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BOTULINUM TOXIN TYPE A IONTOPHORESIS FOR POSTBURN HYPERTROPHIC SCAR

By

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CHAPTER I

INTRODUCTION

A hypertrophic scar (HS) is a skin condition characterized by excessive fibrosis with disordered collagens from skin fibroblasts. HS usually develops during the wound healing process after deep-thickness trauma or burn injury and hinders normal function, resulting in physical, psychological and aesthetic problems for patients. Major risk factors for HS formation include gender, age, genetic predisposition, immunological responses of the patient, type of injury, wound size and depth, anatomical site and mechanical tension on the wound. HS proliferate exponentially for 2–18 months after injury **(Shirakami et al., 2020).**

Hypertrophic scars are more common in burn injuries and occur in 30–90% of patients with burn injuries. Delay (>3 weeks) in wound healing increases the risk of hypertrophic scarring, which is typically restricted to the confines of the initial injury and does not recur after excision. Decreased collagenase activity in hypertrophic scars leads to perturbed collagen production and degradation, resulting in bundles of crosslinked collagen oriented parallel to the epidermal surface. In particular, mature type I collagen expression is reduced and type III collagen is over-synthesized **(Jeschke et al., 2020).**

Botulinum toxin A is produced by anaerobic spore-forming bacteria and is used for various therapeutic and cosmetic purposes **(Samizadeh and De Boulle., 2018).**

It is a potent neurotoxin that blocks neuromuscular transmission. Some authors have reported that botulinum toxin type A can minimize scar formation by reducing muscle tension during wound healing, causing the fibroblast cell cycle to be paused in a non-proliferative state and influencing TGF-β1 expression **(Lee and Janget., 2018).**

Statement of the problem:

Does Botulinum toxin type A iontophoresis help in minimizing postburn hypertrophic scars?

Purpose of the study:

The main objective of the study is to evaluate the therapeutic effect of Botulinum toxin type A iontophoresis in improving postburn hypertrophic scars.

Significance of the study:

Hypertrophic scars result from fibroblast proliferation which is responsible for the enhanced collagen production that leads to excessive scarring. By decreasing the tension in the scar, botulinum toxin causes local fibroblasts to gradually change their functional status to proliferate slower, secrete less biologically active mediators and synthesize less extracellular matrix and collagen resulting in the improvement of hypertrophic scars **(Elhefnawy., 2016).**

One of the most significant drawbacks of BTX-A injection is pain. Iontophoresis, as an alternative modality for drug delivery, has several advantages. It is a painless modality with a reduction of systemic adverse effects due to localized drug administration and its convenience to the patient **(Montaser-Kouhsari et al., 2014).**

Delimitations:

This study was delimited in the following aspects:

1. Subjects:

Seventy-six patients with postburn hypertrophic scar will be randomly divided into two equal groups each one has 38 patients.

2. Equipment and tools:

2.1. Measurement equipment:

- Sonography **(Reinholz et al., 2016).**
- Patient and Observer Scar Assessment Scale (POSAS) **(Reinholz et al., 2016).**

2.2. Therapeutic equipment:

- Iontophoretic device.

Hypothesis:

It will be hypothesized that:

Botulinum toxin type A iontophoresis may help in minimizing postburn hypertrophic scars.

Basic Assumptions:

It will be assumed that:

- All patients will receive the same kinds of medication and the same nursing care.
- All subjects are free from any old previous hypertrophic scars.
- All subjects will continue in the study.
- All patients will follow the instructions during the treatment

Definitions of terms:

The following terms are defined for clear understanding of the terminology used in the present study:

Botulinum toxin type A (BTA):

Botulinum toxin type A (BTA) is an exotoxin of the anaerobic spore-forming bacterium, *Clostridium botulinum*. It had been extensively used in medicine owing to its ability to inhibit the release of acetylcholine which is a helpful tool for treating the hyperactive muscles **(Elsaie., 2021).**

Hypertrophic scar:

A hypertrophic scar is a wide, thickened, and often raised scar that develops at the site of skin injury that can be itchy and typically does not extend beyond the boundary of the original wound. A leading cause of hypertrophic scar formation is the mechanical tension on the wound **(Zhang et al., 2020).**

Iontophoresis:

It is a method for transdermal drug delivery based on the transfer of charged molecules using a low-intensity electric current. This original route of drug delivery is non-invasive and presents several advantages in comparison to the usual passive transdermal administration, such as faster release of the drug into the skin, the capacity to deliver macromolecules and better control of the delivered dose. Depending on the properties of the molecule, systemic administration can also be achieved without first-pass metabolism **(Roustit et al., 2014).**

CHAPTER II

REVIEW OF RELATED LITERATURE

The review of the related studies and literature of the main concept of this study has been presented under the following headings:

1.1- Wound healing process:

The classical model of wound healing involves three distinct, but overlapping phases that follow a time sequence: the inflammatory phase, the proliferative phase and the remodelling phase.

