"A SPLIT FACE COMPARATIVE INTERVENTIONAL STUDY TO EVALUATE EFFICACY OF FRACTIONAL CARBON DIOXIDE LASER AGAINST COMBINED USE OF FRACTIONAL CARBON DIOXIDE LASER AND PLATELET RICH PLASMA IN THE TREATMENT OF POST ACNE SCARS."

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LIST OF ABBREVIATIONS

NO	ABBREVIATIONS	EXPANSION
1	CO2	Carbon dioxide
2	PRP	Platelet rich plasma
3	PPAR	Peroxisome proliferator activated receptors
4	IL	Interleukin
5	TNF	Tumor Necrosis Factor
6	TGF-B	Transforming Growth Factor Beta
7	NF-κβ	Nuclear factor kappa beta
8	AP	Activator protein
9	15-HETE	15-hydroxyeicosatetranoic acids
10	cAMP	Cyclic Adenosine Monophosphate
11	TLRs	Toll like receptors
12	PRRs	Pattern recognition receptors
13	PAMPs	Pathogen associated molecular patterns
14	MUFA	Monounsaturated fatty acids
15	ASRS	Acne Scar Rating Scale
16	ECCA	Echelle d'evaluation Clinique des Cicatrices d'acne
17	SCARS	The Self-assessment of Clinical Acne Related Scars
18	FASQoL	Facial Acne Scar Quality of life
19	PDL	Pulsed Dye Laser

20	**KTP**	**Potassium Titanyl Phosphate**
21	**IPL**	**Intense pulsed-light**
22	**TCA**	**Trichloroacetic acid**
23	**CROSS**	**Chemical Reconstitution of skin scars**
24	**RF**	**Radiofrequency**
25	**Er:YAG**	**Erbium: yttrium-aluminium-garnet**
26	**Nd:YAG**	**Neodymium: yttrium-aluminium-garnet**
27	**MTZs**	**Microthermal Zones**
28	**MENDs**	**Microscopic Epidermal Necrotic Debris**
29	**PMMA**	**Polymethylmethacrylate**
30	**PDGF**	**Platelet derived growth factor**
31	**VEGF**	**Vascular endothelial growth factor**
32	**EGF**	**Epidermal growth factor**
33	**FGF**	**Fibroblast growth factor**
34	**IGF**	**Insulin like growth factor**
35	**CTGF**	**Connective tissue growth factor**
36	**PRF**	**Platelet rich fibrin**
37	**ACD**	**Acid citrate dextrose**
38	**CPD**	**Citrate phosphate dextrose**
39	**RBCs**	**Red blood cells**
40	**WBCs**	**White blood cells**

41	**RCF**	**Relative centrifugal field**
42	**VAS**	**Visual Analog Scale**
43	**HIV**	**Human Immunodeficiency Virus**
44	**HBV**	**Hepatitis B Virus**

ABSTRACT

INTRODUCTION

Acne scarring is a major cause of disfiguring scars over face. It causes not only cosmetic issues but also psychological problems. There is no standard treatment for acne scars. Various therapeutic modalities have been found efficacious, among which laser therapy is a major treatment option. Platelet rich plasma has been long used as an adjuvant along with fractional carbon dioxide laser for the treatment of post acne scars.

AIMS AND OBJECTIVES

To compare the effectiveness and safety of combined use of platelet rich plasma and fractional carbon dioxide laser against fractional carbon dioxide laser alone in the treatment of acne scars.

MATERIALS AND METHODS

This is a split face(right-left) comparative study including 32 patients with moderate to severe scars according to Goodman and Baron qualitative grade. The patients underwent three sessions of fractional carbon dioxide laser along with platelet rich plasma and fractional carbon dioxide laser alone on right and left sides of the face respectively at an interval of 6 weeks. Goodman and Baron qualitative and quantitative grades were used for the evaluation of results along with visual analog scale (VAS) for patient satisfaction and physician assessment of scars. Adverse effects following the procedure was also evaluated and compared.

RESULTS

There was significant improvement of scars over both sides of face, but the difference between the right and left side of face was not statistically significant. With the help of Chi square test, qualitative score was compared between both sides of the face.

