A Comparative Study To Assess The Efficacy Of Fractional Carbon Dioxide Laser And Combination Of Fractional Carbon Dioxide Laser With Topical Autologous Platelet Rich Plasma In Post Acne Atrophic Scars

by

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Dissertation submitted to

In partial fulfillment for the degree of

DOCTORE OF MEDICINE

IN

DERMATOLOGY

the Guidance of

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2019

LIST OF ABBREVIATIONS

ACD-A - Anticoagulant citrate dextrose solution formula A

ARs - Androgen receptors

CaCl - Calcium chloride

CO_2 - Carbon dioxide

CW - Continuous wave

DA – Dermabrasion

DQLI - Dermatological Quality of Life Index

DHT – Dihydrotestosterone

DHEAS – Dehydroepiandrosterone sulphate

FGF-2 – fibroblast growth factor 2

IGF-I – Insulin like growth factor 1

MDA – Microdermabrasion

MMPs – Matrix metalloproteinase

PDGF – Platelet derived growth factor

PRP – Platelet rich plasma

P-PRP – Pure Platelet rich plasma

L-PRP – Leucocyte Platelet rich plasma

SHBG - sex hormone binding globulin

TEM – Transverse electronic mode

TGF – transforming growth factor

VEGF – vascular endothelial growth factors

VAS- Visual analogue scale

ABSTRACT

Background

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit prevalent in adolescent population. Acne can manifest as inflammatory or non-inflammatory lesions. Inflammatory acne can produce permanent scarring which causes low academic performance, unemployment, depression and suicide.

Although challenging to treat, various modalities both non-energy based and energy based can be used for acne scars. However, the introduction of fractional carbon dioxide laser and platelet-rich plasma therapy for atrophic scars has opened up new avenues for management of acne scars

Objective

To evaluate the efficacy of fractional carbon dioxide laser alone and in combination with topical platelet rich plasma in treating post acne atrophic scars.

Method

A hospital based prospective, double blinded, randomized, comparative study was conducted. A total of 33 cases of post acne atrophic scars of moderate to severe grade attending outpatient department of tertiary care hospital were included in the study. The enrolled patients were allotted into 2 groups. Detailed history and clinical examination were performed and recorded and score of acne scars using Goodman and Baron qualitative grading and quantitative scoring chart was calculated. Patients in GROUP A were treated with fractional carbon dioxide laser ablation followed by topical platelet-rich plasma. Patients in GROUP B were treated with fractional carbon dioxide laser ablation only. Baseline investigations and clinical photographs were taken. Three laser sessions were performed 4 weeks apart and patient were assessed (clinical photographs, scar score, adverse effects,

VAS) at every visit. At the end of 12 weeks, baseline and post treatment photographs were compared for final assessment.

Results

Out of the 33 patients enrolled in the study, 25 patients completed the study.

Mean change in score at the end of the study was higher in subjects of Group A (4.17) when compared to those in Group B (3.15) which was statistically insignificant. The mean scar score in Group A reduced from 11.5 to 4 and in Group B from 12 to 3. This reduction of scar score in both groups was highly significant ($P<0.0001$).

Mean VAS for patient's assessment of improvement was higher in subjects in Group A (4.08) when compared to those in Group B (3.46). The change in scar score irrespective of treatment was the highest in patients with rolling scars with a mean of 4.2 followed by boxcar scars with a mean of 3.89 and the lowest in ice-pick scars with a mean of 2.33 which was highly significant. Erythema, erythema with oedema, pain and hyperpigmentation was more in subjects in Group B.

Conclusion

Combination therapy with platelet rich plasma is more efficacious in reducing the appearance of scars, reducing adverse effects of laser therapy and better patient satisfaction by the means of subjective improvement as compared to monotherapy with fractional carbon dioxide laser. Fractional carbon dioxide laser gives better response for rolling and boxcar scars as compared to ice pick scars.

Keywords: fractional carbon dioxide laser, platelet rich plasma, post acne atrophic scars

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INTRODUCTION

INTRODUCTION:

Acne vulgaris is prevalent in 90% of adolescent population. It is a chronic inflammatory disorder of the pilosebaceous unit. In 12-14% of these patients acne persists even in adulthood. In a world where both women and men are conscious about their appearance, acne poses as a psychological and social burden of high gravity.[1]

Acne can manifest as inflammatory or non-inflammatory lesions. Inflammatory acne can produce permanent scarring, excoriations, post-inflammatory erythema and dyspigmentation, the severity of which may depend on delay in treatment of acne patients.[1, 2] Scarring occurs by the healing of all forms of active acne, extending from comedone, papules, pustules to nodulocystic acne.[2]

The predominance and severity of acne scarring has not been well calculated.[1, 2] However, severe scarring caused by acne is an element of peril for low academic performance, unemployment, depression and suicide. This stands true particularly in adolescents since face is most commonly involved. In a clinical assessment of acne scarring and its frequency, it was estimated that predominant facial scarring occurs to certain extent in 95% of patients. In a survey of acne patients by Kubba *et al.*, 49% patients reported having scars whereas on clinical evaluation, 14% of females and 11% of males were found to actually have scarring.[3]

Broadly, acne scars are classified into atrophic and hypertrophic scars. Atrophic acne scars have been additionally categorized as ice pick, rolling and shallow or deep boxcar scars.[2]

In dermatology we often face the challenge of successfully improving acne scars to the satisfaction of patients. Various modalities can be used for improvement of acne scars. These can be distributed into non-energy centred techniques and energy centred techniques. Efficacious treatment of facial acne scars remains a difficult task at hand. However, the

introduction of fractional carbon dioxide (CO_2) laser and platelet-rich plasma (PRP) therapy for atrophic scars has opened up new avenues for management of acne scars.

The fractional CO_2 laser thermally modifies a portion of the skin, leaving intervening zones of normal skin intact, which rapidly regenerate the ablated columns of tissue.[4]

In spite of being unparalleled in results, fractional CO_2 laser is followed by side effects like erythema, oedema, infections and risk of developing hyper or hypo pigmentation making it unappealing for patients.[5]

Autologous platelet rich plasma provides with a full array of potential bioactive growth factors and chemokines released upon platelet activation which aid in quick wound healing. Topical platelet rich plasma application after fractional CO_2 laser causes rapid reduction in erythema, oedema and also reduces post laser hyper or hypo pigmentation. Platelet-rich plasma is also known to actively reduce atrophic acne scarring making these two modalities synergistic in nature.[6]

Therefore, this study is conducted to gauge and compare the effectiveness and safety of fractional CO_2 laser resurfacing and combination of fractional CO_2 laser resurfacing and topical platelet rich plasma to provide the best possible treatment and relief to patients anguished by post-acne atrophic scars.

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1. To evaluate and compare the efficacy of fractional carbon dioxide laser alone and in combination with topical platelet rich plasma in treating post acne atrophic scars.

REVIEW OF LITERATURE

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History:

ACNE is not a disease of modern age alone. The first description of acne can be found in the Sushrut Samhita under Kshudra Roga as Mukha Dushika. Mentions are found about this condition in ancient Egyptian writings in the Pharaoh and various treatments used by them. Some sources say that it was Emperor Justinian's physician, Aetius Amidenus, who used the word 'ACNE' for the first time during sixth century A.D. Word ACNE comes from word 'AKME'. It is actually corrupt form of the word AKME, which means 'PRIME OF LIFE'.[7]

Epidemiology:

Acne vulgaris, also called as 'pimples', by common people, is a universal disease affecting about 9.4% population globally. [8] Around 90% people get affected in their teens & in some, it persists till adulthood. In India, prevalence rate is about 50.60% in males and 38.13% in females, in age group of 12-17 years.[9] However, its sequel i.e. scarring continues in adulthood. Difference in male and female prevalence is often reported, but it is probably due to a social bias.

Studies have also shown difference in the prevalence of acne in rural and urban areas as well. It is believed to be more common in urban population, suggesting the role of lifestyle and diet in the occurrence of acne. Around 20% have severe acne which can result in permanent physical as well as mental scarring.

Definition:

Acne is a chronic inflammatory disease of pilosebaceous units seen predominantly in adolescents, characterized by seborrhea & pleomorphic lesions like open and closed comedones, erythematous papules, pustules, nodules and cysts. In several cases, scarring will arise which can be either mild or severe, depending upon the degree of inflammation. The condition is most common between 12-25 years of age. Acne is undoubtedly one of the most common disorders encountered by the dermatologists.[10]

Beginning at a young age, its prevalence varies from 30-100% in teenage years. Post-acne scarring is common sequelae of acne. Even though the potential factors causing acne scarring are well documented, both prevention and treatment of acne scars remains a challenge faced by dermatologists.

In a study by Chuah *et al.*, the approximate age of onset of acne was 16 years with earlier onset in women and duration of the acne was approximately 96 months with longer duration in women. Most patients delayed treatment by 1 year even though 100% had post-acne scarring over the face. The Dermatological Quality of Life Index (DQLI) was 5.6, which was comparable to other debilitating diseases like rosecea, Dariers disease and Hailey-Hailey disease.[11]

Post acne scars are known to be detrimental to the quality of life of young adults.[11] Acne and acne scars both affect areas which are rich in sebaceous glands like face, back and chest. One out of every seven patients found their acne scars disfiguring.[1]

Unfortunately, acne scarring has a profound psychosocial impact. Patients suffering from acne scars develop a feeling of handicap or physical disfigurement which takes a toll on their self-esteem, self-confidence in performing daily activities and ability to fit into a social environment. It may also have a negative impact on the chances of future employment due to psychosocial disability. Even though acne scar severity may be proportional to acne severity,

delay in treatment as well as genetic factors,[12] the psychological burden and distress imposed upon an individual may be disproportionately abundant compared to the disfigurement.

Therefore, early prevention and prompt treatment to minimize the appearance of acne scars effectively remains the backbone to better the quality of life in patients suffering with post acne scars.[13]

Precipitating /aggravating factors for acne:

1) Hormonal [14]

Periods of excessive hormonal activity, like menstrual cycle and puberty, possibly will contribute to the development of acne. An escalation in androgens during puberty follows enlargement of follicular glands and increased sebum production (Figure 1). Similar effect is shown by use of anabolic steroids. It has also been seen that acne develops earlier in females than males, which may be due to earlier onset of puberty in girls. Hormones implicated in acne are:

- Androgens, testosterone, dihydrotestosterone (DHT) and dehydroepiandrosterone sulfate (DHEAS)
- Insulin- like growth factor 1 (IGF-I).

Late onset acne is not uncommon. Acne vulgaris in adult women can be due to any underlying condition of pregnancy, polycystic ovary syndrome, hirsutism, or Cushing's syndrome.

Acne climacterica**:**

It is menopause associated acne which follows as the production of the endogenous acne inhibitory ovarian hormones estradiol and progesterone fails, allowing the unopposed action of the acnegenic hormone testosterone.

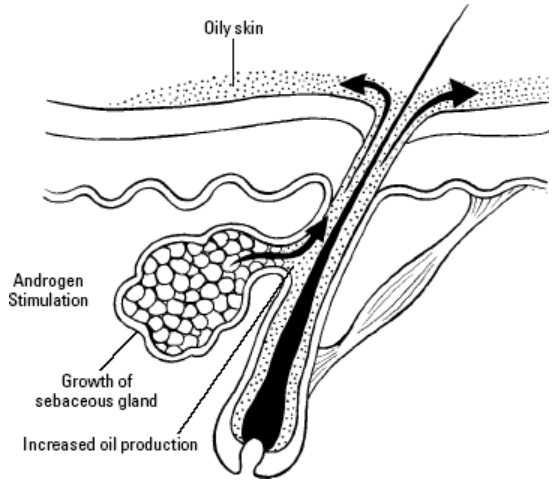


Figure 1: Growth of sebaceous gland.

2) Genetics

Positive family history has been seen in patients with severe persistent acne.[15] In monozygotic twins, sebum excretion rate is the same. The inheritance of predisposition is probably polygenic, as the disease defies classic Mendelian inheritance pattern.

3) Cosmetics

Heavy use of cosmetics can cause flare up of acne. It may be due to blocking of the pilosebaceous orifices by the chemicals. External application of oil, pomades (pomade acne) etc. can cause acne.

4) Sweating

Acne patients usually notice aggravation of acne by sweating. Hot and humid climate aggravates acne, due to increased sweating which causes ductal hydration.[16]

5) Menstrual flare

Premenstrual flare is probably due to altered hydration of pilosebaceous epithelium. [17]

6) Sunlight

There is no scientific evidence that sunlight improves acne, the improvement seen may be due to the cosmetic effect of tanning.

7) Stress

There exists a 'stress-acne-stress' cycle. Stress causes deterioration of acne. Acne itself causes stress. Under stress, body secretes stress hormone cortisone, which causes increased production of testosterones, in turn resulting in sebaceous gland stimulation and paving way for acne formation. Patient's meddling with the lesions causes aggravation of lesions.

8) Smoking

Smoking causes exacerbation of acne. Smoke contains polycyclic aromatic hydrocarbons and arachidonic acid, which induce phospholipase A dependent inflammatory pathway.[18] Smokers also consume diet containing high saturated fat and lower polyunsaturated, linoleic acid compared to those who do not smoke.

9) Diet

Foods with high glycemic index, milk products, sweets, chocolates etc. are known to exacerbate acne. They cause hyperinsulinemia leading to increased androgen synthesis.[19]

10) Drugs

Steroids, anticonvulsants, isoniazid, pyrazinamide, lithium, risperidone, vitamin B12 are known to induce acne like eruptions.

11) Racial differences

Although acne affects all population, few racial differences are known. Japanese are

said to be less affected than Americans. Also cystic acne is more common among the Whites.

Pathogenesis of acne:

Pathogenesis of acne vulgaris is multifactorial. Main factors involved are:

1) Seborrhea

2) Ductal hyperproliferation

3) *Propionibacterium acnes* colonization (*P. acnes*)

4) Inflammation

Seborrhea:

Sebaceous glands and sebum:

Sebaceous gland is a holocrine gland (Figure 2). They are present over most of the body, sparsely over the dorsum of hands and feet and almost never over palms and soles. Sebaceous glands are greatest in both size and number on face, scalp, upper trunk, external ear canal and perineal regions.

There are between 400-900 glands/ cm^2 over scalp, forehead, face and chin.[20] At other sites, there are less than 100 glands /cm^2. Sebaceous glands are active in newborns, but later they involute and become non-functional until puberty. Sebum is a compound mix of lipids, consisting of glycerides, free fatty acids, wax esters, squalene, cholesterol esters and cholesterol. It has fungi static properties.

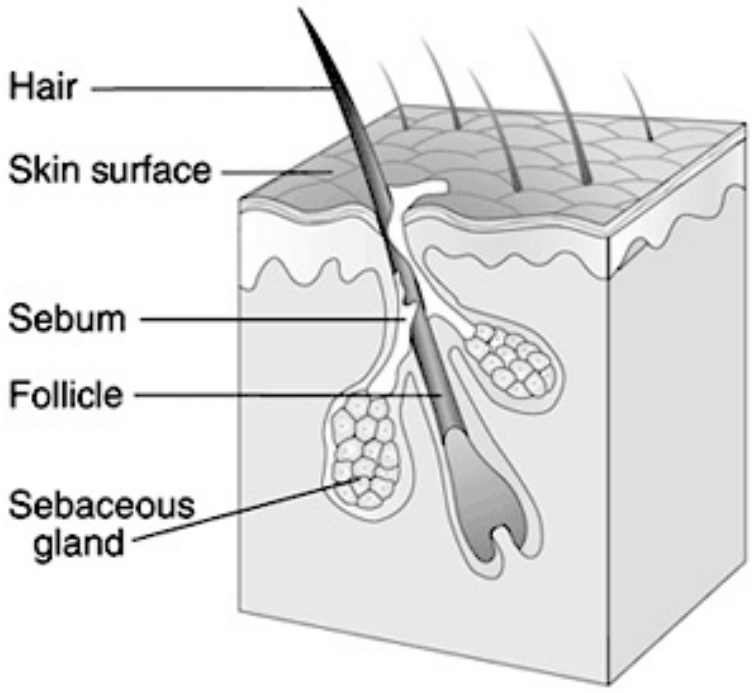


Figure 2: Structure of hair follicle.

Excess sebum production is a pre-requisite for acne formation. [21] The level of sebum production associates well with the severity of acne. This sebaceous activity is under the control of androgens, which can be of gonadal or adrenal source. In androgen insensitive subjects, there are no androgen receptors, there is no sebum production and no acne develops. The dehydroepiandrosterone sulphate is converted to dihydrotestosterone (DHT) and this binds to sebocyte resulting in excess sebum and comedones in acne prone patients. Androgens control function by binding to nuclear androgen receptors (ARs) present in the sebaceous gland.

Dehydroepiandrosterone sulphate (DHEAS)

Dihydrotestosterone (DHT)

Nuclear Androgen Receptors (AR) over sebocytes

Increased sebum production

Changes in the ductal microenvironment

Acne

Androgen affinity for the pilosebaceous unit is well-documented. Testosterone and DHT mediate their action via single nuclear androgen receptor, with dihydrotestosterone (DHT) as the most dynamic ligand. Sebum emission fluctuates from one follicle to another. In acne patients, there is an obvious difference in discrete follicular sebum emission. This hypothesizes that certain follicles might be susceptible to acne with peripheral (end organ) reaction to androgens being the major influence. Unusually elevated plasma DHT and urinary 5α-androstanediols, deliberated as markers of skin androgen metabolism is reported in a few female acne patients. The effect of androgen on sebaceous activity is independent of serum hormone levels.