The first phase of wound healing is the inflammatory phase which starts immediately after tissue injury and lasts for approximately 2–3 days after injury. Coagulation cascades, complement activation and platelet degranulation prevent further fluid and blood losses by creating platelet plugs and a fibrin matrix. The immune system and inflammatory reactions are activated to prevent infection and remove devitalized tissues. Neutrophils are recruited by chemotactic factors produced by platelet and bacterial degranulations, and monocytes are recruited and differentiated into macrophages 2–to 3 days after injury **(Lee and Janget., 2018).**

The second phase of wound healing is the proliferative phase. This phase of new tissue formation occurs approximately 2–3 days after tissue damage and may last for 3–6 weeks. Active cellular proliferation and migration characterize this phase. Keratinocytes migrate to the damaged dermis; new blood vessels grow inward within the damaged tissue; and new capillaries replace the fibrin matrix with granulation tissue via the actions of macrophages and fibroblasts. Granulation tissue forms a new substrate for keratinocyte migration. Keratinocytes proliferate and mature within granulation tissue along the wound margin, restoring the protective function of the epithelium. In the late proliferative phase, a portion of the

fibroblasts differentiates into myofibroblasts in association with macrophages. Fibroblasts and myofibroblasts produce extracellular matrix (ECM), mainly in the form of collagen; this accumulated collagen forms most of the eventual scar. Other constituents of ECM include elastin, hyaluronic acids and proteoglycans. Myofibroblasts, which contain actin filaments, have contractile properties and help bring the edges of the wound together over time **(Lee and Janget., 2018).**

Once wound closure is accomplished, the final remodelling phase commences. This phase is characterized by the degradation of excessive tissue, transforming immature healing products into a mature form. Remodelling may last for a year or more. Excessive ECM is degraded and remodelled from type III collagen, the main component of ECM present during the early wound healing process, to mature type I collagen **(Lee and Janget., 2018).**

1.2- Hypertrophic scar formation:

An imbalance between the proliferation and degradation of extracellular matrix components leads to excessive scar formation. Risk factors for this include delayed epithelialization, wound infection, specific anatomic locations, or genetic predispositions **(Jeschke et al., 2020).**

On a molecular level, increased activity regarding scar proliferation is commonly mediated through inflammatory cells that secrete TGF-β1 and β2 which induce a fibrogenic response within the scar tissue. At the same time, scar remodeling stimulated through matrix metalloproteases and TGF-β3 is decreased. Excessive scar formation is dependent on both the severity of the inflammation and the type of the immune response **(Jeschke et al., 2020).**

2.1: Botulinum toxins:

Botulinum neurotoxin (BoNT) is produced naturally by Clostridium botulinum, an anaerobic, Gram-positive, spore-forming bacillus. Seven

serotypes of BoNT have been discovered (A to G), with only types A and B available for therapeutic usage. BoNTs have been used for spasticity, depression, hyperhidrosis, migraines, ageing of the neck, face, and decolletage on and off label in the medical and cosmetic areas since their commercial availability **(Naik et al., 2021).**

2.2: BoNT-A formulations:

BoNT-A is synthesized as macromolecular protein complexes in nature. The BoNT-A protein has a molecular weight of 150 kDa.

