxin (BNT), the biologically active component of botulinum toxin (BT), is a large double-stranded protein susceptible to various physical and chemical influences. All BT type A (BT-A) drugs are stored as powders allowing shelf lives from 24 to 36 months. After reconstitution, the specified shelf life is reduced to 8–24h ^[5].

Mechanism(s) of Action of Botulinum :

- Botox It is a neurotoxic protein produced by *Clostridium botulinum*, a Gram-positive bacterium. The name (Botox) came from the term Botulinum Toxin and it is abbreviated (BoNT or BTX) ^[6].
- To be able to use a toxin as a drug, it must be isolated, purified, and then stabilized.
- The BoNT molecule is synthesized as a single chain (150kD) and then cleaved to form the dichain molecule with a disulfide bridge (Fig.1). The light chain (~50 kD - amino acids 1-448) acts as a zinc (Zn^{2+}) endopeptidase similar to tetanus toxin with proteolytic activity located at the N-terminal end. The heavy chain (~100 kD - amino acids 449-1280) provides cholinergic specificity and is responsible for binding the toxin to presynaptic receptors; it also promotes light-chain translocation across the endosomal membrane ^[7].

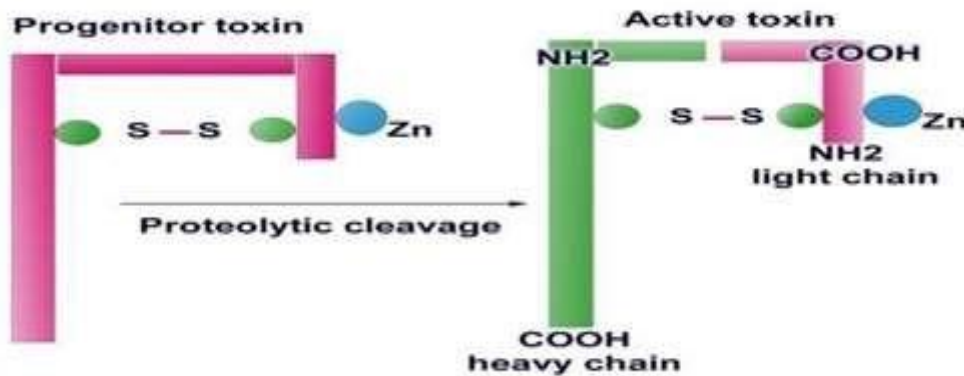


Figure 1. Botulinum toxin structure (schematic diagram)

Action of Botulinum Neurotoxin in Blocking Neurotransmitter Release:

Botulinum toxin consists of two chains, light and heavy chains, that are connected through a disulfide bond. [8].

Identifying the blockade of Ach by BoNTA has sparked the interest of some researchers to look into other potential neurotransmitters that are blocked by BoNT. In one of the active areas, pain research, the analgesic action of BoNTA was investigated extensively [9]. BoNTA shows analgesic efficacy in various pain conditions, such as musculoskeletal pain [01], dental medicine [00], and neuropathic pain .

The mechanism of action of Botox includes the following four main steps:

1. The first step is binding of the toxin to specific receptors on the surface of the presynaptic cells [01].
2. The second step is internalization, which is an energy-dependent receptor-mediated endocytic process. In this step, the plasma membrane of nerve cells in vaginitis around the toxin-receptor complex, [01].
3. The third step is translocation. Following internalization, the disulfide bond is cleaved and the 50kDa light chain of the toxin is released across the endosomal membrane of the endocytic vesicle into the cytoplasm of the nerve terminal [01].
4. The last step is blocking. The light chain of serotypes A and E inhibit the release of acetylcholine by cleaving the cytoplasmic protein [01] Following the injection, the toxin diffuses into the tissue until it binds selectively and reversibly in the presynaptic terminal of the neuromuscular junction



and then attaches to the specific protein-membrane responsible for acetylcholine excretion^[01]. The toxin immediately inhibits the release of acetylcholine in the neuromuscular junction causing relaxation of local muscles resulting in reduced facial wrinkles/lines ^[01-01].

Formulation

Two preparations of botulinum toxin A exist: Disport® and Botox® Botulinum toxin type A (BT, BT-A) for therapeutic purposes is marketed as on a botulinum toxin A (ONA, Botox. Botox consists of 7 types of neurotoxins; however, only toxins A and B are used clinically. Botox A is used for several disorders in the field of medicine, particularly in dermatology, for cosmetic purposes.³ The first type of Botox introduced to the market was on a botulinum toxin A. In 2002, it was recommended to be used as a cosmetic treatment for glabellar frown lines by the Food and Drug Administration (FDA) (^[08-09]).