Sebaceous glands in a few areas demonstrate unusually elevated 5α-reductase activity. 5α-reductase has been isolated from sebaceous glands from various body sites supporting the end-organ hyper-responsiveness theory for acne.

Mechanisms of increased sebum production under androgen effect

- Excess androgen production
- Increased free circulating androgens which may be accompanied by virtual reduction of sex hormone binding globulin (SHBG).
- Increased response of target cells
- Increased capacity of receptors to bind androgens.

Sampling from skin lipids has revealed that patients with acne are inclined to have greater level of squalene and wax esters, while fatty acids are at lower levels. Linoleic acid is suggestively reduced in epidermal and comedonal lipids, relating with ductal hyperproliferation.

Ductal Hyperproliferation:

The infundibular portion becomes hyperkeratotic .The stimulus to this hyperkeratosis may be androgens or due to the irritating effect of sebaceous lipids. There is also increased cohesion of keratinocytes. This leads to formation of a plug in follicular ostia in which bacteria, sebum and keratin accumulate. As a result, there is dilatation of follicle leading to microcomedone formation. Numerous influences have been involved in inducing keratinocyte hyperproliferation. These comprise of sebaceous fatty composition, androgens, cytokines produced locally and bacteria. Among abnormal sebaceous lipids, linoleic acid levels are of importance. Among the cytokines produced by keratinocytes, levels of Interleukin-1- alpha (IL-1-α) are important.[22] Certain externally applied chemicals present in cosmetics like isopropyl myristate and propylene glycol may also contribute to comedogenesis.

***Propionibacterium Acnes* Colonization:**

Acne is not an infectious disease, however, in case of inflammatory acne; the blocked pilosebaceous duct may get infected with *P. acnes* when it gets trapped in the cornified plugs within the ducts. *Propionibacterium acnes* causes production of proinflammatory cytokines (like IL-8 & Human –beta-defensin-2) by binding through Toll like receptors.[23] They are responsible for breakdown of triglycerides to free fatty acids which leads to follicular hyperkeratosis. It also produces other chemotactic and pro-inflammatory substances. Powerful hydrolytic enzymes levels arise, making tissue damage inevitable.

Toll Like Receptors are a group of proteins which contribute in body's innate immunity. They are a form of pattern recognition receptor. Thirteen TLRs have been identified in humans. *P. acnes* activates TLR-2 on keratinocytes and monocytes, thus, initiating manufacture of pro- inflammatory cytokines.

Inflammation:

It is the major cause of scarring. Linoleic acid, a deficient compound in acne, might initiate an alteration in the integrity of the barrier function within individual follicles.[24] The follicular basement membrane remains unbroken in spared follicles. Any unknown soluble antigen may prompt inflammation. Inflammation up-regulates sebum production, in genetically predisposed individuals, which elaborates IL-1 alpha, initiating comedogenesis.

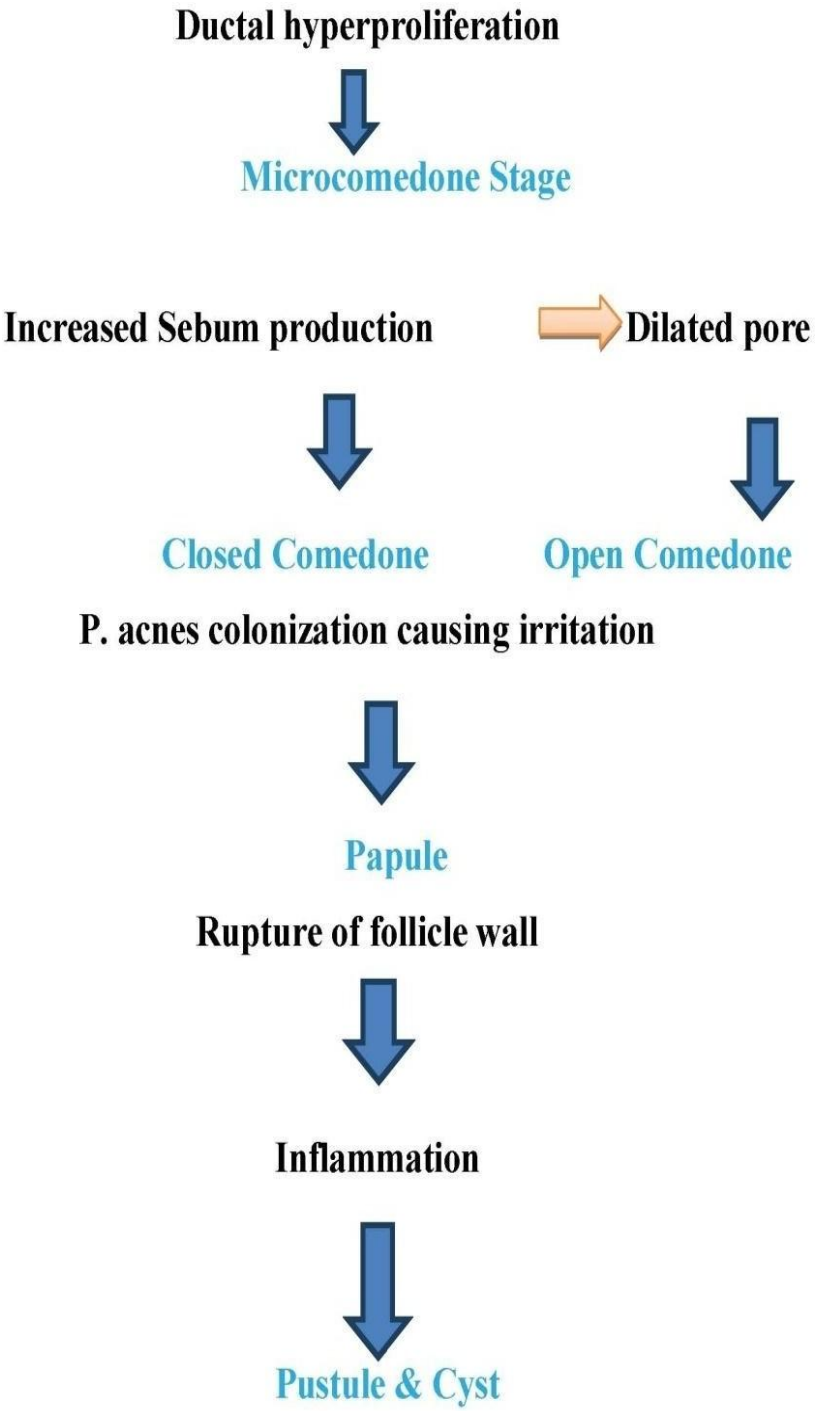


Figure 3: Progression of clinical acne lesions

Recent Concepts:

It has been believed for long that the initial lesion of acne is non – inflammatory. After colonization with *P. acnes,* due to the innate immune response, the inflammation leads to formation of papule, pustule or nodule. However, recent evidence and studies support that inflammation is present during all the stages of acne, may be sub-clinically, even before comedone formation.[25] The spared skin contains eminent levels of CD3+ and CD4+ T cells in the perifollicular and papillary dermis. Lipoperoxidation brings about modification of sebum composition which can affect keratinocytes proliferation and differentiation. Lipid peroxidation products can induce production of cytokines and activate of peroxisome proliferators- activated receptors (PPARs). Regulation of inflammation is transcribed by PPARs.

Clinical Features:

Acne presents as non-inflammatory and inflammatory lesions (Figure 3)

Comedones are the non- inflammatory lesions - They are the characteristic early lesions.

Types of comedones include:

Open comedones- They are also known as blackheads. Dome shaped papules with dilated follicular outlets filled with keratin. The visible black color is due to oxidation of keratin and melanin deposit.

Closed comedones- They are also known as whiteheads. Around one mm in diameter, skin coloured and there is no visible follicular opening. They can be visualized properly in suitable illumination and by stretching the skin.

Submarine comedones- They are larger than 0.5 cm in diameter. They lie further deeply into skin. These may be the foundation of continuing inflammatory nodular lesion.

Sandpaper comedones- Multiple, very small white heads, commonly over forehead. There is a rough, gritty feeling on touching.

Secondary comedones – They are caused by contact to dioxins, pomades, topical steroids. Inflammatory lesions include superficial lesions like papules and pustules (5 mm or less in diameter) & deep lesions like pustules and deep nodules. Nodules have been seen more frequently in males. They may be hemorrhagic or exudative, leading to disfigurement.

When sinuses are formed between pustules and nodules, it gives rise to distressing cosmetic disfigurement and inevitable scar formation.[26] Scarring typically trails deep-seated inflammatory lesions; however, superficial inflamed lesions in predisposed patients may also have the same outcome.

These events stimulate inflammation; rupture of follicle and perifollicular abscess formation leading to healing process mediated my immuno-inflammatory cells.

Wound healing in acne:

Wound healing of active acne takes places in 3 stages:

1. Inflammation
2. Formation of granulation tissue
3. Remodeling of matrix

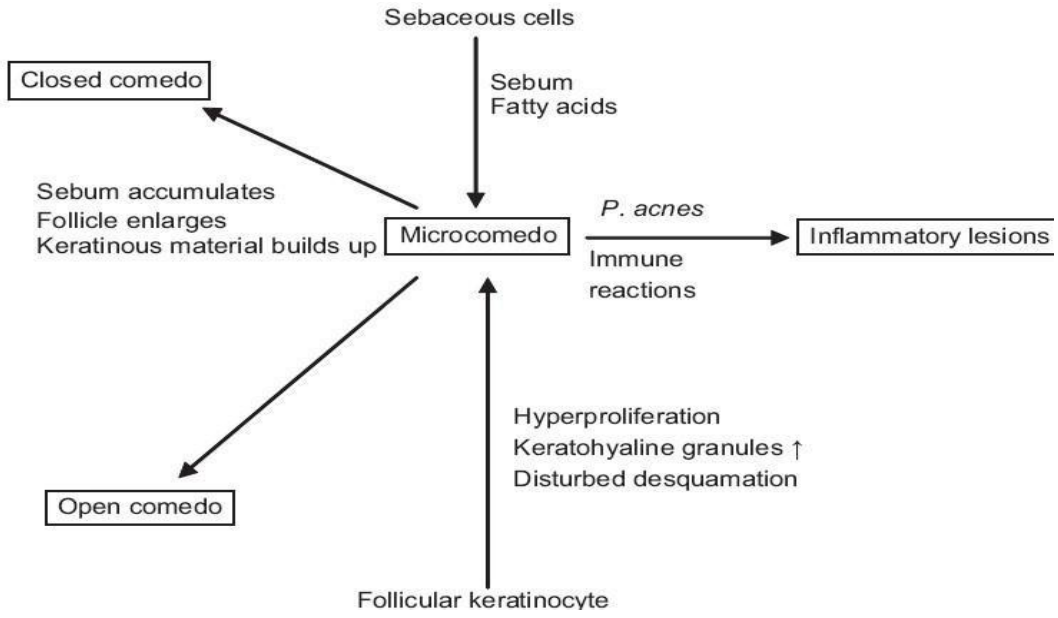


Figure 4: Pathogenesis of clinical lesions of acne

Melanogenesis may also be stimulated simultaneously leading to post acne hyperpigmentation. Due to inflammation, a disproportion of matrix metalloproteinase (MMPs) and tissue inhibitors of MMPs results in either atrophic scars due to poor response or hypertrophic scars due to exuberant response.[1]

Morphological classification of acne scars:

Acne scars are divided into atrophic and hypertrophic scars. Atrophic scars are formed due to loss of tissue, while hypertrophic scars are formed by excessive collagen synthesis. Atrophic scars can be described as following types; these are rolling and boxcar scars.[1, 3] (Table 1)(Figure 5)

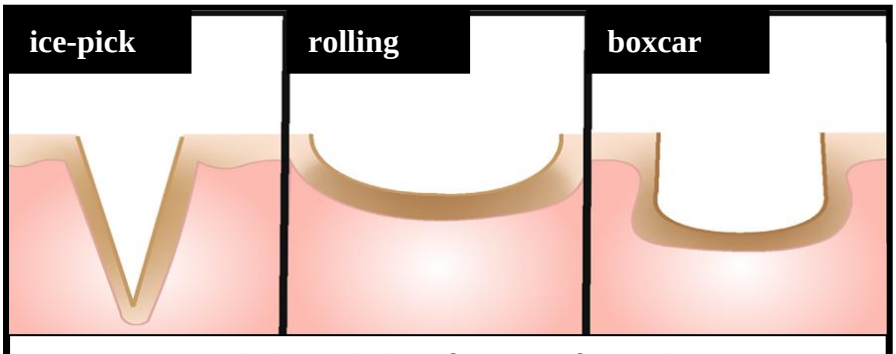


Figure 5: Morphological classification of atrophic acne scars

Table 1: Morphological classification of atrophic acne scars	
Acne scars subtypes	**Clinical features**
Ice-pick	Ice-pick scars are narrow (<2 mm), deep, sharply marginated epithelial tracts that extend vertically to the deep dermis or subcutaneous tissue.
Rolling	Rolling scars occur from dermal tethering of otherwise relatively normal-appearing skin and are usually wider than 4 to 5 mm. Abnormal fibrous anchoring of the dermis to the sub cutis leads to superficial shadowing and a rolling or undulating appearance to the overlying skin.
Boxcar Shallow <3mm diameter >3mm diameter	Boxcar scars are round to oval depressions with sharply demarcated vertical edges, similar to varicella scars. They are clinically wider at the surface than ice-pick scars and do not taper to a point at the base. They may be shallow (0.1–0.5mm) or deep (≥0.5mm) and are most often 1.5 to 4.0mm in diameter.
Deep <3mm diameter >3mm diameter	

Qualitative and quantitative acne scar grading and scoring system by Goodman:

Goodman developed a simple qualitative global acne scar grading system based on disease load based on patient's perception of severity and lesion morphology for more accurate grading and comparative analysis of acne scars over the world.[27] (Table 2)

Goodman and Baron also developed the Quantitative Global Acne Scarring Grading System, constituting the quantity and type of scar based on a point system. "The numerical

scoring scale is subdivided based on severity as follows: macular and mild atrophic scars receive 1 point; moderately atrophic scars 2 points; severely atrophic scars 3 points; hyperplastic scars 4 points. The severity score is then multiplied by a factor depending on the lesion count (1 point if lesion count\10, 2 points if between 11 and 20, and 3 points if greater than 20). The final score ranges from 0 to 84." [2] (Table 3)

Table 2: Goodman's global qualitative post acne scar grading system

Grade	Level of disease	Clinical features
1	Macular	These scars can be erythematous, hyper- or hypo pigmented flat marks. They do not represent a problem of contour like other scar grades but of color.
2	Mild	Mild atrophy or hypertrophy scars 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 men or normal body hair, if extra facial.
3	Moderate	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 men or body hair if extra facial, but is still able to be flattened by manual stretching of the skin (if atrophic).
4	Severe	Severe atrophic or hypertrophic scarring that is evident at social distances greater than 50 cm and is not covered easily by makeup or the normal shadow of shaved beard hair in men or body hair if extra facial and is not able to be flattened by manual stretching of the skin.

Table 3: Quantitative acne scar grading system by Goodman			
Atrophic scar severity	**Point value (A)**	**Lesion count**	**Point value (B)**
Macular/mild	1	<10	1
Moderate	2	11-20	2
Severe	3	>20	3
Total point score	A		B

Treatment options for post acne scars:

Several treatments exist to lessen the appearance of scars. Firstly, it is imperative to decrease as far as possible the time and intensity of inflammation. This is where acne treatment comes into play. They can be distributed into energy centred modalities and non-energy centred modalities.[12]

Non energy based modalities:

1. Pharmacological management

- Isotretinoin:

  It is a synthetic vitamin A derivative which regulates follicular hyper keratinization and reduces inflammation thereby reducing the likelihood of scarring in acne patients. It is recommended to achieve a cumulative target dosage of 120–150 mg/kg, typically over a period of 5-6 months.[28]

- Topical retinoid:

  Topical retinoids increase the collagen synthesis in the dermis and bring about an improvement in the quality of elastic fibres. This mechanism of action is beneficial for management of acne scars. It is popular as monotherapy for comedones and as an adjuvant for inflammatory disease.[28]

2. Procedural management

- Chemical peels:

It is a method by which various chemicals are used to bring about peeling of the skin. They damage the skin to allow a controlled wound healing process. Sloughing of dead cells takes place and there is subsequent re-epithelialization of the skin. This process increases the manufacture of collagen, elastin and glycosaminoglycans in the dermis.