The three botulinum toxin formulations that have been approved by the US Food and Drug Administration and are well known in the Western hemisphere are as follows:

- Onabotulinum toxin A (ONA; Botox®/Vistabel®; Allergan Inc., Dublin, Ireland)
- Abobotulinum toxin A (ABO; Dysport®/Azzalure®; Ipsen, Paris, France/Galderma, Lausanne, Switzerland)
- Incobotulinum toxin A (INCO; Xeomin®/Bocouture®, NT 201; Merz Pharmaceuticals GmbH, Frankfurt, Germany) **(Samizadeh and De Boulle., 2018).**

2.2: Mechanism of action of BONTs:

The most recognized mechanism of action of BoNTs is the inhibition of neurotransmitters (acetylcholine, norepinephrine, substance P, calcitonin gene-related peptide [CGRP], and glutamate) release at the presynaptic neuromuscular junction. However, BoNTs can affect both sympathetic and parasympathetic functionality since acetylcholine is also a neurotransmitter of the autonomic nervous system. Furthermore, recent evidence shows the effect of BoNTs on different human cell types, both neuronal and non-neuronal cells. These latter include epidermal keratinocytes, mesenchymal stem cells from subcutaneous adipose, neutrophils and macrophages, dermal fibroblasts, mast cells, sebocytes

and vascular endothelial cells. Thus, several clinical applications are emerging in dermatology **(Guida et al., 2018).**

2.3: Effect of BoNT A in hypertrophic scars:

Hypertrophic scars occur mainly as a result of the tension exerted on wound edges. Tension can, directly and indirectly, lead to hypertrophic scars. BoNT A works by inhibiting the release of acetylcholine in the neuromuscular junction which indirectly causes neuromuscular transmission, leading to muscle paralysis and thus reducing wound tension. As a result of this and other mechanisms, BoNT A can be used not only as treatment but also as prevention of hypertrophic scars. BoNT A may directly modulate the activity of fibroblasts by altering apoptotic, migratory, and fibrotic pathways on pathological scars and thus improve their appearance **(Kasyanju Carrero et al., 2019).**

3.1: Transdermal iontophoresis:

Iontophoresis is a method of introducing ionized and neutral drugs into the skin by using an electric current. As shown in **(Fig. 1)**, during iontophoresis, electromigration and electroosmosis are the principal mechanisms of enhancing drug transport across the skin into the systemic circulation. Electromigration refers to the ordered movement of ionized drug molecules in the presence of the applied electric field. Therefore, charged drugs are forced across the skin by electronic repulsion of similar charges. Cationic drugs can permeate through the skin by using a positively charged working electrode. Similarly, anionic drugs can cross skin by negatively charged electrodes. Electroosmosis is the fluid moving in a direction in the presence of electric field, forming an electroosmotic flow and driving the movement of hydrated ions. At physiological pH, skin has a slight negative charge. Thus, electroosmotic flow occurs from anode to cathode **(Wang et al., 2021).**