Botulinum toxin Broadcast and Use:

Several products are marketed around the globe with different product characteristics. The chronological footprint tracking of the US-marketed botulinum neurotoxins approved by the FDA ^[20-21] shows OnabotulinumtoxinA (Botox) by Allergan Inc., approved for several conditions such as

1. **Dystonias** are a heterogeneous group of disorders characterized by sustained involuntary muscle contractions, frequently causing repetitive twisting movements, abnormal postures, and pain ^[22].

2. **Spasticity** describes the combination of a central paresis together with various forms of muscle hyperactivity, including dystonia, rigidity, and spasms often associated with pain.

3. **Autonomic Disorders.** Botulinum neurotoxins inhibit neuroexocytosis from cholinergic nerve terminals of the sympathetic and parasympathetic autonomic nervous systems.

4. **Urologic Pathologic Conditions.** The therapeutic applications of BoNT in urology include detrusor sphincter dyssynergia, lower urinary tract symptoms due to benign prostatic hyperplasia, and detrusor over activity (both neurogenic and idiopathic).

5. **Pain.** Despite current availability of a large number of analgesic drugs, management of chronic pain is still a challenge for clinicians.

6. **Other Applications.** The therapeutic possibilities of BoNTs are manifold and certainly not yet exhausted.



7. Gastroenterology and proctologic disorders.

8. Depression is a psychiatric condition affecting millions of people worldwide and it can be treated with many psychotropic substances.

9. Dental treatment by injecting doses of botulinum toxin directly into the masseter muscle^[20-21]

Botox applications in Cosmetic Uses:

BoNT/A1 can be used to reduce all wrinkles in all skin areas of the face and the neck as long as they are based on increased muscle tone. Best results are obtained when BoNT is combined with filler substances to correct non dynamic aspects of the skin lines^[23].

The present major use of BoNT/A1 in humans is related to a variety of cosmetic indications^[24]. Recently published statistics from the International Society of Aesthetic Plastic Surgery show that BoNT/A1 injection is the most popular of all cosmetic procedures worldwide^[25]. Estimate suggests that approximately half of them medical production of BoNT/A1 is used in aesthetic medicine. The serendipitous observation that BoNT/A1 smoothed facial lines when used therapeutically^[26] led to study the toxin effect on glabellar lines, which are perceived as a sign of aging and of negative emotions. The repetitive contraction and activity of the procerus and the corrugator supercilia muscles involved in facial expression is mainly responsible for glabellar lines and can be attenuated by BoNT/A1 injection into three to five sites of these muscles^[27].

Adverse effects:

A key feature of BoNT/A1 is a minimal effect even at short distances from the site of injection, a property that ensures optimal results in most of the clinical applications. However, the possibility of local diffusion or potential leakage into the systemic circulation exists and depends on a number of factors including volume and speed of injection, dose, and site of injection. could cause ptosis, heavy brow, or a frozen face in facial aesthetics^[28].

Importantly, the long and very extensive use of BoNT therapy indicates that it is remarkably safe when administered by authorized medical personnel, using licensed toxin preparations. In contrast, the handling of BoNTs by unskilled/non authorized persons or the use of counterfeit or unapproved agents that have permeated the market worldwide, or their combination, may place



patients at risk for potentially devastating consequences. In fact, four cases of iatrogenic botulism have been described following cosmetic injection with high dose of an unlicensed BoNT/A1 preparation [29].

Adverse effects Injections with botulinum toxin are generally well tolerated and side effects are few. Generalized idiosyncratic reactions are uncommon, generally mild, and transient. There can be mild injection pain and local edema, erythema, transient numbness, headache, malaise or mild nausea. The most feared adverse effect is temporary unwanted weakness/paralysis of nearby musculature caused by the action of the toxin [30-31].

Botulinum toxin should not be used in patients with neuromuscular transmission disorders . Concomitant use of aminoglycoside antibiotics and spectinomycin should be avoided because these drugs may potentiate the effect of the toxin. Patients with dysphagia should not be treated either. Botulinum toxin therapy should be avoided during pregnancy and lactation, even if there are some anecdotal reports about its non-harmfulness [32-33].