Different chemical peels possess variable depth of penetration into the skin[4] (Table 4). Peels are a patient- friendly modality due to their relative non-invasiveness, simultaneous improvement in skin pigmentation, tone and texture. Side effects comprise of dyspigmentation and scars seen more comply with deeper peels. Fitzpatrick skin types IV-VI should be treated cautiously with peels given the propensity to cause hyperpigmentation.[28]

Table 4: Classification of peeling agents based on depth of penetration

Depth	**Peeling agents**
Very superficial	• 30-50% Glycolic acid • Jessners solution 1-3 coats • 10% TCA
Superficial	• 50-70% Glycolic acid • Jessners solution 4-10 coats • 10-30% TCA
Medium depth	• 70%Glycolic acid • 35-50% TCA
Deep	• Phenol 88%, Baker-Gordon phenol formula

- Subcision / punch excision:

  It is indicated in rolling scars. Subcision is a technique in which a needle is passed into the skin below the scar and a fanning motion is used to separate the tethered epidermis from the dermis. A blood clot then occupies the area to retain skin elevation from the fibrous tissue beneath and facilitate the formation of new collagen in the created space.[12, 28] Drawbacks include: bleeding, bruising, infection, acne exacerbation due to disruption of sinus tracts.

  Punch excision method employed for all types of scars uses a punch biopsy (size is dependent on scar diameter) to cut out the scar tissue. The exposed area is then sutured and permitted to heal. The fresh scar generated is less obvious than the previous atrophic acne scar. A replacement graft can be used after punch excision of a deep scar.[12]

- Dermabrasion/ Microdermabrasion:

  Dermabrasion (DA) and microdermabrasion (MDA) are facial resurfacing techniques. Dermabrasion used for deeper scars penetrates up to the papillary and reticulate dermis causing complete removal of the epidermis, inducing structural protein remodeling.

  Microdermabrasion only removes the stratum corneum superficially, causes acceleration of natural exfoliation of skin and is hence used for superficial scars only.[12, 28]

- Micro needling/ derma roller:

  It is a device that punctures the skin multiple times using fine needles for conditions such as post-acne atrophic scars and burn scars. This technique can be used for moderate to severe acne scars.[12, 28]

- Dermal fillers/ soft tissue augmentation:

Dermal fillers are impeccable in superficial atrophic scars (rolling type). It involves the injection of materials into the dermis or sub dermis to deliver confined volume improvement. They encourage collagen production simultaneously. Various fillers with diverse characteristics are classified based on biodegradability.[28] Materials used for fillers include hyaluronic acid, calcium hydroxyapatite, poly-L-lactic acid.[12, 28]

Energy based modalities:

Laser systems are dynamic technologies that permit for a wide array of skin problems to be addressed. Laser treatment of acne scars is amplified by appropriate scar categorization. The choice of laser wavelength and treatment parameters are influenced by several qualities of the scar, including size, colour, texture and prior treatments.[4] (Table 5)

Table 5: Laser intervention modalities for atrophic acne scars

Laser used	**Atrophic Scar type**
Non-ablative	Type1-3 rolling scars
Fractional erbium	Type 1-2 scars
Fractional carbon dioxide	Type 1-4 scars
QS- Nd: YAG	Pigmentation in Q switch mode and type 1-2 scars in quasi pulse mode

LASER [29]

Laser is an acronym for "Light Amplification by Stimulated Emission of Radiation". The pioneering work in the dermatological application of laser was done by Dr. Leon Goldman who is considered to be the Father of laser medicine. A substantial comprehension of lasers and light sources as well as laser physics is mandatory for their ideal usage.

Laser Characteristics: [30]

Laser radiation is part of the electromagnetic spectrum as it is a light form. Laser light contrasts from standard light like sunlight or incandescent light on numerous aspects.

Laser is formed of *monochromatic photon* i.e. light from a given source is of a single wavelength. This property enables lasers to selectively target chromophores in the skin with a corresponding single wavelength.

Laser light is *coherent,* i.e. the waves of energy stay in phase with one another both in space and in time.

Laser light is also *collimated* i.e. the laser beam component waves are highly parallel, producing a narrow beam that can be propagated for long distances with minimal divergence or convergence.

Parameters of Laser Light: [30]

The interaction of laser energy with the tissue is subject to a number of factors including power, spot size, and duration of exposure, wavelength and tissue properties. In order to achieve consistency in performance the following parameters have to be considered.

1. Energy that is contained in the light is expressed in joules.
2. Power is defined as the time rate at which energy is emitted by the laser and is measured in watts (joules/second).
3. Irradiance or power density is the concentration of the beam of light and is expressed as the power applied per unit area (watts/cm_2). This determines the ability of a laser to

coagulate, vaporize or incise tissue.

4. Fluence or energy density is the actual amount of energy applied to the unit area of target tissue and depends on the exposure time.

Fluence (joules/cm^2) = Power x exposure time (seconds) / area (cm^2). Smaller the radius of the laser beam (spot size), higher is its fluence.

5. The wavelength of light emitted by a laser gives it its characteristic color (green, red, yellow) within the visible range and is measured in nanometers (nm).

6. Pulse duration is the amount of time the laser energy is applied (ns, ms).

7. Pulse frequency is measured in hertz. (Hz = 1 pulse / second). It is the repetition rate of pulse.

8. Transverse electronic mode (TEM) is the distribution of energy across the laser beam diameter.

9. Modes of output (i.e. delivery): The light is delivered in two broad categories described according to whether the beam is delivered in a continuous wave (eg. Carbon dioxide – CO_2 laser) or in a pulse wave.

a. In the continuous wave (CW) mode, there is an uninterrupted (constant) beam of radiation of relatively low power. Energy is delivered at some mean level (power density, spot size) continuously for as long as the operator wants (e.g. argon laser, CO_2 laser). The advantages are that larger areas can be treated rapidly with lesser time consumption. The disadvantage is its reduced safety profile and thus more chance of side effects and complications.

b. In the pulse mode, the continuous beam can be interrupted (on and off) to form pulses when light is emitted in spurts of low energy but with peak powers. This is achieved by cutting the beam either with simple mechanical shutters, electronically operated switches, mode locking, Q switching or through controlled pumping and discharge. These

mechanisms produce a single pulse, multiple pulses or pulse trains with a width or duration ranging from milli to several hundred micro to nano seconds. Simple pulsed lasers produce the same peak power as continuous wave, whereas super-pulsed and ultra-pulsed lasers produce higher peak power with lesser pulse durations. In Q-switched mode the pulse is of very short duration with further increase in the peak power. The pulse systems allow improved energy delivery systems allow improved energy delivery systems with uniform dose of radiation across the treatment area as compared to CW lasers. There is a high safety profile because these systems minimize the thermal injury by allowing for tissue "cooling phase" during the "off" portion of the cycle. However the disadvantages is that it is more time consuming than CW systems.

Selective Photothermolysis: [31]

It was introduced in 1983 by Anderson and Parrish.[32] The theory of selective thermolysis refers to laser energy being absorbed by a target chromophore without significant damage to surrounding tissue.

Selective photothermolysis can be achieved by:

1. Producing a beam of light with a wavelength that is preferentially absorbed by that chromophore.
2. Keeping the fluence high enough to thermally alter the target.
3. By shortening the pulse duration to less than the thermal relaxation time of the chromophore, thereby allowing it time to cool by conducting heat to surrounding tissues – thus preventing damage to them.

CO_2 Laser System:[33]

CO_2 laser was introduced in 1964 and is referred to as the 'work horse' owing to its widespread usage as a cutting ablative laser. In the 1980's and the early 1990's continuous wave (CW) CO_2 lasers were used for photo-aged skin. Several new CO_2 laser systems have been introduced with high peak power short pulse duration and rapidly scanning devices to treat a variety of dermatological conditions.[34]

The CO_2 laser produces light (10 600 nm) which is relatively poorly absorbed by the chromophore, water, therefore, penetrates relatively deeply (30 μm).[35]

Laser medium – A mixture of CO_2, nitrogen and helium (1:1.5:4)

Optical Cavity – Encloses the lasing medium and amplification process takes place here

Energy Source – Required for the amplification process. This may be direct electric current, radio frequency waves, optical flash or energy derived from chemical reaction

Delivery System - Brings the laser beam from the machine to the patient. It uses a series of articulating joints and mirror to produce far infrared light of longer wavelength.

Mechanism of action:[36]

- CO_2 laser radiates imperceptible far infrared light at the wavelength of 10,600 nanometer, either in a continuous wave or pulse/super-pulsed/ultra-pulsed mode.
- The depth of penetration is 0.1 mm per pass and 90% of it is selectively absorbed by tissue water which is its target chromophore.
- Based on the principle of selective thermolysis, the target chromophore gets heated and thermally damaged by the absorbed laser energy.
- When the heating is speedy all the tissue water is vaporized as steam and the tissue structures explode due to rapid thermal expansion of water, sharp waves and cavitations

effects (tissue ablation). This tissue debris together with steam forms the "plume".

The vaporization occurs in the most superficial layers of tissue (zone of vaporization). Below the vaporized layer lies a plane of tissue heated between 70^{O}C to 100^{O}C resulting in tissue necrosis (zone of irreversible damage). Deeper to this, there is a zone of tissue that is heated between 35^{O} C to 70^{O} C (Zone of reversible damage) resulting in shrinkage of collagen. All these thermal effects are responsible for the simple tissue changes which are observed: ablation, tightening, remodeling, and re-epithelialization. If numerous pulses quickly impact the same tissue (pulse stacking) then substantial cumulative thermal damage occurs resulting in deeper wound and scarring.

Fractional photothermolysis or fractional CO_2 laser is a technology developed by Anderson and Manstein that ablates fractions of skin as an alternative to the entire skin, the immediate effect of which is vaporization of the epidermis and superficial papillary dermis. There is also thermal damage to a band of underlying dermis, together with collagen denaturation and contraction. In the healing period, regeneration occurs from surrounding skin and from adnexa. This is followed by the formation of a band of dermal fibrosis, often referred to in the cosmetology as 'collagen remodelling'.[35]

The elements affecting the tissue damage by laser are:

- The power reaching the tissue.
- Spot size: This can be changed either by defocusing or focusing the beam by changing its distance from the treated area. Spot size of 2-5mm is classically utilized for vaporization.
- Thermal relaxation time (time between heating of tissues and cooling of tissue).

Clinical Applications: The CO_2 laser is used to perform various types of surgeries through its two modes of action i.e. either the excisional / cutting mode or vaporizational / ablative mode.

- The excision mode employs a focused small spot size beam of high power to generate satisfactory intensity to cut soft tissues.
- A large beam of low power is used in vaporizing mode to precisely ablate the soft tissue superficially.

Both these actions are achieved either with continuous mode or pulse / super- pulse / ultra-pulse system of lasers.

Indications:

- Facial scars[37] – acne, chicken pox, herpes zoster, traumatic.
- Hyperkeratotic lesions – Lichen simplex chronics, papular lichen amyloidosis, porokeratosis, and linear verrucous nevus.
- Appendageal lesions - neurofibroma cylindroma, syringoma, adenoma sebaceum, [38] multiple trichoepitheliomas, sebaceous hyperplasia, epidermal nevi.
- Vascular lesions – lymphangioma circumscriptum, [39] angiokeratoma, pyogenic granuloma.
- Premalignant and malignant lesions – actinic keratosis, actinic chelitis, leukoplakia, Bowen's disease, [40] extra mammary Paget's disease, erythroplasia of Queyrat, superficial basal cell carcinoma.
- Stable vitiligo - Therapeutic adjuvant (spot) and before grafting.
- Pigmentation – Melasma, melanosis, tattoos, lentigenes, etc.
- Miscellaneous – photoaging[34], rejuvenation, xanthelasma, [41] keloid.

In a study done by Alster *et al.*[42] for 50 subjects exhibited scar improvement of 70-90%

with fractional CO_2 laser. In another study done by Bernstein *et al.* 50-75% showed improvement. It was found that, 36% of patients reported transient hyperpigmentation resolving spontaneously within 12 weeks. Milia were seen in 14% patients; patients remained without infections.

Contraindications:[43]

- Active cutaneous bacterial or viral infection
- Ectropion
- Keloidal tendency
- Collagen vascular disease
- Simultaneous UV treatments
- Past radiation therapy to target area

Complications:

Usual events – pain, edema, exudation, discomfort, crusting, erythema.

Adverse effects– persistent erythema, hyperpigmentation, permanent hypopigmentation, exacerbation of acne, secondary infection, allergic contact dermatitis, scarring, ectropion, keloid formation.

Advantages:

- Excellent cosmetic improvement (50%-80%).
- All the scars can be treated at one time.

Disadvantages:

- Expensive
- Sun protection and prolonged post operative cream application
- Permanent hypopigmentation
- Deep ice pick scars cannot be corrected by laser alone.

PLATELET RICH PLASMA

Components of blood:

Blood consists of red blood cells, white blood cells, platelets and plasma.

Plasma**:**

It is relatively clear and yellow tinted constituting 55% of the blood volume. It carries RBCs, WBCs, platelets and contains various hormones, enzymes, proteins and antibodies.

Red Blood Cells**:**

They are large microscopic cells without nuclei making up 40- 50% of total blood volume. Their main role is oxygenation of tissues.

White Blood Cells**:**

They constitute 1% of total blood volume and are a component of body's defence mechanism. They comprise of neutrophils, eosinophils, basophils, monocytes, lymphocyte.

Platelets**:**

They are cytoplasmic fragments of megakaryocytes (a type of white blood cell), which are produced in the bone marrow. They are round or oval in shape, approximately 2 mm in diameter. Platelets do not have a nucleus, however, they contain organelles and granules (α, δ, λ). The α granules hold around 30 bioactive proteins which play a role in hemostasis and tissue healing.

In PRP therapy the basic objective is applying supra- pharmacological dose of platelets directly at the site where tissue regeneration is required. It helps to rejuvenate & regenerate injured tissues and also modulates wound healing.

Definition of PRP:

Platelet-rich plasma (PRP), also called as autologous platelet gel or plasma-rich growth factors and, platelet-concentrated plasma means "abundant platelets that are concentrated into a small volume of plasma."[43]

The unearthing of platelet-derived growth factor (PDGF) in encouraging wound restoration, angiogenesis and skin remodeling brought to notice this fresh autologous therapeutic modality. This mixture of growth factors plays pivotal role in modulation of tissue repair and regeneration.[44]

Degranulation of the pre-packaged growth factors in platelets get "activated" after coming in contact with coagulation triggers leading to its degranulation and hence, release of the pre-packaged growth factors. The secreted growth factors fix themselves to their individual trans membrane receptors present over various cells types. This brings about an internal signal-transduction pathway, unraveling the expression of a normal gene sequence of a cell like cellular division and growth, formation of collagen, osteoid tissue and matrix etc., thereby enhancing the ordinary wound-healing progression.

History of PRP:

PRP has been therapeutically used in dentistry since 1998, and the clinical application of PRP was recently expanded to other fields, including cosmetic medicine.[45] Different ways of concocting PRP have come into being: orthodox blood centrifugation to commercial systems; applied as platelet suspension or as a gel; and improvement in this methodology is an on-going process.

It is a biological product containing platelet-derived factor which when injected into a tissue acts to generate the wound healing process without any actual trauma. It encourages local tissue remodeling and angiogenesis by activation of tissue-resident as well as marrow-derived progenitor/stem cells.

For preparing PRP , two methods have been explained depending upon the number of centrifugation steps used: one is the single spin method and the other is double spin method. The subsequently prepared platelet-rich plasma is stable, in the anti- coagulated

state, for eight hours[46] and requires activation for the release of their content.

PRP is prepared on an out-patient basis just before the procedure. "The process must be carried out under strict aseptic conditions as well as optimum temperature regulations i.e.20-22°C. In order to inhibit platelet aggregation, it is prepared with an anticoagulant, commonly using anticoagulant citrate dextrose solution formula A (ACD-A) [47] or sodium citrate. The platelets need to be sequestered in high concentrations, enough for achieving therapeutic benefit and in a viable state at the same time, so that they can actively secrete their growth factors."

So this recent technology permits us to focus platelets and white blood cells from blood and to prompt the discharge of growth factors by injection directly into injured tissue, encouraging the identical healing process in a directed fashion.

Various growth factors present in PRP are **–**

1) **Transforming growth factor beta (TGF) β1** – mediates angiogenesis. **TGFβ 2-** Acts as chemotactic for fibroblasts, keratinocytes and macrophages. It is also mitogenic for smooth muscle cells and fibroblasts. It also has potential to regulate matrix proteins, collagen and proteoglycans.

2) **Platelet derived growth factor (PDGF) αα** –It is chemotactic for fibroblasts and macrophages. PDGF ββ and αβ – They are mitogenic for fibroblasts, smooth muscle cells and endothelial cells.

3) **Vascular Endothelial Growth Factor (VEGF)-** Chemotactic and mitogenic for endothelial cells.

4) **Epidermal growth factor** – It is mitogenic for fibroblasts, keratinocytes and endothelial cells.

5) **Fibroblast growth factor-2 (FGF-2)-** It has a role in tissue organization and regeneration.

6) **Fibroblast growth factor 9**- Helps in regeneration of hair follicle.

7) **Hepatocyte growth factor** – Helps in regeneration.

PRP preparation by manual double spin method: [48, 49]

According to The American Association of Blood Banks technical manual, first the 'platelet-rich plasma' is divided from whole blood by 'soft or light-spin' centrifugation following which the platelets are concentrated by 'hard or heavy-spin' centrifugation. The basic principle behind the PRP separation procedure is as follows: Different blood components have different specific gravities. So on centrifugation they get separated into different layers.