There was no significant difference in the distribution of qualitative score between both sides, before (p value- 1) and after treatment (p value- 0.9115). By two sample t-test, there was no significant difference in the mean of quantitative score between both the side of face before (p value- 0.63) and after treatment (p value- 0.695). On assessment of VAS score, majority of the patients were very satisfied with the procedure over both the side of face. But there was no statistically significant patient satisfaction over right side as compared to left side (p value- 0.857). In physician assessment of scars, grade 2 improvement which is 26-50% improvement was observed in most of the patients over both sides, but there was no difference in improvement between both sides of face. Erythema, burning sensation, scabbing, edema, and post inflammatory pigmentation were the most common side effects observed. Pigmentation was the only long term adverse effect observed. There was no difference in adverse effects between both sides of the face.

CONCLUSION

Both methods were effective in the management of acne scars. Adding platelet rich plasma to fractional carbon dioxide laser did not produce any statistically significant synergistic effects. Erythema, burning sensation and scabbing was present in almost all patients post procedure. Addition of platelet rich plasma provided no improvement or addition of side effects and no increase or decrease in duration of side effects. Therefore, by avoiding PRP we can reduce additional interventional procedures, overall cost and can lead to an increase in patient compliance.

KEY WORDS: Fractional CO2 laser, PRP, Acne scars.

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INTRODUCTION

Acne is a chronic inflammatory disease of pilosebaceous unit in the skin. Most people in the age group of 15 to 17 years develop some form of acne. 15–20% of patients suffer from moderate to severe type.[1] Presentation varies from increase in sebum production, non-inflammatory lesions like open and closed comedones, inflammatory lesions like papules and pustules to different types of scarring.[2] The pathogenesis of acne include multiple factors.[3]

i. Increase in production of sebum
ii. Formation of comedones due to alteration in keratinization
iii. Propionibacterium acnes (P.acnes) colonizing the follicles
iv. Inflammatory mediators surrounding pilosebaceous unit.

Scarring develops in 95% of patients with acne.[4]

Different types of acne scars are:

- Atrophic, being most common (further divided into ice pick, rolling, and boxcar scars.)
- Hypertrophic
- Keloidal[5]

Inflammatory mediators and enzymatic degradation of collagen and subcutaneous fat forms the pathogenesis of atrophic scar.[5]

Different phylotypes of Propionibacterium acnes exist, which causes activation of epidermal innate immunity differentially, thereby leading to variations in acne severity.(6)

Severity of acne scars depends on the time delayed for the initiation of treatment from the onset of acne lesions thereby further highlighting the need for early and aggressive treatment. Studies show that untreated acne can lead to significant scaring in 3 years.(4)

Treatment modalities of atrophic scars include chemical peels, dermabrasion or microdermabrasion, subcision, needling, radiofrequency, laser treatment, punch techniques, fat transplantation and stem cell therapy.(5)

The laser-resurfacing technique utilises non-ablative, fractional, and ablative technologies. Fractional laser resurfacing technique is gaining more popularity in recent times due to its good clinical outcome, less side effects and decrease in the time taken for recovery.(7)

The Carbon dioxide laser emits an infrared beam (10,600 nm) which is invisible and targets extracellular and intracellular water. Vaporization of skin happens when water containing tissue absorbs this energy.(8)

Platelet-rich plasma is an autologous solution of plasma. It contains platelet concentration about 4 to 7 times the baseline concentration of human platelets and contains alpha and dense granules (rich in growth factors).

Platelet-rich plasma is prepared by collecting patient's blood and then centrifuging it in a centrifuging machine. PRP method can be employed in skin conditions which requires wound healing, and for cosmetic and anti-inflammatory uses.[9]

In this split face study, we compare the efficacy of fractional carbon dioxide laser alone against the combined use of fractional carbon dioxide laser and PRP. The efficacy of PRP along with fractional carbon dioxide laser still remains a controversy as there are several studies that show both for and against the results.

AIMS AND OBJECTIVES

To evaluate and compare

i. **Efficacy**

ii. **Safety**

of fractional carbon dioxide laser alone and combined use of fractional carbon dioxide laser and platelet rich plasma in the treatment of acne scars.