LITURATURE REVIEW

A study in Karachi, Pakistan by Shahlla Imam

In this study they accomplished to assess the knowledge of female students towards Botox therapy for beautification as well as for therapeutic purposes. The study was conducted from June 2018 to December 2018 in different medical colleges. Three hundred and eighty-six female students participated in the study. The response rate was 77.2%. The findings of the present study revealed that 58.3% of the participants were aware of Botox therapy and found it effective in cosmetology. The unexpected side effects, high procedure costs, and lack of cosmetologists were thought to be the main reasons that limit people from using Botox in Karachi-Pakistan. [34]

A study in Riyadh, Saudi Arabia by Eman M. Al Hamdan

Their aim was to evaluate dentists' knowledge and attitude towards using Botulinum toxin and DFs in dental treatment. They used Five hundred questionnaires were distributed in Riyadh city to government and private sectors to determine awareness and attitude towards using Botulinum toxin and DFs in dental clinics for therapeutic and aesthetics uses. A total of 47.8% reported that higher doses are more toxic, and that males need higher doses than females (10.2%). Their conclusion



was, limited knowledge regarding BTX and DFs uses for therapeutic treatment and cosmetic dentistry was found among dentists in Riyadh, Saudi Arabia, and this limited knowledge was the reason for their negative attitude towards application of both materials. [35]

A Study in Singapore by Eng-King Tan

Hemifacial spasm (HFS), a potentially disabling facial condition affects quality of life (QOL) and Botulinum toxin is an effective treatment. Therefore they examined the relationship between knowledge of disease with improvement in QOL following Botulinum toxin treatment in HFS patients. A total of 106 HFS patients were prospectively included. A baseline knowledge questionnaire and a validated disease-specific quality of life scale (HFS-7) were administered before and after botulinum toxin treatment. Their results showed a better educational level was an independent predictor of high knowledge.

Their conclusion was HFS patients with high knowledge of disease reported better improvement in QOL following botulinum toxin treatment. [36]

A study in Birmingham, UK by J Menon and A Murray

The aim of study was to examine such used bottles of Botulinum toxin for microbial growth after 4 h at room temperature, Eleven consecutive. These were subsequently analyzed for microbial growth. No growth was directly obtained from any of the Botulinum toxin bottles during this study. This pilot study suggests that if aseptic precautions are followed during the use of Botulinum toxin, the contents of the bottle remain sterile [37]

A study in São Paulo, Brazil by Teissy Osaki

In this study The authors sought to determine whether bacterial and/or fungal proliferation occurred in vials of reconstituted BTX-A and subsequent storage of the remaining solution under refrigeration for 4 weeks. A portion of the contents of 88 consecutive 100-U vials of BTX-A was administered aseptically to 108 patients for essential blepharospasm. Concluded that storing vials of reconstituted BTX-A for 4 weeks after administration to patients was not associated with detectable growth of bacteria or fungi. [38]

METHODOS

Aims of this Study, to identify the awareness and knowledge of the workers in Benghazi clinic about



Botox injections and the potential growth of Clostridium in Botox vials during the use of injections
The study methods consist of two axes:

A- The questionnaire section:

The cross-sectional study was conducted from February 2023 to March 2023 in different clinics in Benghazi, Libya. The questionnaire was developed after a comprehensive survey of the literature on the use of Botox injections in cosmetology.

The targeted clinics of the study are the first clinic, Al-Sabri, the second clinic, Al-Kish, and the third clinic, Khaled bin Al-Walid. The total of the whole community is 577, while the stratified sample was 233.

Study tools a self-questionnaire. It included three sections. The first section included personal data (age, educational level, job). The second section is social and the third is environmental and cultural.

Study population and inspection, the study population was a sample of the medical staff and their assistants. The multiple random sample method was used, and the questionnaires were distributed and filled out for a period of two weeks.

B- Microbial cultivation of Botox:

We prepared samples of Botox in the laboratory to grow them in Petri dishes from 10/4/2023 to 1/6/2023. The first step was, we have taken five test tubes, 100ul of Botox from the Botox bottle was taken through the insulin needle and put into the first tube, then 50ul of normal saline 0.9 % was added then a serial dilutions on five tubes was preformed, the diluted solution of the first tube it is concentration is 100% the second tube concentration 50% the third tube concentration 25% the fourth tube concentration 12.5% and the fifth tube concentration 6.25% we take 10 ul of each concentration of Botox that we have prepared and put them on Blood agar on a petri culture dish, a Chocolate agar on petri dish and Manconkie agar on a petri dish .

Then the culture pates were put in an incubator for culture for a week and re-implanted sample with the same bottle of Botox and in the same way, with sterilization of a Botox vibrator stopper with alcohol 70%, and the insulin needle was changed, this was repeated every week for four weeks.