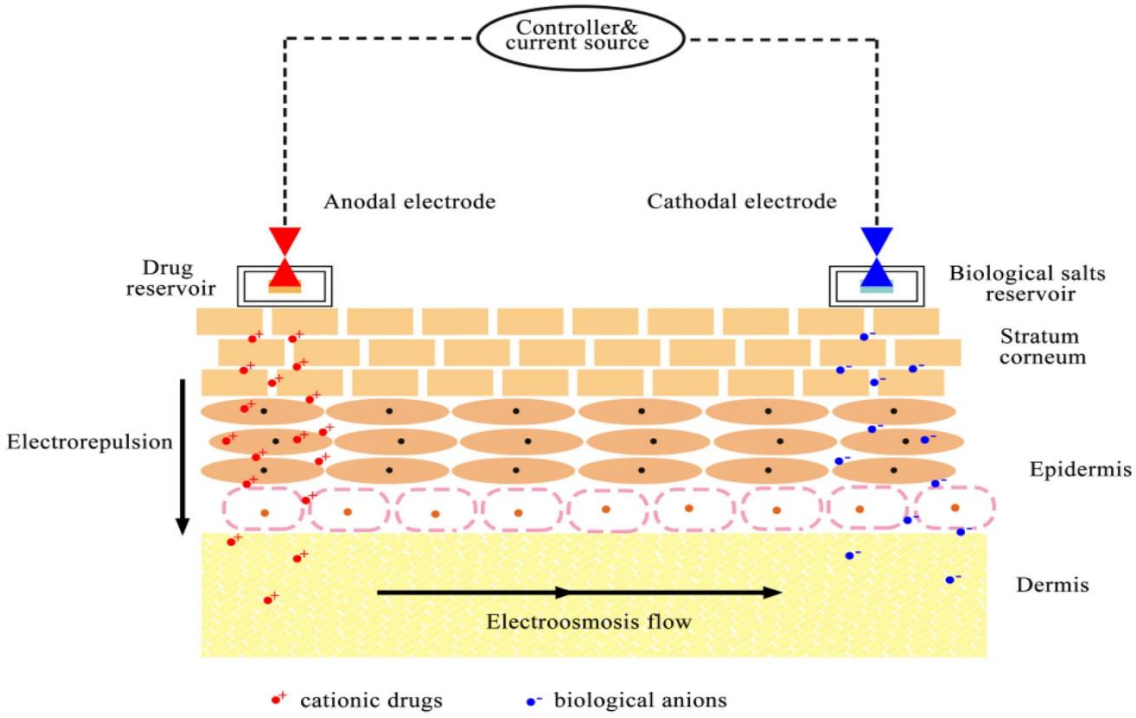

Fig. (1): A schematic diagram of iontophoretic technique **(Adapted from Wang et al., 2021).**

3.2: Factors influencing iontophoretic process:

There are various factors affecting iontophoresis as: The drug used should be water-soluble, of low dosage and susceptible to ionization. Smaller particles are more mobile. Increasing drug concentration results in greater drug delivery, but only to some extent. If buffer ions are present, they compete with the drug, reducing its delivery. The effect of iontophoresis also depends on the tissue on which the electrodes are applied (e.g., thickness, permeability, the presence of pores). In the skin, sweat glands are the most significant way the charges are carried **(Karpiński., 2018).**

3.3: Iontophoretic device:

An iontophoretic device consists of four parts: power source, control circuit, electrodes, and reservoirs. The power source can be connected to the skin through a control circuit and two electrodes (the anode and the cathode). Two electrodes are attached to two reservoirs

(one reservoir containing drug ions and the other containing physiologically compatible salts such as NaCl). Generally, Ag/AgCl is selected as the electrode material in iontophoretic systems **(Wang et al., 2021).**

3.4: Iontophoretic delivery of peptides and proteins:

Iontophoresis transiently increases the skin permeability of peptides and proteins. Peptides and proteins play a determining role in modern therapy. Their potency and specificity make them excellent therapeutic agents; however, their physicochemical properties and stability requirements almost invariably necessitate their administration by subcutaneous, intramuscular or intravenous injection. These act as effector agents that regulate and/or mediate physiological processes, serving as hormones, neurotransmitters and signal transducing factors. It has long been known that iontophoresis can administer therapeutic amounts of biologically active peptides into the body. More recent studies have shown that it is also capable of delivering structurally intact, functional proteins non-invasively into and across intact human skin and becomes the most popular choice of drug delivery **(Dhote et al., 2012).**

CHAPTER III

SUBJECTS, MATERIALS AND METHODS

In this part of the study, the materials and methods will be presented under the following headings: subjects, equipment, procedures of the study and statistical procedures.

1-Subjects (Sample size):

Sample size calculation is performed using G*POWER statistical software (version 3.1.9.2; Franz Faul, Universitat Kiel, Germany) based on data of VSS from the previous study **(Elshahed et al., 2020)** who reported a significant effect of botulinum toxin compared with control in treating hypertrophic scars. The required sample size for this study was 38 subjects per group. Calculations were made using α=0.05, power 90% and effect size = 0.76 and allocation ratio N2/N1 =1.

Seventy-six patients who have postburn hypertrophic scars will participate in this study. Their ages will be ranged from 20 to 40 years. The participants will be selected from the outpatient clinic of the faculty of physical therapy, at Cairo University and randomly distributed into two equal groups.

1.1 Design of the study:

In this study, the patients will be randomly assigned into two equal groups (38 patients for each group).

1.1(a) Group A (Study group):

This group includes 38 postburn hypertrophic scar patients who will receive Botox iontophoresis once monthly for 3 months; in addition to their physical therapy program (Positioning, Stretching exercises,

Pressure Therapy and Massage) 2 sessions per week for 3 months **(Mowafy et al., 2021).**

1.1(b) Group B (Control group):

This group includes 38 patients who will receive traditional physical therapy (Positioning, Stretching exercises, Pressure Therapy and Massage) 2 sessions per week for 3 months **(Mowafy et al., 2021).**

1.2 Criteria for the patient selection:

1.2. a. Inclusion Criteria:

The subject selection will be according to the following criteria:

- Patient with hypertrophic scar (3-6) months after burn healing.
- Age range between 20-40 years.
- Male and female patients will participate in the study.
- All patients have a postburn hypertrophic scar at different body sites.
- All patients enrolled on the study will have their informed consent.

