"A PROSPECTIVE STUDY TO COMPARE RESURFACING OF FACIAL ACNE SCARS IN ADULTS USING FRACTIONAL CO2 LASER AS MONOTHERAPY VS FRACTIONAL CO2 LASER WITH TOPICAL VITAMIN C SERUM IN A TERTIARY HEALTH CARE CENTRE"

By

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DISSERTATION SUBMITTED TO

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(M.D.)IN
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LIST OF ABBREVIATIONS

SL NO	ABBREVATIONS	FULL FORMS
1	ALR	Ablative Laser
2	A F R	Ablative Fractional Resurfacing
3	bFGF	Basic fibroblast growth factor
4	cm^{-1}	reciprocal centimeter
5	CO2	Carbon Dioxide
6	col	Collagen
7	CROSS	Chemical reconstruction of skin scars
8	°C	Degree Celsius
9	DOPA	3,4-dihydroxyphenylalanine
10	3D	Three Dimension
11	ECCA	Echelle d'Evaluation clinique des cicatrices
12	Er:YAG	Erbium Yttrium Aluminium Garnet
13	FDDA	Fractional Deep Dermal Ablation
14	FGF	Fibroblast growth factor
15	FrCO2	Fractional Carbon Dioxide Laser
16	Hz	Hertz
17	J	Joule
18	J/cm^2	Joule per square centimeter
19	k H z	Kilo Hertz

20	KTP	Potassium-titanyl-phosphate
21	MHz	Millihertz
22	mJ	Millijoules
23	ml	Milliliters
24	mm	Millimeters
25	mm^2	Square millimeters
26	ms	Milliseconds
27	mg	Milligrams
28	MNZ	Micronecrotic zone
29	MTZ	Microthermal Treatment Zone
30	MMP	Matrix metalloproteinase
31	μm	Micrometers
32	μs	Microseconds
33	nm	Nanometers
34	Nd:YAG	Neodymium-doped yttrium aluminium garnet
35	%	Percents
36	PLLA	Poly-l-lactic acid
37	PRP	Platelet-rich plasma
38	PDL	Pulsed Dye Laser
39	PIH	Post Inflammatory Hyperpigmentation

40	ROS	Reactive Oxygen Species
41	RF	Radiofrequency
42	YSGG	Yattrium-Scandium-Gallium-Garnet
43	W	Watts

ABSTRACT

Background: Dark skin type has a high inclination to acne scarring and is frequently complicated by persistent erythema or pigmentation. Fractional CO2 Laser injures the collagen with a monochromatic light beam, resulting in neocollagenesis, leading to resurfacing atrophic acne scars; however, prolonged erythema and post-inflammatory hyperpigmentation limit its usefulness, particularly in coloured skin. Vitamin C is famous for its anti-oxidant, anti-pigmentary, and wound healing properties. Laser-assisted drug delivery achieves greater penetration of topical agents. Recent literature demonstrates that ablative lase therapy and topical Vitamin C serum would optimize clinical outcomes in atrophic acne scars.

Objectives: The primary objective was to assess the efficacy and safety of Fractional CO2 Laser as Monotherapy and Fractional CO2 Laser in combination with Topical Vitamin C Serum in treating Atrophic Facial Acne Scars. Secondary objectives were to assess post-laser adverse effects and healing.

Material and Methods:

The study was a double-blinded randomized trial among 76 subjects; 38 in Fractional CO2 laser monotherapy (Group1) and 38 in the Fractional CO2 Laser in combination with Vitamin C serum within 2 minutes immediately after Laser and daily for four months (Group 2). Patients with facial atrophic acne scar belonging to both sexes, within an age group of 18 -40 years, with moderate to severe scar, and willing to undergo treatment and follow up were included in the study. Participants underwent serial photography of the

lesions at baseline, 1 month, 2 months, 3 months, and 4 months from the first treatment session. The visual analog scale (scoring from 0 to 10) was used to record adverse events (erythema and edema) as perceived by participants. The final assessment was made subjectively by a single observer at the last follow-up visit, and a quartile grading scale was used to assess the response objectively. Data were entered into a Microsoft Excel datasheet and was analysed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. The Chi-square test or Fischer's exact test (for 2x2 tables only) was used as a test of significance for qualitative data. Continuous data were represented as mean and standard deviation. MS Excel and MS word were used to obtain various graphs. P-value of <0.05 was considered statistically significant after assuming all the rules of statistical tests.

Results: A statistically significant difference ($p<0.001$) was found between the groups with respect to improvement in the appearance of scars after six months. 42.1% and 52.6% of Group 2 patients had >75% and 50-75% of improvement. 21.1% and 31.6% of patients in Group 1 had >75%, and 50-75% of improvement. >80% of patients were either satisfied or very satisfied in both groups. A statistically significant difference ($p<0.001$) was found between the groups regarding erythema and edema on Day 7 after the CO2 laser therapy session. With a P-value of 0.016, a statistically significant difference was observed between the groups concerning side effects.

Conclusion: The combination of using an ablative fractional CO2 laser and Vitamin C serum in the treatment of atrophic scars has a synergistic effect on their inherent properties in up-regulating new collagen synthesis to improve atrophic scars. Erythema, edema, and post-laser downtime are significantly reduced, with diminished risk of adverse effects.

Keywords: Atrophic, Acne, Scar, Fractional, Healing, Pigmentation, Laser, Vitamin C

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INTRODUCTION

INTRODUCTION

Acne vulgaris is a chronic inflammatory disease commonly found in a patient during adolescent years, young adults and can even persist through later adulthood.[1] It is clinically characterized by pleomorphic lesions like comedones, papules, pustules, nodules, and cysts.[2] In addition, seborrheic areas like the face, upper chest, and back of the trunk are usually affected.[3] The primary pathogenesis of acne is increased sebum production, colonization of *Cutibacterium acnes*, follicular hyperkeratinization, and peri-follicular inflammation, which can damage the skin and result in scarring.[1-4]

Acne was initially presumed to be merely a cosmetic affliction. However, the psychosocial effects of the disease have now been scientifically proven.[5, 6] Studies have shown these effects to improve when acne is treated.[6,7] Acne scars of the face are seen in approximately 20% of teenagers. This facial scarring, especially in males, is identified to be a risk factor for suicide.[4-7]

There are mainly two basic types of scars after the healing of acne.[7] In up to 80 percent of the cases, atrophic scars occur, while the rest develop hypertrophic scars or keloid—various atrophic scars; maybe soft and distensible or fibrotic. [8] For months, these scars tend to sustain a vascular hue before becoming less evident. Atrophic scars can be sub-classified into three subtypes; rolling scar, box scar, and ice pick scar.[7-9] Each has its characteristics and responds differently to the same treatment modality. [7]

Multiple modalities have been introduced in treating acne scars, such as

chemical peels, dermabrasion, laser treatment, dermal grafting, tissue augmentation, needling; there is still no definitive guideline in treating acne scars.[8] Over the last few years, laser therapies for acne scarring have become popular because of their remarkable outcomes.[16] Combining different methods improves the efficacy of acne scars and decreases the downtime and inflammation associated with laser treatment.[7-9]

Lasers for acne scars can be divided into two major categories, the ablative laser, and the non-ablative laser.[10,11] An ablative laser is more efficacious in treating facial imperfections, but its disadvantages, such as peri-procedure discomfort and longer recovery time, have become their most significant limitation.[11-13] On the contrary, a Non-ablative laser is more tolerable with a shorter recovery time, but multiple treatment sessions are required and provide less impressive results than traditional ablative lasers.[14] Examples of Ablative lasers are CO2 lasers, Er: YAG lasers, and Non-ablative lasers are Nd: YAG laser and diode lasers.[11]

The laser operates by remitting a monochromatic light within the scar, which heats and injures the collagen, resulting in neocollagenesis.[10] Fractional laser has been introduced in the field of laser to reduce the disadvantages of those traditional ablative lasers made while trying to maintain the high efficacy in resurfacing the skin.[11,15] The fractional lasers function by delivering the laser beam on the affected skin in 'fractions' by omitting intervening regions of skin untreated.[13-15] This ensures rapid re-epithelisation by the untreated areas; furthermore, the risk of extended and severe adverse effects is

minimized[15].

Resurfacing atrophic acne scars is stimulated by wound remodelling simultaneously with the synthesis of new collagen and elastin utilizing a Fractional CO2 Laser.[12-15] There is an increased generation of Myofibroblasts along with matrix proteins example: hyaluronic acid.[11,12] The restrained dermal injury diminishes the possibility of further scarring due to a high-energy, short-duration exposure of 10,600 nm CO2 laser light that evaporates intra- and extracellular water, causing tissue ablation rapidly to decrease injury.[11-15]

The inhibitory effect of Vitamin C on melanogenesis has been hypothesized.[16,17]Therefore, Vitamin C might be beneficial for post-inflammatory hyperpigmentation following laser skin rejuvenation.[18-20] At the molecular level, the expression of basic fibroblast growth factor (bFGF) was remarkably increased in the treatment of facial acne scars.[17,19] Post-laser use of Vitamin C combined with Vitamin E and Ferulic acid was delivered, resulting in reduced edema compared to the control group. Poor wound healing is associated with Vitamin C deficiency.[16-19] Vitamin C aids in collagen synthesis and additionally has an antioxidant effect.[21] A sufficient supply of antioxidants is present within Vitamin C Serum, which benefits Optimal skin health.[20,21]

In the case of acne scars, the fractional resurfacing CO laser gives excellent results.[22,23] However, the prolonged erythema lasting for weeks, crusting and

oozing, and post-inflammatory hyperpigmentation are recognized side effects associated with these treatment modalities, particularly in the coloured skin, limiting the usefulness of this option.[23,24] The technology of fractional CO2 laser avoids many undesirable effects as only a "fraction" of the skin is shot, and the epidermis and its integrity are not compromised.[23,24] For that, the microscopic wound produced by the laser beams is enclosed by normal, unlasered tissue, the healing is quick, and the unwanted side effects are minimized.[24]

Antioxidants in the skin are considerably exhausted after the epithelium is triggered by fractional CO2 laser therapy.[23-25] Therefore, the utilization of Vitamin C may be impactful to replenish depleted antioxidants and promote wound healing.[25,26] In addition, a laser-assisted delivery system can achieve excellent penetration of Topical Vitamin C Serum deep into the skin.[26-29]

Very few studies have been done where a combination of Fractional CO2 Laser with Topical Vitamin C Serum is used; this study will help us determine their synergistic effect in treating facial acne scars.

AIMS & OBJECTIVES

OBJECTIVES

Aims and Objectives of the Study:

1. To Assess the efficacy and safety of Fractional CO2 Laser as Monotherapy and Fractional CO2 Laser in combination with Topical Vitamin C Serum in the treatment of Atrophic Facial Acne Scars.

2. To Assess post laser adverse effects and healing of Fractional CO2 Laser as Monotherapy and Fractional Co2 Laser in combination with Topical Vitamin C Serum in the treatment of Atrophic Facial Acne Scars.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Scarring on visible areas such as the face and neck can be associated with negative psychological impacts. Acne vulgaris affects 80% of people between the ages 11-30 years old and 5% in adults over 30 years of age.[8,30] Acne can be considered as a chronic disease in view of the older and the most recent definitions of chronicity by the World Health Organization.[31] Multiple treatment modalities have been introduced for the treatment of acne scars.[32]

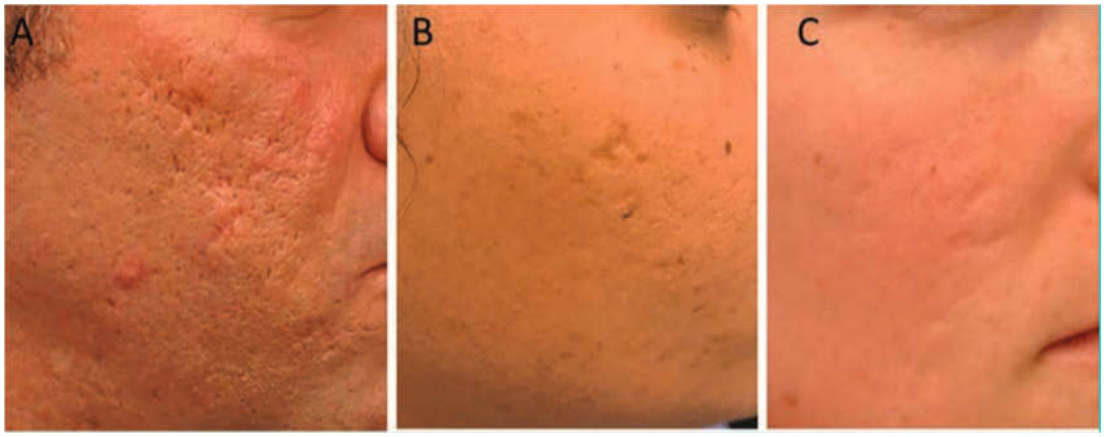
Pathogenesis

The main pathogenesis of acne is an increase in sebum production, colonization of *Cutibacterium acnes*, follicular hyperkeratinization, and perifollicular inflammation, all of which can damage the skin and result in atrophic or hypertrophic scars.[33,34] During the healing process, scars become atrophic due to the net degradation of the collagen fibers. On the other hand, collagen gain causes the scar to become hypertrophic or keloid.[33-36]

Classification

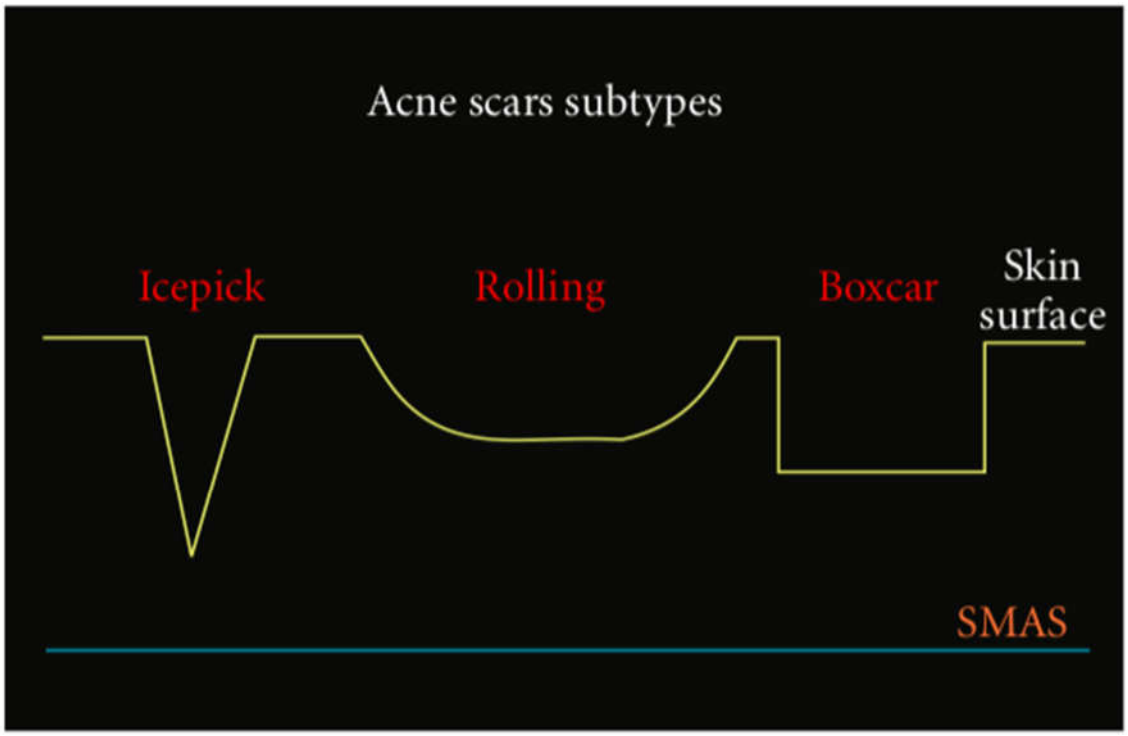
There are mainly two types of the scar; in up to 80 percent of the cases, atrophic scars occur, while the rest develop hypertrophic scars or keloid. According to their morphology, atrophic acne scars are subdivided into three types: icepick, rolling, and boxcar. (Figure 1)[35,36]

Figure 1: The three types of atrophic acne scar: a) Icepick, b) Boxcar, and c) Rolling [35]



Sub-classification of atrophic scars aid the clinicians in choosing an adequate treatment for each type of scar.[36] Icepick scars are very resistant to conventional treatment.[55-57] Icepick scars are V-shaped epithelial tracts with a sharp margin extending downwards to the deep dermis or subcutaneous tissue with less than 2mm. They are the most common subtype and are seen in up to 70 percent of all the scar types.[35,36] In about 20-30 percent of scars, box scars are observed, characterized by round to oval depressions with a diameter between 1.5 to 4.0 mm, and they are the second most common subtype.[32,36] Rolling scars have a unique feature of fibrous anchoring of the dermis down to subcutaneous; they are the least common type, seen in about 15- 25 percent, characterized by superficial shadowing and undulating appearance with a diameter up to 5 mm (Figure 2)[36-43]

Figure 2: Acne scar subtypes (adapted from Fabbrocini and his team)[36]



Evaluation

Commonly used grading scales are the ECCA grading scale and Goodman and Baron qualitative and quantitative global scarring grading system.[39-41]

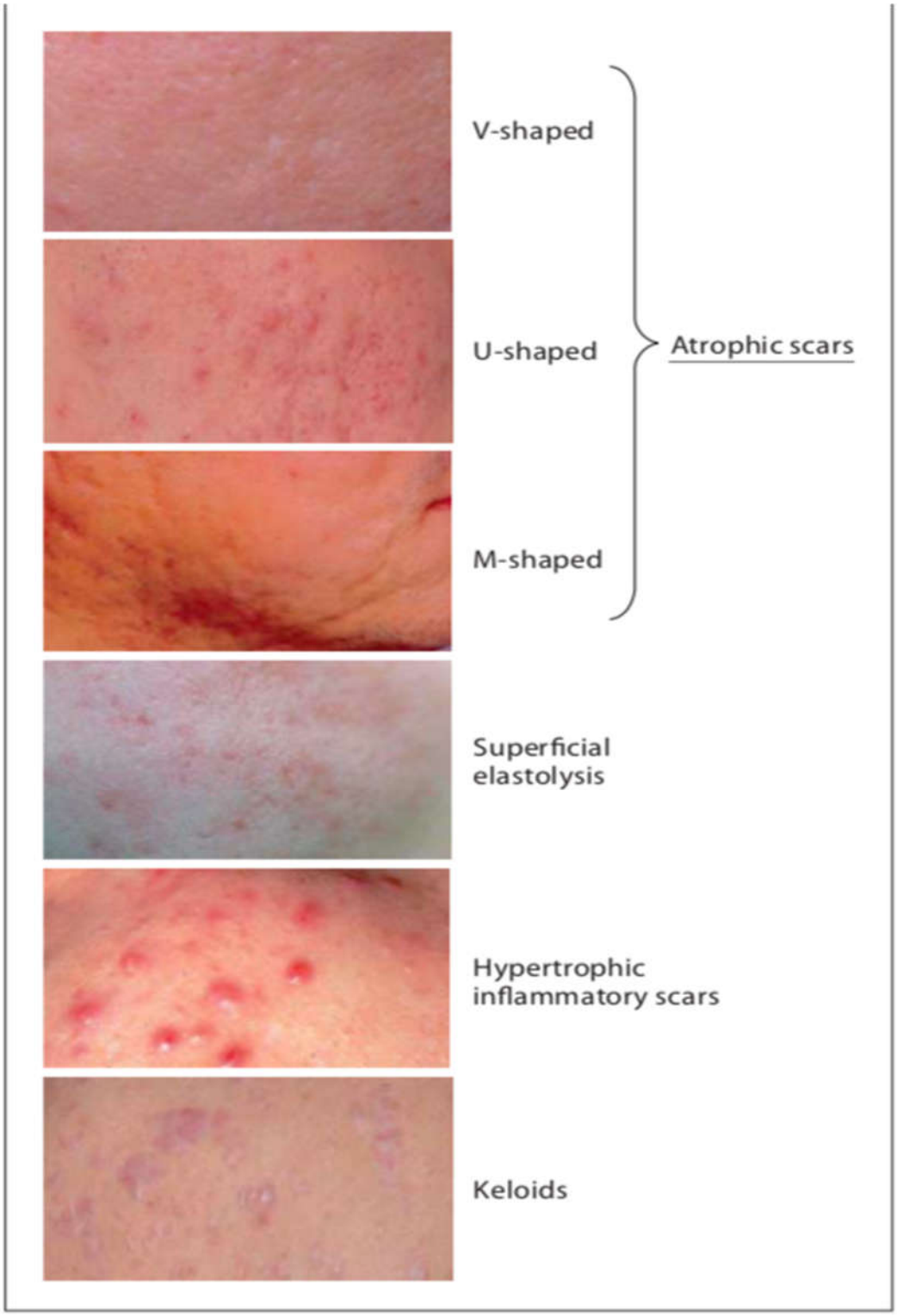
1. **ECCA grading scale**

ECCA grading scale (echelle d'évaluation Clinique des cicatrices d'acné) is a tool designed to evaluate the efficacy of the treatments on acne scars; it helps dermatologists in their everyday lives practice. Grading is composed of 6 items corresponding to 6 types of acne scars, with each kind of scar associated with a quantitative score ranging from 0 to 4 and a weighting factor ranging from 15 to 50. (Table 1) (Figure 3)[39]

Table 1: ECCA grading scale [39]

Description	Weighting factor (a)	Semi-quantitative score (b)	Grading (a × b)
V-shaped atrophic scars, diameter of less than 2 mm, and punctiform	15	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
U-shaped atrophic scars, diameter of 2–4 mm, with sheer edges	20	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
M-shaped atrophic scars, diameter of more than 4 mm, superficial and with irregular surface	25	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Superficial elastolysis	30	0 = absent 1 = mild 2 = moderate 3 = intense	/_____/
Subgrading 1			/_____/
Hypertrophic inflammatory scars, scars of less than 2 years of age	40	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Keloid scars, hypertrophic scars, of more than 2 years of age	50	0 = no scar 1 = a few scars 2 = limited number of scars 3 = many scars	/_____/
Subgrading 2			/_____/
Global score (subgradings 1 + 2)			/_____/

Figure 3: Acne scars assessed by the ECCA grading scale.[39]



2. Goodman and Baron quantitative scarring grading system

Goodman and Baron's quantitative scarring grading system classified scars into four types, i.e., mild atrophic scars, moderate atrophic scars, severe atrophic scars, and hypertrophic/keloidal post acne scars. Atrophic scars are scored according to the number of lesions the patient has. For example, mild atrophic scars are scored less heavily than moderately atrophic scars and, again, lesser than severe atrophic scars. On the other hand, hypertrophic/keloidal post acne scars are scored according to the area of the skin involvement.[41] (Table 2) (Table 3)

3. Goodman and Baron qualitative scarring grading system

Goodman and Baron's qualitative scar grading system concentrates more on the scar morphologies and disease severity by dividing acne scars from the least severe to the most severe into four grades like grade 1, grade 2, grade 3, and grade 4. The patient will be described according to the highest grade, omitting the milder disease if the lesions on the patient's face are sub-classified into two or more grades.[40] (Table 4)

Table 2: Goodman and Baron Quantitative Scarring Grading System[41]

(Grade) Type	Number of lesions: 1 (1–10)	Number of lesions: 2 (11–20)	Number of lesions: 3 (> 20)
(A) Milder scarring (1 point each)	1 point	2 points	3 points
Macular erythematous or pigmented			
Mildly atrophic dish-like			
(B) Moderate scarring (2 points each)	2 points	4 points	6 points
Moderately atrophic dish-like			
Punched out with shallow bases small scars (< 5 mm)			
Shallow but broad atrophic areas			
(C) Severe scarring (3 points each)	3 points	6 points	9 points
Punched out with deep but normal bases, small scars (< 5 mm)			
Punched out with deep abnormal bases, small scars (< 5 mm)			
Linear or troughed dermal scarring			
Deep, broad atrophic areas			
(D) Hyperplastic	2 points	4 points	6 points
Papular scars			
(D) Hyperplastic	Area < 5 cm^2	Area 5–20 cm^2	Area > 20 cm^2
	6 points	12 points	18 points
Keloidal/hypertrophic scars			

Table 3: Assessment of Goodman and Baron Quantitative Scarring Grading System[41]

Assessment of	Goodman and Baron Quantitative Scarring Grading System
0-5	Minimal Reduction in GSGS Score
5-10	Moderate Reduction in GSGS Score
10-15	Good Reduction in GSGS Score
>15	Very Good Reduction in GSGS Score

Table 4: Goodman and Baron Qualitative Scarring Grading System[40]

Grade	*Level of disease*	*Characteristics*	*Examples of scars*
1	Macular disease	Erythematous, hyper- or hypopigmented flat marks visible to patient or observer irrespective of distance.	Erythematous, hyper- or hypopigmented flat marks
2	Mild disease	Mild atrophy or hypertrophy that may not be obvious at social distances of 50 cm or greater and may be covered adequately by makeup or the normal shadow of shaved beard hair in males or normal body hair if extrafacial.	Mild rolling, small soft papular
3	Moderate disease	Moderate atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair if extrafacial, but is still able to be flattened by manual stretching of the skin.	More significant rolling, shallow "box car," mild to moderate hypertrophic or papular scars
4	Severe disease	Severe atrophic or hypertrophic scarring that is obvious at social distances of 50 cm or greater and is not covered easily by makeup or the normal shadow of shaved beard hair in males or body hair (if extrafacial) and is not able to be flattened by manual stretching of the skin.	Punched out atrophic (deep "box car"), "ice pick", bridges and tunnels, gross atrophy, dystrophic scars significant hypertrophy or keloid

Treatment

In the same visit, combination therapy may be necessary for treating atrophic acne scars to achieve the best result. Multiple modalities have been introduced to treat atrophic acne scars, such as chemical peels, dermabrasion, laser treatment, dermal grafting, tissue augmentation, needling, excision, and fat transplantation.[8-10] Each type of acne scar responds differently to each treatment option. Therefore physical examination to evaluate each patient is an essential procedure.[44](Figure 4)

The first step of treating acne scars is to decrease the redness with vascular lasers such as PDL, IPL, or KTP. Therefore initial assessment of residual erythema is done at the first visit, following that clinician addresses whether the patient has generalized or individual scars.[8] The scars are then treated as per the most suitable option. Lasers and resurfacing agents remain the mainstay of treatment for generalized areas of scarring. For individual scars, injectable fillers or surgical procedures are more appropriate.[43] (Figure 5)