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REVIEW OF LITERATURE

Acne vulgaris is a common skin condition which can be recognized easily. Almost all people suffer from acne at some point of their life.[2] In those people with a positive family history, the presentation is severe and it occurs earlier than others who do not have a family history.[1]

Distribution of acne is more in face, neck, shoulders, upper chest and back regions which can be attributed to the increase in density of pilosebaceous units in these areas.[2]

Acne can be noninflammatory or inflammatory. Noninflammatory can be open or closed comedones. Inflammatory lesions include papules, pustules and nodules which can later lead to cyst and sinus tract formation.[1] If treatment delays, it can lead to acne scar formation.[5]

Patients with severe nodular lesions suffer from pain, psychological problems and feeling of social embarrassment.[10]

EPIDEMIOLOGY

Acne develops in almost 90% of boys and 80% girls during their adolescent age. For girls, the incidence of acne is in its peak during 14 to 17 years and for boys it is 16 to 19 years. Moreover, 80% of people in age group of 11 to 30 years develop some form of acne.[11] In about 50% of people, acne can continue into adulthood.[12]

According to a study conducted by Lucky et al puberty has a positive corelation with severity of acne in boys and was found that 50% of boys in the age group of 10-11 years had more than 10 comedones.[13]

Another study by lucky et al showed that acne was present in 78% of girls in the age group of 8 and 12 years.[14]

Sharma RK et al conducted a study among adolescents in North India. According to their study, out of 1032 students included in the study, 81.9% had mild acne, 17.1% had moderate acne and severe type was present in 0.9%.[15]

Adityan B et al in their study found out that 39.5% developed scarring post acne. Grade 1 acne was found in 60.2%, grade 2 in 27.5%, grade 3 in 2.6%, and 30% had grade 4 acne.[16]

According to a study conducted by V Goulden et al, prevalence of acne was 54% and 40% in women and men respectively and that post acne scars was found in 14% of women and 11% of men. [17]

Husain et al in his study found out that the mean age group was 16 years and that there was no much gender differences in prevalence in adolescence. His study also showed that with increasing age, the prevalence of acne also increases.[18]

ETIOLOGY

The various factors that can predispose a person to develop acne are:

- A family history of acne
- Genetics
- Polycystic ovary syndrome [2]
- Androgens (testosterone, dehydroepiandrosterone sulfate and dihydrotestosterone) [19]
- Propionibacterium infection

- Ethnicity
- Stress and picking
- Hyperinsulinemia
- Obesity [(1)]
- Skin surface occlusion by greasy substances, sweating and clothes.[(2)]
- Monomorphic acne due to anti-epileptic drugs.
- Anti-cancer drugs like Gefitinib and anabolic steroids can cause acneiform eruptions.
- Hot and humid environment.
- Chloracne due to exposure to dioxin.
- Smoking
- Few evidences support the role of diet, skin hygiene and sunlight in acne.[(2)]

PATHOGENESIS

Cytokines along with genetic and environmental factors has been implicated to play an important role in acne pathogenesis.[(19)]

Four main factors which are significant in the development of acne are:

- ✓ Release of inflammatory mediators,
- ✓ Follicular hyperkeratinisation with follicular plugging,
- ✓ Colonization of follicle by Propionibacterium acnes,
- ✓ Increase in the production of sebum.[(12)]

A recent hypothesis in acne pathogenesis suggests a combined action of PPAR ligands and androgens in the pilosebaceous unit in genetically predisposed individuals

thereby resulting in increase in sebocyte proliferation, sebum excretion and change in quality of sebum.[20]

In addition to this, various studies have implicated the role of diet, nutrition, genetics, oxidative stress in the pathogenesis if acne.[21]

Role of inflammatory mediators

Inflammation is considered as a key factor in acne pathogenesis.[19] Activation of keratinocyte proliferation is considered to be triggered by an increased activity of pro-inflammatory cytokine(interleukin-1).[22] mRNA gene levels of cytokines (TNF-α, IL-1, IL-8,IL-10) which are regulated by Nuclear factor kappa beta (NF- κβ) are remarkably upregulated in acne affected skin as compared to the uninvolved normal skin.[19] Metalloproteinases, β-defensin 4, IL-8 and granulysin are also involved in inflammatory acne lesions.[23]