As the red blood cells are heaviest they settle at the bottom, then come the white blood cells and the topmost deposit is of platelets as they are lightest. In the first step aim is to separate the plasma from rest of the components. This is done by a slow centrifuge, after which platelets get concentrated just above the buffy coat. In the later stage, spin is rapid so that platelets get concentrated and settle down at the lowermost portion of the tube.

Almost three fourth of the supernatant is thrown out and the platelet- rich pellet is re-suspended in residual plasma. Calcium chloride ($CaCl_2$) or thrombin may be added as an "activator" to activate the platelets.

Maximum secretion of growth factors occurs within ten minutes of activation, so the activated PRP must be used as early as possible. There is variability in the yield of platelets obtained depending upon the methods employed, rate and time of spin, anticoagulant used and even size and shape of the container. The platelet yields may vary from 4 to 7 times the baseline. To assure the viability of platelets the temperature should be maintained between 20- 22^{o} C. Trypan blue staining can be used to confirm the viability.

Double spin method is used preferably over single spin method, as studies have shown that the single spin method failed to achieve the therapeutic levels of platelets. As there are different protocols, devices and centrifuge speeds for preparing PRP, different types of platelet concentrates are obtained. It was Ehrenfest *et al.* who first proposed a classification for the platelet concentrate.[50]

It was classified into four types depending upon the leucocyte and fibrin content:

1) **P-PRP (Pure Platelet Rich Plasma) -** This can be prepared by collecting only the buffy coat alone after the first soft spin. Very few leucocytes will be present.

2) **L-PRP(Leucocyte and Platelet Rich Plasma)** – This type contains mostly platelets with few but appreciable amount of leucocytes. There is difference in collection of PRP. After soft spin, plasma, buffy coat and topmost layer of RBC is harvested. Later, after hard spin, lowest fraction of product is harvested which contains all platelets and few leucocytes.

3) **P-PRF (Pure Platelet Rich Fibrin)** –This is obtained by mixing PRP with activator and incubating it for some time so that a stable platelet rich fibrin clot is formed .

4) **L-PRF (Leucocyte and Platelet Rich Fibrin)** – In this no anticoagulant added and no activator required. First blood is collected without any anticoagulant and centrifuged without delay. The process results in three layers. L-PRF layer is formed in middle and harvested.

Concentration of platelets in PRP:

The average concentration of platelets in blood is 200,000 ± 75,000/μL. For a preparation to be labeled as "PLATELET RICH", the concentration of platelets should rise to level of five to ten times the base line.[51]

Nowadays in the market, various automated devices are available for preparing PRP. However, these devices are expensive compared to manual methods and the commercial interest of the manufacturers can deteriorate the quality of platelet concentrates.

Indications of PRP in dermatology:

1) **Alopecias**–PRP has been used as incubation medium in Follicular Unit Transplant as well as mesotherapy in androgenetic alopecia.[52, 53] Significant hair growth has been seen in alopecia areata and telogen effluvium.[54]

2) **Skin rejuvenation** – PRP has become very popular in aesthetic medicine. It is shown to remove photo damaged extra cellular matrix and induce synthesis of new collagen. PRP can be applied topically under occlusion or given as intradermal injections.[54]

3) **Acne scars and contour defects**– PRP has been used as mesotherapy alone or in combination with other modalities like derma roller or laser resurfacing.[55]

4) **Wound ulcers and connective tissue disease associated ulcers**- Stasis ulcers, trophic ulcers, diabetic ulcers, venous ulcer, lipodermatosclerosis and traumatic ulcers have shown good healing after treatment with PRP.[56, 57, 58]

5) **Striae distensae**[57]- Kim *et al*. used an intradermal radio- frequency device along with injectable PRP as tissue augmentation through its needle electrode.

6) **Lichen Sclerosus**[59]-PRP along with autologous fat transfer is a novel technique in management of lichen sclerosis of vulva.

Safety of PRP:

Autologous PRP is quite safe. The mitogenic effect of PRP is limited to the standard healing procedure. PRP remains non-mutagenic as whatever growth factors it delivers, act through signal transduction only.[60] These growth factors fail to move into the cells or nucleus. There may be limited injection site reactions like transient erythema or pain. Secondary infection is rare when the procedure is carried out under strict septic precautions. Since PRP is autologous, there is no risk of transmission of hepatitis B, C or HIV.

Review of fractional CO_2 and PRP in dermatology literature:

Monotherapy with intradermal PRP for acne scars has been reported to be beneficial.[5] Recently, topical as well as intradermal PRP injections have been used for acne scars combined with laser therapy with varying results. As fractional CO_2 laser creates intermittent cutaneous thermal injury and PRP aids in wound healing, merging both would undoubtedly result in additive benefit.[5, 61] Lack of studies in an Indian setting is a driving force to undertake this study.

A split-face trial of sixteen patients by Faghihi *et al.* in Iran with one side treated with fractional CO_2 laser alone and the other half treated with a combination of fractional CO_2 laser with intradermal PRP showed overall better clinical improvement of acne scars on the side treated with PRP after 2 sessions one month apart. It also showed lesser side effects on the PRP side than the side treated with laser alone.[62]

A study in 2014 compared the efficacy of autologous platelet rich plasma injected intradermally and applied topically after fractional CO_2 laser and that of fractional CO_2 laser alone in atrophic acne scars in Cairo. Thirty patients underwent a split-face trial for three sessions and the results were evaluated at six months. Outcomes showed combining PRP and CO_2 laser gave superior results and lesser down time. Treatment with topical PRP had significantly lesser pain scores.[5]

An Egyptian study of 20 patients by Abdel Al *et al.* was conducted recently where patients were treated for acne scars by fractional CO_2 laser and the right side of the face was treated with topical PRP post laser. The right side of the face showed excellent improvement in comparison to the left side along with high patient satisfaction on the right side.[63]

In another study by Kar and Raj measuring the efficacy of fractional CO2 vs. fractional CO2 with topical platelet-rich plasma in management of acne scars, there was a significant improvement on both side of the face. Edema and pain was significantly lower on

side treated with fractional CO2 laser combined with PRP compared with only fractional CO_2.[64]

Shah *et al.* evaluated the efficacy and safety of intradermal PRP combined with fractional CO_2 laser for the treatment of post acne scars through a comparative split face study. The study concluded that the patient rated significantly lower scar severity scores when treated with PRP as an adjuvant to fractional CO_2 laser for post acne scars with lower edema and persistent erythema in the PRP treated side compared to the placebo side at the end of 4 months. [65]

A study on combination of PRP and fractional CO_2 laser to increase the efficacy of acne scar by enhancing collagen production and for modulating of laser-induced inflammation by Min *et al.* enrolled 25 patients. The results reported were increased efficacy and reduced laser induced inflammation. It was also concluded that PRP causes both dose and time dependent increase in fibroblast activity which was confirmed histopathologically. [66]

On the basis of the various studies available, it gives us the idea that the PRP can indeed improve the quality of acne scars treated with the ablative fractional CO_2 laser and decreases the duration of the side effects due to laser like edema and erythema.[67]

With the available literature on fractional CO_2 laser followed by PRP for acne scars, it is unmistakable that it is a safe and a promising tool in the treatment for atrophic acne scars. Moreover, fractional CO_2 laser and PRP are both efficacious procedures which can be used to attain great results and patient satisfaction. The side effects of fractional CO_2 laser like prolonged erythema, edema and post inflammatory hyperpigmentation can be alleviated by using topical PRP. The present study is undertaken to determine the efficacy of combination of topical PRP with fractional CO_2 laser compared with fractional CO_2 laser alone.

METHODOLOGY

METHODOLOGY:

SOURCE OF DATA

Patients with post acne atrophic scars of moderate to severe grade, attending outpatient department of Dermatology, Venereology and Leprosy of B.L.D.E.(DU)'s Shri. B.M. Patil Medical College Hospital and Research Centre, Vijayapura, were enrolled for the study.

Period of study:

The study was conducted during the period of November 2017 to June 2019.

Study design:

A hospital based prospective study.

METHOD OF COLLECTION OF DATA:

A total of 33 patients of age group of 18 years and above with post acne atrophic scars of moderate to severe grade were enrolled for the study.

Inclusion criteria:

1. Patients with atrophic acne scars of moderate to severe grade

Exclusion criteria:

1. Patients with active acne
2. Patients with macular/mild atrophic acne scars
3. Predisposition to keloid formation/ hypertrophic scar formation
4. Patients active bacterial or viral infection
5. Patients who have undergone fractional CO_2 laser ablation in the past 1 year

Methods:

Detailed history with respect to the onset and duration of acne and scarring, any treatment for acne/acne scars received within past 6 months and pre-existing medical conditions were recorded.

Initial clinical examination of the patient was done by one of the investigators to determine the grade and score of acne scars using Goodman and Baron qualitative grading and quantitative scoring chart. These findings were recorded in the proforma (first visit record). Informed consent for the study was undertaken from all the patients.

Thirty three cases of atrophic acne scars of moderate to severe grade were allocated alternately into two groups. Patients in GROUP A with 17 patients were treated with fractional carbon dioxide laser ablation followed by topical platelet-rich plasma. Patients in GROUP B with 16 patients were treated with fractional carbon dioxide laser ablation only. Baseline investigations and clinical photographs were taken. The end point of the study was 3 laser ablation sessions.

Three laser sessions were performed 4 weeks apart and patient were assessed before the first session, at 4 weeks, 8 weeks and at the end of 12 weeks. Clinical photographs and acne scar score/change in acne scar score was recorded at baseline, after 4 weeks, 8 weeks and at the end of 12 weeks prior to successive sessions for patients in both the groups. At the end of 12 weeks, baseline and post treatment photographs were compared for final assessment.

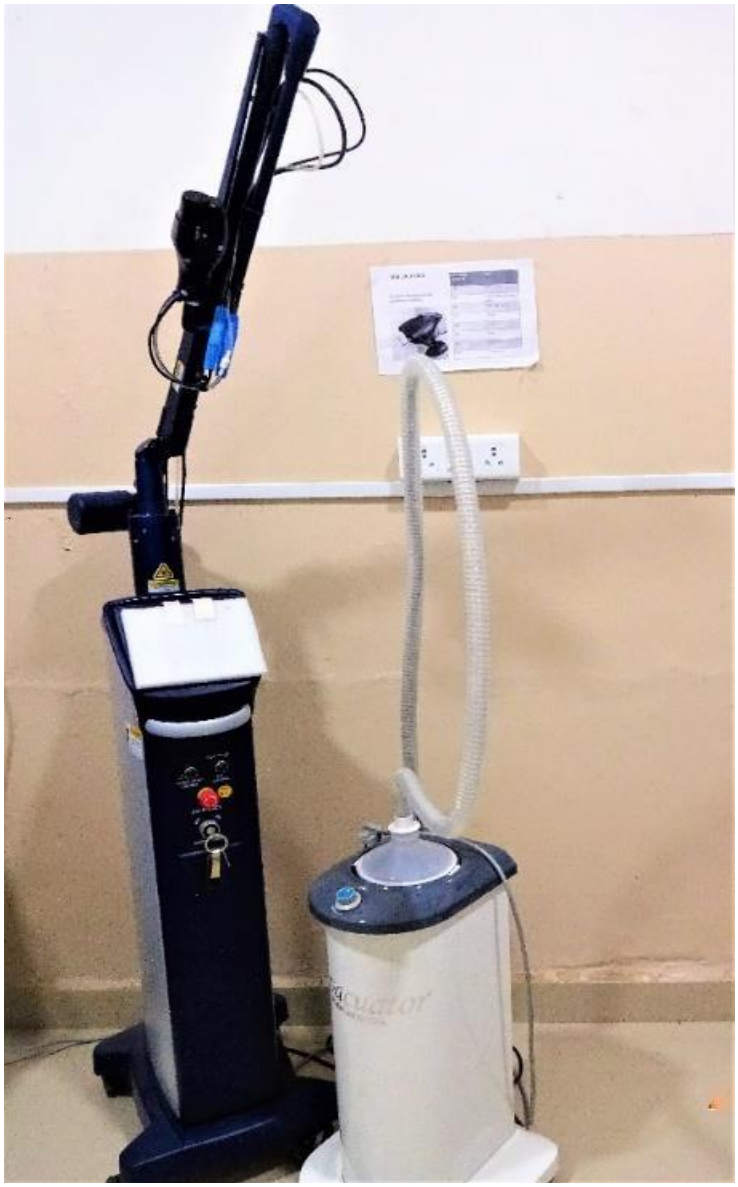
Methodology:

Equipments:

i. *eCO_2 laser:* The fractional CO_2 Laser (Lutronic Corporation, Goyang, Korea) is a newly upgraded fractional CO_2 system with wavelength of 10,600nm which has a high absorption rate to intra/extracellular water causing tissue vaporization (Figure 6).

The acne scars were treated with fractional CO_2 Laser 10,600nm at 4-weekly intervals for 3 consecutive sessions. The treatment settings were as follows: a pulse energy of 50-100mJ and a spot density of 50-100 spots cm^2 in the static mode; 1-2 passes were delivered using a 120-density tip with beam size of 120μm and peak power of 30W. The laser was irradiated to the entire area containing acne scars.

Figure 6: Fractional carbon dioxide laser



ii. *Method of Platelet-rich plasma preparation:* Two-stage centrifugation process (double-spin method) was employed in the preparation of PRP. Whole blood samples (10ml) will be drawn from the patient and transferred into a tube prefilled with citrate

anticoagulant solution. The mixture was centrifuged at 1600rpm for 7 minutes (first spin). After the first spin, the lower red blood cell portion was discarded and supernatant containing platelet-poor plasma and buffy coat was centrifuged again at 4000rpm for 5 minutes (second spin). The lower $1/3^{rd}$ of this solution provided approximately 2ml of autologous platelet-rich plasma for topical application.

***Procedure**:*

The face was cleaned with mild cleanser before the procedure. Topical anaesthetic containing lidocaine 2.5% + prilocaine 2.5% was applied under occlusion and left for 1 hour. The topical anaesthetic was completely removed. The affected areas were irradiated with fractional CO_2 laser using the same standard parameters in patients of both groups.

After the laser, patients in GROUP A received topical autologous platelet-rich plasma application. All patients were instructed to avoid washing their face for 6 hours after the procedure, direct sunlight, heat or friction on the treated areas. Similarly, two more sessions were performed 4 weeks apart using the above mentioned protocol.

***Follow -up*:**

The patients in both groups were asked to come for next laser session after 4 weeks and 8 weeks and thereafter for assessment at the end of 12 weeks. During each visit investigator recorded the findings related to treatment response like reduction/ improvement in acne scars (acne scar score), patient satisfaction and general perception regarding the treatment. Clinical photographs were taken in identical settings and lighting at every follow up before successive laser session. Any adverse effects related to therapy was recorded in the proforma after immediately after laser ablation (short term) and at each follow up before successive session (long term). At the end of 12 weeks the final response was evaluated according to the above- mentioned procedure.

***Efficacy evaluation*:**

The 2 primary efficacy parameters were assessed:

i) Objective assessment- Change from baseline acne scar score using Goodman and Baron quantitative scoring system at the end of 12 weeks(done by the investigators as per above protocol).

ii) Subjective assessment- Patient assessment of improvement of acne scar after at the end of 12 weeks; patients in both the groups will be asked to mark their improvement on a 10 inch long 'visual analog scale (VAS).'

INVESTIGATIONS:

Following investigations were done:

1. Complete blood count
2. Random blood sugar (RBS)
3. HIV, HbSAg testing.

STATISTICAL ANALYSIS:

1. The data obtained were entered in a Microsoft Excel sheet, and statistical analysis was performed using statistical package for the social sciences (Version 17).
2. Results are presented as Mean±SD, counts and percentages and diagrams.
3. Results were compared using Independent t test /Mann Whitney U test, paired t test, repeated measures of ANOVA and One way Anova.
4. Categorical data were compared using Chi square test.
5. For all tests, significant was achieved at $p<0.05$

RESULTS

RESULTS:

A hospital based prospective, double blinded, randomized, comparative study was conducted from November 2017 to June 2019. A total of 33 cases of post acne atrophic scars were included in the study. The enrolled patients were allotted into 2 groups:

-Group A (fractional CO_2 laser with topical PRP)

-Group B (fractional CO_2 laser only)

Age and Gender distribution

Among the 33 patients enrolled, 21 (63.6%) were male and 12 (36.4%) were female. There was no significant difference in the gender distribution between the two groups (Table 6, Figure 7)

Table 6: Gender distribution.