RESULTS

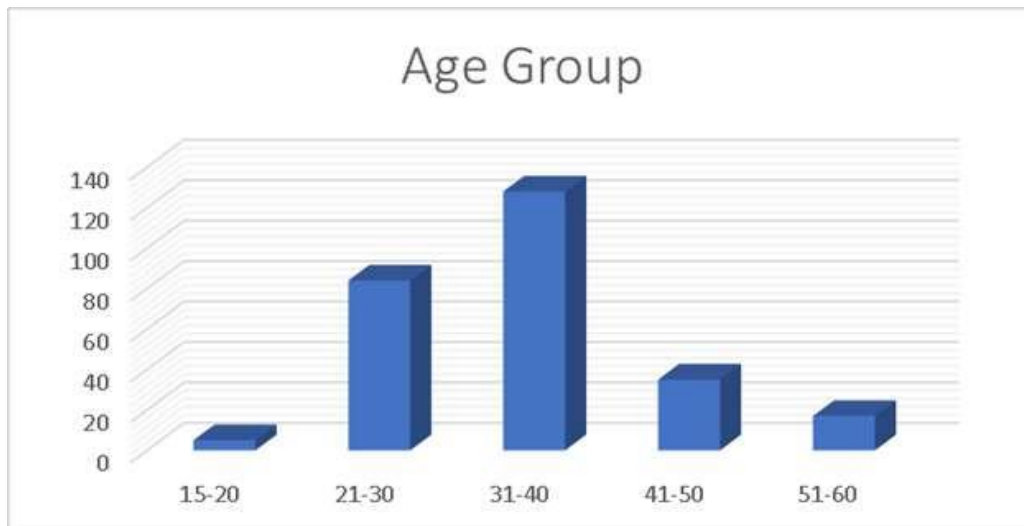


Figure 2: The age of the study participants.

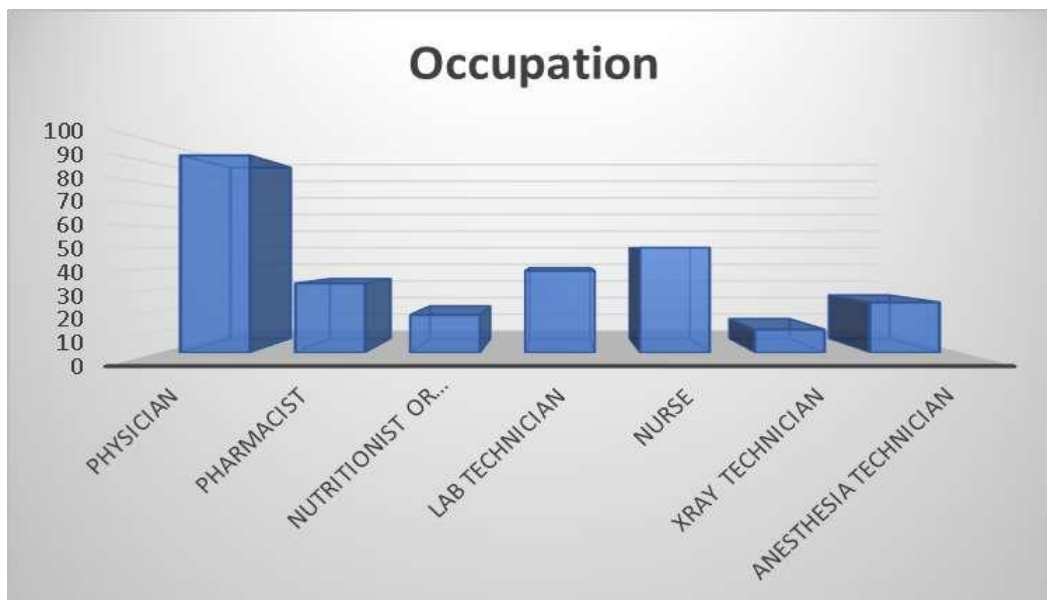


Figure 3: Occupations of the study participants.