Each type of scar responds to each treatment differently; fractional photothermolysis is the only effective treatment in all kinds of scar. In contrast, other treatment modalities can provide more negligible or no effect in one type of scar.[43-45] Ablative laser resurfacing is one of the most popular choices in treating generalized atrophic acne scars because of its ability to reduce all types of scars compared to other treatment modalities. Ablative lasers may lack efficacy in some scars.[46-48]

Additionally, techniques such as subscision and tissue augmenting agents can provide effective treatment in all types of scars, but less in comparison to fractional photothermolysis and are also more suitable in patients with individual scars.[49,50] (Table 5)

Figure 4: Diagram of Acne Scar Subtypes[36]

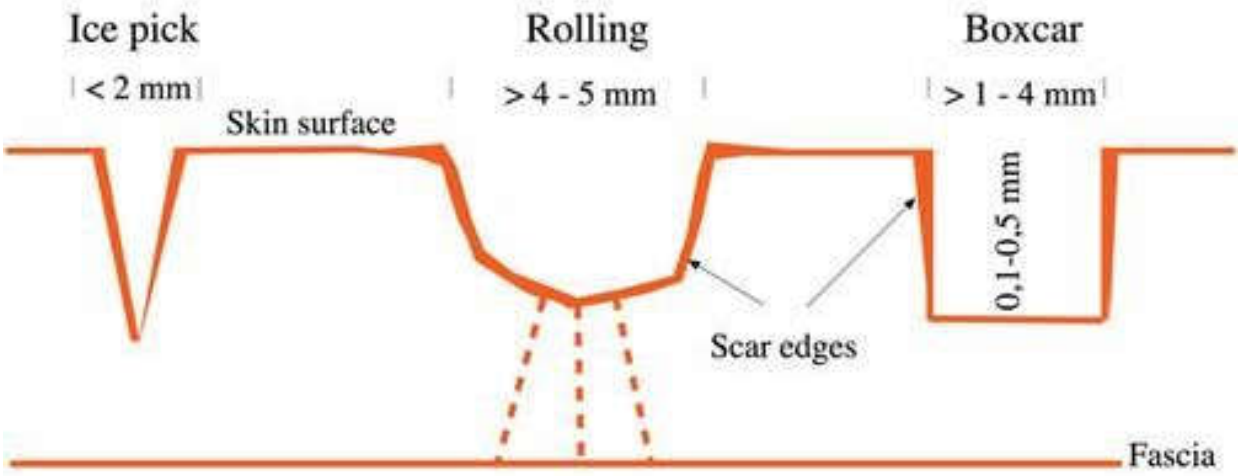


Figure 5 : Acne scarring treatment algorithm[43]

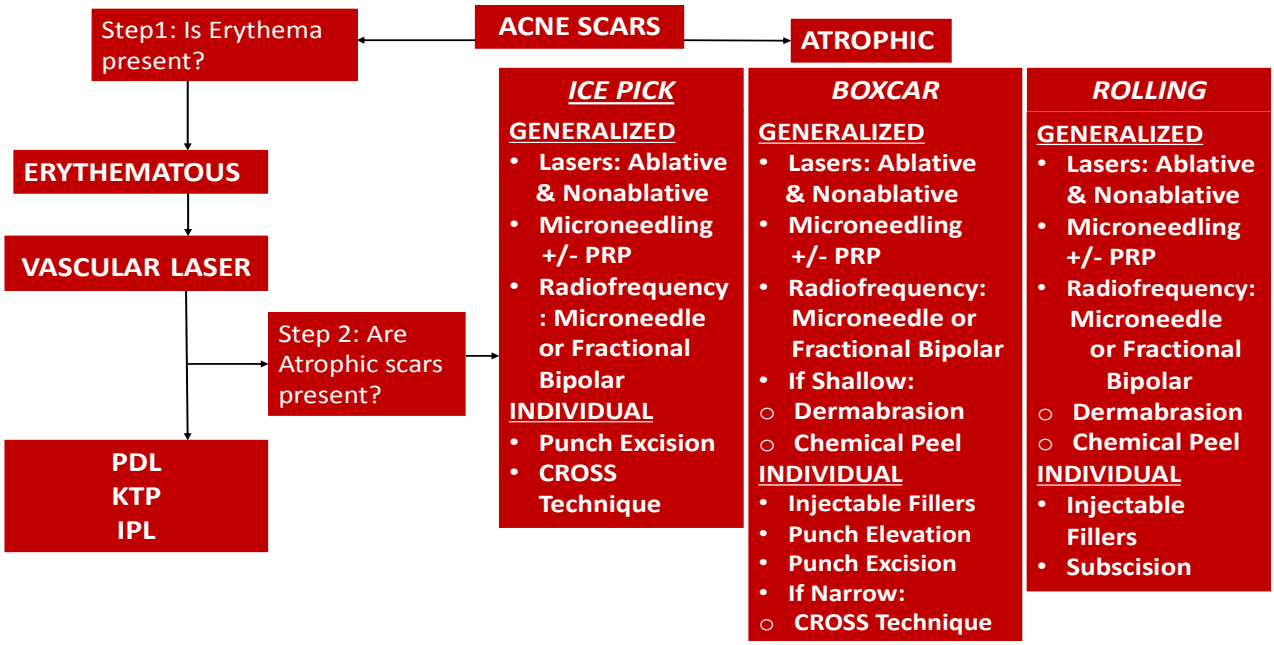


Table 5: Procedures to select by lesion type of Scars[49,50]

TABLE 2. Procedures to select by lesion type of scars			
TREATMENT	ICE PICK SCARS	ROLLING SCARS	BOXCAR SCARS
Chemical peels TCA CROSS technique	++ ++	– –	++ ++
Dermabrasion/microdermabrasion	+	–	+
Laser Ablative and nonablative laser Fractional photothermolysis	– ++	++ ++	++ ++
Punch techniques Punch excision Punch elevation Punch replacement grafting	++ – ++	– – –	+ ++ –
Tissue augmenting agents	+	++	+
Needling	–	++	++
Subcision	+	++	+
++ = Effective, + = less effective, – = not effective			

A physician must consider the following while treating acne scars[45-49]

- Cost
- Severity of lesions
- Physician expectations
- Patient expectations
- Side effects
- Duration between treatment sessions.

The aim of treatment is improvement in the appearance of the scar, not for a complete cure.[46-49] The current treatment modalities of atrophic acne scar include chemical peels, subcision, punch techniques, dermabrasion/microdermabrasion, dermal punch grafting, tissue augmented grafting (fat transplantation), other tissue augmented grafting (such as autologous collagen, bovine collagen, hyaluronic acid injection), platelet-rich plasma, plasma skin regeneration, and laser skin resurfacing.[49-52] The treatment options may be categorized into five main categories: medical management, procedure management, surgical management, tissue augmentation, and combination therapy.[45-52]

1. Medical Management

Topical retinoids, topical/injectable corticosteroids, and other topical or injectable substances such as vitamin A, vitamin C, vitamin E, zinc, colchicine, cyclosporine, honey, and onion extract, and 5-fluorouracil are the current medical options available.[10, 51-54]

Topical retinoids are helpful mainly in treating very superficial scars and also preventing scars.[55] Hypertrophic scars and keloid are commonly treated with intralesional corticosteroids, reducing cellular adhesion molecules and enzymes related to the inflammatory process.[53,56] Hypertrophic scars, keloids, and post-acne pigmentary changes focus on medical management, but the atrophic scars need other forms of intervention. [50-56]

2. Surgical Management

The surgical treatments are mainly recommended for icepick, boxcar, and rolling scars.[54] There are multiple available techniques for surgical management in atrophic acne scars.

(1) Punch Excision

The icepick and deep boxcar scars primarily utilize punch excision to eliminate the deeper portions, enabling fewer passes of laser treatment; therefore, it is beneficial.[37] The diameter of the scar walls should be equal to the diameter of the punch biopsy instrument.[57] The distance between two lesions should be at least 4-5 mm to prevent excess traction and lack of

proper wound eversion.[58] Punch elevation or elliptical excision is preferred to avoid a "dog-ear" appearance if the scar requires a larger punch.[31,95-98]

(2) Punch Grafting

Punch Grafting is a combination of punch excision followed by full-thickness skin grafts used to treat deep icepick acne scars.[95, 96] Mismatch in color and thickness of grafts with normal surrounding skin is one of the disadvantages of this method.[57,58]

(3) Punch Elevation

Initially, the punch biopsy instrument, whose size fits the inner diameter of the scar, is used to explore the scar down to the subcutaneous tissue. Subsequently, the scar base is carefully elevated slightly higher than the surrounding skin.[57] This technique is best employed for shallow and deep boxcar scars with normal skin appearance on the scar base.[58] The absence of risk of colour or texture mismatch is the main advantage of this method.[55-58]

(4) Subcision

The technique uses a hypodermic needle probing beneath the lesion through the puncture movement and causes the breaking of the papillary dermis from the fibrous connections of the SMAS and produces minor injury that commences to the wound healing process (Figure 6 and 7).[59] It is the idealistic choice for rolling or depressed scars. However, subcision is not a true incision. This method may require multiple attempts or sessions.[60,61]

Most authors recommend combining the surgical with other modalities for a better treatment outcome. However, these are mainly used for initial debulking.[10, 37]

Figure 6: Subcision technique done by undermine the fibrous connection under rolling scars [35]

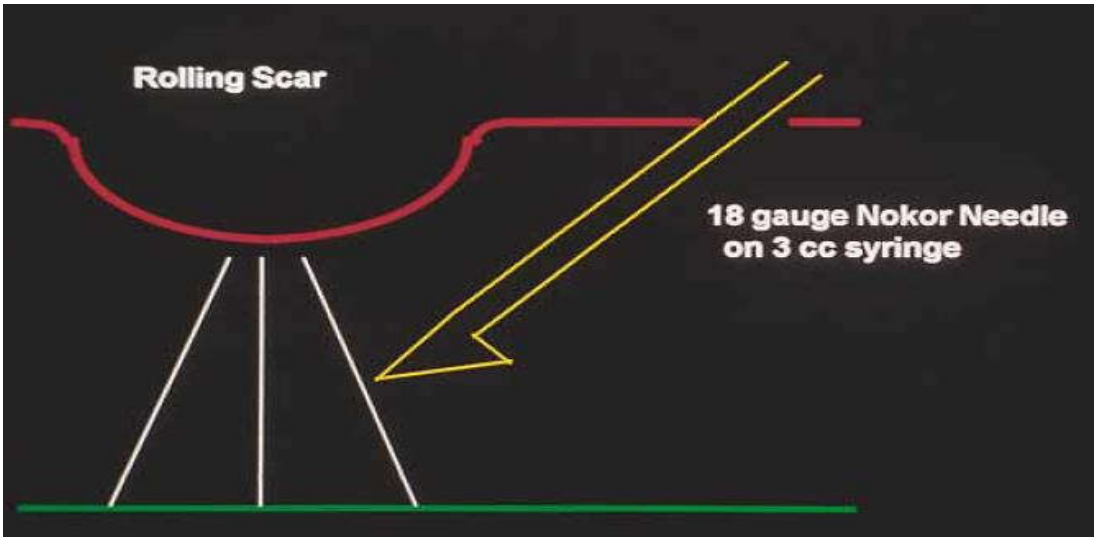
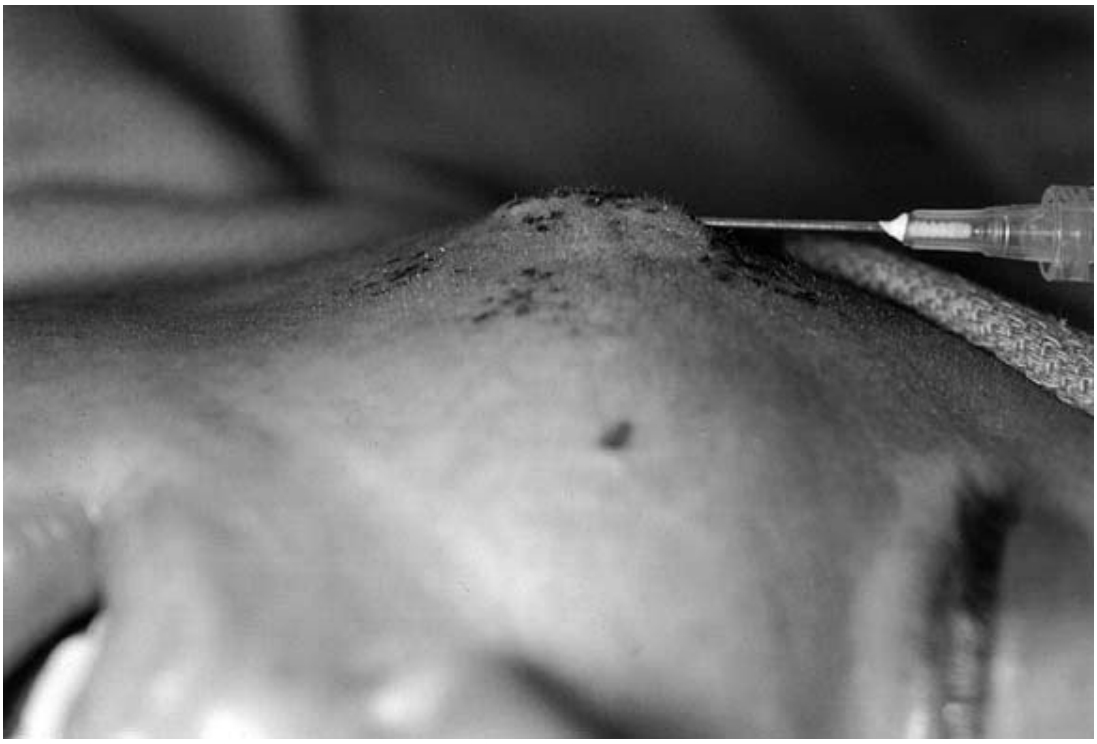


Figure 7: Piston-like motion: the technique for release the fibrous bands by advance the needle through them. [35]



3. Procedural Management

The general group of therapies consists of chemical peels, dermabrasion and microdermabrasion, electrodesiccation, cryosurgery, and laser treatment in treating atrophic acne scar.

(1) Chemical Peels

A good candidate for this procedure is a patient with mild scars.[37] The mechanism damages a part or entire epidermis, with or without the dermis, leading to exfoliation and removal of superficial lesions, stimulating the epidermal and dermal tissue remodeling. However, various treatments are required for efficacy. [61, 62] Combination therapies are generally used to improve effectiveness.[62] Chemical peeling is a popularly practiced procedure in the outpatient clinic. Chemical peels can be divided into four different types-

CROSS (chemical reconstruction of skin scars) technique/ dot peeling, with high strength of TCA, is a beneficial and easy office procedure.[59] It is best fitted to treat ice pick or small boxcar scars. A fine toothpick is used to apply 65-100% TCA to the bottom of scars, leading to collagenization and filling up the atrophic scars.[63, 64]

(2) Dermabrasion and Microdermabrasion

The technique mechanically ablates damaged skin to promote re-epithelialization using a high-speed diamond cylinder brush or manual silicone carbide sandpaper.[65-67] The re-epithelialization occurs by migrating stem cells from adnexal structures to the healing Surface.[14, 68] The superficial treatment eliminates the epidermis, and the deep treatment removes the epidermis and papillary or reticular dermis[30, 69]. The treatment modality produces a clinically significant improvement in skin texture and acne scars.[43] Microdermabrasion is less invasive than dermabrasion as it is a more superficial painless form of dermabrasion but has a lesser effect than dermabrasion and, therefore, cannot manage deep scars.[36] The drawback of dermabrasion is the scarcity of adnexal structure in the neck, chest, and back areas that are not ideally suited for treatment.[65-68]

(3) Cryosurgery and Electrodessication

Cryosurgery causes physical damage by locally enhancing the cell damage and thrombosis of the vessel in lesions using a liquid nitrogen spray. Skin atrophy or hypopigmentation are the possible side effects.[70] Cryosurgery is mainly used for treating keloids and hypertrophic scars.[118] The mechanism of electrodesiccation is thermal tissue damage and coagulation by using electrical probes.[53] Electrodesiccation helps shape the edge of boxcars scars and usually is adjunctive treatment because of the significant risk of developing new scars.[70]

(4) Combination Therapy

There are multiple approaches to combining acne scars treatment modalities, commonly chemical peels, with procedure management. Dot peeling TCA, followed by subcision, and, in the end, fractional laser resurfacing are commonly employed triple combination therapy that is a safe and efficient treatment of a variety of scars for a duration of 12 months.[10, 58-69] Twice every 2-3 months, dot peeling and subcision, were performed, and fractional laser resurfacing was conducted in the interval of 3-4 weeks.[61]

Currently, many studies concentrate on platelet-rich plasma (PRP), an autologous concentration of human platelets contained in a small volume of plasma as one of the combination procedures.[66,71] Ibrahim ZA and his colleagues found higher efficacy and safety with a combination of microdermabrasion and autologous PRP in treating acne scars rather than alone.[71] With fractional laser treatment, such as FrCO2, PRP is also a good combination for skin rejuvenation.[72] The benefit of the combination was evident in numerous aspects, including the rapidity and degree of improvement of the atrophic acne scars, lesser side effects, and shorter post-operative recovery groups.[10,36, 73]

(5) Tissue Augmentation

There are many available autologous, non-autologous biologic, and non-biologic tissue augmentation agents used to treat atrophic scars.[74] as classified in Table 6.

Table 6: Classification of Derma fillers[74]

Filler class	Average clinical efficacy	Examples
Temporary	3–18 months	Hyaluronic acid and collagen
Semi-permanent	Up to 24 months	Poly-l-lactic acid and calcium hydroxylapatite
Permanent	Many years if not lifelong	Silicon, polyacrylamide, polymethacrylate, and hydroxyethylmethacrylate

Dermal fillers efficiently treat rolling scars.[36, 74] There are two techniques- First, fillers are directly given beneath individual scars. Second, they are used for increasing volume into the laxity or deep tissue atrophy to accentuate the appearance of acne scars such as Poly-l-lactic acid (PLLA) or calcium hydroxyapatite.[74-76] It is hypothesized that PLLA stimulates endogenous fibroblast and subsequently causes collagen production.[126]

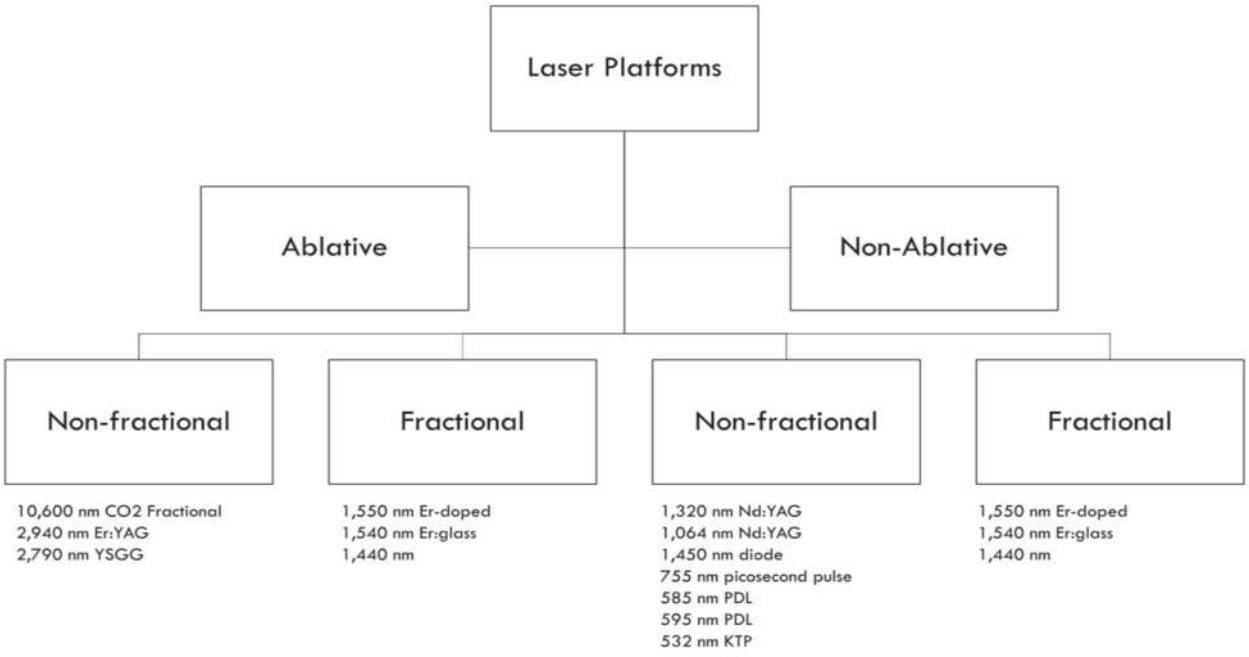
Various tissue augmentation agents used in the past are at high risk of developing side effects.[127] Hyaluronic acid is a glycosaminoglycan polysaccharide and a component of the extracellular matrix.[74-76] Many types of Hyaluronic acid fillers differ in their percentage of cross-linking, cross-linking technology, the ratio of hyaluronic acid and bound water, hardness, viscosity, modulus, swelling, particle size, gel to fluid ratio, and ease of injection.[77] The microinjection of low viscosity hyaluronic acid is a valuable

technique for treating superficial depressed scars.[78] Currently, hyaluronic acid is recommended as it can improve the longevity of treatment by stimulating fibroblasts to produce collagen and reduce the risk of immunogenicity and hypersensitivity.[77,78] Unavoidable side effects are mild-to-moderate pain during injection.[78]

(6) Laser Treatments for Acne Scars

The laser resurfacing, ablative, sub-ablative and non-ablative, have been proved to be helpful for patients with boxcar and rolling scars for homogenized overall skin texture.[24, 79]

Table 7: Overview of Lasers for Acne Scar[79]

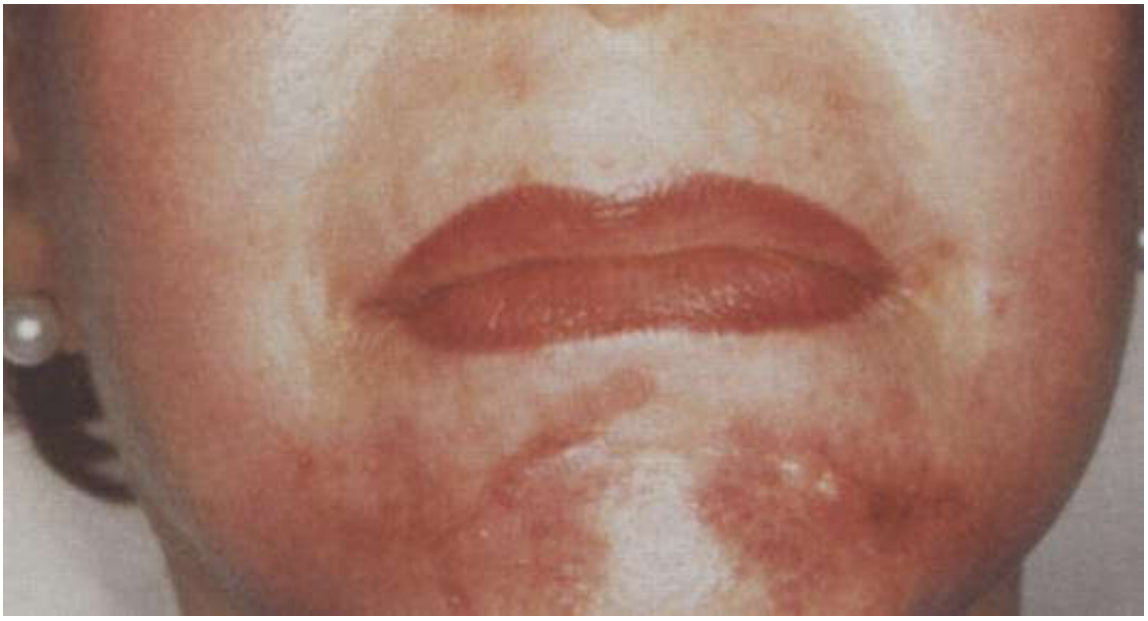


(1) Ablative Laser

The most commonly used ALR are CO_2 laser and Erbium: Yttrium-Aluminum-Garnet laser (Er:YAG). The CO_2 laser was first developed in 1964 by Patel and colleagues in the Bell Labs, USA.[80] It emits infared beam at the 10,600 nm wavelength which is strongly absorbed by water-containing tissue.[81-87] It cause tissue evaporation and may cause immediate contraction of the ablated areas by denaturing existing old collagen.[88-93] The continuous wave CO_2 laser is the gold standard in ablative lasers.[94-99] The indication for using CO_2 laser (absorption coefficient 800 cm^{-1}) for treatment are actinic and seborrheic keratosis, wart, skin tags, epidermal and dermal melanocytic nevi and xanthelasma.[100-112]

Other conditions that have been shown to respond to CO_2 laser resurfacing include dermatofibroma, rhinophyma, severe photodamage, sebaceous gland hyperplasia, syringoma, actinic cheilitis, angiofibroma, depressed scar, hypertrophic/keloid scar, neurofibroma, pyogenic granuloma, and pearly penile papules.[112-117] The minor complications that frequently occur are PIH, milia formation, perioral dermatitis.[118-123] More serious complications include skin infection (bacteria, virus, candida), hypopigmentation, and persistent erythema. The most severe complications are hypertrophic scarring (Figure 8), disseminated infection, and ectropion. [85,123-127]

Figure 8: Hypertrophic scars developed after underwent CO_2 laser resurfacing[85]



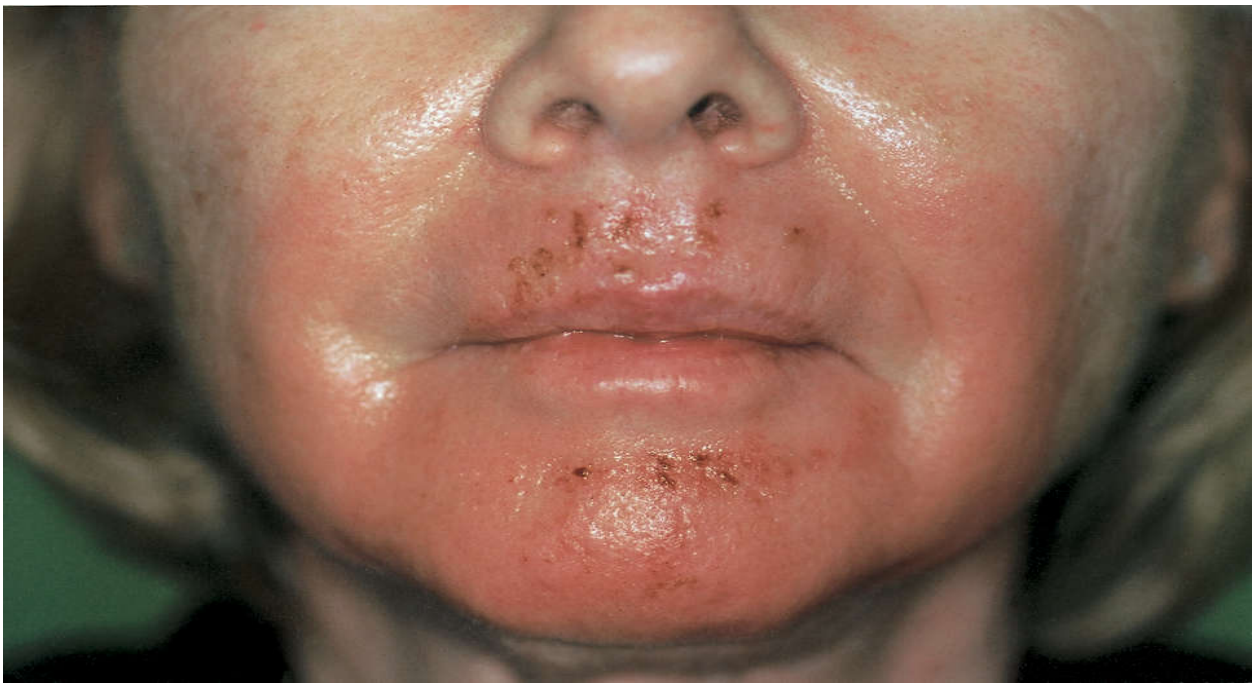
The short-pulse, high-peak power, rapidly continuous wave CO_2 and normal mode Er:YAG laser are developed after the traditional CO_2 laser, they are increase in the accuracy for ablate the tissue layers.[13]

The Er:YAG laser emits light at the 2,940 nm wavelength in the infrared range which is closer to the peak absorption range of water and produce greater absorption coefficient than CO_2 laser result in more precise ablation of skin and less side effects (12,800 cm^{-1},12-18 times more efficiently absorbed by water than CO_2 laser).[128,129] The pulse duration of Er:YAG is 250 μs which is much shorter than that of CO_2 laser, resulting in decrease thermal effect and less effect of hemostasis.[129] In spite of greater absorption coefficient, both CO_2 laser and Er:YAG laser has similar efficacy from the

comparative study, the reason may be the reduction of amount of collagen contraction due to the reduction of collagen reduction.[130] The variable pulse Er:YAG laser is developed to correct the disadvantages of conventional Er:YAG laser by add hemostasis ability and dermal collagen remodeling induction. No significant different in efficacy of old and new version of Er:YAG. [131] The Er:YAG can be used for the treatment of dyschromia, photoaging, scars, wrinkle, coarse skin texture and skin laxity.[128-131]

However, there is controversy that the CO_2 laser may have superior advantage in tightening skin tissue while producing more adverse events (oozing, bleeding, crusting, prolonged downtime post procedure (about one week or more) (Figure 9), acne, persistent erythema, post inflammatory hyper-, and hypopigmentation and infection.[107-129]

Figure 9: Patient underwent fulfill ablative resurfacing to treat perioral wrinkles with single pass of CO_2 laser show diffuse erythema and crusting.