1.2.b Exclusion Criteria:

Exclusion Criteria related to iontophoresis (Sheikh and Dua., 2020):

- Patients with prior medical histories of cardiac arrhythmias.
- Patients with cardiac pacemakers.
- Patients with orthopaedic implants.
- Areas of skin with lesions and impaired sensation.
- During pregnancy and breastfeeding.
- Patient with diabetes mellitus.

Exclusion Criteria related to the delivered drug itself (Elshahed et al., 2020):

- Patients with a history of hypersensitivity or adverse reactions associated with (BTX_A).
- Recent BTA administration 6 months before the study.
- Any subject complaining of psychiatric disorders or neurological disorders such as myasthenia gravis.

2-Equipment:

2.1-Measurement equipment:

2.1. (a)Sonography

A high-resolution B-image sonogram of every patient will be taken before and after treatment using an 11-Mhz receiving transducer of Logiq P6 Pro (GE Healthcare, Solingen, Germany). This method allows a good penetration depth of up to 40 mm into the skin and a resolution of around 158 micro m. It has been successfully utilized for objectively measuring pathological scars **(Reinholz et al., 2016).**

2.1. (b) Patient and Observer Scar Assessment Scale (POSAS)

POSAS is an adequate tool to objectively measure scar quality and the therapeutic success of the applied scar treatment. It consists of two parts; one for the patient (Patient scale; POSAS Patient) and one for the physician (Observer scale; POSAS Observer). Both contain six items on a 10-point rating scale and an extra category “Overall Opinion”. All characteristic features of the pathological scars are covered by the questionnaire: vascularity, pigmentation disorders, relief/texture, thickness, pliability, surface area, pain, and itching/pruritus. The latter items, in particular, concern the well-being of the patients **(Reinholz et al., 2016).**

2.2-Therapeutic equipments:

2.2. (a) Iontophoretic device:

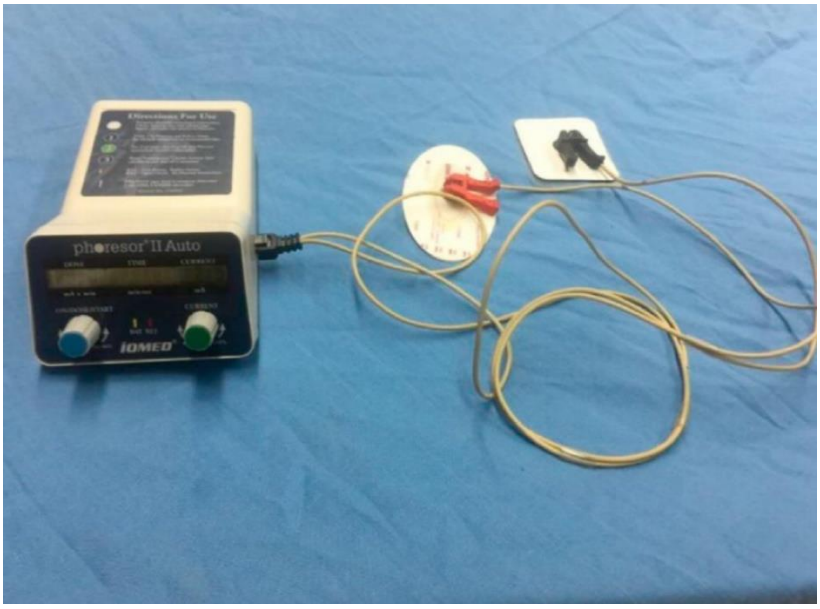

Fig. (2): The iontophoresis device **(Adapted from Ibrahim et al., 2021).**

2.2. (b) Botulinum toxin type A:

- Botox Allergan ®(Irvine, CA, USA) will be used .

It is 100 U vacuum-dried powder in a single-use vial for reconstitution diluted in 2 mL of sterile, preservative-free 0.9% saline to constitute a solution at a concentration of 4 U/0.1 mL) **(Elhefnawy et al., 2016).**

3-Procedures of the study:

3.1-Measurement procedures:

Both sonography and POSAS will be used for assessment of hypertrophic scar pre treatment and post treatment (after 3 months then after 6 months as follow up).