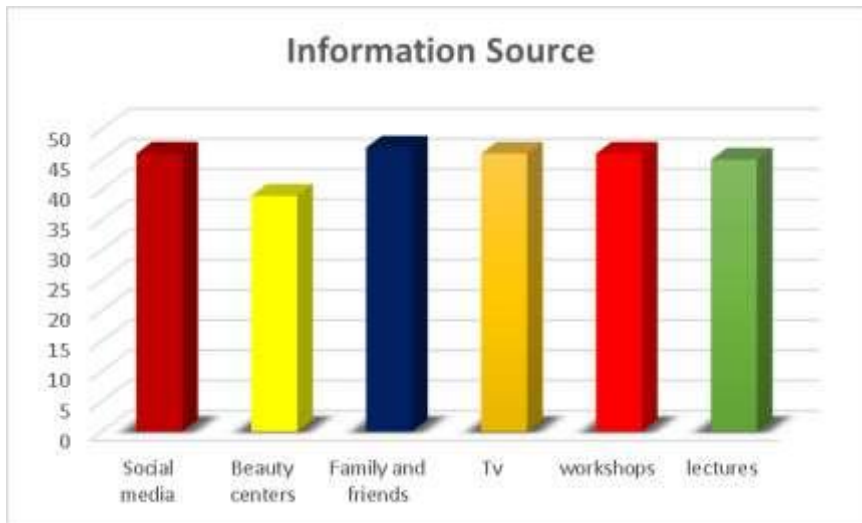


Figure 4: Sources of information about Botox Injection.

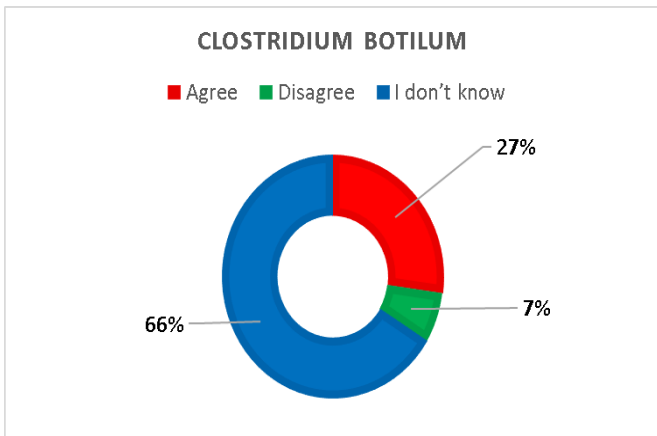


Figure 5: knowledge of extraction injections Botox from Clostridium botulinum bacteria

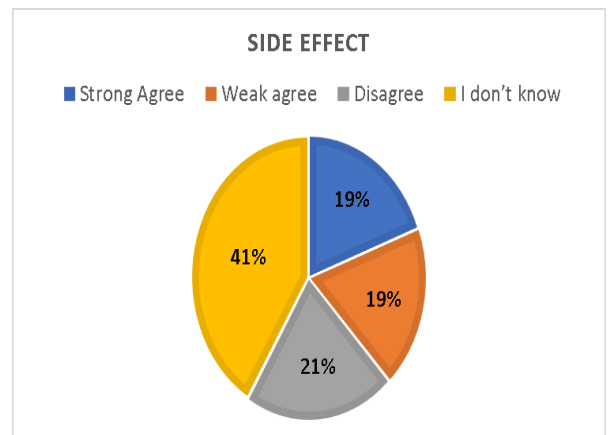


Figure 6: Side effect - Botox human health and environment

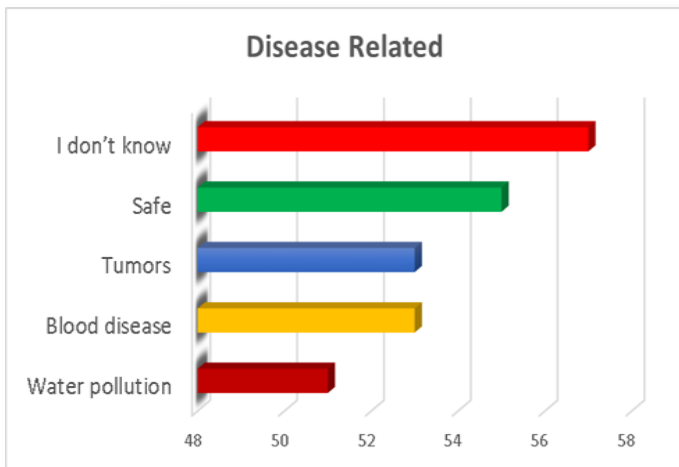


Figure 7 : Graph represents what are the most common effects associated with Botox contamination

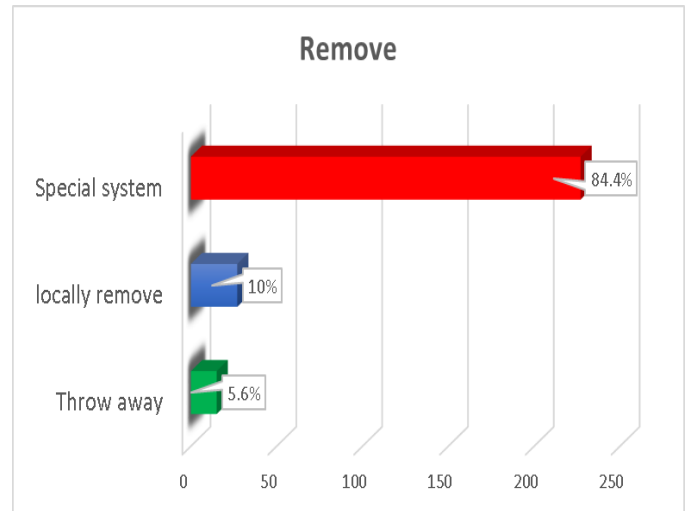


Figure 8: The following graph represents the knowledge of how to dispose of leftover needle after using them