(2) Non-ablative Laser

Due to the complications of ablative lasers, most of patients tend to receive the skin resurfacing treatments with non-ablative lasers which produce less side effects. The most common used are Neodymium-doped yttrium aluminum garnet Nd:YAG, 1540-nm erbium glass laser and diode lasers.[133] They remain intact tissue surface and stimulate new collagen formation. The 532-nm potassium titanyl phosphate (KTP) laser and the 585 to 595-nm pulsed dye lasers (PDL) also used for rejuvenation, however there are much better for the vascular lesions but little effects on collagen and elastin.[45-56] Q-switched Nd:YAG 1064-nm laser also has been shown to stimulate dermal remodeling for either original version and combined with a carbon particle solution version.[30-50] Although the non-ablative procedures produce minimize side effects, the improvement is not as impressive as in ablative laser resurfacing.[53-65] The ablative and non-ablative systems for acne scars treatment are summarized in Table 8.

Table 8: Ablative and non-ablative systems for acne scars treatment[10,13]

LASER TYPES	WAVELENGTH (nm)	FLUENCE (J/cm2)	PULSE DURATION	SPOT SIZE (mm)
ABLATIVE LASERS				
CO2 laser (Ultrapulse 100 W, individual pulse)	10,600	5-7	<950μs	3 Collimated beam
CO2 laser (Scanned >20 W, spiral or rasterized scan)	10,600	5-15	0.03–0.52-sec scan duration 300–1000 μs dwell time	0.6- to 15-mm scan size with 0.1- to 0.25-mm focused beam
Er:YAG (Individual pulses)	2,940	0.001- 0.05	300 μs to 10 ms	2- to 7-mm collimated or focus beam
NONABLATIVE LASERS				
KTP laser	532	15	20 ms	10
PDL	585/595	3/6-8	350 microns/6 ms	5/10
Nd:YAG	1064/1320	50/18	50/18 ms	12/6
Diode	1450	8-14	250	6
Er:glass	1540	Up to 126	3.3 ms	4

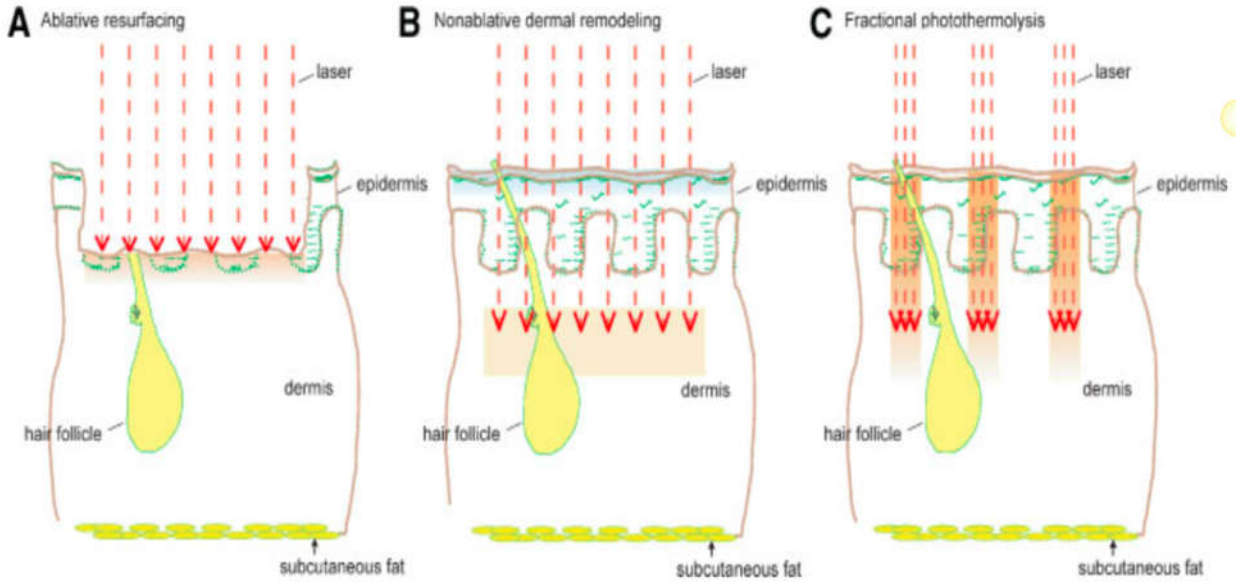
(3) Fractional Photothermolysis

'Fractional Photothermolysisis' is a term given by Rox Anderson, MD, and Reliant Technologies (Mountain View, Calif).[134] Ablative FP is a recently developed technology that is an effective modality and overcomes the disadvantages of conventional ablative laser.[135]

The FP technique creates the MTZ, characterized by microscopic wounds, as demonstrated in Figure 10, surrounded by uninjured tissue. MTZs resemble multiple minute dots on the skin, but they are revealed as columns or

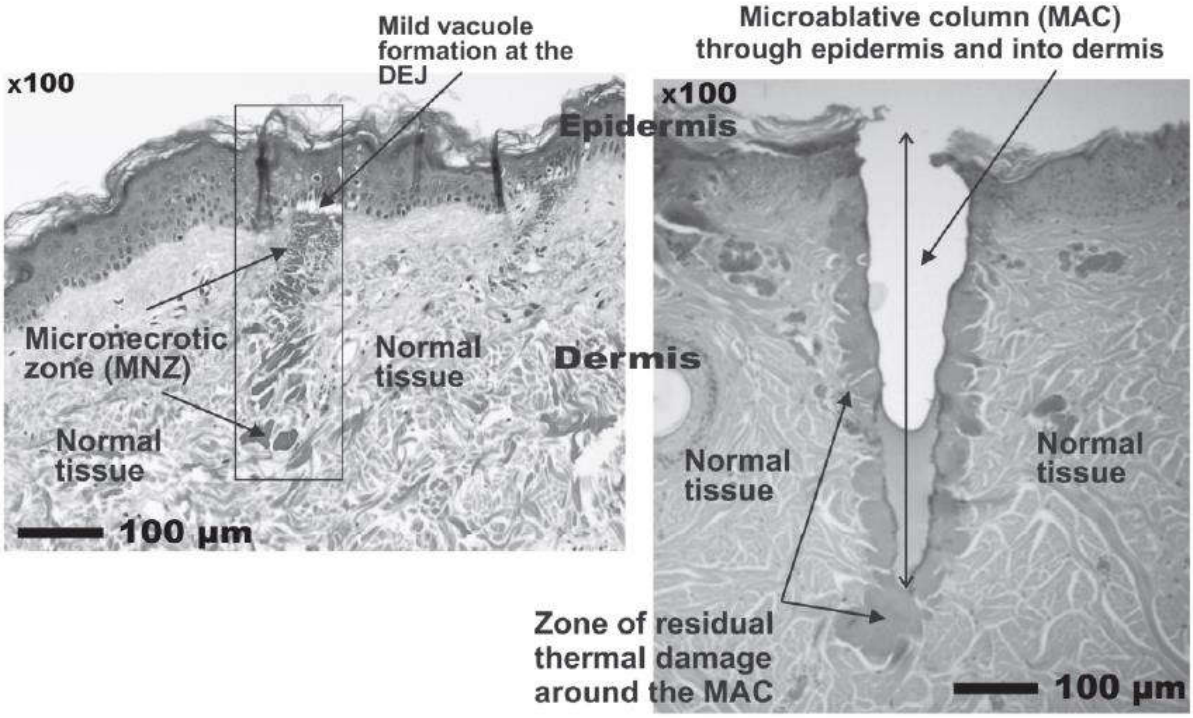
cylinders of the epidermal and dermal thermal damage on histological examination. After laser radiation, tissue commences healing rapidly. The benefits of this technique are a short recovery period and fewer side effects such as PIH, post-inflammatory hypopigmentation, scarring.

Figure 10: Comparison of ablative skin resurfacing, nonablative dermal remodeling, and FP[135]



The fractional lasers are classified as nonablative and ablative fractional resurfacing (AFR) lasers with different histological characteristics. The nonablative fractional lasers create a nonablative micronecrotic zone (MNZ) surrounded by unaltered, normal tissue, while the AFR forms micro ablative columns (MAC), affecting both epidermis and dermis. The re-epithelialization is usually achieved in 2-3 days.[80] (Figure 11).

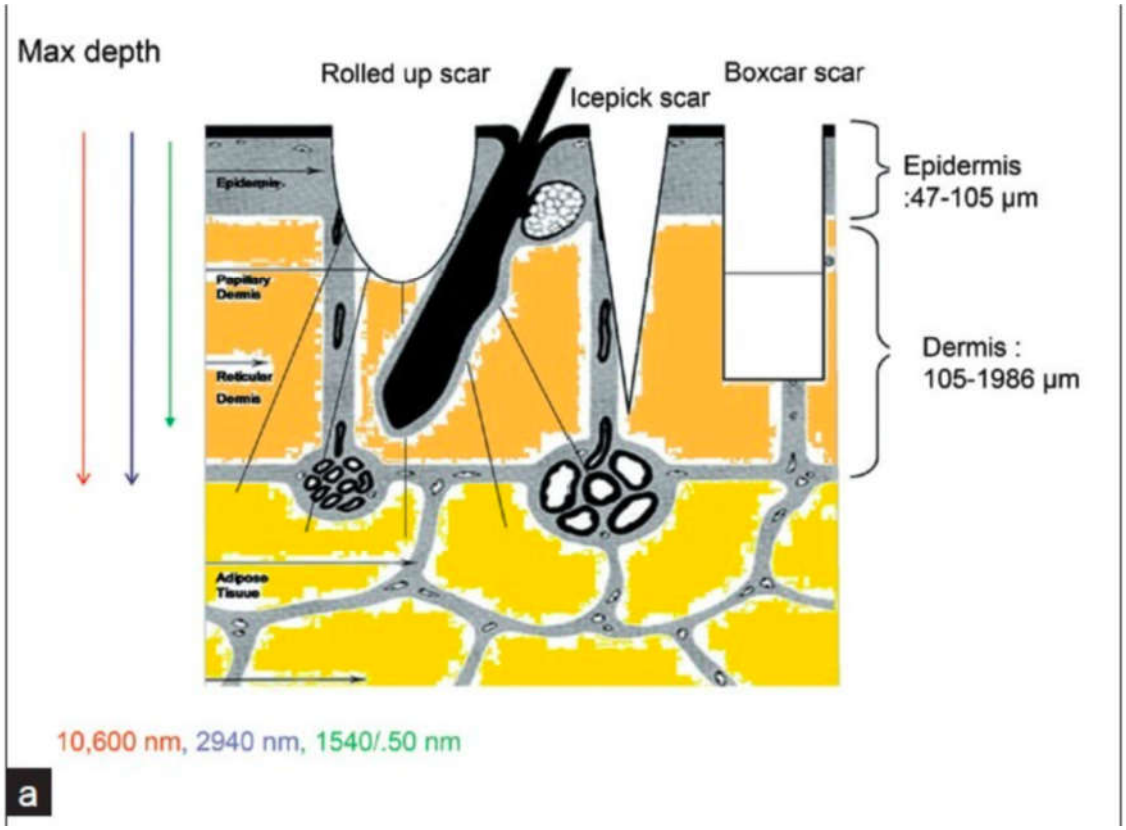
Figure 11: The difference of histological skin response after treatment with non- ablative fractional lasers (left picture) and AFR (right picture). The non-ablative fractional lasers produce non-ablative MNZ surrounded by unaffected normal tissue. The AFR produce MAC which involve both epidermis and dermis.[80]

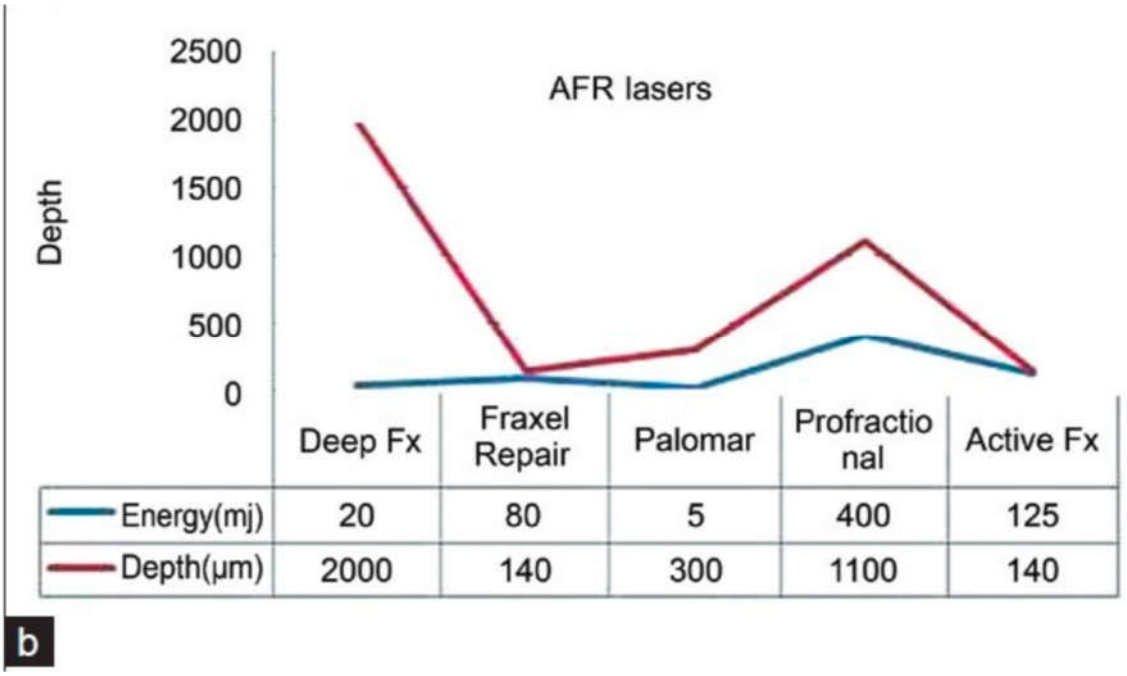


The indications of fractional technology are chronic photodamage, skin laxity, rhytides, multiple types of scarring: acne scars, hypopigmented scars, surgical scars, and facial melasma.[13] Atrophic acne scars are frequently treated with fractional erbium glass 1550/1540 nm laser as a nonablative fractional laser, and fractional 2940- nm Er: YAG laser along with fractional 10,600-nm CO2 laser as ablative fractional laser resurfacing (AFR).[135, 136] Most fractional lasers have depth- with a ratio of MTZ about 4-5. The more depth reached by fractional lasers, the more the laser's efficacy achieved for the deep scars.[135] (Figure 12) Before treating acne scars, it is essential to consider the depth of scars. Since maximum scars are mixed type (Figure 12a), the ultimate efficacy of fractional lasers depends on the most predominant type of scars and laser used. [137]

Using a higher density performs more remarkable improvement in texture, tone, and dyspigmentation.[137] Moreover, the increase of pulse energies and fractional deep dermal ablation (FDDA) treatment, the novel combined technology that adds deep ablation to thermal ablation, leads to increased scarring improvement.[130-137]

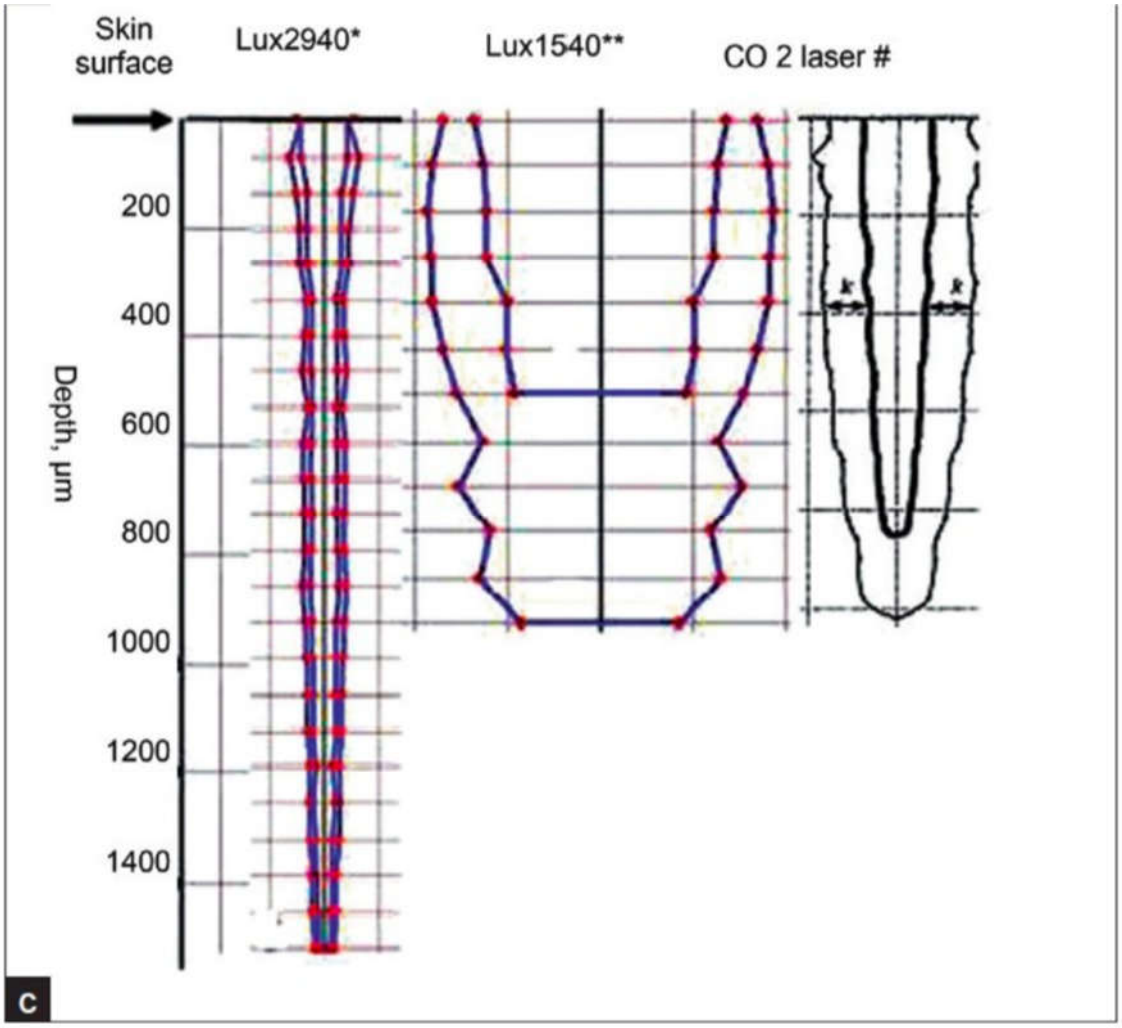
Figure 12: (a) A representation of the types of acne scars and the mean depth of penetration of fractional lasers on the facial skin (based on both *ex vivo* and *in vivo* data). (b) A comparison of the dose and depth achieved with AFR. DeepFX, ActiveFX and Fraxel repair are fractional CO_2 lasers while the Palomar and Profractional are fractional Er:YAG lasers. (c) A comparative MTZ lesion reconstruction plots of three fractional lasers. The plotted shapes have an outer and inner diameter corresponding to the zone of coagulation and ablation. *k* represents the zone of necrosis.[136]





	Deep Fx	Fraxel Repair	Palomar	Profractional	Active Fx
Energy(mj)	20	80	5	400	125
Depth(μm)	2000	140	300	1100	140

b



c

(4) Sub-ablative devices

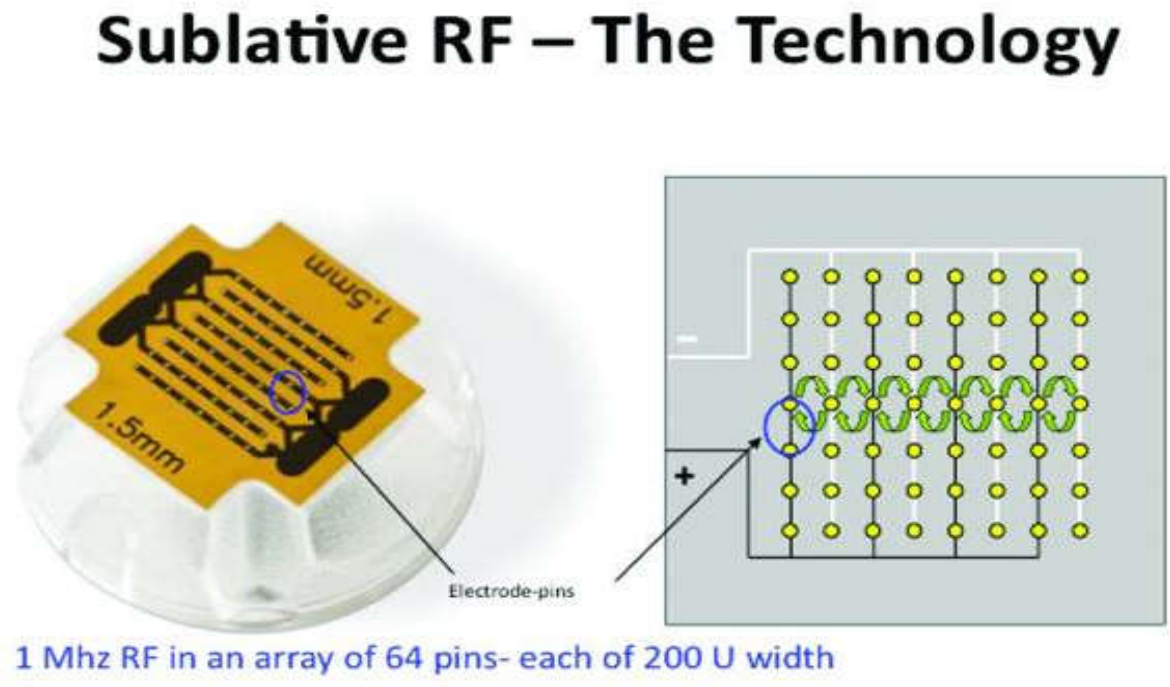
Sub-ablative resurfacing employs fractional bipolar radiofrequency to create small epidermal wounds much wider in the dermis than epidermis (pyramidal shape).[134] The radiofrequency devices generate electric current using electromagnetic radiation in the range of 3 kHz to 300 MHz and produce heat through tissue impedance.[138] The impedance in the epidermis is higher than the dermis due to reduced water content, so the flow of electrical current is predominantly found in the dermis. The thermal damage in the deep dermal collagen stimulates the denaturation and contraction of collagen fibers, which brings the skin tightening effect.[139] Due to most of the thermal damage zones occurring in the dermis, this fractional RF device produces fewer side effects than fractional ablative lasers.