3.1. (a)Sonography

High frequency ultrasound is the most common used technique for scar assessment. Its inferior resolution compared to optical equivalents is mitigated by its superior penetration depth allowing thickness analysis, even in severe scar thickening. The working mechanism is based on refection of sound waves of structures with different acoustic impedances and the analysis of the refection time to determine the depth of the structure. The penetration depth ranges from the upper dermal layers to full-thickness skin and subcutaneous structures, depending on the employed frequency **(Téot et al., 2020).**

3.1. (b) Patient and Observer scar Assessment Scale (POSAS):

The scar will be rated numerically on a ten-step scale by both the patient and doctor on six items: vascularity, pigmentation, thickness, relief, pliability, and surface area on the Observer Scale. The Patient Scale consists of pain, itchiness, color, stiffness, thickness, and irregularity of the scar. One of the reasons POSAS was chosen for scar evaluation is because it is the only scar assessment tool to include a component for patients to fill in. Furthermore, its distinctive feature of reflecting subjective symptoms like pain and pruritus and because of its appropriateness for everyday practice **(Kant et al., 2018).**

Also, The Arabic version of the (POSAS) is an easy-to-administer, simple, reliable and valid tool for assessment of burn scar and for use on Egyptian population. It is advised to be used in clinical practice as well as scientific researches **(Nossier et al., 2018).**

Observer Scar Assessment Scale

	normal skin 1	2	3	4	5	6	7	8	9	10 *worst scar imaginable*	
Vascularization	o	o	o	o	o	o	o	o	o	o	
Pigmentation	o	o	o	o	o	o	o	o	o	o	Hypo ☐ Mix ☐ Hyper ☐
Thickness	o	o	o	o	o	o	o	o	o	o	
Relief	o	o	o	o	o	o	o	o	o	o	
Pliability	o	o	o	o	o	o	o	o	o	o	

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Total score Observer Scar Scale:

Patient Scar Assessment Scale

	No, no complaints 1	2	3	4	5	6	7	8	9	10 *Yes, worst imaginable*
Is the scar painful ?	o	o	o	o	o	o	o	o	o	o
Is the scar itching?	o	o	o	o	o	o	o	o	o	o

	No, as normal skin 1	2	3	4	5	6	7	8	9	10 *Yes, very different*
Is the color of the scar different?	o	o	o	o	o	o	o	o	o	o
Is the scar more stiff ?	o	o	o	o	o	o	o	o	o	o
Is the thickness of the scar different?	o	o	o	o	o	o	o	o	o	o
Is the scar irregular?	o	o	o	o	o	o	o	o	o	o

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Total score Patient Scar Scale:

(Adapted from Draaijers et al., 2004).

3.2-Therapeutic procedures:

3.2. (a) Procedures of iontophoretic device (Ibrahim et al., 2021):

Iontophoretic drug delivery system (Phoresor IIAuto,Model PM850, IOMED). The active electrode is placed directly over the marked area. The dispersive electrode was applied to the skin 6 inches distal from

the active electrode (Figure 3). The dose required is selected on the device; depending on the subject's tolerance, the current intensity is gradually increased, ranging from 2 to 4 mA.

3.2. (b) -Procedures of Botox iontophoresis (Elhefnawy et al., 2016) An intralesional injection of botulinum toxin type A (Botox Allergan ®, Irvine, CA, USA).

1. 100 U vacuum-dried powder in a single-use vial for reconstitution diluted in 2 mL of sterile, preservative-free 0.9% saline to constitute a solution at a concentration of 4 U/0.1 mL) will be administered once a month for a total period of three months.
2. The dose will be adjusted to 2.5 U/cm2 of the lesion.
3. The dose shouldn't exceed 100 units per session.

3.2. (c) Physical therapy program for both groups:

Positioning:

Positioning should begin immediately post injury and maintain during the entire process. Positioning should be carried out together with proper ROM training, otherwise, a prolonged fixed position will also result in reduced ROM and contracture. Positioning could be achieved through various modalities including pads, pillows, headboard, foam pads, splints and restraint belts **(Cen et al., 2015).**

Stretching and early mobilization:

Patients should be encouraged to get out of bed and exercise as soon as they are fit enough to do so. Therapeutic exercise encompasses ambulation of joints, consideration of neurovascular integrity, improving cardiovascular and respiratory capacity, coordination, balance, muscle strength and endurance, exercise performance and functional capacity.