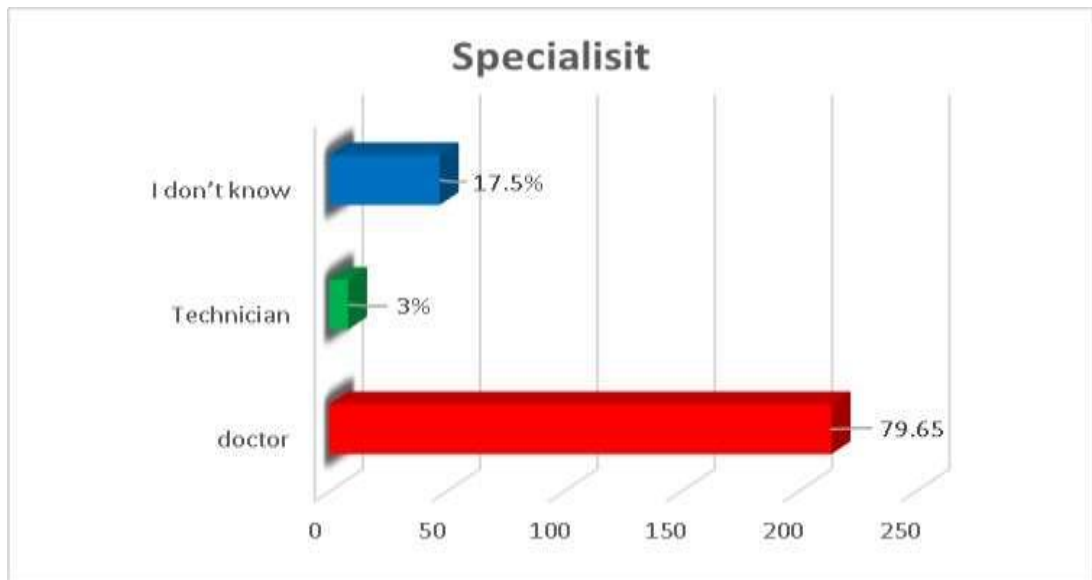


Figure 9: The following graph who is supposed to inject a Botox needle.

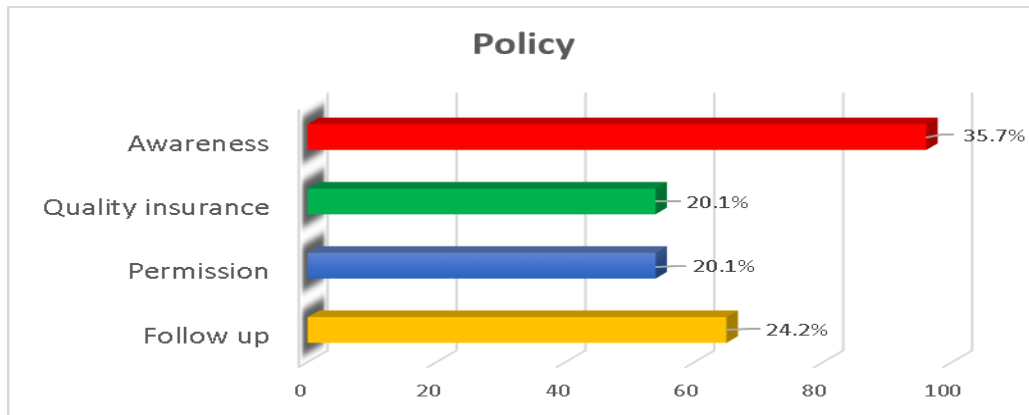


Figure 10: What is the policy adopted by the Ministry of Health for the proper and safe use of Botox injections?