There is increase in the expression of IL-8 and activator protein (AP)-1, which further attracts circulating inflammatory cells into the tissue. Active lipid mediators like leukotrienes, prostaglandins and 15-hydroxyeicosatetraenoic acids (15-HETE) also act to produce inflammation. Due to decrease in intracytoplasmic cAMP levels by phosphodiesterases, there is a favourable expression of pro-inflammatory cytokines namely TNF-α, IL-1, IL-8, IL-12 and IL-23.[24, 25]

Comedogenesis and remodelling of pilosebaceous unit are activated by Interleukin-1. Neutrophils gets attracted to the site of inflammation with the help of Interleukin-8. Invasion of Gram-positive organisms leads to the production of Interleukin-12 with the help of monocytes, which in turn will cause expression of

anti-microbial peptides like defensins (implicated in the evolution of the acne lesion).[26]

Toll-like receptors (TLRs)

Toll-like receptors are pattern recognition receptors (PRRs). TLRs causes activation of innate immune responses with the help of keratinocytes, neutrophils, natural killer cells, monocytes, macrophages, and dendritic cells such as Langerhans cells. TLR-2 and TLR-4 and are considered specific in the pathogenesis of acne. Propionibacterium acnes causes activation of multiple pathways which will trigger nuclear factor (NF)-κβ transcription factor which in turn lead to the production of inflammatory cytokines such as IL-1, IL-6, IL-8, IL-10, IL-12 and TNF-α. In addition, TLRs also causes release of β defensin 1 and β defensin2.[27]

Cytokines mediated by Toll-like receptors causes induction of matrix metalloproteinases which leads to triggering of inflammation, dermal matrix destruction and later leading to formation of scar.[19]

Follicular hyperkeratinisation with subsequent plugging of the follicle

P.acnes causes activation of TLR which results in release of Interleukin-1α from infundibular keratinocyte, thereby initiating comedogenesis. This is considered as an important step in the natural course of acne.[28] IL-1α not only helps in comedone formation but also has a role in sebocyte hyper-cornification. Increase in follicular keratinisation and a decrease in desquamation of infundibular keratinocytes leads to formation of microcomedones. Microcomedones are the precursor lesions and forms a plug of keratin at the follicular infundibulum.[19]

Alternation in quality of sebum, increase in levels or sensitivity of androgens, increase in P. acnes growth and local cytokine mileu are the factors that lead to epithelial hyperproliferation. Epithelial hyperproliferation causes comedone formation.[29]

An aggregation of microorganisms enclosed by an extracellular polysaccharide lining which is secreted by the bacteria is called a Biofilm. It acts like a biological glue and has a role in microcomedone formation.[29]

Propionibacterium acnes follicular colonization

P. acnes is an anerobic Gram-positive bacteria. They are present as a normal commensal in the sebaceous follicle and contribute in the development of inflammatory acne lesions. Propionibacterium granulosum and Propionibacterium avidum are the other types of Propionibacterium that are involved in development of acne.

Enzymes such as proteinases, hyaluronidases, lipases and chemotactic factors are released by P. acnes. These enzymes play an important role in the inflammatory cascade. [30]

Modulation of Th1/Th2 response and activation of monocyte-derived dendritic cell maturation helps in triggering of immune reactions.[19]Innate immune response of the host is activated by TLRs and by recognizing pathogen-associated molecular patterns (PAMPs).[31]P. acnes also stimulates inflammasome formation, which triggers the activation of caspase-1, IL-1β and IL-18 (involved in the development of produce the inflammatory papules).[32]

Role of sebum production

Important events in the pathogenesis of acne are increase in sebum excretion, alteration in oxidant/antioxidant ratio and change in lipid composition.[33] Previous studies have found a decrease in the linoleic acid levels in patients developing acne.[34] Presence of lipoperoxides in sebum is the main hallmark in patients with acne. It is produced because of peroxidation of squalene and a reduction in the Vitamin E levels.[35]

Keratinocyte proliferation and differentiation can be altered by both lipoperoxides and monounsaturated fatty acids (MUFA). Production of pro-inflammatory cytokines and activation of peroxisome proliferator-activated receptors (PPAR) are brought about by peroxides.