Exercise also helps the patient to experience a general feeling of wellbeing and a sense of confidence and achievement **(Procter., 2010).**

Massage therapy:

Massage therapy, manual or mechanical (i.e., compressed air, threadlike showers, vacuotherapy, etc.), is standard therapy in rehabilitation centers specializing in the treatment of scars and burns. Although there is no scientific evidence, it has been shown that massage therapy not only reduces scar-related pain and itching, but also increases range of motion, reveals patients' anxiety and improves their mood and mental status. Mechanical compression is shown to reduce collagen synthesis by several mechanisms. These include decreased blood, oxygen and nutrients delivery to the scar, MMP-28 (matrix metalloprotease-28) downregulation and increase of PGE2 (prostaglandin-E2), which activates collagenase **(Arno et al., 2014).**

Pressure therapy:

pressure therapy is believed to work by reducing perfusion and oxygen concentrations reaching the scar surface resulting in reduced collagen synthesis. Moreover, pressure on scars reduces hydration, decreases angiogenesis, and induces apoptosis. A variety of materials can be used to provide pressure such as custom-fitted splints and adhesive plaster fittings while the efficacy is mostly dependent on the scar location with limbs and trunks being most appropriate for pressure therapy. Pressure garments can help reduce pain and itching associated with scars **(Elsaie., 2021).**

4-Statistical procedures:

Descriptive statistics:

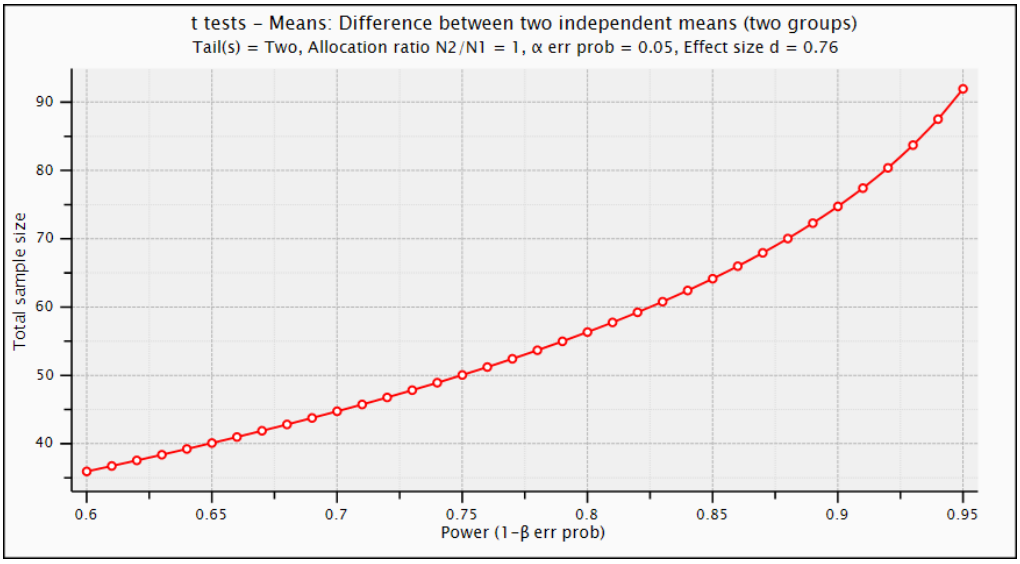
In this study, the descriptive statistics (the mean and the standard deviation) will be calculated for all subjects in all groups of the study to determine the homogeneity of the groups.

Analytical statistics:

Comparison will be made by student's t-test to compare the variables between all groups of the study. Paired t-test was used to compare before and after treatment in the same group. A value of $p \leq 0.05$ will be considered statistically significant **(Maronna et al., 2006).**

Sample size:

Sample size calculation is performed using G*POWER statistical software (version 3.1.9.2; Franz Faul, Universitat Kiel, Germany) based on data of VSS from the previous study **(Elshahed et al., 2020)** who reported a significant effect of botulinum toxin compared with control in treating hypertrophic scars. The required sample size for this study was 38 subjects per group. Calculations were made using $\alpha=0.05$, power 90% and effect size = 0.76 and allocation ratio N2/N1 =1.



Test family: t tests

Statistical test: Means: Difference between two independent means (two groups)

Type of power analysis: A priori: Compute required sample size – given α, power, and effect size

Input Parameters

Tail(s)	Two
Determine => Effect size d	0.7636043
α err prob	0.05
Power (1−β err prob)	0.9
Allocation ratio N2/N1	1

Output Parameters

Noncentrality parameter δ	3.3284740
Critical t	1.9925435
Df	74
Sample size group 1	38
Sample size group 2	38
Total sample size	76
Actual power	0.9074163

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