Additionally, this pattern of injury offers the benefit of the nonablative therapy with a mild degree of epidermal damage; the reports of adverse effects are similar across all nonablative laser studies included discomfort, transient erythema, and mild edema. It can be used safely in dark skin types due to the absence of chromophore-specific targets.[140] However, the combination of Fractional RF and fractional laser treatment increased PIH incidence to 6.5% .[141]

Microneedle RF comprises multiple parallel rows of microneedles or electrodes arranged in a bipolar array. Fractional RF devices include a handpiece applicator with a disposable tip. The different tips define the types of fractional RF. The superficial fractional RF is non-invasive RF technology that creates a close circuit in each pair of electrodes running parallelly on the

tips. Figure 13, identical to the bipolar current generated from each pair of microneedles in a microneedle RF.[141] The broad applications of fractional RF in dermatology include skin laxity, rhytides, cellulite, acne vulgaris, and scarring.[133-142] For example, Vermeer and his team used sublative fractional bipolar RF for treating patients with moderate to severe acne scars in 3-5 treatment sessions. Three months post-treatment, 50% were very satisfied, 50% of patients were satisfied with the overall improvement.[142]

Figure 13 Superficial fractional RF tip.[140]



Characteristic of skin injuries after irradiation

The Micro-ablative column stretches from the epidermis and upper dermis, with sparing of unaffected normal tissues. Zone of ablation is wide in the base of the wound extending from epidermis to upper dermis that narrow in the opening and MTZ with columns of altered collagen (MNZ), entire stratum corneum stays intact. The commonly used for treatment of atrophic acne scars are fractional erbium glass 1550/1540 nm laser for non-ablative fractional laser, and fractional 2940- nm Er:YAG laser and fractional 10,600-nm CO_2 laser for ablative fractional laser resurfacing (AFR).[136] The indication for this fractional technology are the following, chronic photodamage, skin laxity, rhytides, multiple types of scarring: acne scars, hypopigmented scars, and surgical scars and facial melasma.[13]

Table 9: Comparison of AFR, Sub-ablative resurfacing and non-AFR after irradiation.[140]

	ABLATIVE FRACTIONAL LASER RESURFACING	SUB-ABLATIVE RESURFACING	NON-ABLATIVE FRACTIONAL LASER RESURFACING
Characteristic of skin injuries after irradiation	Micro-ablative column extending through epidermis and upper dermis, with sparing of unaffected normal tissues	Zone of ablation extending from epidermis to upper dermis that narrow in the opening and wide in the base of the wound	MTZ with columns of altered collagen (MNZ), entire stratum corneum remains intact.

Fractional CO2 laser and its application

(1) Safety of fractional CO2 laser

The safety and adverse event of fractional CO2 laser has been reported in many studies despite its high safety profile compared to the traditional ablative laser. Although some adverse events may be unavoidable, the patient will still experience erythema, edema, and PIH, but it can be resolved within one month after treatment.[80-94]

No machine before has been able to improve the skin appearance as evident as a CO2 laser. Thus, most dermatologists regard the full-fill ablative tradition CO2 laser as a gold standard for resurfacing.[143] Following Anderson and Parrish's discovery of the selective photothermolysis concept, the FrCO2 laser was developed to deliver results similar to fully ablative resurfacing while sustaining an acceptable safety profile.[136, 143]

(2) Mechanism of Action

Fractional carbon dioxide laser has combined the traditional carbon dioxide laser, which is 10,600 nm, with the fractional photo-thermolysis (FP) intervention technology. This way, the patient will experience lesser downtime such as transient erythema, crusting, and post-inflammatory pigmentation after each laser session, while still gaining the high efficacy of the traditional CO2 laser. Nevertheless, some patients will still face the inflammation associated with laser treatment but in a lesser portion.[92-104] The target chromophore of FrCO2 is principally tissue water, as same as CO2

laser, and the vital target structures are keratinocytes, collagen, and vessel. Skin ablation using a FrCO2 typically ends in a classic quadruple zone response. First, from the position of laser contact, there is a zone of ablation (deep to the upper dermis) adjacent to the zone of necrosis (thin eschar layer), then the zone of coagulation and hyperthermia (as demonstrated in Figure 14) [144].

A fluence of 5 J/cm2 must be delivered to avoid excessive thermal damage, with a pulse duration of less than 1 ms, which is the thermal relaxation time of the skin.[37-55]

Hantash and colleagues demonstrated the column of thermal coagulation immediately after FrCO2 laser treatment on ex vivo skin. Post-treatment, the ablative zone was replaced entirely with invaginating epidermal cells at 48 hours. The advantage of creating a microabrasion zone is a route for transepidermal drug delivery methods in a proper time (<48 hours).[145]

The collagen denaturation conventionally occurs at 66.8°C; after that, the collagen rapidly shrinks and contracts to one-third of its length. The tightening effect following FrCO2 laser treatment is from collagen contraction and shrinkage. The collagenase, which degrades the fragmented collagen, initiates the wound healing process that first appears as a fast reconstruction of the epidermis by migrating cells from adnexal structures.[146]

The period of dermal remodeling is up to 6 months following the treatment.[14]

The dimensions of the microscopic ablation zone are dependable on the energy level employed. Ablation depth was significantly increased in a linear pattern with increasing energy levels. One study conducted in 2011 compared the morphologic dimension of the MTZs when using low (50mJ), medium

(100mJ), and high energy settings (300mJ). Outcomes illustrated that the MTZs reached the superficial dermis, mid-dermis, and deep dermis with low, medium, and high energy settings, respectively (as shown in Table 10). The dermal remodeling lasts for up to 4 weeks, which should be the minimum period between treatment sessions, and higher energies (300mJ) may provoke granuloma formation.[85-100]

Figure 14 : Microscopic ablation zone (Abl. Depth = ablation depth, rae = radius of epidermal ablation, nz = necrosis zone, cz, = coagulation zone)[144]

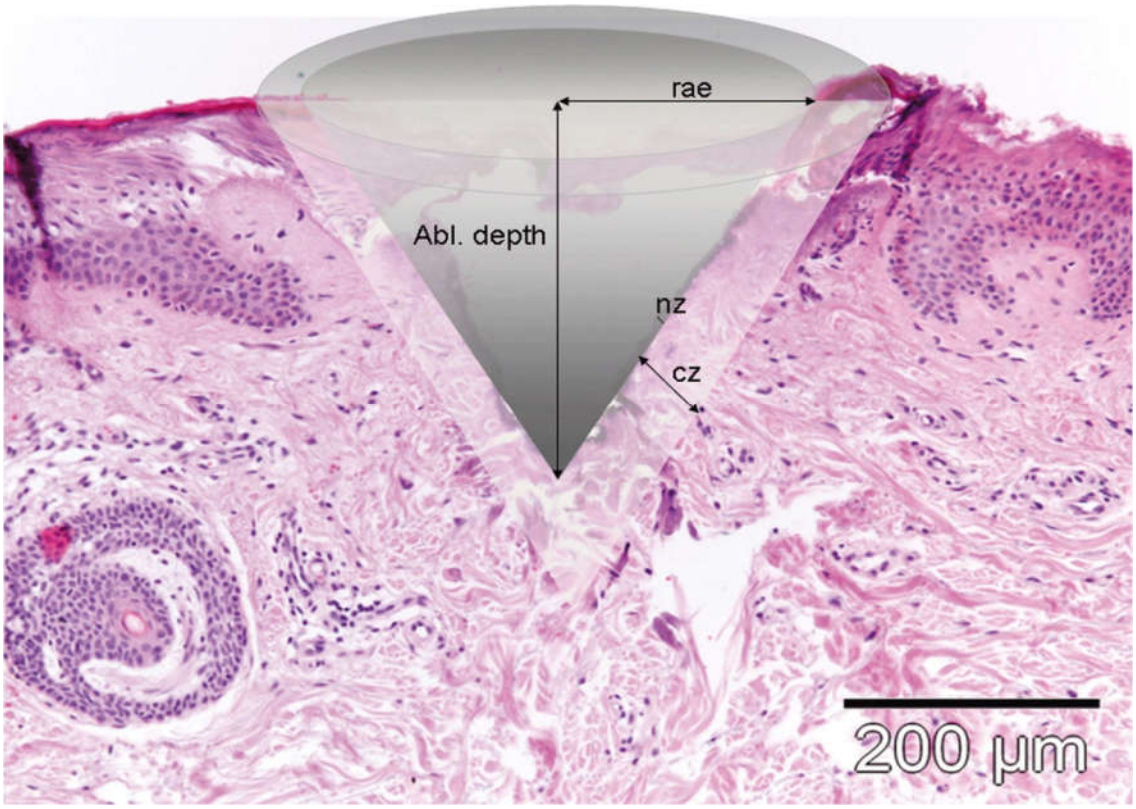


Table 10 Morphometric dimension of MTZs in response to three different treatment protocols.[144]

	ABLATION DEPTH (µM)	ABLATION WIDTH EPIDERMAL (µM)	ABLATION WIDTH DERMAL (µM)	COAGULATION ZONE (µM)	NECROSIS ZONE (µM)
(A) Low setting	100 ± 33.6	224 ± 36.5	4.9 ± 16.3	68.9 ± 15.7	1.6 ± 3.6
(B) Medium setting	167 ± 58.9	260.7 ± 60.2	110 ± 74.5	50.5 ± 14.7	6.7 ± 4.4
(C) High setting	451 ± 161.7	397.5 ± 111.5	166.6 ± 41.3	58.8 ± 13.0	8.3 ± 4.6
(A) *vs.* (B)	*p = 0.004*	*p = 0.003*	*p = 0.001*	*p = 0.032*	*p = 0.010*
(B) *vs.* (C)	*p = 0.001*	*p* = 0.121	*p* = 0.424	*p* = 0.076	*p* = 0.965
(A) *vs.* (C)	*p < 0.001*	*p = 0.001*	*p < 0.001*	*p* = 0.159	*p = 0.004*

(3) The Role of Fractional Carbon Dioxide Laser in Dermatology

The clinical applications of FrCO2 may divide into cosmetic and therapeutic applications.

(A) Cosmetic Applications

Photoaging and rhytides, periorbital resurfacing and blepharoplasty, laser-assisted drug delivery (triamcinolone, platelet-rich plasma, epidermal growth factor, tranexamic acid, and vitamin C), post-acne scars, keloid/hypertrophic scars, melasma, and striae utilize FrCO2 laser for cosmetic purposes.[30, 56, 147]

(B) Therapeutic Applications

The application of FrCO2 laser therapy in actinic keratosis, Bowen's disease, xanthelasma, seborrheic keratosis, warts, syringoma rhinophyma, and onychomycosis are employed for therapeutic purpose.[80-92,148]

(C) Fractional Carbon Dioxide Laser for Acne Scars Treatment

FrCO2 has excellent efficacy for treating acne scars in many studies. However, after undergoing FrCO2, most patients anticipate the downtime, including white frosting, which may last for 5 to 10 minutes immediately following laser irradiation, accompanied by moderate to marked erythema and edema that ordinarily persist for 24 hours.[24-33] Typically, superficial crusting occurs, and re-epithelialization entirely disappears in 5 to 7 days, depending

on the laser's density and energy. PIH can be observed after the crusts slough off, generally around 1 or 2 weeks after the procedure.[49-54]

Although FrCO2 provides excellent efficacy and fewer complications than non fractionated ablative laser treatment, yet adverse effects still occur (relatively uncommon).[37, 149] William M. Ramsdell has explained the complications of FrCO2 including, infection (Staphylococcus, Pseudomonas, Klebsiella, and Enterobacter), koebner phenomenon, contact dermatitis, scarring, ectropion, dyschromia, prolonged erythema, acne eruption, and milia. Still, the possibility to acquire these complications may depend on the proper patient selection, pre-and post-operative care, and proper operative techniques.[150] In 2010, Manuskiatti and his colleagues found that FrCO2 appeared to be effective and well-tolerated for treating atrophic acne scars in Asians, but mild PIH was found to be the side effect in 92% of the subjects.[149] A study evaluated the efficacy and safety of FrCO2 in the treatment of acne scars, inferred that FrCO2 promises to be an effective tool in the technologies for acne scar treatment, and the side effect found was post-treatment erythema.[83] Furthermore, Chan and his team have reported the prevalence and risk factors of PIH after practicing fractional resurfacing in Asians. The result showed that both the density and energy of the treatment determine the risk of PIH in dark-skinned patients.[151]

To conclude, much clinical success and safety have been published in utilizing a fractional laser to treat atrophic acne scar; a few side effects are still inevitable such as scarring, PIH, and persistent erythema.[83, 140-151] In addition, PIH is the most common adverse effect of post FrCO2 treatment, especially in dark-skin individuals, including most of the Asian population.[150]

POSTOPERATIVE CARE

There are four stages of dermal wound healing in Medium-depth and Deep resurfacing procedures: (1) Inflammation and Coagulation; (2) Re-epithelialization; (3) Fibroplasia and matrix formation; and (4) Collagen remodeling.[36, 55]

Pronounced edema following procedures may reduce the appearance of scars; this leads to a wrong judgment of an exaggerated improvement. Hence, counsel and advise the patient that continuous collagen remodeling will be for several months, facilitating clinical progress.[80-88] The healing period is directly proportional to the depth of the resurfacing procedure. Therefore, minimal downtime is sufficient for superficial resurfacing procedures as there is no dermal wound healing present.[100-115]

The severity of the erythema and desquamation is dependent on the techniques and wounding agent employed in the resurfacing procedure.[56-60] During healing, regular washing with a mild cleanser, moisturizers, and sunscreens should suffice. Following resurfacing procedures, there is a longer

healing time, and more intensive postoperative care is essential.[82-85]

Occlusive dressings may be used during the first three postoperative days, which functions as a biologic dressing until peeling occurs. Then, the patient is directed to soak the areas four times daily with warm compresses and to apply an emollient after each soak and during the intervening periods as necessary. It is preferred to use 0.25% acetic acid solution (one tablespoon of white vinegar added to one pint of warm water) for the soaks because the mild acidity is physiologic for healing granulation tissue. It is also has a mild debriding and has antibacterial effects, especially against Pseudomonas spp. and other Gram-negative organisms. Occlusive emollients, such as petrolatum speed the process of re-epithelialization and reduce the tendency for delayed healing.[89-98] The emollients are additionally helpful in wound debridement and the prevention of crust formation and infection. Follow-up should be scheduled regularly to monitor the patient's postoperative course. At every visit, instructions for wound care are reviewed with the patient, and any questions are answered.[120-132]

Following the procedure, edema begins to appear almost immediately and progressively worsens during the first 48 hours. Aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and oral steroids can be administered preoperatively and during the first 24 hours postoperatively to alleviate discomfort and reduce swelling.[55-57]

Gently debride the crusts with saline soaks and moistened cotton tips. The patient should be instructed to regularly apply an occlusive ointment to areas around the mouth, eyelids, and hairline that are not adequately covered by the

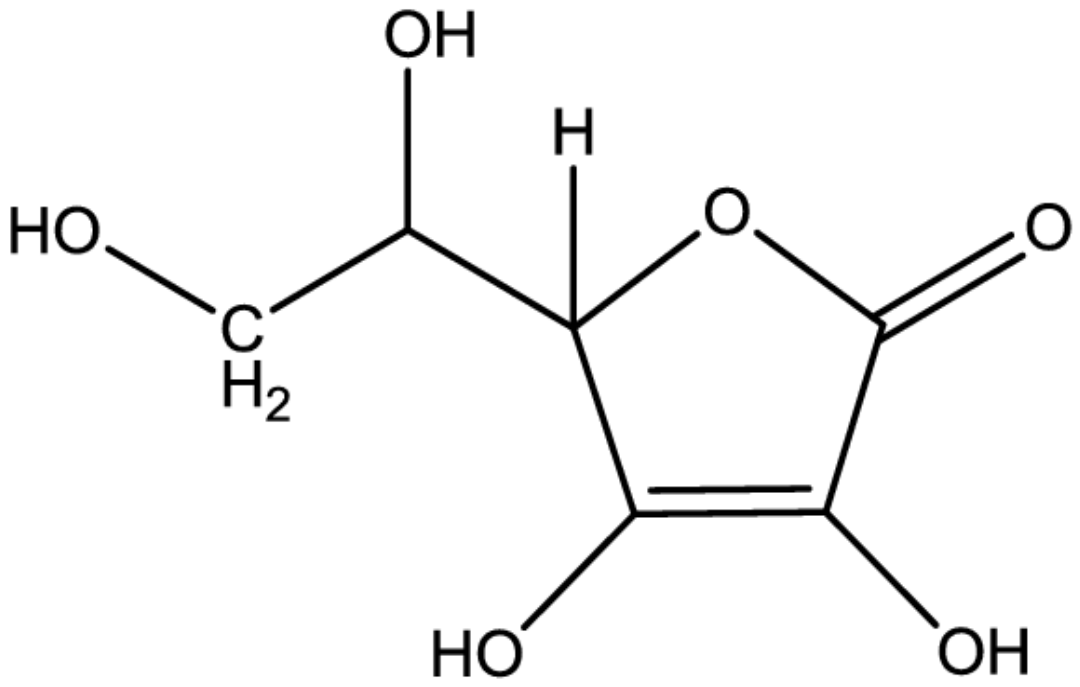
dressing.[70-79] Occlusive biosynthetic dressings have been shown to enhance collagen synthesis and hasten re-epithelialization in superficial wounds. They also minimize the amount of discomfort and prevent repeated soaks by the patient during the first few days after the procedure. Strict sun avoidance is critical for months.[20-32]

VITAMIN C

1. Structure

Vitamins are organic substances that maintain metabolic functioning in the body. Ascorbic acid (vitamin C, Figure 15) is the most plentiful and most typically water-soluble nonenzymic antioxidant in human tissue.[152] The chemical structure of ascorbic acid determines its physical and chemical properties. It is a weak, water- soluble, unstable organic acid which can be easily oxidized or destroyed by light, aerobic conditions (oxygen), high temperatures, alkali, copper, and heavy metals.[153] Vitamin C has several functions on the skin for example collagen synthesis, depigmentation and antioxidant activity.[154] Topical Vitamin C Serum may aid in the wound-healing process and minimize the posttreatment downtime.[155]

Figure 15. Chemical structure of ascorbic acid.



2. Metabolism

An insufficient amount of ascorbic acid is absorbed orally despite the high dose because an active transport mechanism limits the absorption of ascorbic acid in the intestine. Therefore, the bioavailability of ascorbic acid in the skin is insufficient following oral administration. Consequently, we require topical routes, exhibiting that the usage of local application aids in healing and better tissue reconstruction.[27, 29]

Table 11: Vitamin C content of skin in comparison to other tissues[155]

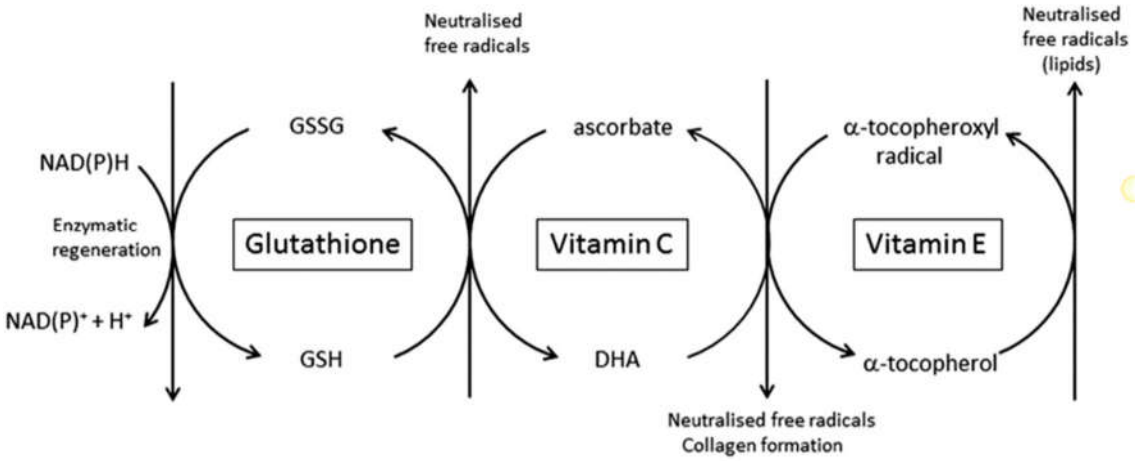
Tissue	Vitamin C Content (mg/100 g Wet Weight)
Adrenal glands	30–40
Pituitary glands	40–50
Liver	10–16
Spleen	10–15
Lungs	7
Kidneys	5–15
Heart muscle	5–15
Skeletal muscle	3–4
Brain	13–15
Skin-epidermis	6–64
Skin-dermis	3–13

3. USES OF VITAMIN C

a. Antioxidant Properties

In biological systems, Vitamin C reduces both oxygens- and nitrogen-based free radicals and, therefore, acts as an antioxidant. It protects the skin by neutralizing reactive oxygen species (ROS) generated. A set of complex biochemical events occur in an orchestrated cascade to repair the damage if there is a skin injury.[156, 157] Ascorbic acid protects the skin by sequential donation of electrons by neutralizing free radicals since the oxidized forms of ascorbic acid are relatively unreactive. Therefore, the availability of ascorbic acid in the skin is reduced on repeated exposure to UV light. Ascorbic acid is often combined with another redox partner, such as vitamin E, in skincare formulations to slow oxidative degradation.[155, 157]

Figure 16: Antioxidant Role of Vitamin C[155]



b. Photoprotection

Topical ascorbic acid can exert photoprotection against UVR due to its antioxidant and anti-inflammatory properties. A by-product of filaggrin: Trans-urocanic acid in the skin acts as a chromophore for photons of solar radiation leading to singlet oxygen formation, triggering a cascade of events that forms 'ROS'.[157] They can cause damage to nucleic acids, proteins, and cell membranes as they are highly toxic and unstable molecules. Furthermore, ROS triggers the signal transduction cascade inducing upregulation of factors, such as activation protein-1 (AP-1) and nuclear factor-b, and downregulation of transforming growth factor-β (TGF-β). As a result, Matrix Metalloproteinases (MMPs) are upregulated, which degrades collagen, reduces collagen production and, increases elastin accumulation.[158,159]

Clinical manifestations of this process are photoaging pigmentation, telangiectasias, coarse texture, deep wrinkles, and solar elastosis. Sunscreens are only partially effective in blocking free radicals produced on UV exposure. Vitamin C inhibits the activation of AP-1, which reduces MMP production and collagen damage.[158]

Cd1a-expressing Langerhans cells are reduced due to acute and chronic UV exposure. These are antigen-presenting cells in the epidermis, which are responsible for a protective immune response.[155-160] Vitamin C- containing topical solutions prevent the reduction of Cd1a-expressing Langerhans cells upon UV radiation. Therefore ward protection against UV-induced immunosuppression.[155]

UV- induced reactive oxygen species causes mutations on the p53 gene, affecting the repair of damaged deoxyribonucleic acid (DNA) and leading to programmed cell death (apoptosis).[156] UV-induced erythema and thymine dimer mutations add to photo carcinogenesis. 10% topical vitamin C reduces UVB-induced erythema by 52% and apoptotic sunburn cell formation by 40 - 60%. 19. Vitamin C solutions reduce UV-induced thymine dimers, thereby possibly diminishing the risk of photocarcinogenesis.[27,155,160]

c. Anti-aging

Currently, medicine recognizes signs of aging by degenerative changes in the skin—for example, wrinkles, age spots, and skin flaccidity that

occur mainly due to oxyradical damage. 10% topical vitamin C reduced photoaged scores and improved wrinkling in a study.[153-157] Vitamin C regulates gene expression antioxidant enzymes, including those involved in DNA repair.[15-159] Ascorbic acid and its derivatives, such as 3-O-ethyl ascorbate or tetra-isopalmitoyl ascorbate, are used as anti-aging cosmetic products.[160]

d. Anti-pigmentary

Ascorbic acid also plays a role as anti-pigmentary agent. It interacts with copper ions at the active site of the tyrosinase enzyme thereby inhibiting the action of the enzyme. Tyrosinase is the main enzyme responsible for converting tyrosine into melanin, thereby decreasing melanin formation.[28] Tyrosinase catalyses the hydroxylation of tyrosine to dihydroxyphenylalanine (DOPA) and the oxidation of DOPA to its corresponding ortho-quinone. [29]Vitamin C prevents skin hyperpigmentation due to its ability to interfere with the action of tyrosinase, the rate-limiting enzyme in melanogenesis. The vitamin's ability to decrease the ortho-quinones generated by tyrosinase is plausibly responsible for melanin synthesis inhibition.[30] A clinical study examining the effect of a topical formulation containing 25% ascorbic acid and a chemical penetration enhancer reported a significant decrease in pigmentation caused by melasma after 16 weeks. [31]

e. Wound Healing

Fibroblasts require Ascorbic acid to synthesize stabilized collagen, which is fundamental in wound healing—ascorbic acid functions as a cofactor for various enzymes, such as lysyl hydroxylase and prolyl hydroxylase.[154] In the case of Vitamin C deficiency, unstable collagen yields a weak framework for repair, making wound healing difficult.[155] Ascorbic acid levels are usually low in older patients, contributing to slower and more difficult wound healing. Ascorbic acid prevents reduced proliferative capacity of in fibroblasts in older people.[154-161]In addition, vitamin C increases the proliferation and migration of dermal fibroblasts which is vital for efficient wound healing.[162] By stimulating regulatory hydroxylases, vitamin C also regulates the stabilization and activation of the hypoxia-inducible factor (HIF)-1, a metabolic sensor that guides the expression of hundreds of genes linked with cell survival and tissue remodelling, including collagenases. Finally, vitamin C synthesizes Glycosaminoglycan as part of extracellular matrix formation.[154-161]

f. Topical Formulations

L-ascorbic acid is a hydrophilic, unstable, and charged molecule; its penetration into the skin is inadequate due to the hydrophobic nature of the horny layer. Therefore, for optimal penetration of the epidermal barrier, aqueous formulations of ascorbic acid need to be at a pH that is below the pKa (4.2) of ascorbic acid itself.[162]