The various factors which regulate the function of sebocytes are ligands of receptors expressed in sebocytes (androgens and estrogens, PPAR ligands and neuropeptides (NP), liver-X receptor ligands) histamines, retinoids and vitamin D. [19]

All the events together initiate inflammatory process, perifollicular abscess, rupture of follicular unit, and later resulting in wound healing. Any injury to the skin can result in wound healing process.

Wound healing is a complex process. It involves extracellular matrix, soluble chemical mediators, parenchymal resident cells (keratinocytes, fibroblasts, endothelial cells, nerve cells) and infiltrating blood cells such as lymphocytes, monocytes, and neutrophils.

Scars can be atrophic or hypertrophic and they occur in the site of injury.

The process of wound healing has 3 stages:

- Inflammation,
- Granulation tissue formation,
- Matrix remodelling. [36,37]

Type of scar depends on the abnormal production and destruction of collagen during the process of healing. Atrophic scars are common and is seen in 80-90% of cases wherein there is a degradation of collagen.[6]

CLINICAL FEATURES

The clinical features of acne are seborrhoea (excess sebum), non-inflammatory lesions (open and closed comedones), inflammatory lesions (papules and pustules), and various degrees of scarring. Severe nodulocystic acne consists of multiple nodules and cysts.[2]

Micro-comedones

⬇

Closed and open comedones

⬇

Inflammatory lesion

⬇

Postinflammatory erythema,

⬇

Post-inflammatory hyperpigmentation,

⬇

Scarring. [6]

Permanent disfiguring scars can occur if the inflammation is severe.[38]

TYPES OF SCARS

Two basic types of scars occur due to net gain (Hypertrophic scar) or loss of collagen (Atrophic scar). Atrophic scars contribute to the majority of scars as compared to hypertrophic scars and keloids.[39]

Hypertrophic scars are more common during the first few months after injury and they are restricted by the margins of injury. It can be associated with pruritis, pain and limitation in movement. On the otherhand, keloids have a tendency to mature normally and can extend beyond the margins of injury. In Keloids there is a disproportionate production and accumulation of collagen.[11]

Atrophic Acne scars are divided into 3 types:

- Icepick scars,
- Rolling scars, and
- Boxcar scars (further divided into shallow (0.1-0.5mm) and deep (more than or equal to 0.5mm).[38]

Icepick scars

These scars are punctiform, narrow and deep with a diameter of 2mm. The opening of the scar is wider than the infundibulum thereby forming a “V” shape.

Rolling scars

Rolling scars are characterised by tethering of dermis to the subcutaneous tissue. Their diameter is more than 4-5mm and has a rolling appearance, giving an “M” shape.

Boxcar scars

Boxcar scars are round/oval scars characterised by sharp vertical. They are wider at the opening as compared to icepick scars and lack the tapering V shape. They have a "U" shape appearance. Boxcar scars can be either shallow or deep.[39]

Sinus tracts, hypertrophic scars, and keloidal scars are the other less common scars.[38]

ACNE SCAR GRADING:

The various modalities of acne scar grading are:

- Lesion counting
- Subjective self-assessment
- Acne Scar Rating Scale (ASRS)
- Evaluator-based qualitative and quantitative scarring grading systems
- Echelle d'Evaluation Clinique des Cicatrices d'acne (ECCA)
- Global Scale for Acne Scar Severity (SCAR-S) and
- Imaging.[40]

Lesion counting:

The individual scars are counted and a score is given depending on the number of each scar. Disadvantages of this method are that it is tiresome, time consuming and other parameters like size and color are not taken into consideration. Moreover, the subtype of scar does not alter even after treatment.[40]

Subjective self-assessment:

The subjective self-assessment method helps the physician to know about the perspective of patients and it helps in routine practices.

Two modalities of subjective self- assessment are

- ✓ The Self-assessment of Clinical Acne-Related Scars (SCARS)

  It is a questionnaire which can rate the acne scar severity depending on how it seems on a mirror. It is a 5 item questionnaire
- ✓ Facial Acne Scar Quality of Life (FASQoL) questionnaire

  It evaluates the influence of acne scars on the mental health and day to day activities. It is a 10 item questionnaire.