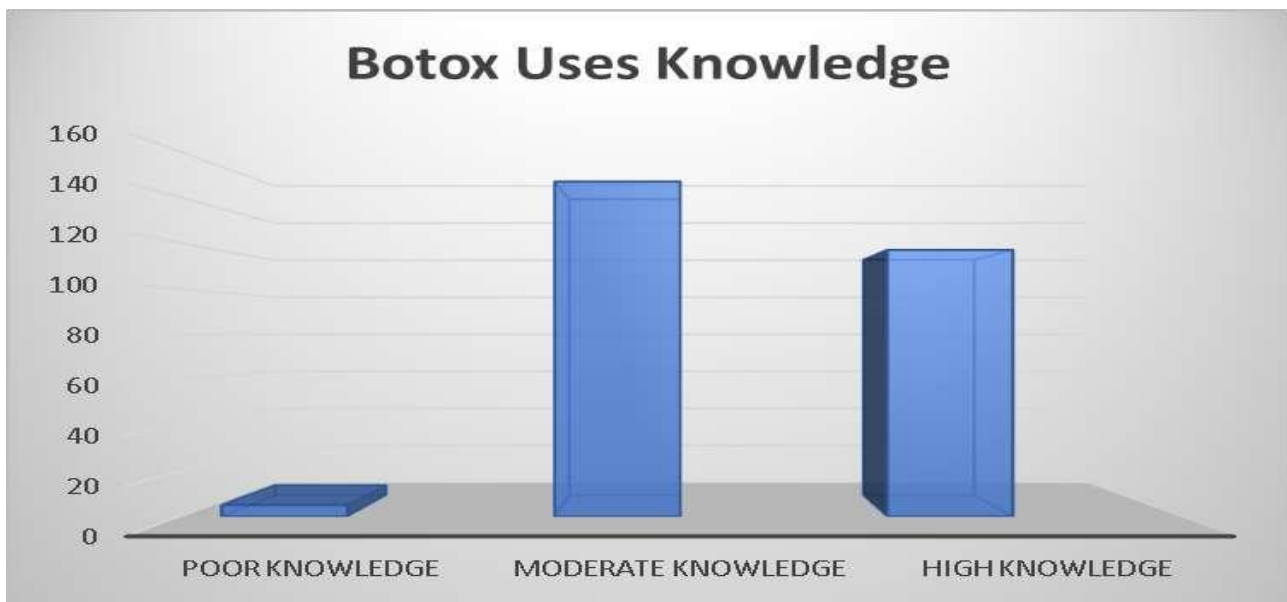


Figure 11: Knowledge of Botox Use and Safety the average answers to three levels of knowledge are weak, medium and good

Table 2: The relationship between the Education level with knowledge of the Botox uses.

knowledge	Education				Total
	preparatory school	High school	graduate	postgraduate	
poor knowledge	0	0	4	1	5
moderate knowledge	4	9	97	37	147
High knowledge	10	25	96	14	117
Total	14	34	169	52	269

Table 3: Indicates that there is no growth of any botulinum toxin bacteria.

No. of Vials	Preparation of BTX-A	Reconstitution on Liquid	Storage Duration (Location)	Culture Media	Results
5	OnabotulinumtoxinA	Preserved saline	New	Blood agar, chocolate agar, MacConkey agar	No bacterial contamination
5	OnabotulinumtoxinA	Preserved saline	Up to 1 wk (F)	Blood agar, chocolate agar, MacConkey agar	No bacterial contamination
5	OnabotulinumtoxinA	Preserved saline	Up to 2 wk (F)	Blood agar, chocolate agar, MacConkey agar	No bacterial contamination
5	OnabotulinumtoxinA	Preserved saline	Up to 3 wk (F)	Blood agar, chocolate agar, MacConkey agar	No bacterial contamination
5	OnabotulinumtoxinA	Preserved saline	Up to 4 wk (F)	Blood agar, chocolate agar, MacConkey agar	No bacterial contamination

DISCUSSION

A- Questionnaire section:

Botox injections, facelifts, and eyelid surgery, to name a few, from cosmetic procedures to the face, have become a magic potion for many women, and some note that the procedures are very risky.

In our study on the extent of women's health awareness of the medical staff and medical assistant by injecting Botox into the city of Benghazi, in three complex clinics, the highest value was for the age group (31-40) by 130%, while in India Where the awareness of cosmetic dermatology procedures among health workers was (20 - 30), it was 65.2% [39]. While in another study to Botox, a cross-sectional survey among students in Karachi, Pa. showing the highest in any age group, from (25 - 29), with a percentage of 194% (50.3) [40]. In our study about the extent of women's health awareness of the medical staff and the paramedic by Injecting Botox in the city of Benghazi, in three combined clinics, and the most participating category with us from the medical staff was the category of female doctors.

When asking women from the target group about the source of their information about Botox injections, we found in the study that (46%) of the medical and paramedical staff of women were the source of their information from social media and friends. In another study conducted at the University of Saudi Arabia [35]. The media was the most common source of information mentioned by students (79%).