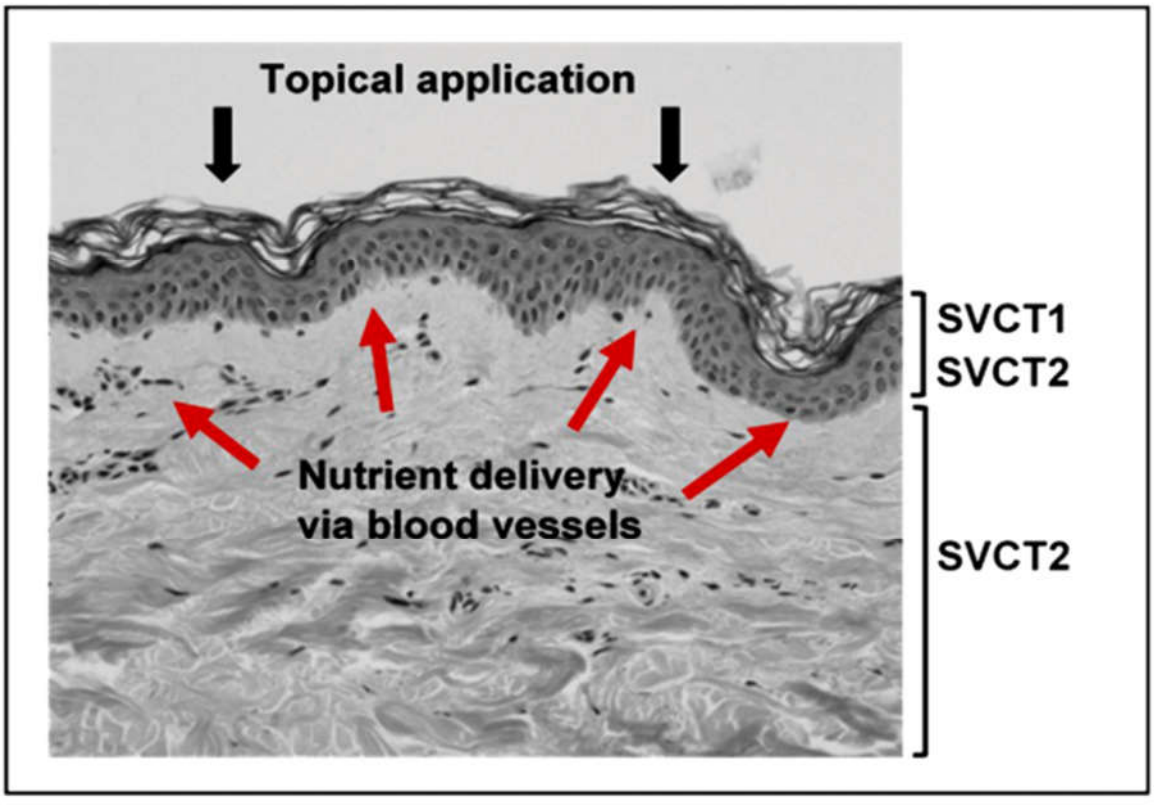
L-ascorbic acid is also combined with ferulic acid, thus stabilizing the molecule to reduce a pH lower than 3.5. The rate of ascorbic acid degradation is due to High pH or temperature, dissolved oxygen, and catalytic amounts of metal ions.[27] Thus, strategies such as encapsulation, low pH, oxygen-impermeable packaging, and the inclusion of electrolytes and other antioxidants are employed.[155-160]The more stable and easier derivatives to formulate are Ascorbyl 6-palmitate, tetra-isopalmitoyl ascorbate, magnesium ascorbyl phosphate, sodium ascorbyl phosphate, ascorbyl 2-glucoside, ascorbyl 2-phosphate-6-palmitate, and 3-O-ethyl ascorbate.[153-161] Ascorbyl 2-glucoside is one of the essential L-ascorbic acid derivatives because of its resistance to reduction and oxidation. It is also easily degraded by a-glucosidase to release L-ascorbic acid and glucose.[155] Patients who apply topical ascorbyl 6-palmitate to burns caused by UV radiation obtained a reduced redness by 50%, proposing that the derivative acts as an antioxidant and anti-inflammatory agent.[160-162] Ascorbyl phosphate is a free radical scavenger, a photoprotective agent that increases collagen production levels. Ascorbyl 2-phosphate 6-palmitate can penetrate the skin and be converted to ascorbic acid after delivery, showing skin absorption limited by topical application.[159-162]

g. Safety

Ascorbic acid is safe at high usage levels for long periods due to its solubility in water. No adverse effects have been observed in any well-controlled studies reviewed that indicated daily intakes of more than

100 times the U.S. recommended daily amount of vitamin. Any excess consumption tends to excrete from the body in the urine.[155] This is the reason why concentrations of ascorbic acid and other water-soluble vitamins can rarely accumulate to toxic levels.[156-159]

Figure 17: Delivery of Nutrients to the Skin. SVCT1 and SVCT2 are the transporter proteins of Vitamin C. Red arrow indicate nutritional flow from blood vessels in the dermis to the epidermal layer. Nutrients delivered by topical application have to penetrate stratum corneum.[155]



MATERIALS AND METHODS

MATERIALS AND METHODS

(1) Source of data:

This study was conducted in outpatient clinic of Dermatology, Venereology and Leprosy in R L Jalappa Hospital and Research Centre attached to Sri Devaraj Urs Medical College, Tamaka, Kolar from January 2020 to July 2021.

(2) Study Design:

Prospective Study

(3) Sample size calculation:

Sample Size was estimated based on excellent and good results seen in acne scars patients by treatment with Fractional CO2 laser as monotherapy[12]. The observed proportions as 68.3% expecting an improvement of 25% in excellent and good results with Fractional CO2 Laser in combination with Topical Vitamin C Serum with 95% confidence and α error of 5%, estimated sample size per group was 38.

Formula

$H_o : P_1 = P_2$; $H_a : P_1 \neq P_2$

$$n = \frac{\left\{ Z_{1-\frac{\alpha}{2}} \sqrt{2\,\bar{P}\,(1-\bar{P})} + Z_{1-\beta} \sqrt{P_1\,(1-P_1) + P_2(1-P_2)} \right\}^2}{(P_1 - P_2)^2}$$

Where,

$$\bar{P} = \frac{P_1 + P_2}{2}$$

P_1 : Proportion in the first group

P_2 : Proportion in the second group

α : Significance level

$1-\beta$: Power

Proportion in group I = 0.683

Proportion in group II = 0.933

Risk difference = -0.25

Power(%) = 80

Alpha Error(%) = 5

Side = 2

Required sample size for each arm = **38**

(4) Statistical Analysis:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. **Chi-square test or Fischer's exact test** (for 2x2 tables only) was used as test of significance for qualitative data. Continuous data was represented as mean and standard deviation. **Independent t test** was used as test of significance to identify the mean difference between two quantitative variables.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs.**P value** (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel**,** SPSS version 22 **(**IBM SPSS Statistics, Somers NY, USA) was used to analyze data

(5) Method of collection of data (including sampling procedure)

(1) Inclusion criteria:

1) Age group between 18 and 40 years
2) Moderate to Severe acne scars (as per Goodman and Baron's acne scar grading scale)

(2) Exclusion criteria:

1) Patients with a predisposition to keloid, active infection, herpes, HIV, HBV infection.
2) Patients on oral isotretinoin use in preceding 6 months.
3) Patients with diabetes mellitus, collagen vascular disease.
4) Patients with h/o ablative or nonablative laser skin resurfacing within the preceding 12 months.
5) Pregnant or lactating women
6) Patients with bleeding diathesis
7) Patients with unreasonably high expectations were excluded from this study. But we will provide them with all treatment options and their success rates and use multiple modalities if necessary.

(3) **Sample size:**

Sample size per group is 38.

38 participants will be treated with Fractional CO2 Laser Monotherapy and other 38 participants will be treated with Fractional CO2 Laser in combination with Topical Vitamin C Serum.

Methodology:

All patients satisfying the inclusion criteria will be divided into two groups as follows

GROUP 1- participants will be treated with Fractional CO2 Laser

GROUP 2- participants will be treated with Fractional CO2 Laser in combination with Topical Vitamin C Serum within 2 minutes immediately after laser and daily for 4 months.

All participants were treated with Fractional CO2 laser at an interval of 4 weeks for 3 sessions. A topical anaesthetic, containing a mixture of lidocaine-2.5% w/w + prilocaine-2.5% w/w in a cream base will be applied for 1 hour on the treatment area. After satisfactory anaesthesia was achieved, the treatment area will be cleaned with a mild cleanser followed by 70% ethanol solution. Eyes were protected with eye shields. Fractional CO2 laser treatment was then delivered to each atrophic scar present. Ice pack was applied immediately.

Participants underwent serial photography of the lesions at baseline, 1 month, 2 months, 3 months and follow up after 4 months from the first treatment session with same assessment scaling and questionnaire. Lighting and positioning was kept identical for all serial photographs. Each participant was instructed to evaluate his/her overall satisfaction with the treatment at end of 1st and 4th month of the treatment using a quartile grading system which defined 0 as unsatisfied, 1 as slightly satisfied, 2 as satisfied, or 3 as very satisfied. The visual analog scale (scoring from 0 to 10) was used to record adverse events (erythema and edema) as perceived by participants on days 0, 2, 4, 6, 8, 15 and 30 after each treatment session.

Finally, the occurrence of other possible side adverse events including secondary infection, acneiform eruption, dyschromia and new scar formation was assessed. The final assessment was made subjectively by a single observer at the last follow-up visit, and a quartile grading scale was used to assess the response objectively. A score of 0, 1, 2 and 3 will be given if the response was <25%, 25-50%, 51-75% and >75%, respectively. Sunscreen with spf 50 was prescribed for daily use in the morning and afternoon. Counselling was done for all patients.

RESULTS

RESULTS

The study included 76 participants, Group1 had 38 participants treated with Fractional CO2 Laser, and Group 2 had 38 participants treated with Fractional CO2 Laser in combination with Topical Vitamin C Serum within 2 minutes immediately after laser daily for four months.

In both groups, a maximum number of patients were in age groups of between 21-25 years, followed by 26-30 years. Table 12 depicts the distribution of patients according to the age group between Group 1 and Group 2. No statistically significant difference (p=0.964) was found between the groups with respect to age. (Graph 1).

Females in Group 1 were 21/38 (55.3%), and in Group 2 were 20/38 52.6%) whereas there were 17/38 (44.7%) of males in Group 1 and 18/38 (47.4%) of males in Group 1. There was no statistically significant difference (p=0.818) found between groups with respect to sex. (Table 13) (Graph 2) (Graph 3).

27/38 (71.1%) and 23/38 (60.5%) of patients were unmarried in Group 1 and Group 2, respectively. There was no statistically significant difference (p=0.818) found between groups with respect to marital status. (Table 14) (Graph 4). There was also no statistically significant difference (p=0.560) found between groups with respect to occupation. (Table 15) (Graph 5).

Table 16 and Graph 6 Illustrate that >65% of patients were Fitzpatrick skin type IV in Group 1 as well as in Group 2 while <8% of patients were Fitzpatrick skin type II in both the groups and rest had Fitzpatrick skin type III. However, There was also no statistically significant difference (p=0.744) found between groups with respect to Fitzpatrick skin types.

With a P-value of 0.788, there was no statistically significant difference found between groups with respect to grading of the scar. Although 28/38 (73.7%) and 30/38 (78.9%) of patients had Grade 4 scars in Group 1 and Group 2, respectively, and 10/38 (26.3%) and 8/38 (21.1%) of patients had Grade 3 scars in Group 1 and Group 2, respectively. (Table 17) (Graph 7).

A statistically significant difference ($p<0.001$) was found between the groups with respect to improvement in the appearance of scars after six months. 16/38 (42.1%) and 20/38 (52.6%) of Group 2 patients had >75% and 50-75% of improvement compared to Group 1 where 8/38 (21.1%) and 16/38 (31.6%) of patients had >75%, and 50-75% of improvement was seen. (Table 18) (Graph 8).

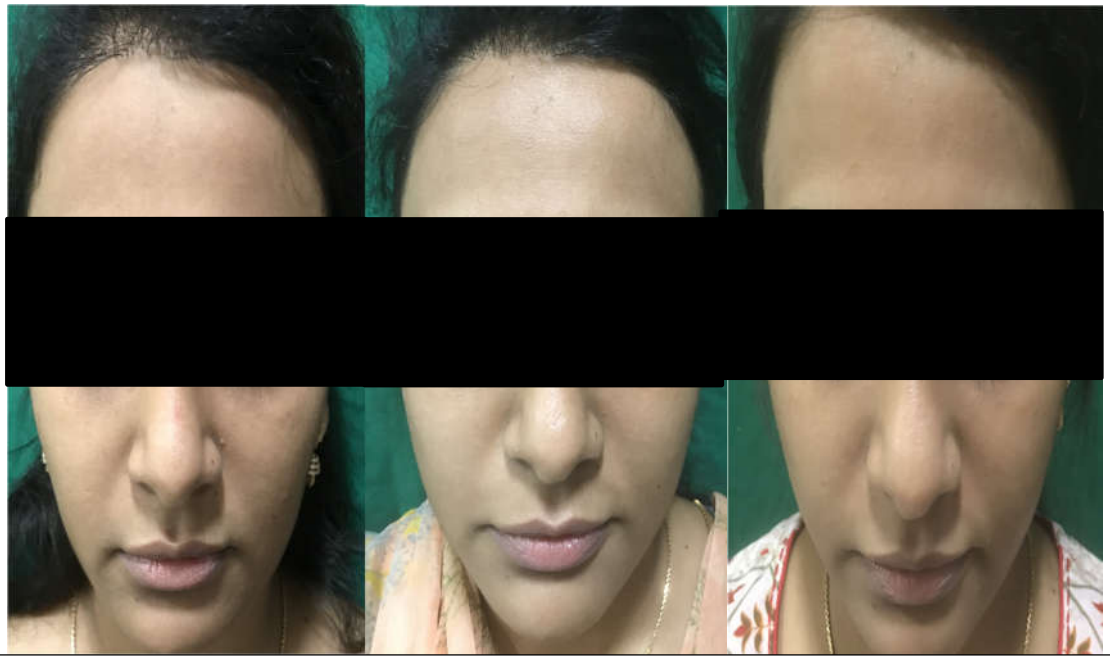
As far as patient satisfaction is concerned, >80% of patients were either satisfied or very satisfied in both groups. Therefore, there was no statistically significant difference ($p=0.192$) found between the groups with respect to patient satisfaction. (Table 19) (Graph 9).

Table 20 and Graph 10 show no statistically significant difference between the two groups with respect to edema on Day 1 at the first session, second session, and third session following CO2 Laser therapy. However, there was a statistically significant difference between the two groups regarding edema on Day 7 at the first session, second session, and the third session of CO2 Laser therapy.

Table 21 and Graph 11 represent no statistically significant difference found between the two groups with respect to erythema on Day 1 at the first session. Nevertheless, following the second session and third session of CO2 Laser therapy, we found a statistically significant difference ($p<0.001$) between the two groups regarding erythema on Day 1. Furthermore, during treatment, we observed a statistically significant difference ($p<0.001$) between the two groups with respect to erythema on Day 7 at the first session, second session, and third session after CO2 Laser treatment.

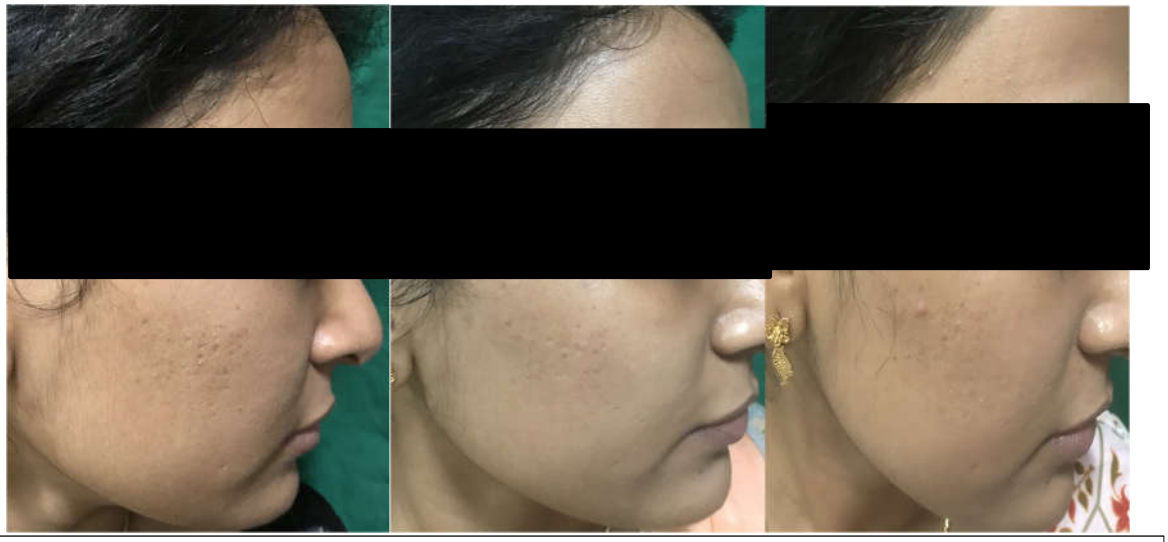
Finally, 7/38 (18.4%) of patients in Group 1 developed Post Inflammatory Hyperpigmentation (PIH), but it was resolved within three months after receiving treatment for PIH. 2 patients from Group 1 and 1 patient from Group 2 developed Acneiform eruption, which also resolved on treatment. With a P-value of 0.016, there was a statistically significant difference found between groups with respect to side effects, as depicted in Table 22 and Graph 12.

FIGURE 18 : GROUP 1- Case 1 Front Face Profile



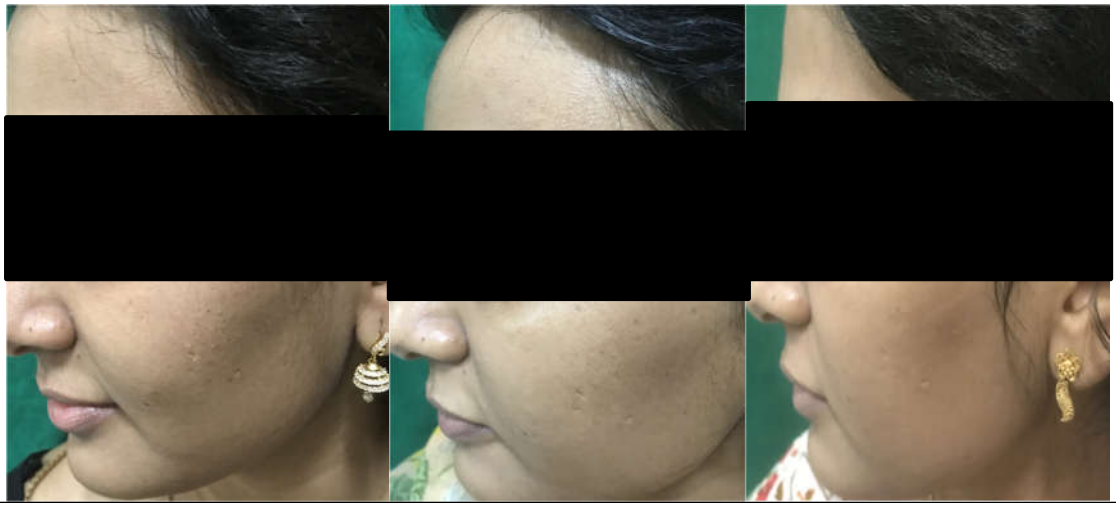
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 19 : GROUP 1- Case 1 Right Face Profile



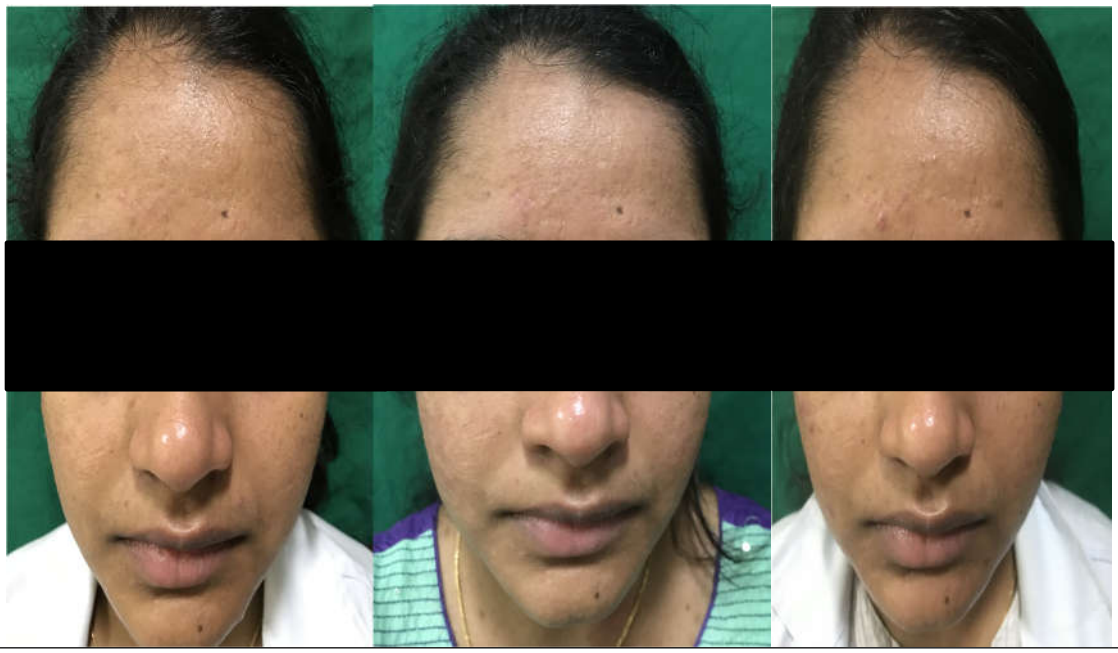
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 20 : GROUP 1- Case 1 Left Face Profile



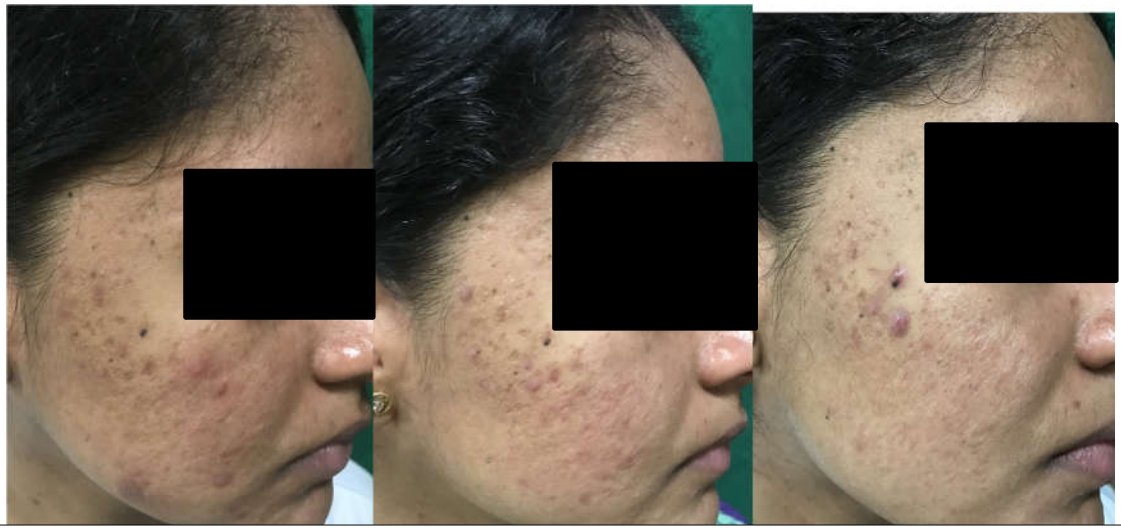
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 21 : GROUP 1- Case 2 Front Face Profile



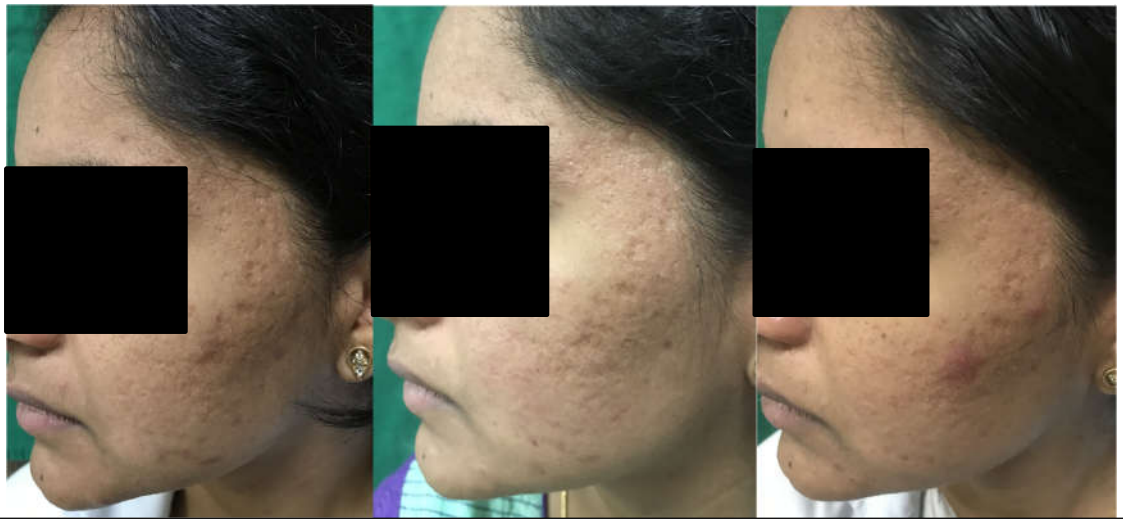
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 23 : GROUP 1- Case 2 Right Face Profile