Subjective self -assessment is an unreliable method as compared to grading scales.[41,42]

GLOBAL ACNE SEVERITY SCORING:

Acne scar rating scale (ASRS)

The scale is categorized as:

1- minimal,

2- mild,

3 -moderate,

4- severe.

Here each scar is given an individual score depending on the visibility and depth. This method does not include any pigment changes.[43]

Evaluator-based qualitative and quantitative global scarring grading systems

According to this system, acne scars are graded based on scar severity into grades 1 to 4.

The grading system also classify acne scars depending on its distribution. If there is only focal involvement of a single area it is categorized as "A" and if two or three areas with discrete involvement is present then it is categorized as "B". [40]

Table 1: Global Acne Scarring Classification by Area of Involvement and Major Scar Type[40]

			Number of cosmetic units involved	
Grade	Grade description	Subgroup	A (focal, single cosmetic unit)	B (discrete, 2–3 cosmetic units)
1	macular	Erythematous	1A	1B
		Hyperpigmented	1A	1B
		Hypopigmented	1A	1B
2	mild	Atrophic	2A	2B
		Hypertrophic	2A	2B
3	moderate	Atrophic	3A	3B
		Hypertrophic	3A	3B
4	severe	Atrophic	4A	4B
		Hypertrophic	4A	4B

Table 2 : Goodman And Baron Qualitative Scar Grading System[44]

Grade of Acne Scar	Level of Disease	Clinical Features
1	Macular	Erythematous, hyperpigmented or hyperpigmented patches.
2	Mild	Mild hypertrophic or atrophic scars. Scars may not be visible at a distance of 50 cm or more. Can be covered up by makeup, shadow of beard hair in males or body hairs if it is extrafacial.
3	Moderate	Moderate hypertrophic or atrophic scars which is visible even at a distance of 50 cm or more. These scars cannot be covered up easily by makeup or shadow of beard hair and extrafacial body hair. On manual stretching the scar flattens.
4	Severe	Severe hypertrophic or atrophic scars. Obvious at a distance more than 50 cm. Cannot be covered up by makeup or by the shadow of beard hair or extrafacial body hairs. On manual stretching the scars will not flatten.

A Quantitative Global acne scar grading system was also developed by Goodman and Baron which is a point based system and instead of category, uses the type and quantity of scars.

The scale based on numerical system is again divided based on severity:

macular and mild atrophic scars - 1 point;

moderately atrophic scars - 2 points;

severely atrophic scars- 3 points;

hyperplastic scars- 4 points.

The severity score is multiplied by a factor. The factor depends on the lesion count (1 point if lesion count is less than 10, 2 points if lesion count is between 11 and 20, and 3 points if more than 20).The end score can range from 0 to 84.[44]

Table 3 : Quantitative Acne Scars Grading System

Grade (Type)	1(1-10 lesions)	2 (11-20 lesions)	3 (>20 lesions)
(A) Mild Scarring (1point each) Macular erythematous or pigmented Mild atrophic dish like	1 point	2 points	3 points
(B) Moderate Scarring (2 points each) Moderately atrophic dish like Punched out with shallow base Shallow with broad atrophic areas	2 points	4 points	6 points
(C) Severe Scarring (3 points each Punched out with deep, normal bases Punched out with deep abnormal bases Linear or troughed scar Deep, broad atrophic scar	3 points	6 points	9 points
(D) Hyperplastic Papular Keloidal	2 points 6 points (area<5cm^2)	4 points 12 points (area 5-20cm^2)	6 points 18 points (area>20cm^2)

Echelle d'evaluation clinique des cicatrices d'acne (ECCA):

ECCA is another quantitative scale and it depends on the total of number and type of scar

The scar type is further divided based on the morphological features. The lesion count determines the extend of the scar and the categorization is made on a 4 point scale. The total of the type of scar and extend of the scar gives the final score (ranges from 0 to 540).[45]

0 -no scars,

1 - less than five scars,

2 - between five and 20 scars,

3 - more than 20 scars.