	Frequency (Percentage)
Female	12 (36.4%)
Male	21 (63.6%)
Total	33 (100%)

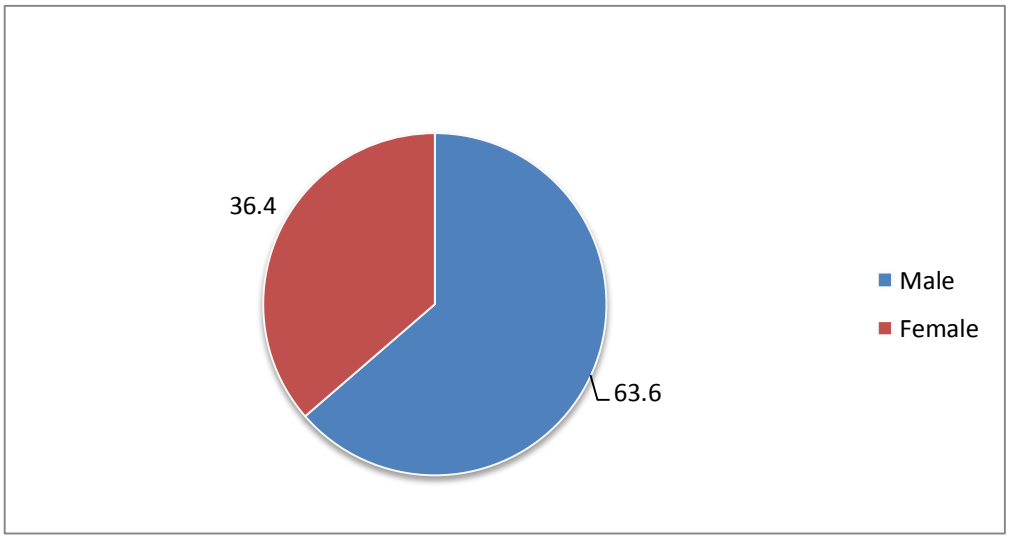


Figure 7: Gender distribution.

Mean age, duration of acne and duration of scars distribution

Mean age of the patients was 24.36 ± 4.37 years. The mean acne duration in the patients included was 4.7 ± 2.25 years. The mean duration of scars in the patients included was 2.74 ± 1.57 years. There was no significant difference in these variables between the two groups. (Table 7)

Table 7: Mean age, duration of acne and duration of scars.

	Mean ± SD
Age in years	24.36 ± 4.37
Acne duration in years	4.7 ± 2.25
Scar duration in years	2.74 ± 1.57

The distribution of patients based on type of treatment received for acne

Out of 33 patients in the study, 18 patients had received topical antibiotics, 7 patients had received OA, 2 patients had received topical retinoids and 2 patients had received oral retinoids for acne. Only 1 patient had received intralesional triamcinolone for acne. A combination of the treatments mentioned above was present in 8 patients. The distribution of treatment type for acne in subjects included in the study is presented in Figure 8.

One patient each had undergone derma roller and fractional CO_2 laser treatment previously for acne scars. None of the patients had keloidal tendencies, active acne, cutaneous infections or other dermatosis at the time of enrolment.

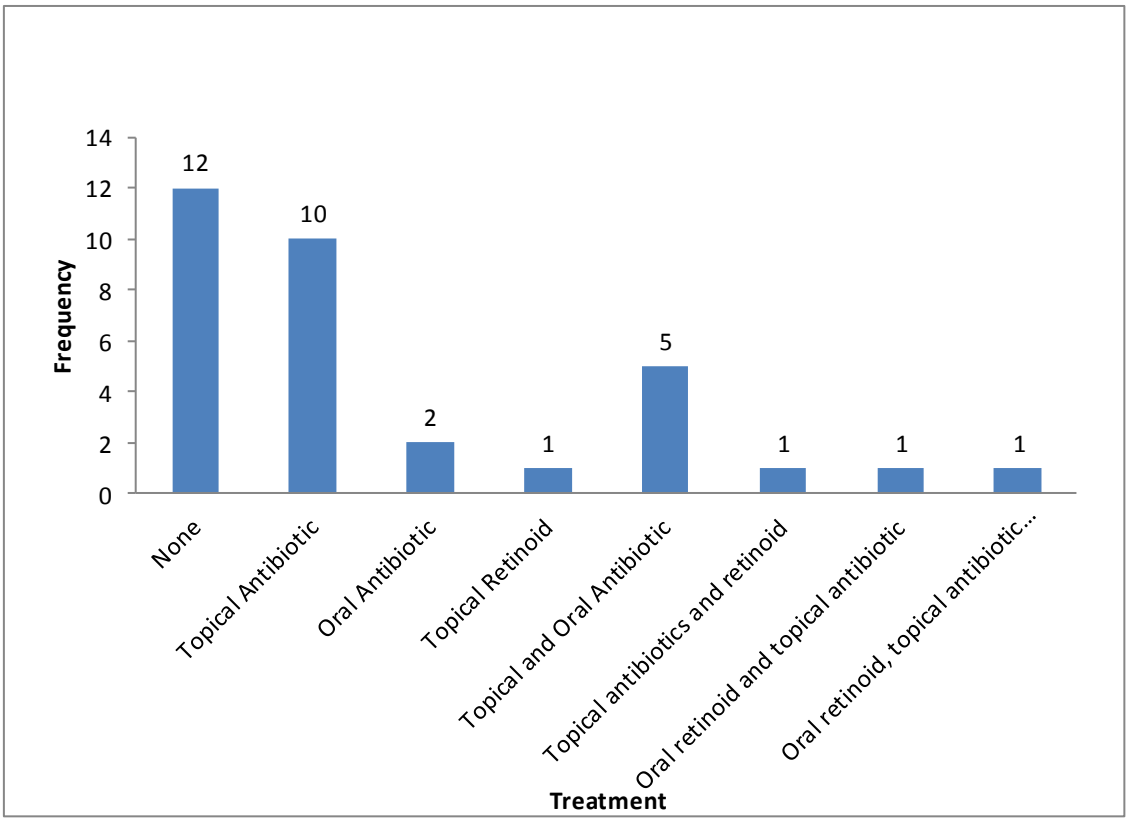


Figure 8: The distribution of patients based on type of treatment received for acne

Distribution of patients according to Fitzpatrick skin type

Among the patients enrolled, 60.6% had Fitzpatrick skin type IV, 30.3% had skin type V followed by type III skin type in 9.1% of patients. (Table 8, Figure 9)

Table 8: **Distribution of patients according to Fitzpatrick skin type**	
	Frequency (Percentage)
III	3 (9.1%)
IV	20 (60.6%)
V	10 (30.3%)
Total	33 (100%)

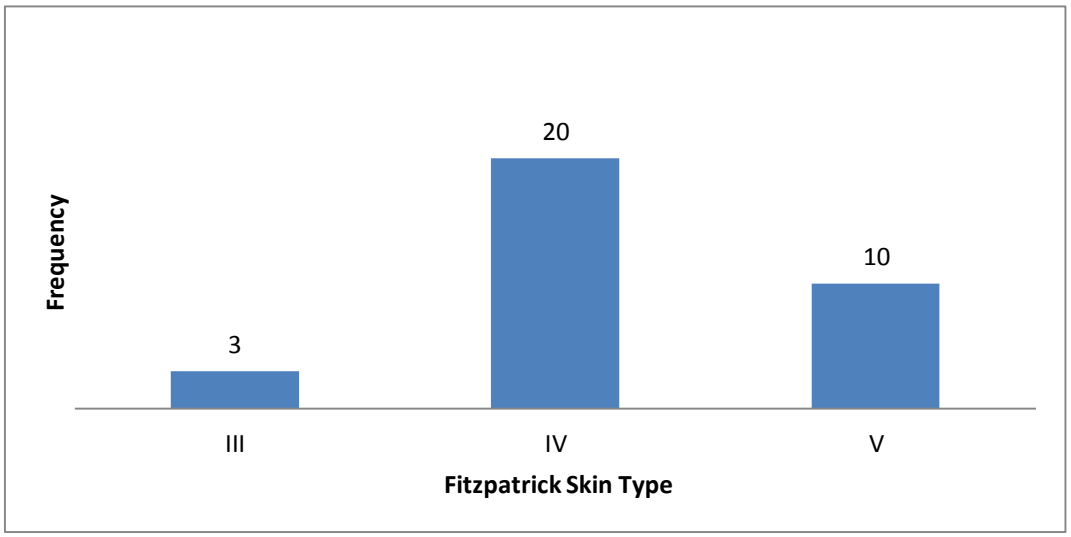


Figure 9: Distribution of patients according to Fitzpatrick skin type

Type of scars

Out of the 33 patients enrolled, 14 (42.4%) had predominantly rolling type of scars, 13 patients (39.4%) had boxcar type of scars followed by 6 (18.2%) with ice pick type of scars (Table 9, Figure 10). There was no significant difference between the predominant type of scars in Group A and B. [X^2=0.5159, P=0.7726]

Table 9: Type of scars.	
	Frequency (Percentage)
Rolling	14 (42.4%)
Ice-pick	6 (18.2%)
Boxcar	13 (39.4%)
Total	33 (100%)

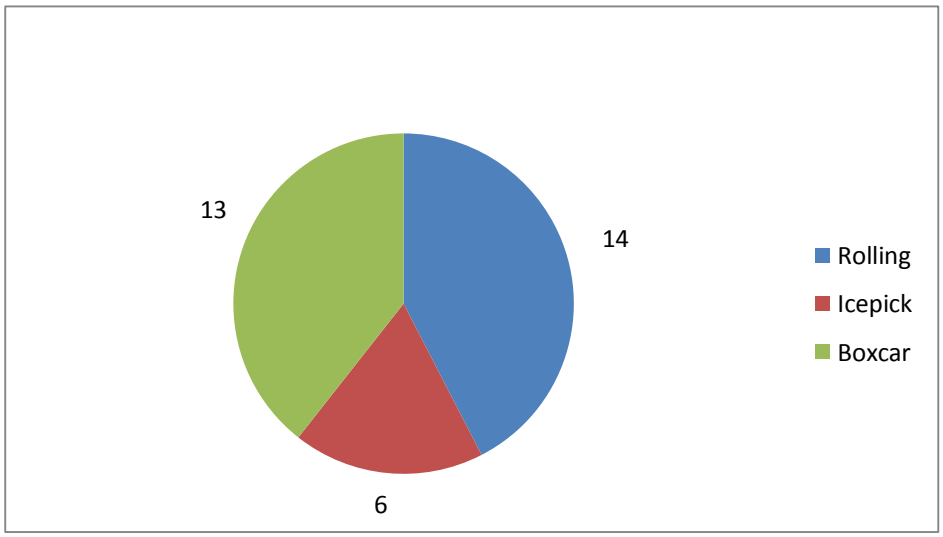


Figure 10: Type of scars.

Mean scar score at baseline

Mean scar score at baseline was higher in Group A (12.41) subjects when compared to those in Group B (11.75). However, this difference was insignificant (Table 10).

Table 10: Mean scar score at baseline.

	Type of treatment	N	Mean ±SD	p value
Scar score baseline	Group A	17	12.41 ±3.792	0.635
	Group B	16	11.75 ±4.139	
Independent t test was used for analysis. $p<0.05$ is considered significant.				

Out of the 33 patients enrolled in the study, 25 patients completed the study and 8 patients were lost to follow up due to unknown reasons. We discontinued treatment for 1 patient due to adverse effect of laser in the form of nodular acne.

Mean scar score at the end of the study

Mean change in score at the end of the study was higher in subjects of Group A (4.17) when compared to those in Group B (3.15). This was not statistically significant (Table 11).

Table 11: Mean change in score at the end of the study				
	Type of treatment	N	Mean± SD	p value
Change in score at the end of study	Group A	12	4.17 ±1.528	0.129
	Group B	13	3.15 ±1.676	
Independent t test was used for analysis. p<0.05 is considered significant.				

Comparison of baseline scar scores and change in the scar scores at the end of the study

The mean scar score in Group A reduced from 11.5 to 4. This was highly significant (P<0.0001). The mean scar score in Group B reduced from 12 to 3. This was highly significant (P<0.0001) (Table 12).

Table 12:Baseline scar scores and change in the scar scores study					
Groups	**Baseline scar score**		**Scar scores at the end**		p value
	Mean	SD	Mean	SD	
Group A	11.08(11.5)	3.45	4.17(4.0)	1.53	p<0.0001 HS
Group B	12.08(12)	4.48	3.15(3.0)	1.68	p<0.0001 HS
HS: Highly significant					

VAS for patient's assessment of improvement

Mean VAS for patient's assessment of improvement was higher in subjects in Group A (4.08) when compared to those in Group B (3.46). This was not significant (Table 13, Figure 11).

Table 13: Mean VAS for patient assessment of improvement				
VAS for patient assessment of improvement (out of 10)	Type of treatment	N	Mean ±SD	p value
	Group A	12	4.08 ±1.443	0.228
	Group B	13	3.46 ±1.050	
Independent t test was used for analysis. p<0.05 is considered significant.				

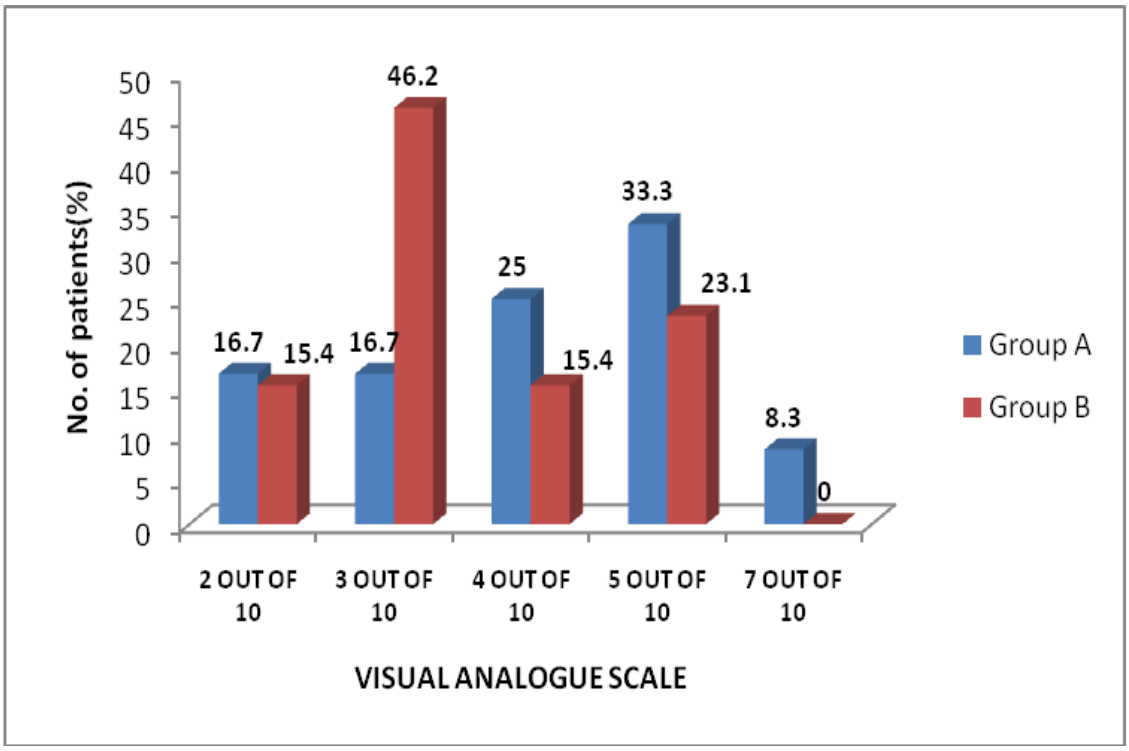


Figure 11: Mean VAS for patient assessment of improvement

Frequency of adverse effects

Out of the 33 patients enrolled, immediate adverse effect were seen in 21 patients. Twenty patients developed erythema, 8 patients developed oedema and 1 patient had pain after the procedure (Table 14, Figure 12).

Table 14: Frequency of Immediate Adverse Effect	
	Frequency (Percentage)
None	8 (24.2%)
Erythema	13 (39.4%)
Oedema	1 (3.0%)
Erythema, Oedema	7 (21.2%)
Pain	1 (3.0%)

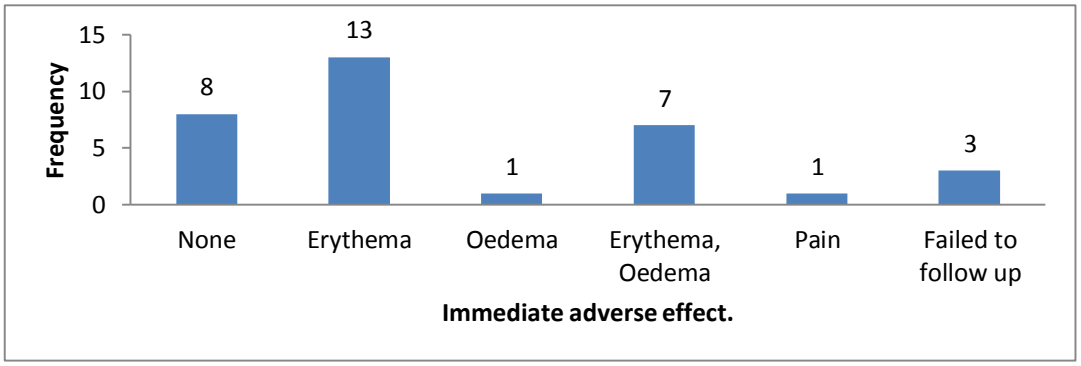


Figure 12: Frequency of Immediate Adverse Effects.

Erythema, erythema with oedema and pain was more in subjects of Group B. There was no significant difference in immediate adverse effects between two groups (Table 15).