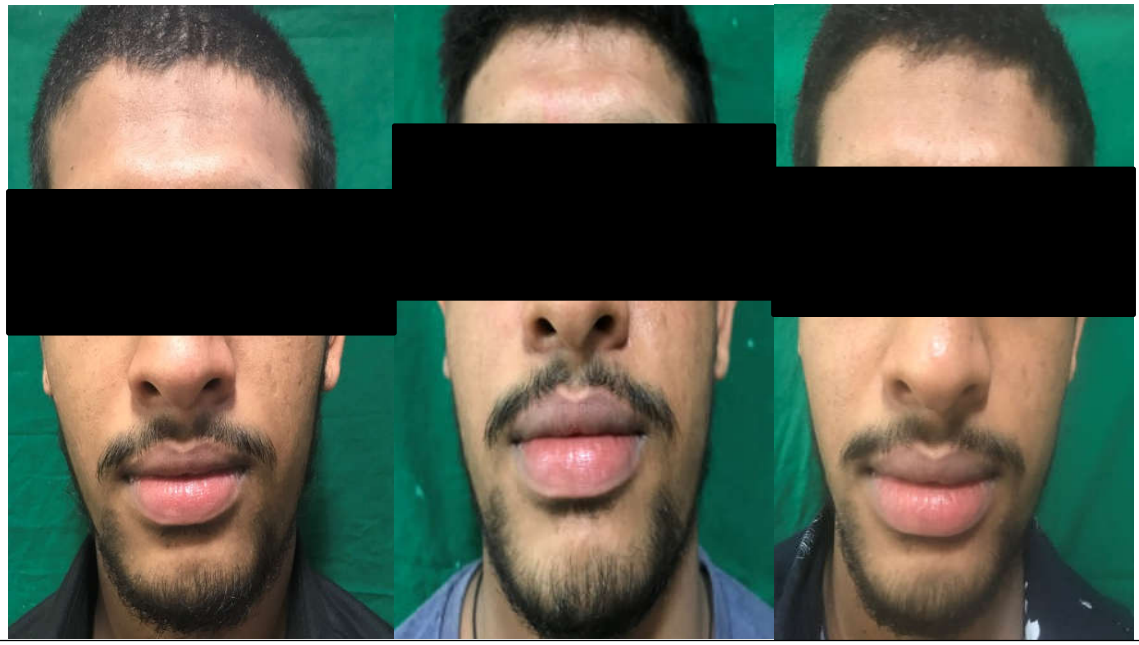
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 22 : GROUP 1- Case 2 Left Face Profile



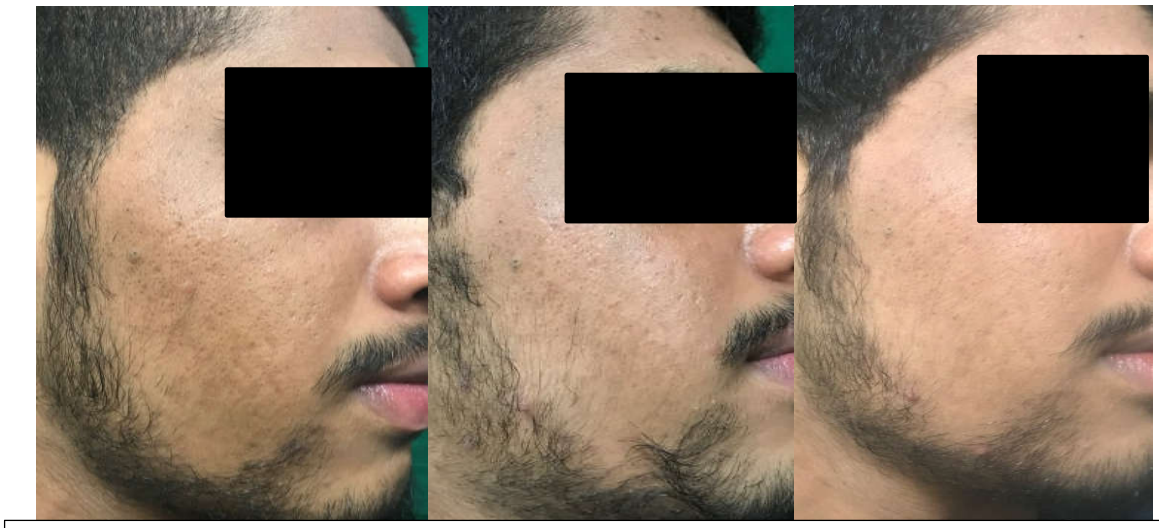
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 23: GROUP 2- Case 1 Front Face Profile



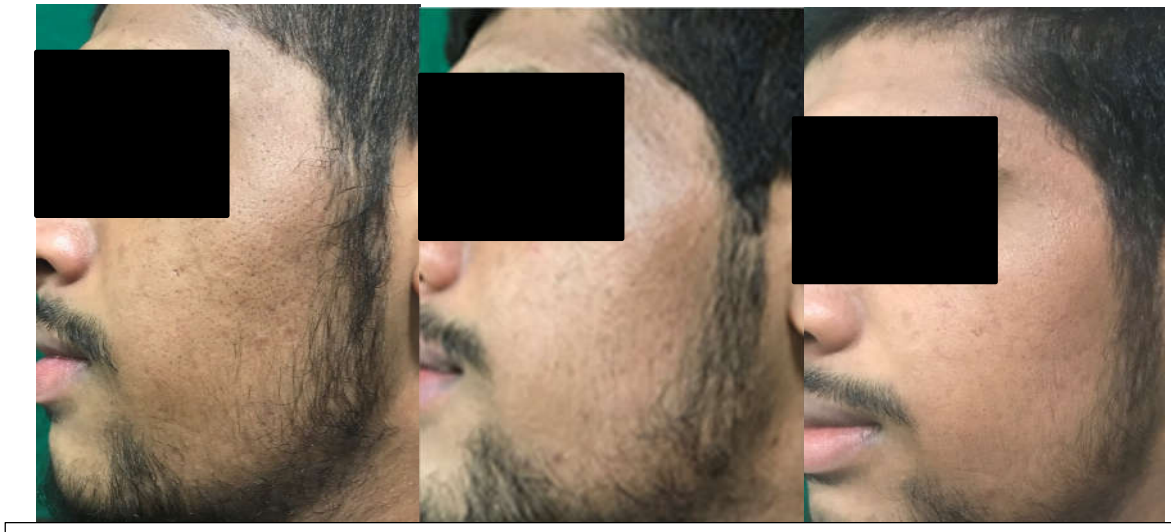
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 24: GROUP 2- Case 1 Right Face Profile



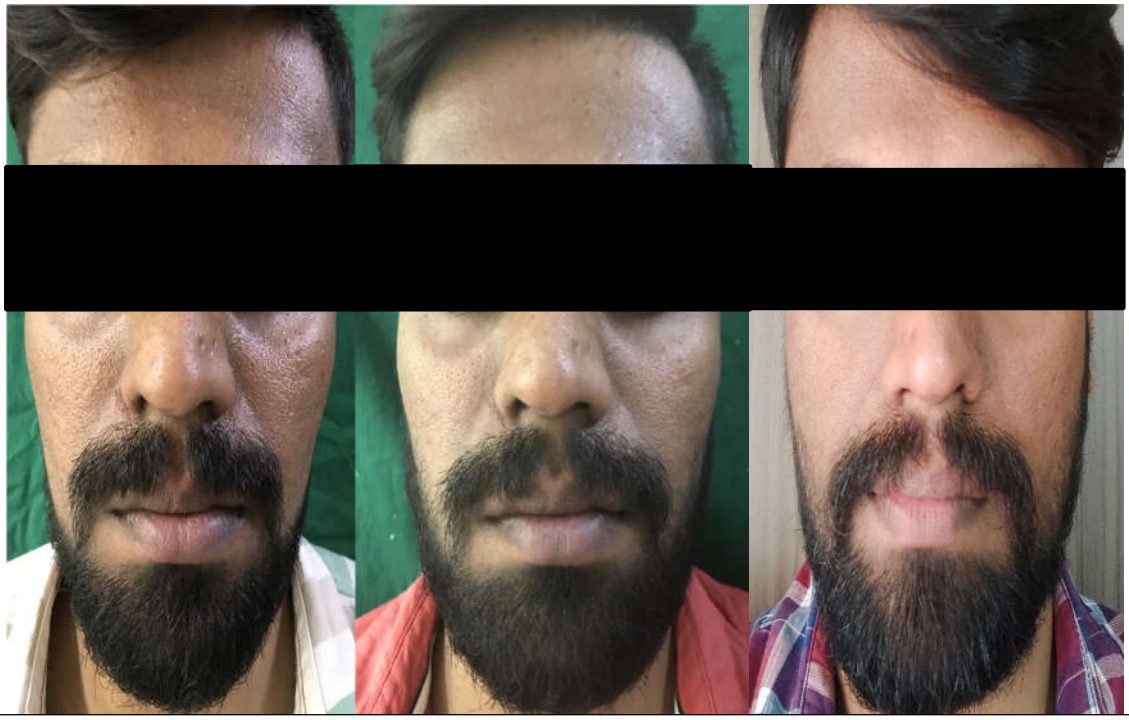
BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 25: GROUP 2- Case 1 Left Face Profile



BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 26: GROUP 2- Case 2 Front Face Profile



BASELINE	THREE MONTHS POST TREATMENT	SIX MONTHS POST TREATMENT

FIGURE 27: GROUP 2- Case 2 Right Face Profile

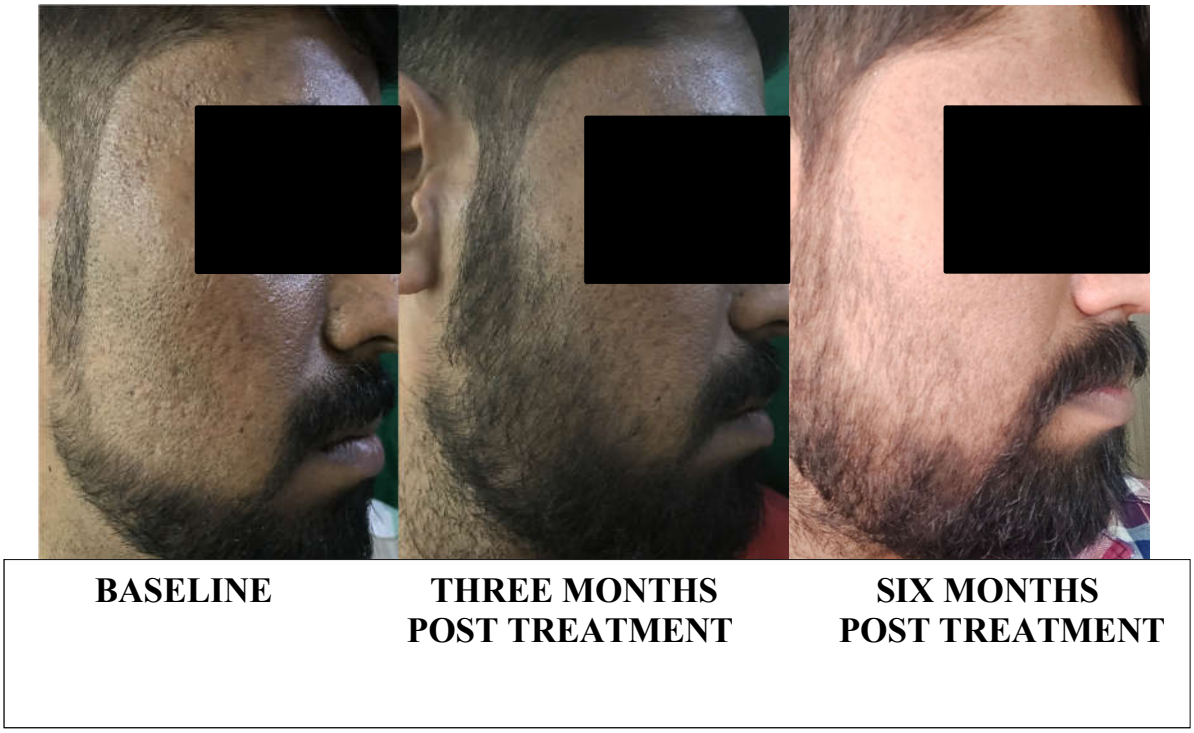


FIGURE 28: GROUP 2- Case 2 Left Face Profile

Table 12: Distribution of subjects according to age group between the groups.

	Group 1	Group 2
<20yrs	5	5
	13.2%	13.2%
21-25yrs	17	15
	44.7%	39.5%
26-30yrs	12	14
	31.6%	36.8%
>30yrs	4	4
	10.5%	10.5%
Total	38	38
	100.0%	100.0%

Graph 1 : Distribution of subjects according to age group between the groups.

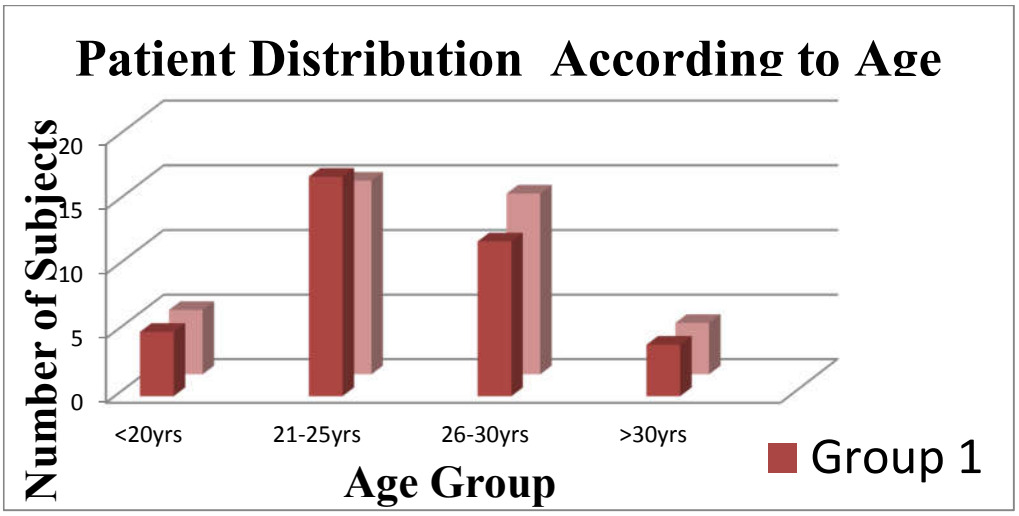
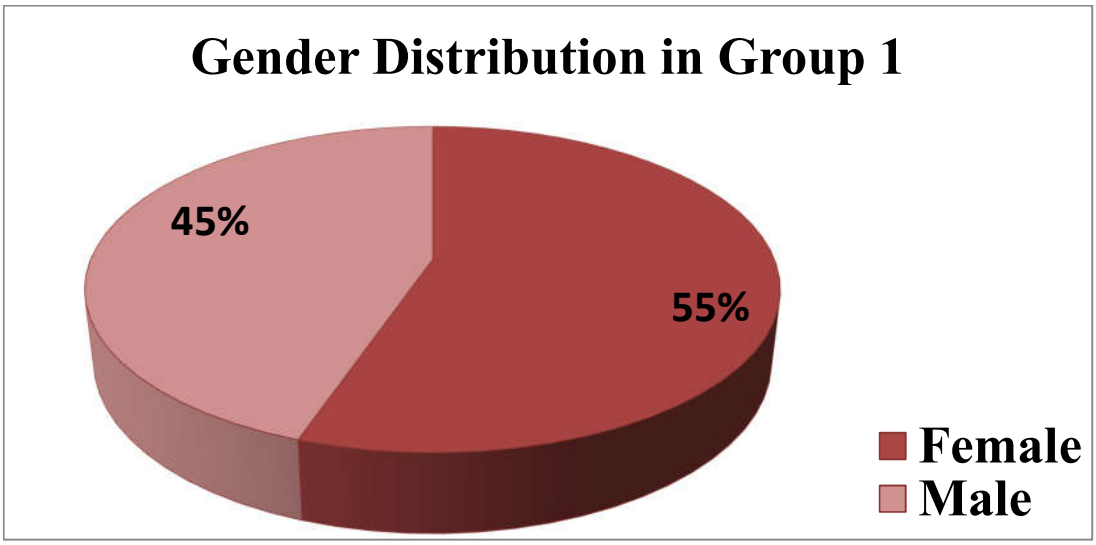


Table 13: Distribution of subjects according to gender between the groups.

	Group 1	Group 2
Female	21	20
	55.3%	52.6%
Male	17	18
	44.7%	47.4%
Total	38	38
	100.0%	100.0%

Graph 2: Distribution of subjects according to gender in group 1



Graph 3: Distribution of subjects according to gender in group 2

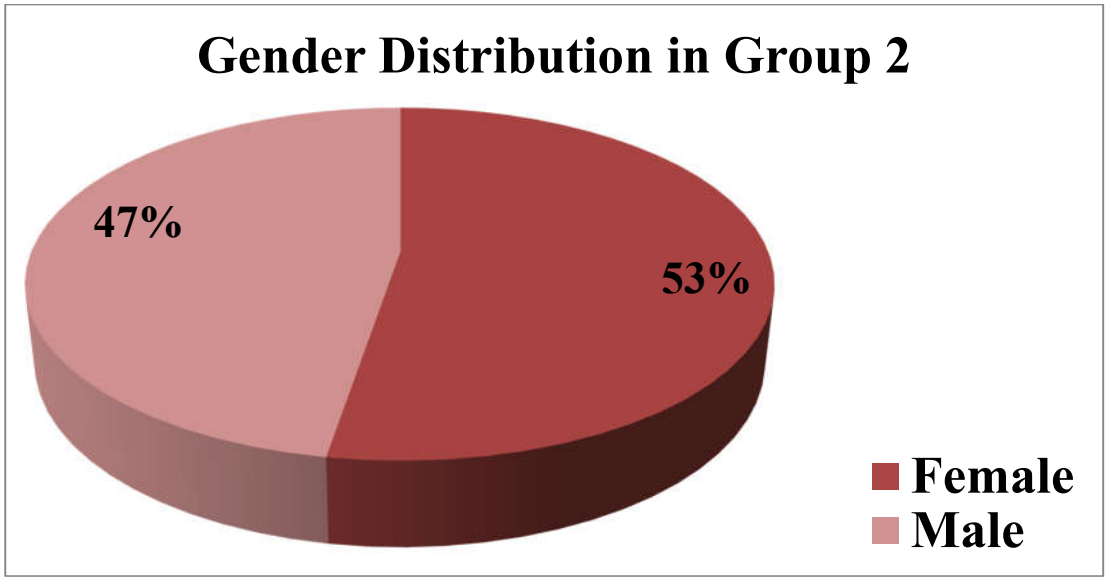


Table 14: Distribution of subjects according to marital status between the groups.

	Group 1	Group 2
MARRIED	11	15
	28.9%	39.5%
UNMARRIED	27	23
	71.1%	60.5%
Total	38	38
	100.0%	100.0%

Graph 4: Patient Distribution according to marital status between the groups.

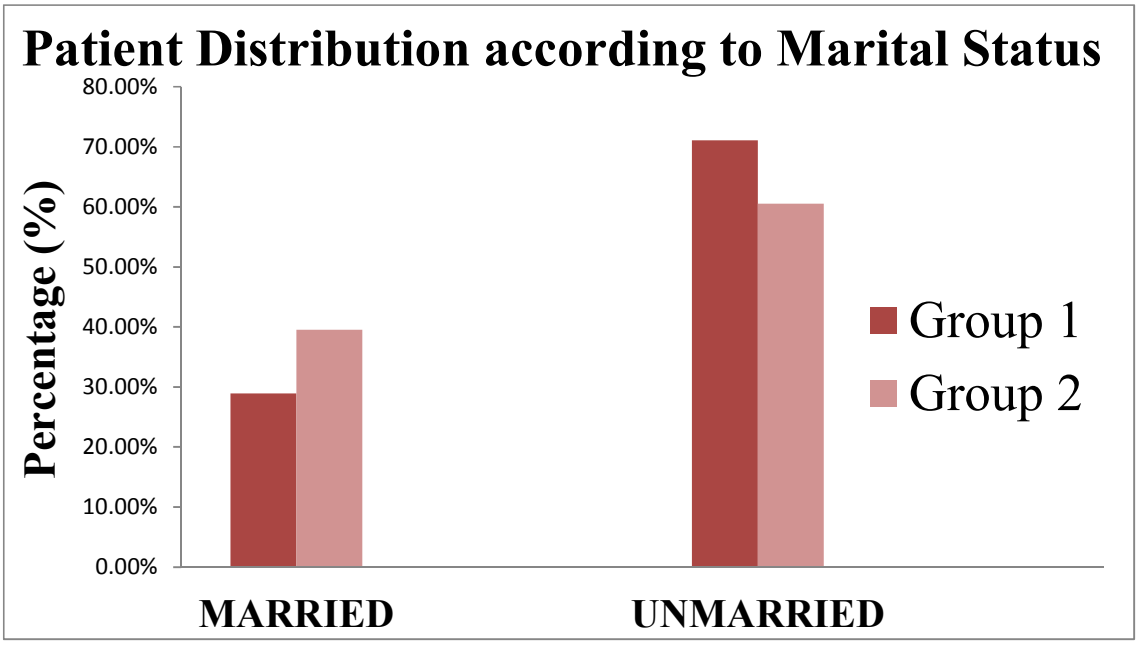


Table 15:- Distribution of subjects according to occupation between the groups.

	Group 1	Group 2
Professionals	19	18
	50.0%	47.4%
Skilled worker	3	1
	7.9%	2.6%
Unskilled worker	4	5
	10.5%	13.2%
Housewife	1	4
	2.6%	10.5%
Student	11	10
	28.9%	26.3%

Graph 5: Patient Distribution according to occupation between the groups.

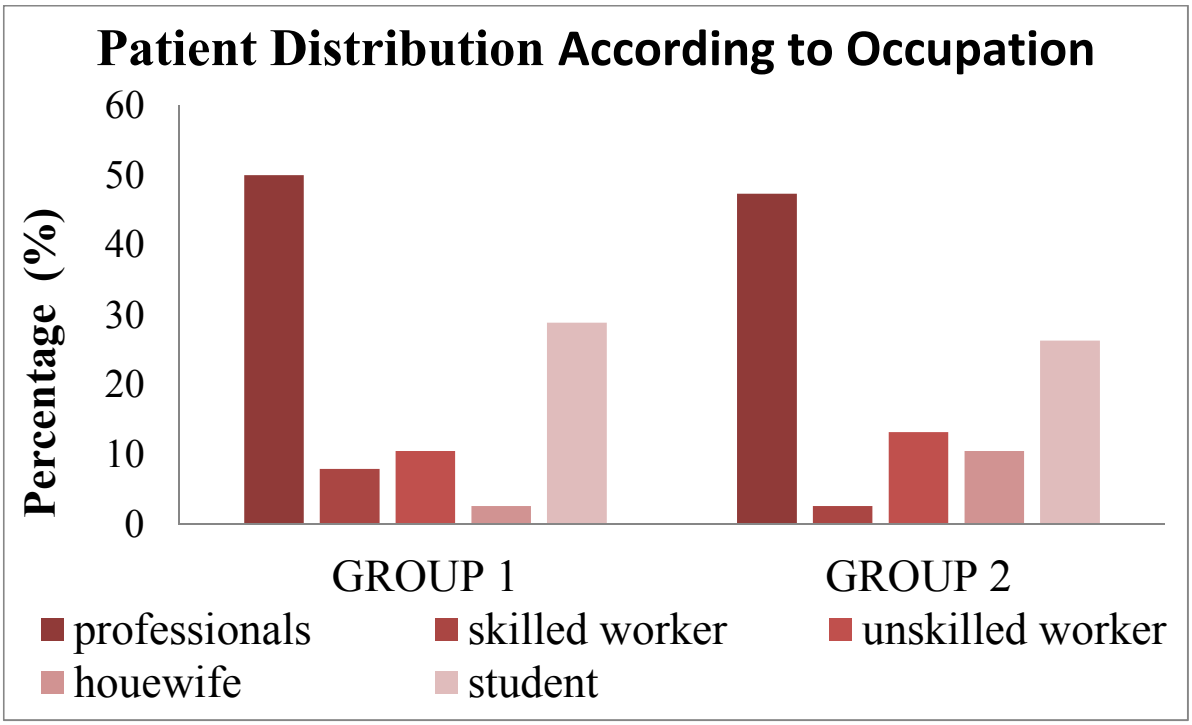


Table 16:- Patient Distribution according to fitzpatrick skin type between the groups

	Group 1	Group 2
II	3	2
	7.9%	5.3%
III	10	8
	26.3%	21.1%
IV	25	28
	65.8%	73.7%

Graph 6:- Patient Distribution according to fitzpatrick skin type between the groups.

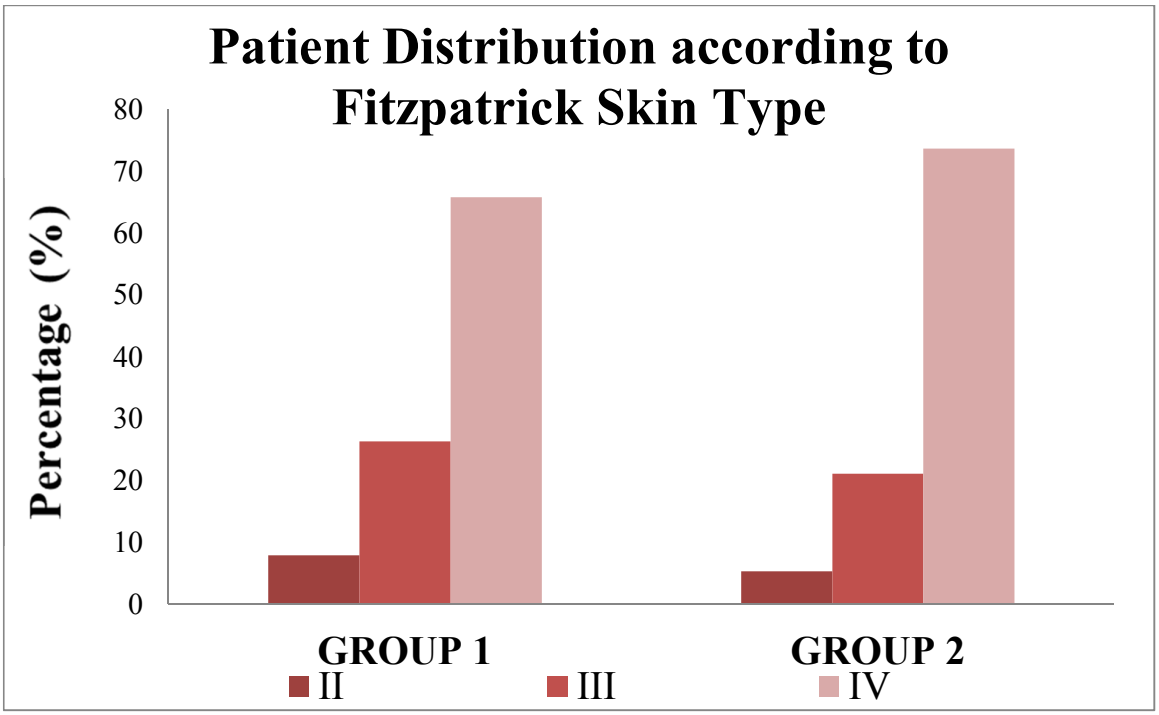


Table 17:- Distribution of subjects according to grading of scar between the groups.

	Group 1	Group 2
3	10	8
	26.3%	21.1%
4	28	30
	73.7%	78.9%
Total	38	38
	100.0%	100.0%

Graph 7: Patient Distribution according to grading of scar between the groups.