When the target group was asked if they had an idea that Botox was extracted from the Clostridium botulinum bacteria (66%), their answer was that they do not know or have no knowledge of the composition of Botox. While (28%) were aware that Botox was extracted from the bacteria Clostridium botulinum. Compared to the study conducted in Chennai on the knowledge of students in the College of Dentistry about Botox injections it was found that (43.9%) were familiar with the composition of Botox, and whom said no (56.1%) because they did not know them.

The toxic effects on human health and the environment in our study . About women's health awareness among the medical staff. The highest value was 41%, I don't know, and the lowest two values 19%, I agree, and 19%, I don't agree, but in another study Chennai Dental College students' opinions about awareness of risks associated Botox were more in the 'no risk' category at 45.4%. [3].



Pollution-related effects and illnesses of Botox on women's health awareness among medical staff in our study , the highest value was 57, I don't know, and the lowest value 51 leakage of waste into water and soil. When Botox was injected among the students of the Chennai Dental College, the students of the Chennai Dental College were aware of the side effects of Botox such as infection, inflammation and swelling. 54.8% (dark blue) said yes ^[3]

According question of how to get rid of leftover needles after using them about the health awareness of women among the medical staff, and the highest value was 84.4%, to get rid of them in special equipment, and the lowest value was 5.6% for throwing them out.

The question about who performs cosmetic dermatological procedures was selected 53.5% for dermatologists and 12.3% for plastic surgeons and dermatologists.

The graph (10), represents the policy that the Ministry of Health can take for the proper and safe use of Botox Injections, the highest value was (35.7%), and the lowest two values were (20.1%).
At the conclusion of the questionnaire study, the table and picture (2,12) were used, respectively, to determine the use and safety of Botox, according to the educational level of the participants, the highest value was 147, medium knowledge about Botox.

B-Microbial cultivation of Botox-A

Microbial cultivation of Botox- Botulinum toxin A.

Results of this pilot study for the sterility of the contents contained in the botulinum toxin used, if aseptic precautions were followed during each use after exposing the bottle to 4 hours at room temperature as is the norm in many clinics.

No direct bacterial growth was obtained from any 20 bottles of BTX-A in this study. This also in consistent with other studies in the Department of Ophthalmic Plastic Surgery, Federal University of São Paulo, Brazil, their results showed no microbial contamination with bacteria, and it is difficult to compare the current study with current findings on this topic because all of these studies involved different methods and different sample sizes, however, all of these studies were free



of contaminated bacteria before 14 weeks of age ^[41]. In Addition to another studies in animal and human models have examined the potency of the reconstituted venom and repeatedly confirmed that this did not change even after two weeks, concluding that the reconstituted venom had the same initial potency and duration of effect even after 2 weeks of storage. ^[42]

CONCLUSION

To conclude the current study indicated that most of the participants, regardless of their age and educational level, did not use Botox injections, and its high cost and insufficient awareness of it led to its non-use.

It was found that there is an urgent need to raise awareness of the dangers of Botox, especially via the Internet, as a high response was observed in our study (online awareness is more widespread and accessible), and also the need to encourage professional bodies in the field of health care to develop strategies to educate their members. In addition to enlightening policy makers about this issue, public awareness is also essential.

In the second aspect of this study, no growth was detected on any plate or inside the broth in injection bottles, even after 2 weeks of storage. Also, reconstituted BTX-A that remained (under sterile conditions) for 4 weeks did not result in any contamination detectable bacteria. Subsequent studies are therefore needed to evaluate more vials of reconstituted stored BTX-A contents, particularly those used for facial rejuvenation, and to evaluate longer storage times and potential growth of contaminants. The results of these studies may support storage of reconstituted BTX-A for longer than the period recommended by some manufacturers.

RECOMMENDATIONS

- 1- Dermatology clinics and cosmetic centers must safely dispose of leftover needles after using them.
- 2- Vials must be stored protocol approved by their manufacturer.
- 3- Strict laws must be put in place for companies and pharmacies to sell needles without a prescription.



- 4- The Ministry of Health must intensify periodic publications and instructions to raise awareness of the use of Botox injections and their effectiveness and dangers.
- 5- For those who wish to experience Botox injections, contact a dermatologist and consultant instead of non-specialized workers in cosmetic centers.

LIMITATIONS

- Lack of sources and references related to the research in Libya. □ The high cost of Botox needles and the lack of research centers.
- Non-cooperation of some of the medical staff in answering a questionnaire, especially the dermatology department

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