Table 15: Immediate adverse effects between groups								
Type of treatment (p value = 0.277)		Immediate Adverse Effect						Total
		None	Erythema	Oedema	Erythema, Oedema	Pain	Failed to follow up	
Group A	Count	3	8	0	3	0	3	17
	% within Type of treatment	17.6%	47.1%	0.0%	17.6%	0.0%	17.6%	100.0%
	% within Immediate Adverse Effect	37.5%	61.5%	0.0%	42.9%	0.0%	100.0%	51.5%
	% of Total	9.1%	24.2%	0.0%	9.1%	0.0%	9.1%	51.5%
Group B	Count	5	5	1	4	1	0	16
	% within Type of treatment	31.3%	31.3%	6.3%	25.0%	6.3%	0.0%	100.0%
	% within Immediate Adverse Effect	62.5%	38.5%	100.0%	57.1%	100.0%	0.0%	48.5%
	% of Total	15.2%	15.2%	3.0%	12.1%	3.0%	0.0%	48.5%
Fischer's exact test was used for analysis. P<0.05 was considered as significant.								

Out of the 33 patients enrolled, long term adverse effects were seen in 10 patients. Hyperpigmentation was seen in 3 patients, pain, erythema and acne was seen in 2 patients each, and secondary infection was seen in 1 patient (Figure 13, Table 16).

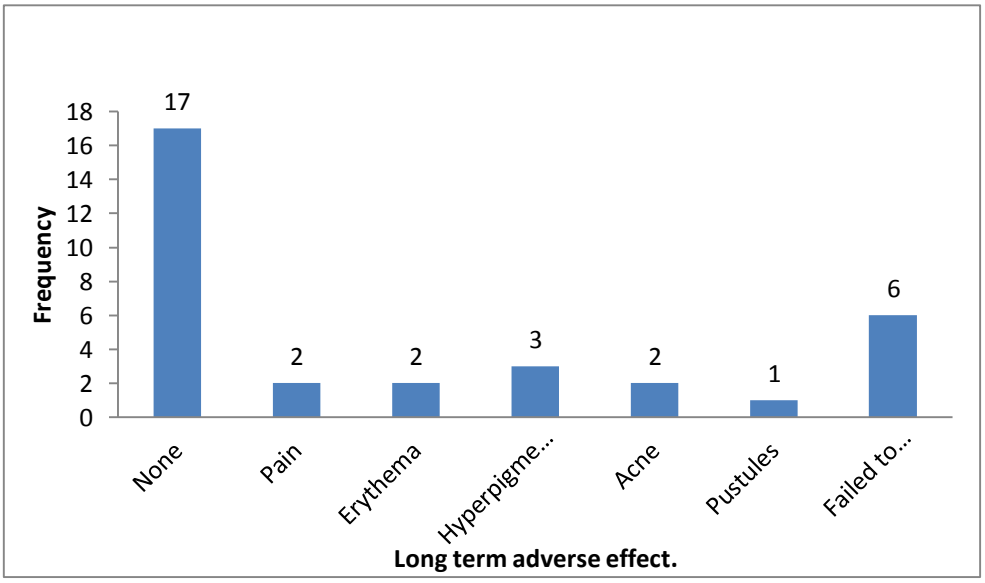


Figure 13: Frequency of long term adverse effects.

Table 16: Frequency of long term adverse effects		
	Frequency (Percentage)	
None	17 (51.5%)	
Pain	2 (6.1%)	
Erythema	2 (6.1%)	
Hyperpigmentation	3 (9.1%)	
Acne	2 (6.1%)	
Pustules	1 (3%)	

Pain and hyperpigmentation was more in subjects in Group B. There was no significant difference in long term adverse effects between two groups (Table 17).

Table 17: Long term adverse effects difference between Group A vs. Group B

Type of treatment (p value = 0.699)		Long term Adverse effect							Total
		None	Pain	Erythema	Hyperpigmentation	Acne	Pustules	Failed to follow up	
Group A	Count	10	0	1	1	1	1	3	17
	% within Type of treatment	58.8%	0.0%	5.9%	5.9%	5.9%	5.9%	17.6%	100.0 %
	% within Long term Adverse effect	58.8%	0.0%	50.0%	33.3%	50.0%	100.0%	50.0%	51.5%
	% of Total	30.3%	0.0%	3.0%	3.0%	3.0%	3.0%	9.1%	51.5%
Group B	Count	7	2	1	2	1	0	3	16
	% within Type of treatment	43.8%	12.5%	6.3%	12.5%	6.3%	0.0%	18.8%	100.0 %
	% within Long term Adverse effect	41.2%	100.0%	50.0%	66.7%	50.0%	0.0%	50.0%	48.5%
	% of Total	21.2%	6.1%	3.0%	6.1%	3.0%	0.0%	9.1%	48.5%

Fischer's exact test was used for analysis. P<0.05 was considered as significant.

Comparison of change in scar scores according to predominant scar type.

The change in scar score irrespective of treatment was the highest in patients with rolling scars with a mean of 4.2 followed by boxcar scars with a mean of 3.89 and the lowest in ice-pick scars with a mean of 2.33. This mean change in scar score based on predominant type of scar was highly statistically significant (p= 0.0428) (Table 18).

Table 18: Comparison of change in scar scores according to predominant scar type.

Predominant type of scars	**Change in scar score**		p value
	Mean(Median)	SD	
Boxcar	3.89(4)	1.49	p=0.0428 HS
Icepick	2.33(2.5)	0.82	
Rolling	4.2(4)	1.87	
HS: highly significant			

Figure 14 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser with PRP)

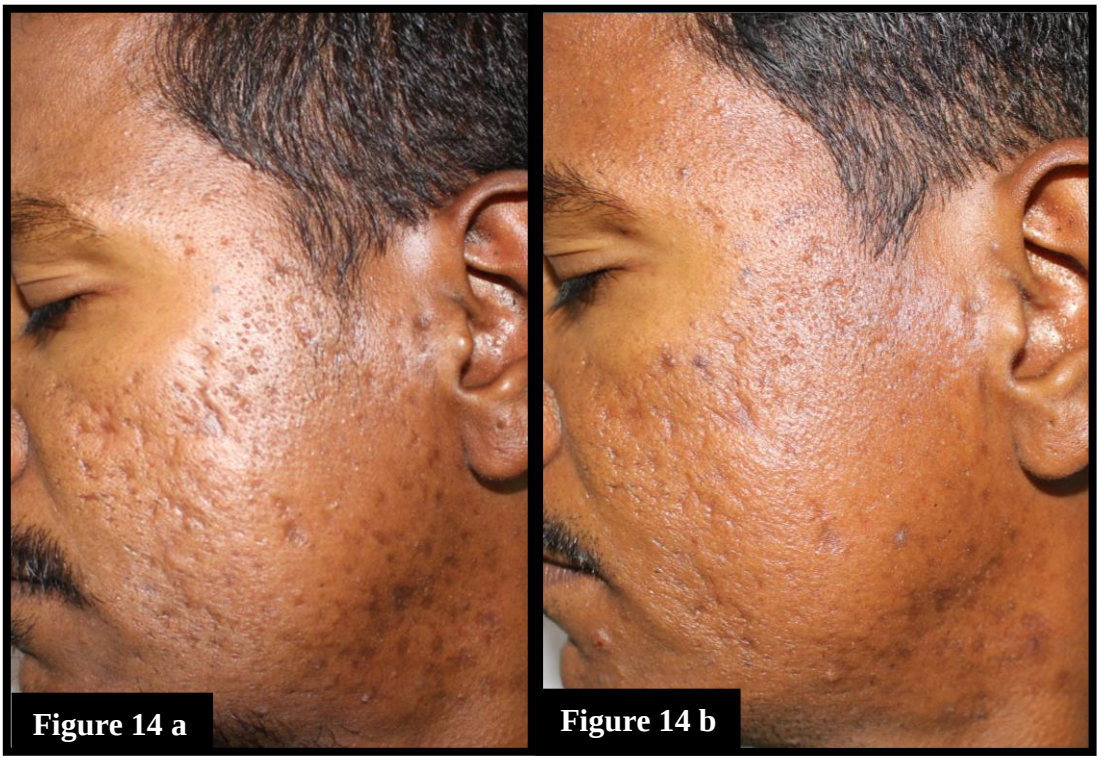


Figure 15 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser with PRP)

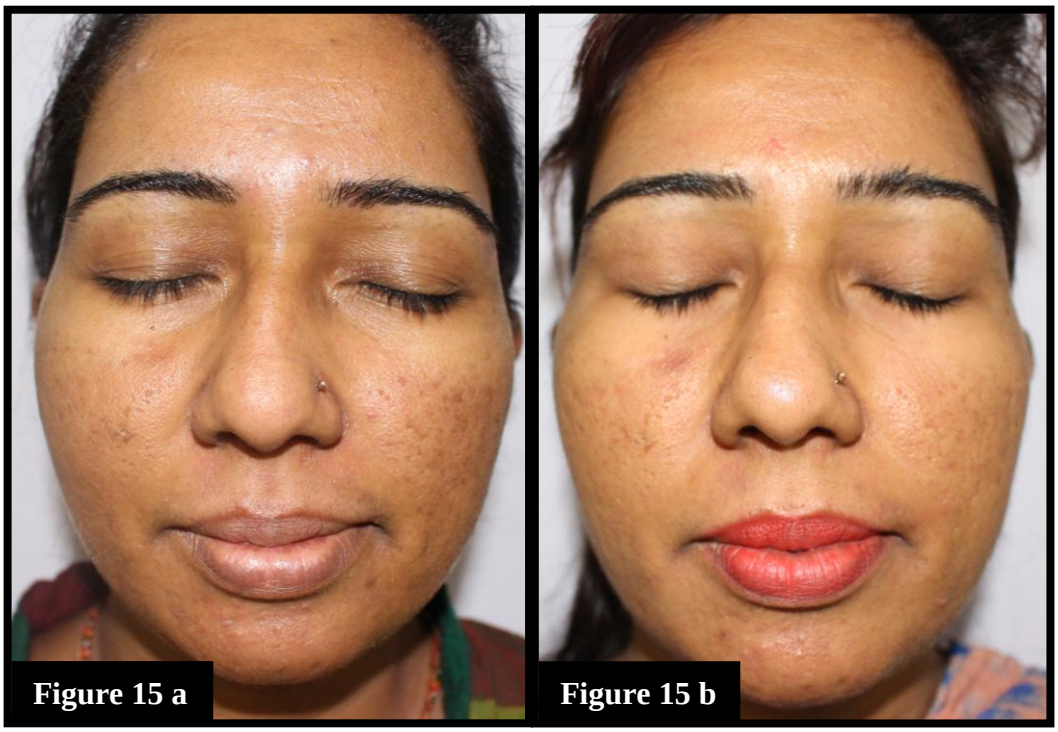


Figure 16 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser with PRP)

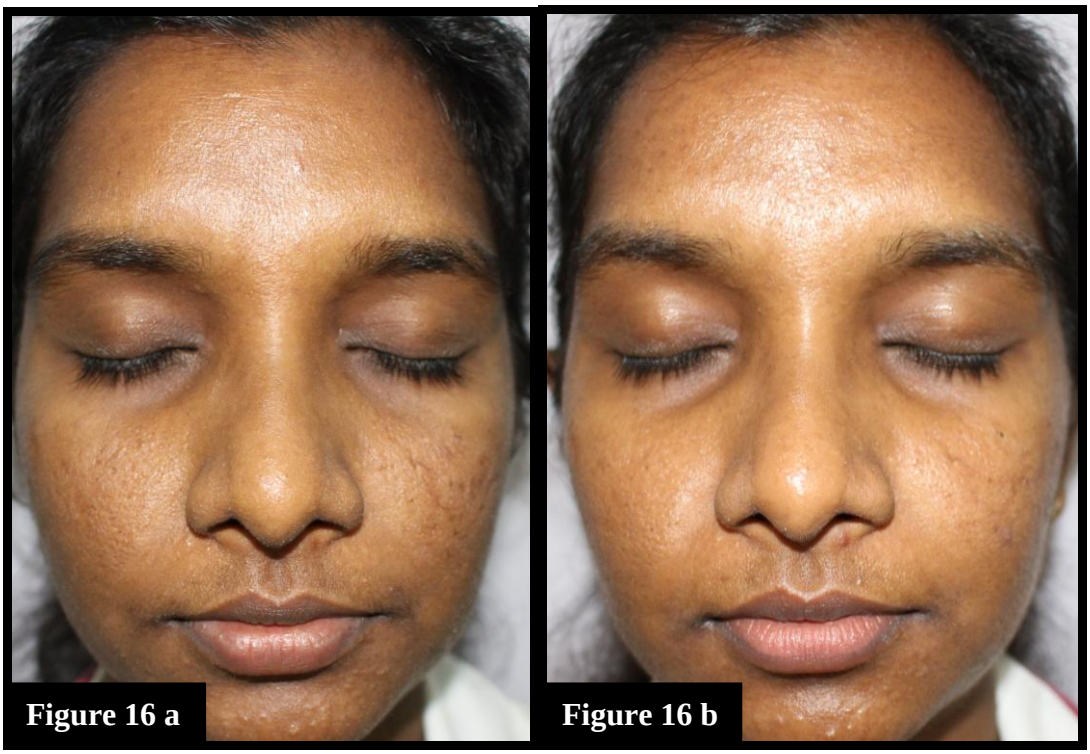


Figure 17 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser with PRP)

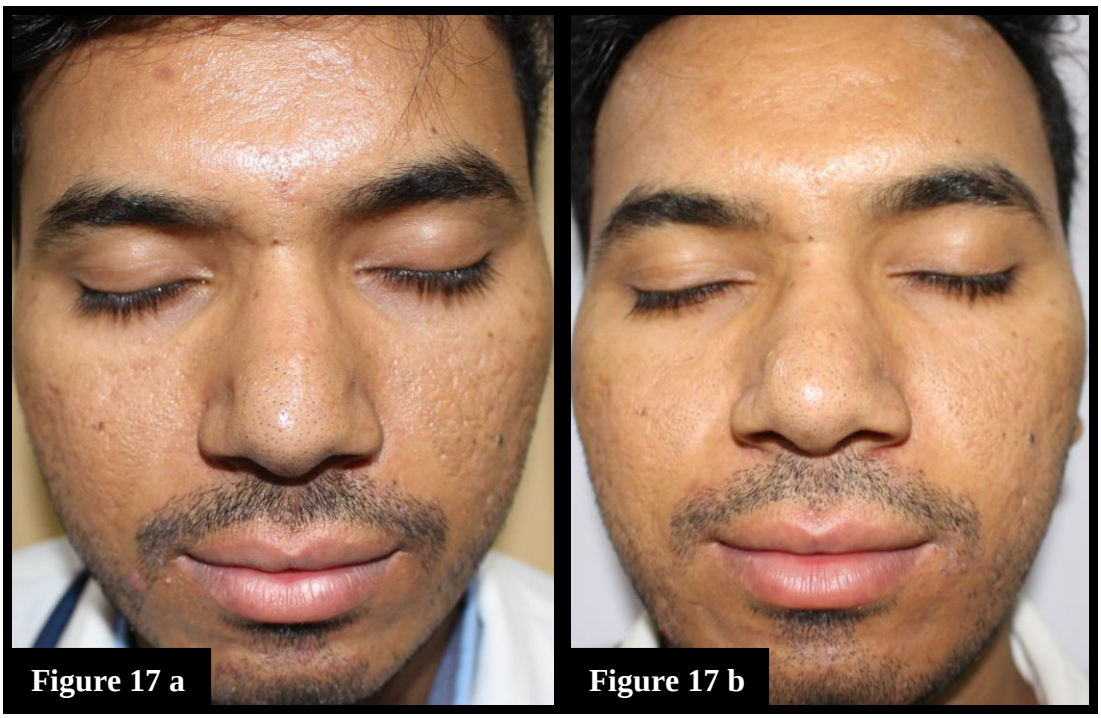


Figure 18 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser only)

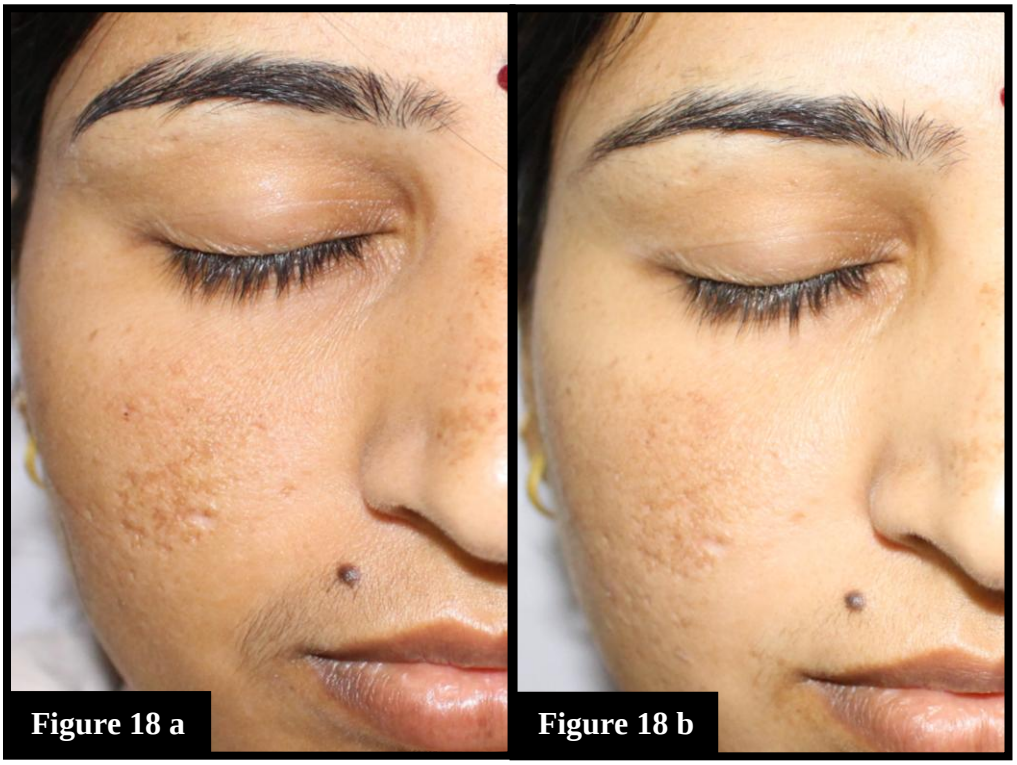


Figure 19 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser only)