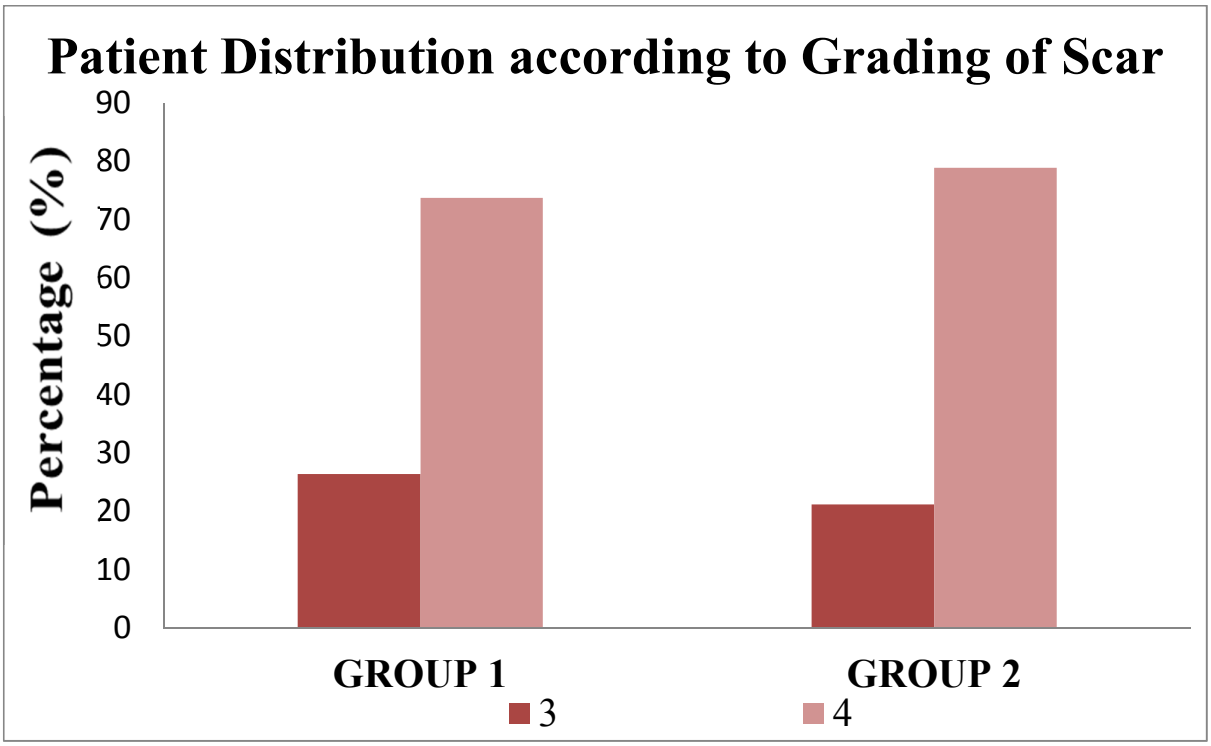


Table 18:- Distribution of subjects according to improvement after 6months between the groups.

	Group 1	Group 2
<25%	4	1
	10.5%	2.6%
25-50%	14	1
	36.8%	2.6%
50-75%	12	20
	31.6%	52.6%
>75%	8	16
	21.1%	42.1%

Graph 8:- Patient Distribution according to improvement in appearance of scars after 6 months

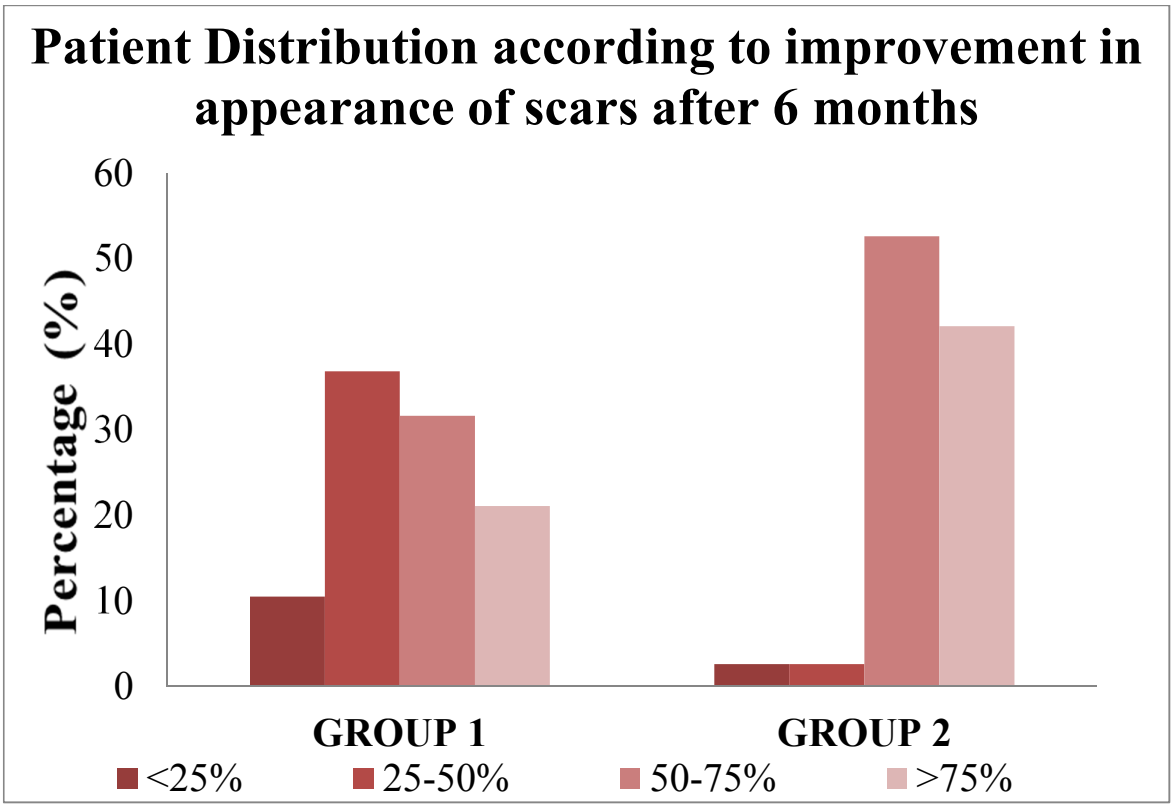


Table 19:- Distribution of subjects according to patient's satisfaction between the groups.

	Group 1	Group 2
Unsatisfied	3	0
	7.9%	.0%
Slightly satisfied	4	3
	10.5%	7.9%
Satisfied	15	12
	39.5%	31.6%
Very satisfied	16	23
	42.1%	60.5%

Graph 9: Patient Distribution according to patient's satisfaction between the groups.

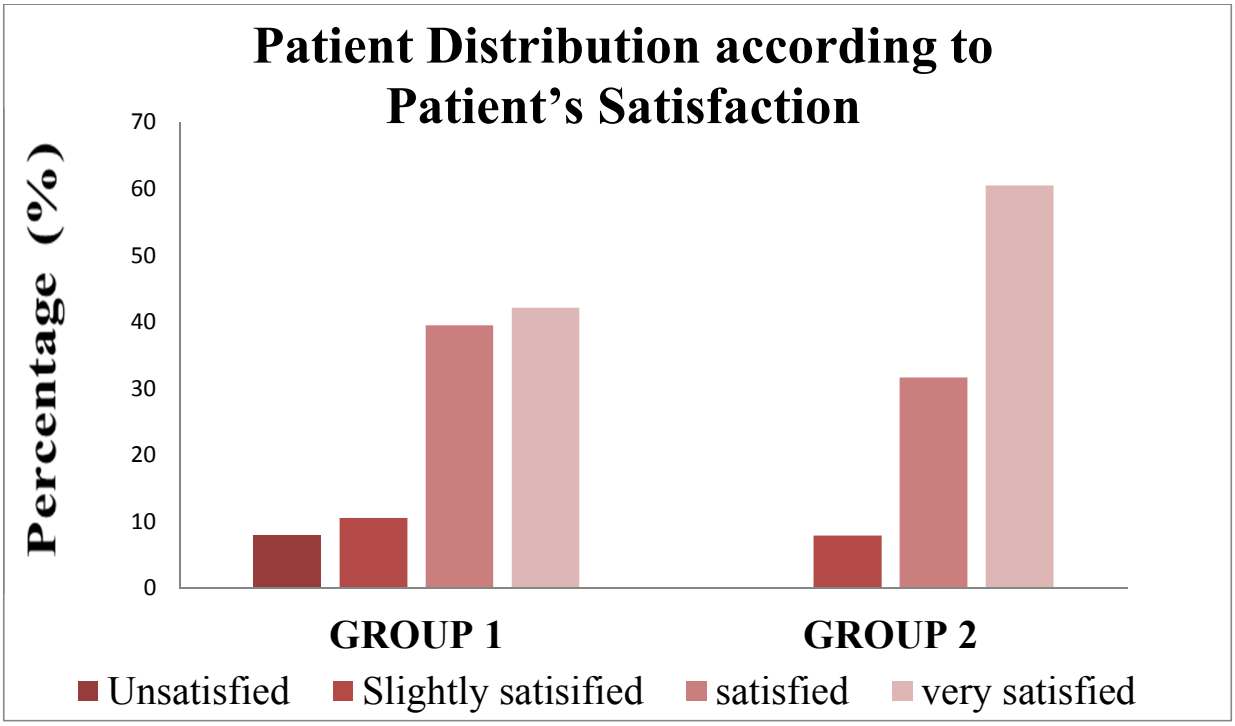
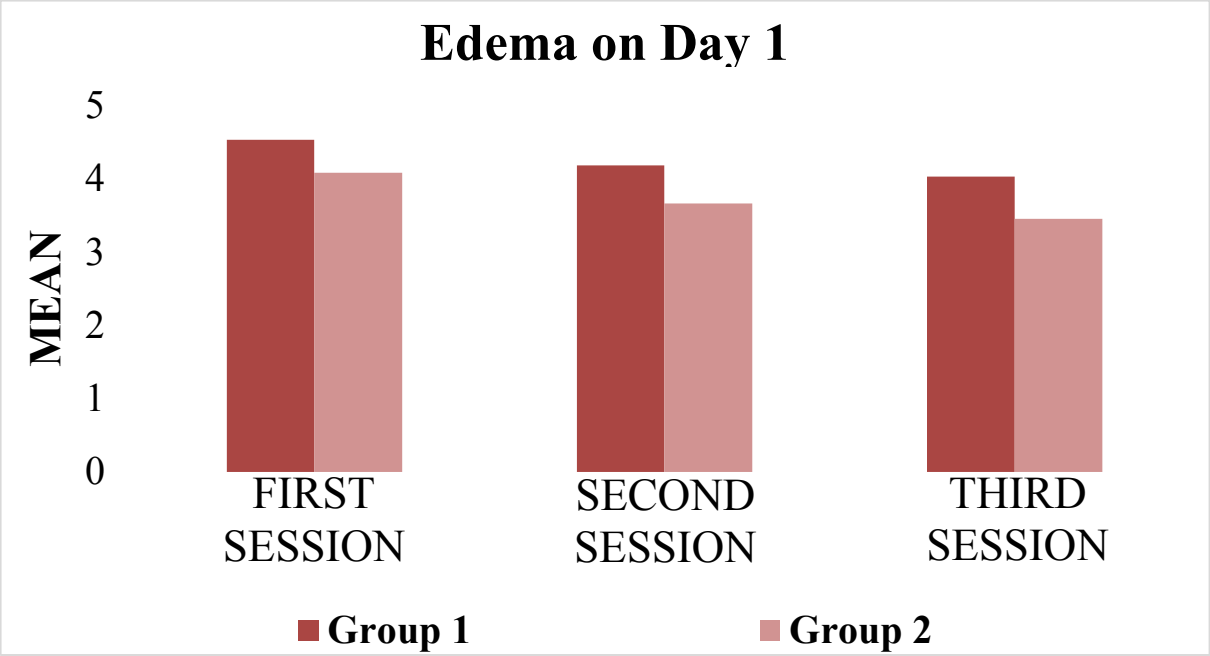


Table 20: Comparison of edema on Day 1 and Day 7 at various session between two groups between the groups.

	Group 1		Group 2		P value
	Mean	SD	Mean	SD	
1st Session Day 1	4.53	1.87	4.08	1.89	0.304
2nd Session Day 1	4.18	1.61	3.66	1.55	0.150
3rd Session Day 1	4.03	1.67	3.45	1.39	0.104
1st Session Day 7	.68	.99	.16	.37	0.004
2nd Session Day 7	.29	.52	.03	.16	0.004
3rd Session Day 7	.39	.82	.11	.31	0.046

Graph 10:- Comparison of edema on Day 1 at various session between two groups between the groups



Graph 11: Graph showing Comparison of edema on Day 7 at various session between two groups between the groups.

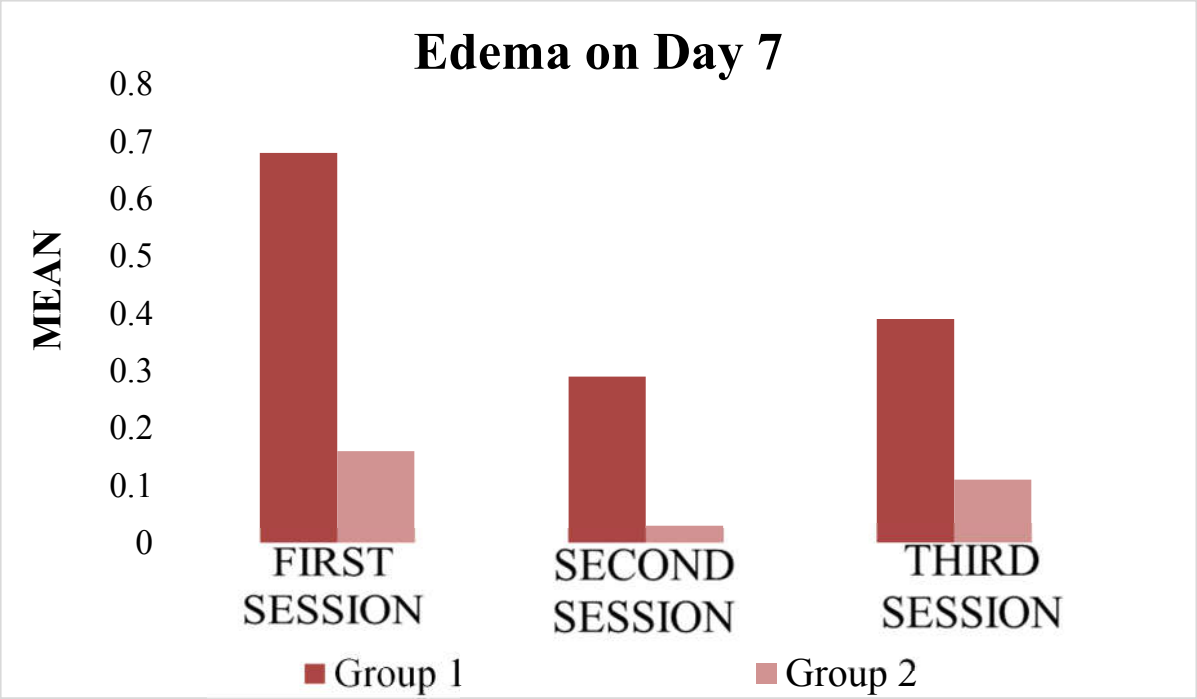
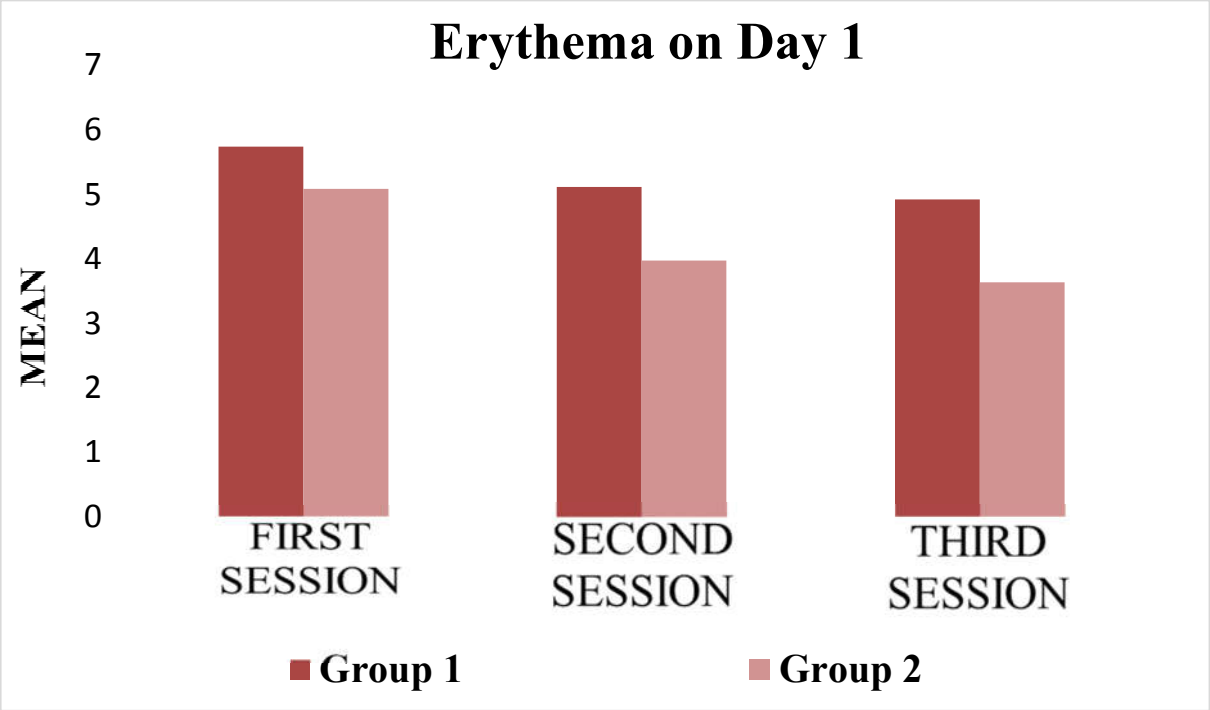


Table 21: Comparison of erythema on Day 1and Day 7 at various session between two groups between the groups.

	Group 1		Group 2		P value
	Mean	SD	Mean	SD	
1st Session Day 1	5.74	1.83	5.08	1.94	0.132
2nd Session Day 1	5.11	1.59	3.97	1.55	<0.001
3rd Session Day 1	4.92	1.62	3.63	1.46	<0.001
1st Session Day 7	1.50	1.18	.66	.75	<0.001
2nd Session Day 7	1.03	.85	.18	.46	<0.001
3rd Session Day 7	.76	.71	.05	.23	<0.001

Graph 12: Comparison of erythema on Day 1 at various session between two groups between the groups.



Graph 13: Graph showing Comparison of erythema on Day 7 at various session between two groups between the groups.

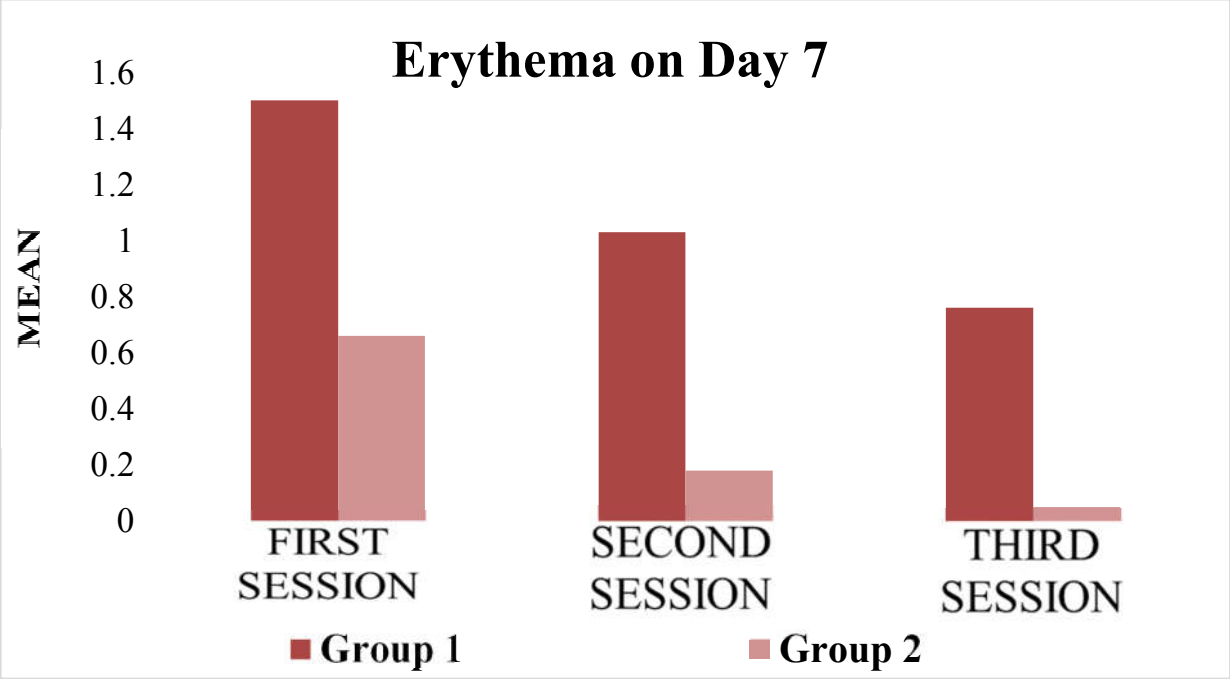
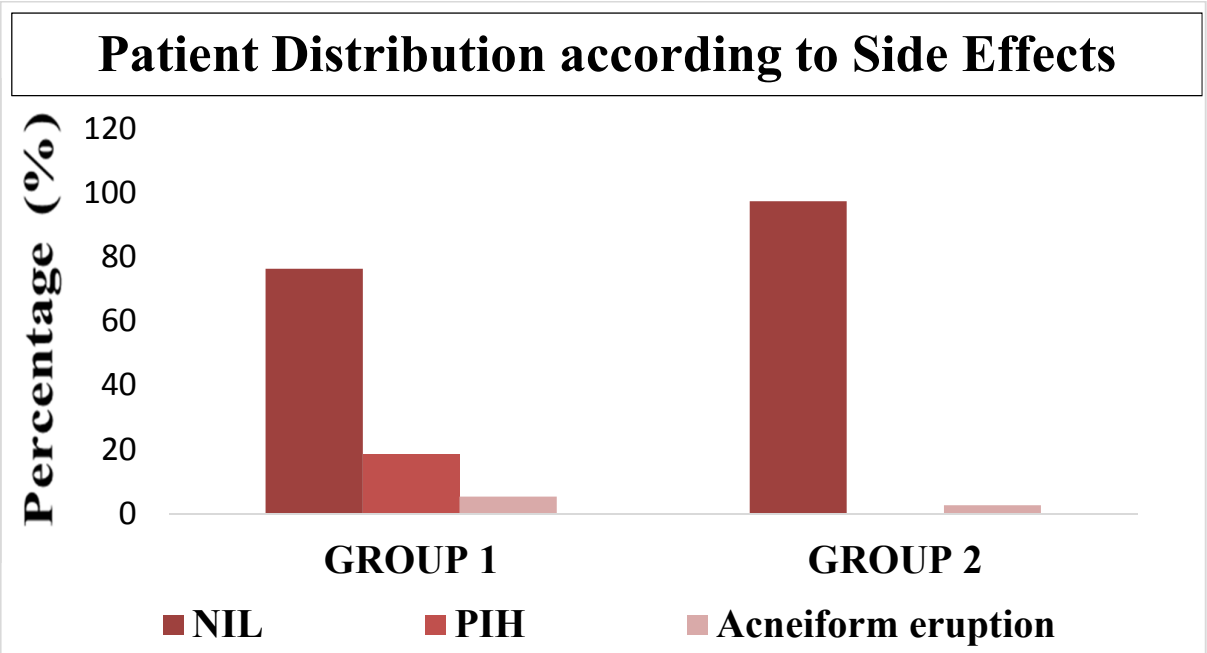


Table 22: Distribution of subjects according to side effects between the groups

	Group 1	Group 2
NIL	29	37
	76.3%	97.4%
PIH	7	0
	18.4%	.0%
Acneiform eruption	2	1
	5.3%	2.6%

Graph 14:- Patient Distribution according to Side Effects between the groups.