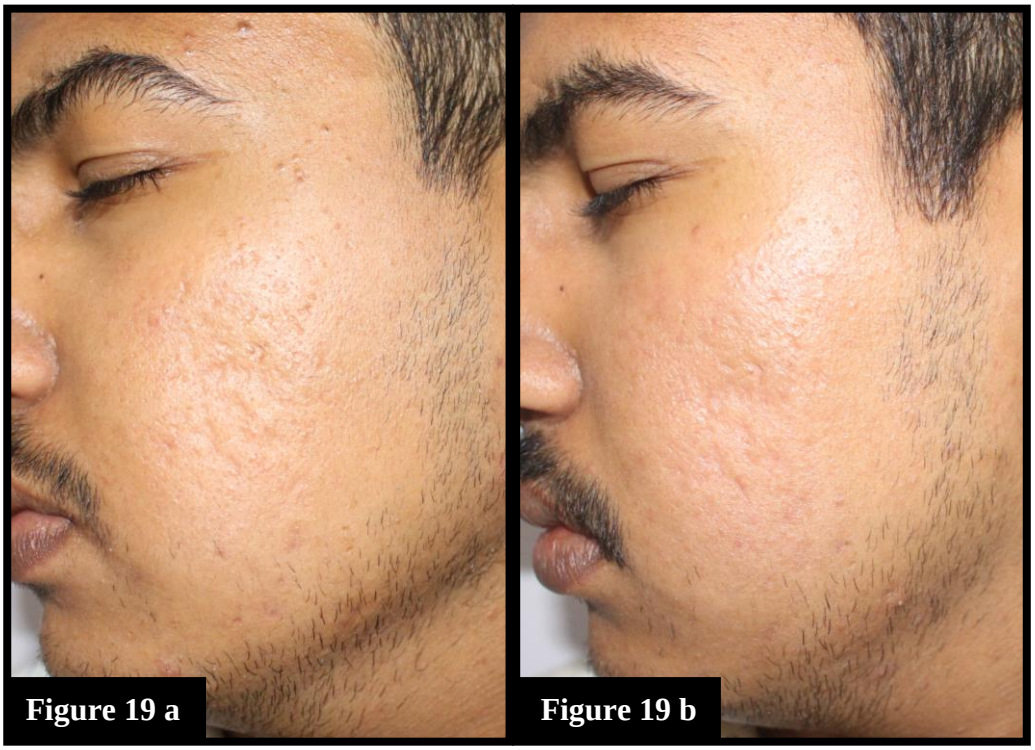


Figure 20 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser only)

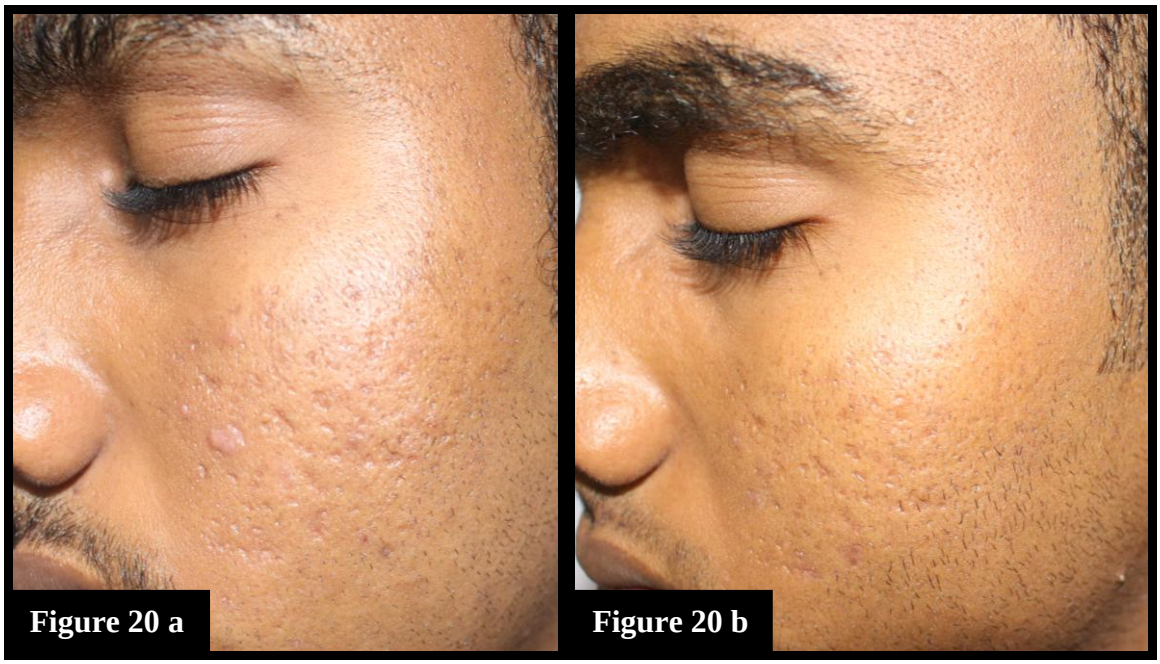


Figure 21 (a): Clinical picture at baseline; (b): Clinical picture at the end of the study (fractional CO_2 laser only)

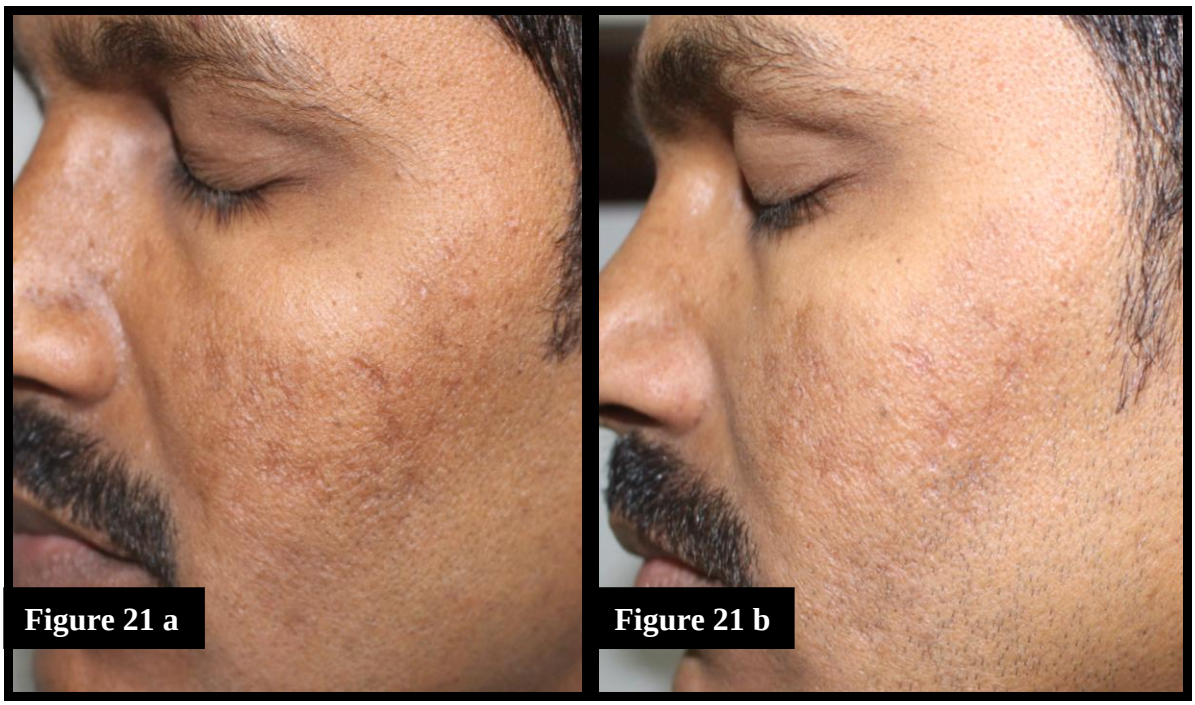


Figure 22: Clinical picture of post laser hyperpigmentation

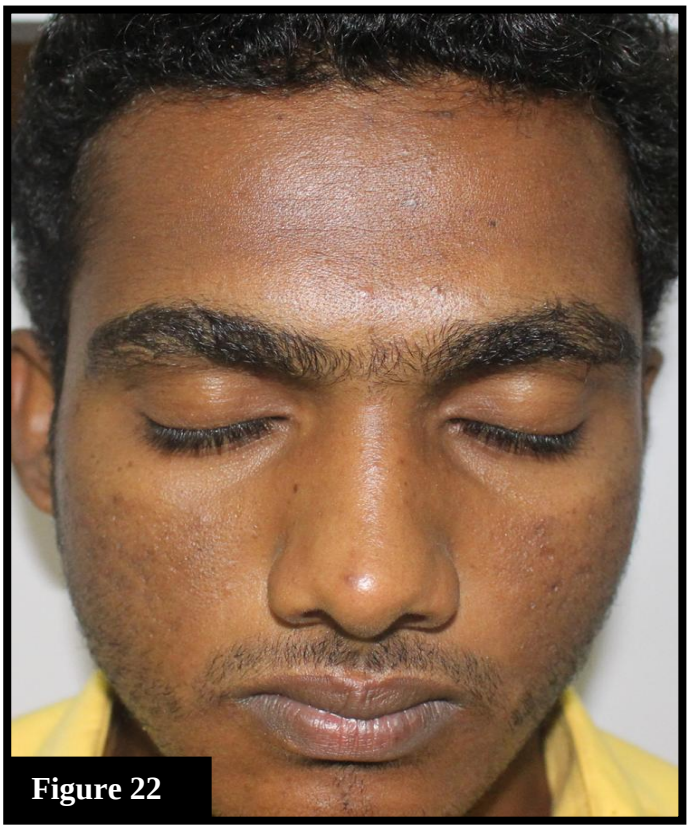
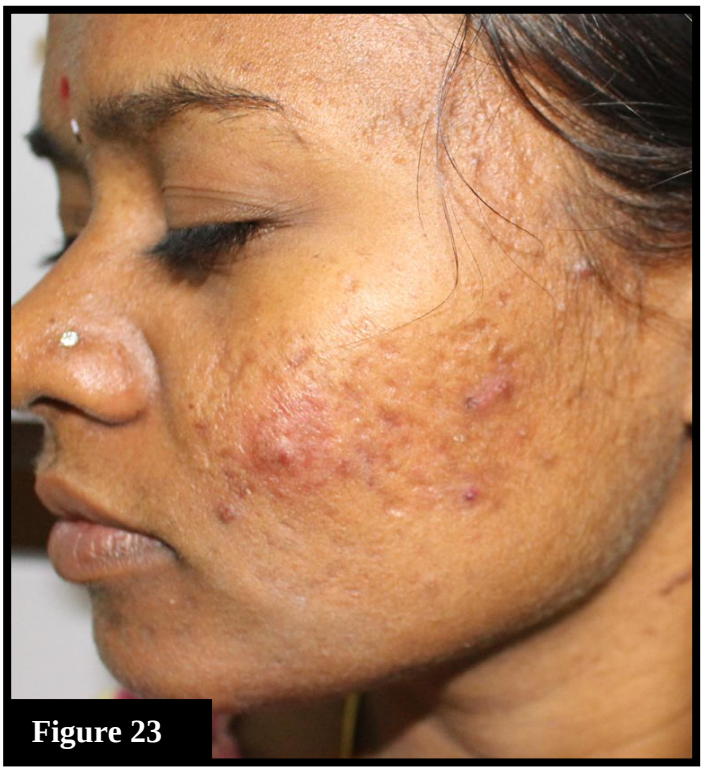


Figure 23: Clinical picture of acne occurring post laser



DISCUSSION

DISCUSSION:

Acne vulgaris is prevalent in 90% of adolescent population. It is a chronic inflammatory disorder of the pilosebaceous unit. In 12-14% of these patients, acne persists even in adulthood. Both women and men are conscious about their appearance and acne poses as a psychological and social burden of high gravity.

Atrophic facial acne scars occur frequently, most commonly as a consequence of severe acne during the teenage years. Many patients seek treatment for the disfigurement resulting from acne, not only because of the obvious variation in skin texture and appearance, but also for the limitations on social interactions, daily activities and self-esteem. Various modalities can be used for improvement of acne scars. These can be divided into non-energy based techniques and energy based techniques.[4]

Our study enrolled 33 patients of post acne atrophic scars out of which 25 patients completed the study. These 25 patients were divided into Group A containing 12 patients who were treated with a combination of fractional CO_2 laser with PRP and Group B containing 13 patients who were treated with fractional CO_2 laser alone for 3 sessions four weeks apart. The patients were followed up at baseline, at every visit and 1 month after the third session for evaluation.

The mean age of the patients included in present study being 24.36 ± 4.37 years, many of them belonged to a younger age group. These findings are similar to a recent study conducted by Chang *et al*.[67] corresponding to the age of onset of acne and, therefore, acne scars.

Comparing with the various surveys conducted previously where there was no gender predilection; in our study males were predominant. Since acne is more severe in boys than in girls, it may have probably resulted in severe acne scarring in males.[1] In the present study, the

duration of acne was found to be 4.7 ±2.25 years, which was similar to a study by Apfelberg.[68] Scars were present in our patients for the duration of 2.74 ±1.57 years.

Where type of acne scars of the patients was simplified as mixed type in a study conducted by Laytong *et al*.,[69] majority of our patients presented with rolling type of scars (n=14) followed by boxcar (n=13) and ice-pick type (n=6). In a study by Mahajan *et al.*, ice-pick type of scar were predominantly present.[61] The skin type distribution showed that the majority were type IV (n=20), followed by type V (n=10) and least weretype III (n=03) which form the predominant skin types found in south India.

The choice of treatment of post acne scars was independent of both the morphological types as well as severity of each scar present on the face as the patients were alternately allotted into Group A and group B. Mean Scar score at baseline was higher in Group A (12.41) subjects when compared to those in Group B (11.75). However, this was not statistically significant.

In the present study, there was a statistically significant improvement in scar score in the patients treated with fractional CO_2 laser ablation followed by topical PRP, after each sitting. Similarly, in the patients treated with the fractional CO_2 laser alone, there was significant reduction in the scare score at every follow-up. From this observation, we can infer that fractional CO_2 laser with or without PRP is an effective treatment modality for post acne atrophic scars as confirmed by similar studies. [30, 42, 66]

On comparison of the mean scar scores improvement at each visit between the two groups, the mean change in scar scores were higher in the fractional CO_2 with PRP group patients compared to fractional CO_2 laser alone, showing an added reduction in the scar score by addition of topical PRP. These finding were similar to studies done by Chang *et al.* and Arsiwala and Desai.[67, 70-72] Based on these outcomes, we can state that fractional CO_2 laser

treatment when combined with PRP gives superior improvement as compared to fractional CO_2 monotherapy.

There was a better mean VAS improvement of the patient post fractional CO_2 laser ablation followed by topical PRP (4.08±1.44) than the CO_2 laser ablation alone (3.46±1.05). Although these results were insignificant, they indicate that subjective improvement in the post acne atrophic scars was better where fractional CO_2 laser treatment was followed by topical PRP as corroborated by various studies. [62, 64, 65]

The immediate adverse effects seen in the patients was erythema (39.4%) followed by erythema and edema (21.2%). Other adverse effects were edema and pain, which were similar to findings documented by the similar studies. [64, 65, 67] Erythema, erythema with oedema and pain was more in subjects treated with fractional CO_2 laser only. There was no significant difference in immediate adverse effects between two groups. Similar to findings reported by Na *et al.,*[70] the present study shows that immediate side effects like erythema, oedema and pain reduce when fractional CO_2 laser treatment is followed by topical application of autologous PRP probably due to the regenerative properties of PRP. [61]

In our study, long term adverse effects were seen in 10 patients. Hyperpigmentation was seen in 3 patients, pain, erythema and acne was seen in 2 patients each, and secondary infection was seen in 1 patient. Treatment with fractional CO_2 laser had to be discontinued for 1 patient as she developed acne after each laser sitting as observed at follow-up. This decision was taken in view of exacerbation of acne which is seen as an adverse effect of fractional CO_2 laser.[43] Pain and hyperpigmentation was seen lesser in patients treated with fractional CO_2 laser followed by topical PRP. There was no significant difference in long term adverse effects between two groups. As validated by numerous studies over the last 10 years, [5, 56, 62, 64, 66] PRP following fractional CO_2 laser therapy leads to a considerable reduction in long term adverse effects like pain and post laser hyperpigmentation (which is

commonly seen in Fitzpatrick skin type III to V) owing to its richness of growth factors responsible for reducing inflammation and hastening the wound healing process. Moreover, we used topical PRP instead of injecting the PRP intradermally, which probably prevented added inflammation due to injection, in turn reducing pain and post laser hyperpigmentation.[70]

In the present study, the mean change in scar score irrespective of treatment was the highest in patients with rolling scars (4.2) followed by boxcar scars (3.89) and the lowest in ice-pick scars (2.33). This mean change in scar score based on predominant type of scar was highly significant. Poorer response of ice-pick scars as compared to rolling and boxcar scars was observed in our study which was similar to a study by Majid and Imran.[73]This is probably attributed to the depth of ice-pick scars and the inability of thermal effect to reach their base for dermal stimulation.