DISCUSSION

DISCUSSION

Laser devices have the advantage of controlling the tissue depth penetration in an organ. The application of fractional ablative lasers to deliver bioactive agents to a patient through tunnels of intended depth into cutaneous tissue has vast clinical implications. This technology programs fractional lasers to intrude the skin's barrier properties, generating deep channels to allow local delivery of cellular agents through the disrupted barrier.[156,157]

Age distribution:

Many studies published that patients in the most prevalent age group seeking treatment for acne scars were between 21-25 years, followed by 26-30 years. A study conducted by T. Pooja and her team observed that 61.6% of subjects were in the 18–24 years age group followed by 31.6% of subjects were in 25–31 years age group.[92] 40% and 30% of acne scar patients in a clinical trial conducted in Punjab, India belonged to age groups between 21-25 years and 26-30 years, respectively.[83] In our study, 42.1.% of subjects were between 20-25 years, followed by 34.2% of patients between 26-30 years. This is in agreement with Al Taweel AI Study and Tenna S Study, where the maximum prevalence of acne scars was witnessed in the age group 20- 30 years.[110,124]

Gender Distribution:

In our study, 55.3% and 52.6% of female patients were present in Group 1and Group 2, respectively. At the same time, 44.7% and 47.4% of male patients were in Group 1and Group 2, respectively. Concurrent to this study conducted by Majid I and Imran S saw 35 female and 25 male patients receive treatment for atrophic acne scars in Jammu & Kashmir, India.[12] Similarly, another study in Cairo, Egypt, commented that 16 female and 14 male patients sought treatment for atrophic acne scar.[99]

Fitzpatrick Skin Type

The majority of studies observed that patients looking for acne scars treatment had Fitzpatrick Skin Type III and Type IV. In a Korean Study, all patients belonged to either Fitzpatrick Skin Type III or Type IV.[94] Our study showed 26.3% and 21.1% patients with Fitzpatrick Skin Type IV in Group and Group 2, respectively. There were 65.8% and 73.7% patients with Fitzpatrick Skin Type IV in Group and Group 2, respectively. In agreement with our study, Wanitphakdeedecha R and his colleagues observed that 11.11% and 86.11% of patients were Fitzpatrick Skin Type III and Type IV, respectively. They also found out that the majority of their patients developed transient PIH. Most Indians are Fitzpatrick Skin Type III and IV and are, therefore, prone to Hyperpigmentation post-procedure. It is valuable to use a Depigmenting agent post-procedure for acne scarring.[102] Pigmented scars in Indian skin, which belong to Fitzpatrick type 3–5, are one of the most striking features. Dark skin type has a huge inclination to acne scarring and is frequently complicated by persistent erythema or pigmentation at the base. Initial erythema is succeeded by the purplish base, which may later pigment.[103]

Grading of Acne Scar

Acne scars respond poorly to medical treatment, and achieving an acceptable level of patient satisfaction remains a challenge. On pretreatment assessment by Goodman and Baron qualitative grading of acne scars, we found 10/38 (26.3%) of patients had grade 3, and 28/38 (73.7%) of patients had grade 4 scoring in Group 1, whereas 8/38 (21.1%) and 30/38 (78.9%) of patients had grade 3 and 4 scoring in Group 1, respectively. Contrary to our study, Neinaa and their team conducted research in which more patients had Grade 3 than Grade 4 acne scars (48.8% of patients had Grade 3, and 36.6%had Grade 4 Acne scars).[97] Mild-to-moderate scars involving epidermis and papillary dermis respond to resurfacing laser or other technologies, whereas the deep scars involving reticular dermis require more aggressive or combination modalities.[66] High-grade acne leads to a high degree of inflammation, as seen with papulonodular and cystic acne; a dermal insult to tissue metalloproteinases is more long-lasting and results in a decrease of tissue leading to atrophic scars.[8]

Post Treatment

(1) Appearance of scar after six months

Most clinical trials have documented CO2 Laser efficacious in treating facial atrophic acne scars. A study on the Indian population receiving CO2 Laser as monotherapy reported 68.3% Good or Excellent Improvement.[12] In our study, Group 2 patients showed 52.6% Good and 42.1% Excellent improvement, whereas Group 1 patients had 31.6% Good and 21.1% Excellent improvement in the appearance of acne scars after six months.

A statistically significant difference ($p<0.001$) was found between the groups with respect to improvement in the appearance of scars after six months.

This is in accordance with a study by Waibel JS and his team, which reported more rapid wound healing post-fractional ablative laser. They found out that Elevated bFGF could be involved in Vitamin C, E, and Ferulic acid-induced rapid wound healing.[157] However, this was incompatible with a study by Chawla S and her colleagues concluded that overall results were better with micro-needling and PRP. Vitamin C combined with micro-needling also showed improvement in firmness and smoothness of skin and post-inflammatory hyper-pigmentation. Excellent response was seen in 18.5% of patients with PRP compared to 7% of patients who received vitamin C.[163] Vitamin C's flux and skin deposition across microdermabrasion-treated skin was approximately 20-fold higher than that across patients intact skin.[21] In our study, >80% of patients were either satisfied or very satisfied in both groups. Therefore, there was no statistically significant difference ($p=0.192$) found between the groups with respect to patient satisfaction.

(2) Post Irradiation Edema and Erythema

The Mean±SD edema was 4.53±1.87 in Group 1 on Day 1 after the first session, while in Group 2, it was 4.08±1.89 . Moreover, there was no statistically significant difference between the two groups with respect to edema on Day 1 after the first session, second session, and third session of CO2 Laser therapy. However, Mean±SD Edema was 0.6 ± 0.99 in Group 1, while in Group 2, it was 0.16± 0.37. There was a statistically significant difference ($p<0.001$) between the groups on Day 7 after the first session, second session, and third session of CO2 Laser therapy.

Consistent with this study, a study reported no significant reduction of bFGF expression five days posttreatment laser therapy with topical Vitamin C, E, and Ferulic acid serum compared to the control group.[157]

Vitamin C, E, and Ferulic acid treatment can block laser treatment-induced down-regulation of bFGF in the skin. bFGF is a glycoprotein, which is widely used in treating wounds and ulcers. Furthermore, bFGF receptor blocker and anti-bFGF antibody significantly decrease proliferative activity in adult and fetal skin fibroblasts, suggesting that bFGF plays a critical role in wound healing.[157]

No statistically significant difference was found between the two groups with respect to erythema on Day 1 at the first session. Nevertheless, following the second and third sessions of CO2 Laser therapy, we found a statistically significant difference between Day 1. Furthermore, during treatment, we observed a statistically significant difference between the groups on Day 7 after the first session, second session, and third session of CO2 Laser treatment. It has been observed that Vitamin C enhances the gene expressions of protein coding genes Col1A1, TGF- beta1, TGF-beta 3, MMP-1, MMP-13, and bFGF, which are involved in wound-healing processes.[157] This insight may help explain the exhibited clinical trends of decreasing postoperative downtime. Thus, Vitamin C treatment may improve wound healing by blocking the downregulation of bFGF by laser treatment.[161] However, the detailed signaling pathway involved in the upregulation of bFGF by Vitamin C therapy needs to be further investigated.

(3) Adverse Effects

Lastly, patients in both groups developed transient adverse effects. For Example- 7/38 (18.4%) of patients in Group 1 developed PIH. In addition to that, 2/38 patients from Group 1 and only 1/38 patients from Group 2 developed Acneiform eruption. With a P-value of 0.016, a statistically significant difference was found between groups with respect to side effects, as depicted in Table 22 and Graph 14. Although ascorbic acid has no UV absorption spectra in the UVA (320 to 400 nm) or UVB (290 to 320 nm) range, topical ascorbic acid can exert photoprotection against UVR because of its antioxidant and anti-inflammatory characteristics. Ascorbic acid also plays a role as an anti-pigmentary agent.[155] It interacts with copper ions at the active site of the tyrosinase enzyme, thereby hindering the enzyme's activity. Tyrosinase is the primary enzyme liable for converting tyrosine into melanin, thereby limiting melanin formation.[157]

CONCLUSION

CONCLUSION

Fractional CO2 Laser as Monotherapy and Fractional CO2 Laser in combination with Topical Vitamin C Serum is effective and safe in treating Atrophic Facial Acne Scars. There are minimal transient Post Laser adverse effects of Fractional CO2 Laser alone, but they can be avoided when combined with Topical Vitamin C serum.

Fractional photothermolysis grants the highest degree of scar amelioration. However, it is essential to realize that a typical Indian patient is prone to PIH following an ablative Laser. Therefore, our results contribute to a potentially new understanding of topical Vitamin C serum in preventing PIH and wound-healing following ablative laser surgery. Furthermore, our results exhibited shorter durations of major side effects after combined therapy.

The erythema and edema post laser therapy are significantly reduced when fractional co2 laser therapy is combined with topical vitamin c serum, and thus, the downtime is also lessened.

The current study infers that applying topical Vitamin C serum after ablative fractional CO2 Laser improves clinical outcomes in patients with atrophic acne scarring and hastens recovery of laser-damaged skin.

SUMMARY

SUMMARY

The study was a double-blinded randomized trial among 76 subjects; 38 in Fractional CO2 laser monotherapy (Group1) and 38 in the Fractional CO2 Laser in combination with Vitamin C serum within 2 minutes immediately after Laser and daily for four months (Group 2). Patients with facial atrophic acne scar belonging to both sexes, within an age group of 18 -40 years, with moderate to severe scar, and willing to undergo treatment and follow up were included in the study. Participants underwent serial photography of the lesions at baseline, 1 month, 2 months, 3 months, and 4 months from the first treatment session. The visual analog scale (scoring from 0 to 10) was used to record adverse events (erythema and edema) as perceived by participants. The final assessment was made subjectively by a single observer at the last follow-up visit, and a quartile grading scale was used to assess the response objectively. P-value of <0.05 was considered statistically significant after assuming all the rules of statistical tests.

A statistically significant difference ($p<0.001$) was found between the groups with respect to improvement in the appearance of scars after six months. 42.1% and 52.6% of Group 2 patients had >75% and 50-75% of improvement. 21.1% and 31.6% of patients in Group 1 had >75%, and 50-75% of improvement. >80% of patients were either satisfied or very satisfied in both groups. A statistically significant difference ($p<0.001$) was found between the groups regarding erythema and edema on Day 7 after the CO2 laser therapy session. With a P-value of 0.016, a statistically significant difference was observed between the groups concerning side effects. The combination of using an ablative fractional CO2 laser and Vitamin C serum in the treatment of atrophic scars has a synergistic effect on their inherent properties in up-regulating new collagen synthesis to improve atrophic scars. Erythema, edema, and post-laser downtime are significantly reduced, with diminished risk of adverse effects.

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ANNEXURES

ANNEXURE I

PROFORMA

Name: **Age:** **Sex:** M/F

OP No.: **Education:**

Address: **Occupation:**

Ph No.:

Email id:

C/C:-

HOPI:-

Drug history:-

Past history:- DM/ HTN/ TB/ Epilepsy/ Asthma/ Atopy.

1 Isotretinoin used
(1) Yes..... Duration ..... (2) No [ ]

2 Herpes infections (1) Yes..... (2) No [ ]

3 Skin resurfacing procedures (1) Yes....when..... (2) No [ ]

4 Skin Malignancy
(1) Yes.....when..... (2) No [ ]

5 Keloid/ Hypertrophic scar (1) Yes..... (2) No [ ]

6 Duration of lesion

(1) < 5 years

(2) 5-10 years

(3) >10 years Duration

Family history:- DM/ HTN/ TB/ Epilepsy/ Asthma/ Atopy.

Personal history:-

Diet – veg/ non-veg/ mixed

Appetite –

Sleep – adequate/ disturbed

Bowel & Bladder –Other habits –

Menstrual History :-

Obstetric History:-

Occupational History:-

1. Exposure to sunlight: yes/ no.
2. Duration of exposure:
3. Type of exposure: intermittent/ continuous/ seasonal.
4. Usage of sunscreen: yes/ no.
5. Effect to daily activity :

   (1) None (2) Mininal (3) Mild (4) Moderate (5) Severe

General physical examination:-

PR = bpm BP = / mmHg

RR = cpm Temperature =

P I C C L E Others –

Systemic examination:-

CVS – RS –

P/A – CNS –

Local examination:-

Skin – Fitzpatrick Skin type -

Hair –

Oral mucosa –

Nails –

Acne Scar

1. Type of acne scars

(1) Ice-pick
(2) Boxcar
(3) Rolling
(4) Ice-pick + Boxcar
(5) Ice-pick + Rolling
(6) Boxcar + Rolling
(7) Ice-pick + Boxcar + Rolling

2. Qualitative Scar grading :

a. Macular Disease b. Mild Disease. c. Moderate Disease d. Severe Disease

3. Pain score and Level of burning/stinging sensation

- Pain score (0-10):

  Right.....Left.....

- Level of burning/stinging sensation:

  Right..... Left.....

Side	Measurement	Before	1 month Post-Treatment	% Change From Baseline	3 months Post-Treatment	% Change From Baseline
Right	Texture(rough)					
	Scar Volume					
	Melanin(average)					
Left	Texture(rough)					
	Scar Volume					
	Melanin(average)					

Adverse effects	1st session		2nd session		3rd session	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
Erythema (0-10)						
Edema (0-10)						
PIH (0-10)						
Oozing/Crusting (Y=Yes, No=Absent)						
Petechiae (Y=Yes, No=Absent)						

Skin Infection (Y=Yes, No=Absent)						

Side	Satisfaction scores at 1 month Post-treatment (0-10)	Satisfaction scores 3 months post-treatment (0-10)	Satisfaction scores at 6 months Post-treatment (0-10)
Left			
Right			

ANNEXURE II

CONSENT FORM

A PROSPECTIVE STUDY TO COMPARE RESURFACING OF FACIAL ACNE SCARS IN ADULTS USING FRACTIONAL CO2 LASER AS MONOTHERAPY VS FRACTIONAL CO2 LASER WITH TOPICAL VITAMIN C SERUM IN A TERTIARY HEALTH CARE CENTRE

I, Mr./Mrs./Ms._____, aged_years, S/D/o___, & a resident of ____

_

_____, do hereby declare that I am voluntarily giving my consent to participate/ let my son/ daughter to participate in the study of "A PROSPECTIVE STUDY TO COMPARE RESURFACING OF FACIAL ACNE SCARS IN ADULTS USING FRACTIONAL CO2 LASER AS MONOTHERAPY VS FRACTIONAL CO2 LASER WITH TOPICAL VITAMIN C SERUM IN A TERTIARY HEALTH CARE CENTRE".

I have been explained in my own language about the nature of my skin condition, its prognosis, the treatment options available & their respective side effects. I have also been explained to my full satisfaction, in my own language about the procedure involved in the study. I have been explained that my refusal to consent is however not going to affect my / my patient's right to receive treatment from the department.

I do hereby declare that I will provide complete medical history of the disease, allow myself/ my patient to undergo clinical examination & allow collection of necessary clinical material by the treating Doctor.

I also hereby accord consent to be photographed as & when necessary for the purpose of the study. However, these photographs have to be used only for teaching purposes, clinical presentations & publications but not for advertisements or any other commercial purposes.

Name of the declarant / guardian _____

Signature of the declarant / guardian _____

Name of the witness: _____

Signature of the witness: _____

Name & Signature of the investigator: _____

Date:_____ Place: SDUAHER, KOLAR.

ANNEXURE III

PATIENT INFORMATION SHEET

Study title: A PROSPECTIVE STUDY TO COMPARE RESURFACING OF FACIAL ACNE SCARS IN ADULTS USING FRACTIONAL CO2 LASER AS MONOTHERAPY VS FRACTIONAL CO2 LASER WITH TOPICAL VITAMIN C SERUM IN A TERTIARY HEALTH CARE CENTRE

Study site: R.L Jalappa Hospital , Tamaka, Kolar.

Aim:

1. To Assess the efficacy and safety of Fractional CO2 Laser as Monotherapy and Fractional CO2 Laser in combination with Topical Vitamin C Serum in the treatment of Atrophic Facial Acne Scars.

2. To Assess post laser adverse effects and healing of Fractional CO2 Laser as Monotherapy and Fractional Co2 Laser in combination with Topical Vitamin C Serum in the treatment of Atrophic Facial Acne Scars.

Please read the following information and discuss with your family members. You can ask any question regarding the study. If you agree to participate in this study we will collect information(as per proforma) from

you. Relevant blood investigations will be carried out if required. This information collected will be used for dissertation and publication only.

All information collected from you will be kept confidential and will not be disclosed to any outsider. Your identity will not be revealed. The expenses required for the above investigations will be funded by the study investigator. This study has been reviewed by the Institutional Ethics Committee and you are free to contact the member of the Institutional Ethics Committee. There is no compulsion to agree to thisstudy. The care you will get will not change if you don't wish to participate. You are required to sign/ provide thumb impression only if you voluntarily agree to participatein this study.

For any further clarification you can contact the study investigator:

Name- Dr.Chandrika Nayyar

Mobile No. - 9561389876

Email ID- dr.chandrikamehta@gmail.com

ANNEXURE IV

KEY TO MASTER CHART

GROUP 1- participants will be treated with Fractional Co2 Laser

GROUP 2- participants will be treated with Fractional Co2 Laser in combination with Topical Vitamin C Serum within 2 minutes immediately after laser and daily for 4 months

AGE GROUP

≤20yrs	1
21-25yrs	2
26-30yrs	3
>30yrs	4

Sex

Female	1
Male	2

Marital status

Married	1
Unmarried	2

Occupation

Professional	1
Skilled worker	2
Unskilled worker	3
Housewife	4
Student	5

Percentage of improvement after 6 months

<25%	1
25-50%	2
50-75%	3
>75%	4

Patient satisfaction

Unsatisfied	1
Slightly Satisfied	2
Satisfied	3
Very Satisfied	4

Side Effects

Nil	1
PIH	2
Acneiform Eruption	3

S.No.	UHID No.	Age Group	Sex	Marital Status	Occupation	Group	Fitzpatrick Skin Type	Acne Scar Score	Improvement after 6 months	Patient Satisfaction
1	733288	1	1	2	5	1	IV	4	3	3
2	591338	3	1	2	1	2	III	3	3	2
3	687821	2	2	2	2	1	IV	4	2	2
4	652508	1	1	2	5	1	III	4	3	2
5	678284	3	1	2	1	1	IV	4	3	3
6	713294	2	1	2	3	1	IV	3	4	3
7	722451	3	1	1	1	2	IV	3	3	3
8	782692	2	2	2	1	2	III	3	3	2
9	794075	2	1	2	5	1	IV	4	4	3
10	792889	2	1	2	1	1	IV	4	2	0
11	788925	3	2	1	1	2	IV	4	3	2
12	592182	2	1	1	4	2	IV	4	3	2
13	550041	2	2	2	1	1	II	3	4	3
14	840687	2	1	2	1	1	IV	3	2	0
15	841318	1	2	2	5	2	IV	4	1	1
16	825787	2	2	2	3	2	IV	4	3	2
17	758672	3	2	1	3	1	IV	4	2	1

18	843435	3	1	1	1	1	II	3	1	1
19	819894	3	1	1	1	2	IV	4	3	1
20	838811	2	2	2	5	1	III	4	1	1
21	839246	2	2	1	3	2	IV	4	3	2
22	589262	3	2	1	1	2	IV	4	3	3
23	693451	2	1	1	4	1	IV	3	2	2
24	531120	2	1	2	1	2	IV	3	4	3
25	839578	3	2	2	1	1	II	4	3	3
26	541808	2	1	2	1	1	IV	4	2	2
27	616818	4	2	2	2	1	IV	4	2	2
28	563913	1	2	2	5	2	III	4	4	2
29	839709	4	1	1	1	1	IV	3	3	2
30	693563	3	1	1	4	2	IV	4	3	3
31	522295	1	1	2	5	2	IV	4	3	2
32	885958	2	1	1	4	2	IV	4	4	2
33	814619	2	1	2	1	1	IV	4	3	3
34	822649	2	2	2	5	1	IV	4	3	2
35	821463	2	2	2	1	1	III	4	3	3
36	849262	2	1	2	5	2	III	3	4	3

37	8489611	3	2	2	3	1	IV	3	4	3
38	877782	3	1	2	1	2	III	3	3	3
39	878220	2	2	2	5	1	IV	4	2	3
40	881816	2	2	2	1	2	IV	4	4	3
41	883919	1	1	2	5	2	IV	4	3	3
42	889804	1	2	2	5	2	IV	4	3	3
43	884304	3	2	1	1	1	IV	4	2	3
44	875233	4	2	1	1	2	IV	4	2	1
45	890987	3	1	1	1	2	IV	3	3	2
46	887777	3	2	2	1	2	IV	4	4	3
47	892312	2	1	2	5	2	IV	4	4	3
48	893169	3	2	2	1	1	IV	4	3	2
49	893434	3	2	2	3	2	IV	4	3	2
50	866845	2	2	2	5	2	IV	4	4	3
51	886275	1	1	2	5	1	III	4	2	2
52	883193	1	1	2	5	1	III	4	3	2
53	879212	4	2	1	3	2	III	4	4	3
54	884287	4	1	1	1	1	IV	4	3	3
55	882702	3	2	2	1	2	IV	4	3	3

56	882024	3	1	1	4	2	II	4	3	3
57	886714	2	1	1	1	1	IV	4	1	1
58	898632	3	2	2	3	1	III	3	2	2
59	866845	4	2	1	1	1	IV	4	3	2
60	887388	4	1	1	3	2	IV	4	4	3
61	897777	3	2	2	2	2	III	4	3	3
62	902345	3	1	1	1	1	IV	4	2	2
63	905024	2	1	2	5	1	III	4	4	3
64	904836	4	1	1	1	2	IV	3	3	3
65	905245	2	2	2	1	2	IV	4	4	3
66	927819	3	1	1	2	1	III	4	2	3
67	906987	3	2	1	1	2	III	4	4	3
68	906544	3	2	1	1	1	III	3	2	2
69	907661	2	1	2	5	2	IV	4	4	3
70	833901	2	2	2	1	1	IV	3	4	2
71	903305	2	1	2	1	2	II	4	4	3
72	883698	1	1	2	5	1	IV	4	4	3
73	914962	2	1	2	5	2	IV	4	4	3
74	919807	2	1	2	1	2	IV	4	4	2

75	926152	3	1	2	1	1	III	4	4	3
76	926786	2	2	2	5	1	IV	4	1	0

S.No.	Group	Oedema (D1 After 1st Session)	Oedema (D7 After 1st Session)	Oedema (D1 After 2nd Session)	Oedema (D7 After 2nd Session)	Oedema (D1 After 3rd Session)	Oedema (D1 After 3rd Session)	Erythema (D1 After 1st Session)	Erythema (D7 After 1st Session)	Erythema (D1 After 2nd Session)	Erythema (D7 After 2nd Session)	Erythema (D1 After 3rd Session)	Erythema (D7 After 3rd Session))	Side Effects
1	1	6	1	5	0	4	1	7	2	6	1	6	1	1
2	2	6	0	4	0	4	0	7	0	5	0	4	0	1
3	1	7	2	7	1	7	1	7	3	6	3	6	2	2
4	1	5	0	5	0	5	0	7	1	6	1	6	1	1
5	1	4	0	4	0	4	0	7	1	7	1	6	1	1
6	1	5	0	5	0	5	0	7	0	6	0	6	0	1
7	2	5	0	4	0	4	0	7	1	5	1	5	0	1
8	2	6	0	4	0	4	0	7	1	7	1	5	0	1
9	1	5	1	3	0	3	0	7	2	5	1	5	1	3
10	1	7	2	6	1	6	1	7	3	6	2	6	1	1
11	2	7	1	6	0	5	1	8	2	6	0	6	0	1
12	2	5	0	4	0	4	0	5	1	4	0	4	0	1
13	1	6	1	6	1	6	0	7	2	6	1	6	0	1
14	1	7	2	7	0	6	1	8	3	8	2	8	2	2
15	2	5	0	4	0	5	0	7	1	5	0	5	0	1

16	2	6	0	4	0	4	0	7	0	4	0	4	0	1
17	1	5	1	5	1	5	1	7	2	5	1	5	1	2
18	1	3	0	3	0	3	0	5	1	4	1	4	0	1
19	2	2	0	2	0	2	0	4	1	3	0	3	0	1
20	1	3	0	3	0	3	0	4	1	4	1	4	0	1
21	2	3	0	3	0	3	0	4	0	3	0	3	0	1
22	2	4	0	4	0	4	0	4	0	4	0	3	0	1
23	1	2	0	2	0	2	0	5	1	4	0	4	0	1
24	2	1	0	1	0	1	0	3	0	2	0	2	0	1
25	1	4	0	4	0	4	0	7	2	5	1	4	0	1
26	1	3	0	3	0	3	0	5	2	4	1	4	1	1
27	1	5	1	5	1	5	1	6	2	5	1	5	1	2
28	2	3	0	2	0	2	0	3	0	3	0	3	0	1
29	1	4	0	4	0	4	0	5	1	5	1	5	1	1
30	2	2	0	2	0	2	0	3	0	2	0	2	0	1
31	2	5	0	5	0	5	0	6	1	5	0	4	0	1
32	2	3	0	3	0	2	0	3	0	3	0	2	0	1
33	1	5	1	5	1	4	0	5	1	5	1	4	1	1
34	1	9	3	7	1	8	2	9	4	8	2	8	1	1

35	1	4	0	4	0	4	0	5	2	5	1	5	1	1
36	2	3	0	3	0	2	0	5	1	3	0	2	0	1
37	1	2	0	2	0	2	0	3	0	3	0	3	0	1
38	2	6	0	6	0	5	0	7	1	6	0	6	0	1
39	1	7	1	7	0	6	1	8	3	7	2	7	1	1
40	2	5	1	5	1	5	1	7	2	5	1	5	1	3
41	2	9	1	8	0	6	0	10	3	8	2	7	0	1
42	2	3	0	3	0	3	0	6	0	4	0	3	0	1
43	1	3	0	3	0	2	0	3	0	3	0	3	0	2
44	2	2	0	2	0	2	0	2	0	2	0	2	0	1
45	2	5	0	5	0	4	0	5	1	5	0	4	0	1
46	2	5	0	4	0	5	0	6	1	4	0	4	0	1
47	2	4	0	4	0	4	0	5	1	4	0	4	0	1
48	1	3	0	2	0	3	0	5	1	5	1	4	1	1
49	2	4	0	4	0	4	0	5	1	4	0	4	0	1
50	2	2	0	2	0	2	0	5	0	4	0	4	0	1
51	1	6	2	5	1	5	0	8	3	6	1	6	1	2
52	1	6	1	5	1	4	0	7	2	6	2	6	1	1
53	2	4	0	4	0	4	0	4	0	4	0	4	0	1

54	1	3	0	3	0	3	0	3	0	3	0	3	0	1
55	2	9	0	7	0	7	0	9	2	7	1	7	1	1
56	2	5	0	5	0	4	0	6	0	5	0	5	0	1
57	1	7	2	6	2	6	2	7	3	7	3	7	2	2
58	1	3	0	3	0	3	0	4	0	4	0	4	0	1
59	1	2	0	2	0	2	0	3	0	3	0	2	0	1
60	2	3	0	2	0	2	0	3	0	2	0	2	0	1
61	2	2	0	2	0	2	0	2	0	2	0	2	0	1
62	1	6	0	5	0	5	0	7	1	7	1	7	1	1
63	1	4	0	4	0	3	0	5	1	4	1	4	1	1
64	2	3	1	3	0	3	1	5	1	4	1	3	0	1
65	2	1	0	1	0	1	0	2	0	1	0	1	0	1
66	1	1	0	1	0	1	0	2	0	2	0	2	0	1
67	2	4	1	4	0	4	0	5	1	4	0	4	0	1
68	1	7	4	5	0	6	4	8	4	7	3	7	3	1
69	2	4	1	4	0	3	1	5	1	4	0	3	0	1
70	1	3	0	3	0	3	0	3	0	3	0	3	0	1
71	2	2	0	2	0	2	0	3	0	2	0	2	0	1
72	1	2	0	2	0	1	0	3	0	2	0	2	0	1

73	2	3	0	3	0	3	0	4	0	3	0	2	0	1
74	2	4	0	4	0	3	0	4	1	3	0	3	0	1
75	1	5	1	5	0	5	0	7	2	7	1	6	1	3
76	1	3	0	3	0	2	0	5	1	5	1	4	1	1