Similarly, a systematic review and meta-analysis conducted by Chang HC *et al.* and Arsiwala and Desai found that clinical improvement subsequent to combination therapy was significantly greater than that of laser alone. Combination of fractional CO_2 laser with topical PRP has synergistic positive effects on the clinical outcome for acne scars and can quicken recovery.[67, 72] The autologous PRP injected or applied into the scar after laser treatment augments the skin with potentially bioactive growth factors and other chemokines, enabling faster wound repair.[7, 71] Studies indicate that there is documented faster reduction of routinely encountered adverse effects like post-laser oedema, erythema and hyperpigmentation (especially in darker skin types) with the use of both topical and intradermal PRP.[66, 70]

Synergizing fractional laser therapy with PRP is recognized to dynamically reduce atrophic acne scarring as documented by many authors.[71, 72] Autologous growth factors and other secretory proteins, chemokines and cytokines released on platelet activation facilitate

restoration of cutaneous tissue by stimulating dermal fibroblasts proliferation and increase type I collagen synthesis at the site of administration. [61]

CONCLUSION

CONCLUSION:

The present study was aimed at assessing the efficacy of combination treatment of acne scars with fractional CO_2 laser ablation followed by topical platelet rich plasma application at the site as compared to CO_2 laser ablation alone. Total of 33 patients were treated under this study of which 21 were males and 12 females. The study was completed by 25 patients and 8 patients were lost to follow up. Mean age of the patients was 24.3 ± 4.4 years.

In present study, the predominant type of acne scars seen were rolling type followed by boxcar and ice-pick type. Patients were treated under two arms (Group A and Group B) with 3 follow-up sittings for the treatment. There was significant improvement in the scar after every follow-up sitting in both groups.

On comparison of the mean scar score improvement at each visit between the two groups, the mean change in scar scores were higher in the fractional CO_2 with PRP group showing an added reduction in the scar score by addition of topical PRP making it more efficacious than monotherapy with fractional CO_2 laser . However, this difference in both the groups of patients was insignificant.

Subjective improvement in the post acne atrophic scars was better when fractional CO_2 laser treatment was followed by topical PRP denoted by a better mean VAS improvement of the patients.

PRP following fractional CO_2 laser therapy leads to a considerable reduction in adverse effects like pain, erythema, oedema, post laser hyperpigmentation (which is commonly seen in darker skin types) owing to its richness of growth factors responsible for reducing inflammation and hastening the wound healing process. Moreover, used of topical instead of intradermal PRP probably prevents added inflammation, in turn reducing adverse effects.

The mean change in scar score based on predominant type of scar was highly significant. Poorer response of ice-pick scars as compared to rolling and boxcar scars was observed in our study attributed to their depth.

Combination of fractional CO_2 laser with topical PRP has synergistic positive effects on the clinical outcome for acne scars and can accelerate recovery. Faster reduction of routinely encountered adverse effects like post-laser oedema, erythema and hyperpigmentation is seen with the use of both topical and intradermal PRP.

Therefore, the study concludes that although fractional CO_2 is an effective modality for treating post acne atrophic scars, combination therapy with PRP is more efficacious in reducing the appearance of scars, reducing adverse effects of laser therapy and better patient satisfaction by the means of subjective improvement. Fractional CO_2 laser gives better response for rolling and boxcar scars as compared to ice pick scars.

SUMMARY

SUMMARY:

A hospital based prospective, double blinded, randomized, comparative study was conducted from November 2017 to June 2019. A total 33 cases of post acne atrophic scars were included in the study. The enrolled patients were allotted into 2 groups:

-Group A (fractional CO_2 laser with topical PRP)

-Group B (fractional CO_2 laser only)

A total of 3 laser sessions were performed 4 weeks apart. The patients were evaluated up at baseline, at every follow up and 1 month after the third session.

- A total of 33 patients were enrolled in the study out of which 25 patients completed the study.
- Males were predominant in our study.
- Most patients belonged to a younger age group with a mean age of 24.36 ± 4.37 years.
- Predominant scar type seen was rolling (42.4%); boxcar (39.4%) followed by ice pick (18.2%) type of scars.
- There was statistical significant reduction in the mean scar score during each follow-up in patients of both groups.
- There was no significant difference in the improvement score of scar between the two groups, but mean change in score at the end of the study was higher in Group A (4.17) as compared to Group B (3.15).
- Fractional CO_2 laser ablation followed by topical platelet rich plasma is more efficacious in treating the acne scars than the CO_2 laser ablation alone.
- Topical PRP following fractional CO_2 laser therapy leads to a considerable reduction in adverse effects like pain, erythema, edema, post laser hyperpigmentation.
- Poorer response of icepick scars as compared to rolling and boxcar scars was observed.

- Combination of technologies, either sequentially or rotationally, may improve the treatment outcome; minimize the side effects or both.

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ANNEXURE

ANNEXURE II

PROFORMA

B.L.D.E.(DU)'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPURA.

Department of Dermatology, Venereology and Leprosy.

A COMPARATIVE STUDY TO ASSESS THE EFFICACY OF FRACTIONAL CO_2 LASER ALONE AND FRACTIONAL CO_2 LASER WITH TOPICAL PLATELET-RICH PLASMA IN THE TREATMENT OF POST ACNE SCARS.

SCHEME OF CASE TAKING

1. General information

Name: SL no:

Age:

Sex: Address:

Marital status: married/unmarried Education:

Occupation: Contact no:

Out patient no: Date:

1. History

Duration of Acne :

Duration of Acne scars :

History of Treatment of acne : A) Medical

B)Surgical/Lasers

History of treatment of acne scars :A) Medical

B)Surgical/Lasers

History of keloidal tendency/ hypertrophic scars : Present/Absent

2. Clinical Examination

- General Physical Examination:

Weight: Pallor :

BP: Clubbing :

PR: Icterus :

Cyanosis :

Edema :

Lymphadenopathy:

Other findings:

- Systemic Examination

Cardiovascular system :

Respiratory system :

Central nervous system :

Abdominal examination :

- Cutaneous examination:

Skin Type : Type I/II/III/IV/V/VI

Active Acne : Present/Absent

Keloid/ Hypertrophic scar : Present/Absent

Active Viral Infection : Present/Absent

Active Bacterial Infection : Present/Absent

Evolving Dermatosis : Present/Absent

Scoring of scars(Goodman's quantitative global Acne scars gradingsystem)

Grade/ Type	No of Lesions	Score/Points
Mild Scarring		
Moderate Scarring		
Severe Scarring		
	Total Score/Points	

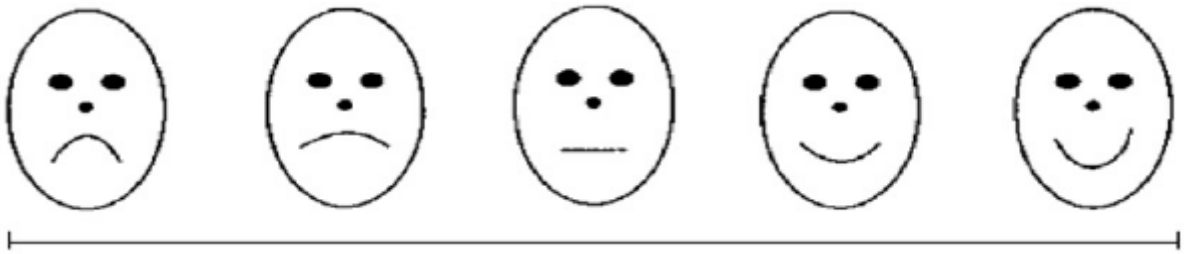
- Investigations

Complete blood count:-	
Total counts	cells/cmm
N/L/E/M/B	%
Hb	gm%
Platelet count	lakhs/cmm
Random Blood Sugar (RBS):-	mg/dl
HIV:-	
HbSAg:-	

	Baseline	1st follow up	2nd follow up	3rd follow up
Procedure				
Total scar score				
Complications Immediate - Long term-				
Change in scar score				

Patient self Assessment Scale of acne scars.

Visual Analogue Scale



0 inches 2 4 6 8 10inches

No impact on Highest impact on

Acne scars Acne scars

ANNEXURE III

CONSENT FORM

B.L.D.E.(DU)'s SHRI B M PATIL

MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE,

VIJAYAPURA-586 103

RESEARCH INFORMED CONSENT FORM

TITLE OF THE PROJECT:- A COMPARATIVE STUDY TO ASSESS THE EFFICACY OF FRACTIONAL CARBON DIOXIDE LASER ALONE AND FRACTIONAL CARBON DIOXIDE LASER WITH TOPICAL PLATELET-RICH PLASMA IN THE TREATMENT OF POST ACNE ATROPHIC SCARS.

PG GUIDE :-

PG STUDENT :-

PURPOSE OF RESEARCH:

I have been informed that this project will assess the efficacy of fractional CO_2 laser alone and fractional CO_2 laser with topical platelet-rich plasma in the treatment of post acne atrophic scars.

BENEFITS:

I understand that my participation in this study will help the investigator to know the effectiveness of fractional CO_2 laser alone and fractional CO_2 laser with topical platelet-rich plasma in the treatment of post acne atrophic scars.

PROCEDURE:-

I understand that relevant history will be taken and I will undergo detailed clinical examination after which treatment will be given.

RISK AND DISCOMFORTS:-

I understand the possible complications that may occur during and after the procedure, i.e., post procedure pain, swelling and erythema at the laser site for 2-3 days.

CONFIDENTIALITY:-

I understand that medical information produced by this study will become a part of my hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purposes no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:-

I understand that I may ask more questions about the study at any time concerned. _____is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:-

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that_____ may terminate my participation in this study at any time after she has explained the reasons for doing so and has helped arrange for my continued care by my own physician, if this is appropriate.

INJURY STATEMENT:-

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient's / relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in patient's own language.

_____ _____

Investigator / P. G. Guide Date

I confirm that …….(Name of the PG guide / chief researcher) has explained to me the research, the study procedures that I undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and I understand this consent form. Therefore, I agree to give my consent for my participation as a subject in this research project.

_____ _____

Participant / guardian Date

_____ _____

Witness to signature Date

ANNEXURE IV

KEY TO MASTERCHART

RX	TREATMENT
SS	SCAR SCORE
A/E	ADVERSE EFFECTS
F	FAILED TO FOLLOW UP
VAS	VISUAL ANALOGUE SCALE
I/L	INTRALESIONAL
A	ABSENT
H	HYPERPIGMENTATION
OA	ORAL ANTIBITOIC
TA	TOPICAL ANTIBIOTICS
TR	TOPICAL RETINOIDS
TOA	TOPICAL AND ORAL ANTIBITOIC
TAR	TOPICAL ANTIBIOTIC AND RETINOID
OR	ORAL RETINOID
I	ICEPCIK
R	ROLLING
B	BOXCAR
E	ERYTHEMA
OE	OEDEMA

ANNEXURE V

SR NO.	OPD NO	NAME	AGE (Y)	SEX	ACNE DURATION	SCARS DURATION	RX FOR ACNE	RX FOR SCARS	KELOIDAL TENDENCY	SKIN TYPE	ACTIVE ACNE	INFECTIONS	DERMATOSIS	SS BASELINE	PREDOMINANT TYPE OF SCARS	SS AT 1ST FOLLOW UP	SS AT 2ND FOLLOW UP	SS AT 3RD FOLLOW UP	CHANGE IN SCORE AT END OF STUDY	IMMEDIATE A/E	LONG TERM A/E	VAS FOR PATIENT ASSESSMENT OF IMPROVEMENT
1A	111444	DEEPIKA	24	F	4Y	1 Y	OA	-	A	V	A	A	A	18	R	16	15	F	-	E, OE	ACNE	-
1B	119075	ANIL	26	M	8 M	4 M	TA	-	A	V	A	A	A	7	I	7	6	6	1	-	-	2
2A	122275	KAVITA	20	F	7Y	4Y	TA	-	A	III	A	A	A	10	R	8	5	4	6	E	-	5
2B	6377	M REDDY	28	M	4Y	2Y	TR	-	A	V	A	A	A	16	B	13	11	11	5	E, OE	PAIN	4
3A	9862	RAJSHEKHAR	26	M	6Y	6Y	-	-	A	IV	A	A	A	17	B	14	13	11	6	E	-	4
3B	141775	SHIVARAJ	18	M	5Y	4Y	-	-	A	IV	A	A	A	11	I	9	8	8	3	E	H	2
4A	145614	MAHANTESH	30	M	8Y	5Y	TA	-	A	V	A	A	A	8	R	7	5	4	4	E, OE	-	7
4B	154855	ARJUN	21	M	3 Y	1Y	TOA	-	A	IV	A	A	A	11	B	F	-	-	-	E	-	-
5A	159204	DEEPAK	30	M	5Y	5Y	TAR	-	A	IV	A	A	A	14	I	12	11	11	3	E	-	3
5B	160886	SUMAN	23	F	1.5Y	1Y	TA	-	A	IV	A	A	A	8	R	F	-	-	-	PAIN	-	-
6A	172343	ALEKHYA	26	F	8Y	2Y	OR, TA	-	A	V	A	A	A	15	R	13	10	9	6	E, OE	PUSTULE	5
6B	170491	MALLU	27	M	6Y	5Y	TOA	-	A	V	A	A	A	17	R	14	12	10	7	E,	H	5

																			OE		
7A	176354	SIDDHU	21	M	2Y	2Y	-	-	A	V	A	A	A	12	B	F	-	-	-	-	-	-
7B	172896	ISHARAT	17	F	3Y	1YR	TOA	-	A	IV	A	A	A	11	I	10	10	9	2	-	PAIN	3
8A	22239	ANITA	25	F	4Y	2Y	TA	-	A	IV	A	A	A	9	R	6	5	5	4	-	-	4
8B	177427	SAGAR	25	M	8Y	2Y	TOA	-	A	V	A	A	A	18	B	16	15	14	4	E, OE	ACNE	3
9A	229341	GOVIND	20	M	5Y	3Y	TOA	-	A	IV	A	A	A	13	B	10	8	8	5	E	-	5
9B	177295	SANJU	21	M	1Y	6M	OR, TA, I/L TRIAM	-	A	IV	A	A	A	14	R	12	11	11	3	E	E	3
10A	251877	MUTTU	22	M	5Y	2Y	-	-	A	IV	A	A	A	6	I	5	3	3	3	E	-	2
10B	236938	RUSHAB	29	M	2Y	6M	TA	-	A	IV	A	A	A	18	B	16	16	15	3	E	-	4
11A	249367	JYOTI	26	F	8Y	4Y	-	-	A	IV	A	A	A	6	B	6	5	5	1	-	-	2
11B	258016	CHANDRASHEKHAR	21	M	5Y	2Y	-	-	A	IV	A	A	A	14	R	12	11	10	4	-	-	5
12A	263766	ARUN	28	M	6Y	5Y	-	-	A	IV	A	A	A	16	R	F	-	-	-	E	H	-
12B	200130	SAVITA	21	F	5Y	2Y	-	-	A	IV	A	A	A	10	I	9	8	8	2	E	-	3
13A	326798	SHWETA	21	F	5Y	3Y	OA	-	A	IV	A	A	A	12	B	10	9	9	3	-	-	4
13B	263037	BASAVARAJ	27	M	5Y	4Y	-	FCO2	A	IV	A	A	A	5	R	5	4	4	1	-	-	3
14A	247913	MEGHANA	21	F	5Y	3Y	TA	-	A	IV	A	A	A	14	B	F	-	-	-	-		
14B	269737	VENKATESH	20	M	2Y	2Y	-	-	A	V	A	A	A	6	R	6	5	4	2	-	-	3
15A	286594	BABY	38	F	2Y	2Y	-	-	A	III	A	A	A	18	R	F						
15B	426302	ABHISHEK	21	M	6Y	4Y	TA	-	A	IV	A	A	A	10	B	8	7	6	4	OE	-	5
16A	468951	BHIMARAY	30	M	8Y	5Y	-	-	A	IV	A	A	A	12	R	10	8	7	5	E	-	5
16B	445462	VANI	24	F	2Y	2Y	TR	-	A	III	A	A	A	12	B	10	F			E, OE	-	
17A	45720	SANTOSH	27	M	8Y	3Y	TA	DERMA ROLLER	A	V	A	A	A	11	B	10	8	7	4	E	E	3.