This method helps to evaluate the extent of scarring for each type of scar. The disadvantages of this method are that it is restricted to face, time intensity and the relevance of the score ranges are not determined. [39,45]

Global scale for acne scar severity (SCAR-S):

The scale include face, trunk, and back. It also includes both hypertrophic and atrophic scar. A six point scale is used for assessment of scar severity. The six categories (1 to 6) include features ranging from clear to being extremely severe. Face, chest and back are individually assessed and later added together to give the end score.[46]

Quantitative measurement of acne scars based on imaging:

Recently visual inspection of the extend of scar has become more popular due to its objectivity and reproducibility.[40] Different imaging techniques have been developed

Previous studies showed that color images can be processed to quantify scars.[40] Another study used 3D images taken with the help of special cameras to set up a topographic map. Quantitative measurement (volumetric) of individual scar is then calculated with the help of a computer.[47]

Colour image based method is helpful in evaluating redness and pigmentation but unlike the volumetric measurement does not help in follow up of scar.[40]

Ultrasound imaging of the skin can be used to assess the thickness of skin tumor, atrophic scar thinning, scleroderma and striae distensae.[48]

PSYCHOLOGICAL IMPACT OF ACNE SCARS

Acne scars can cause distress in young adults and can lead to major psychological damage. Mostly individuals belonging to younger age group suffer from this psychological distress. Depending on the severity of acne, the scars can be permanent which can affect the psyche of the individually negatively for quite a long time.[49]

The impact due to Scar has been found to have correlation with multiple major areas of a person's life such as: self-esteem, self-trust, awareness of treatment of the disease, functionality and comfort of the appearance of a person, ability to function efficiently in the society and psychological damage. [50]

TREATMENT OF ATROPHIC ACNE SCARS

There are multiple therapeutic options for acne but the treatment plan should always be individualized for each person depending on the various features and type of scar. Various treatment methods include subcision, needling, dermabrasion, chemical peels, punch techniques, non-ablative lasers, fillers, autologous fat transfer.[51]

The treatment modalities and the number of visits depends on the scar type of the patient and differs from one person to other. Moreover, combining multiple treatment methods are advisable.[52]

FOR SCAR ASSOCIATED ERYTHEMA

For Scar associated erythema, pulse dye laser (PDL) and other laser devices can be used effectively.[6] Gold standard therapeutic method for scar associated erythema is pulse dye laser (PDL).[53]

PDL results in improvement of redness by causing destruction of dermal vascular components by way of selective thermolysis. Oxyhemoglobin is the main chromophore of PDL which lies within subcutaneous vessels. Oxyhemoglobin absorbs yellow and green light range with peaks being at 418, 542 and 577nm.[54] PDL at the range of 595-600nm (long pulsed PDL) has a low risk of purpura post procedure because of slow heating of target vessels.[55]

PDL also helps in collagen remodelling and leads to stimulation of fibroblast by increasing the transforming growth factor beta (TGF-B).[56]

Other devices found helpful in treating scar associated erythema are the potassium titanyl phosphate (KTP, also known as frequency-doubled Nd:Yag) and Intense pulsed-light (IPL).[57,58]

FOR ATROPHIC SCARS:

CHEMICAL PEELS:

Chemical peeling causes destruction of outer layers of skin that are damaged by applying chemical on to the skin. The process results in increase in exfoliation.[39]

The level of penetration varies between different agents. Therefore, chemical peels are divided based on the level of necrosis on histopathology (Table: 4)

Table 4: Types of Chemical Peels

Depth of penetration	Histologic level	Peeling agents
Very superficial	Stratum corneum is involved.	30-50% Glycolic acid applied for 1-2minutes. 1-3 coats of Jessner solution. 1 coat of 10% TCA
Superficial	Epidermis or part of epidermis anywhere between stratum granulosum to basal layer is involved.	50-70% Glycolic acid applied for 2-20 minutes. 4-10 coats of Jessner solution, 10-30% TCA
Medium	Epidermis and all or part of papillary dermis is destroyed.	Glycolic acid 70% for 3-30 minutes. TCA 35-50% CO2 plus TCA 35% (Augmented TCA) Jessner solution plus TCA 35% Glycolic acid 70% plus TCA 35%
Deep	Involvement of epidermis and papillary dermis, extending into the reticular dermis	Phenol 88% Baker-Gordon phenol formula

Previous history of keloids, cold sores, scarring tendencies and any excessive radiation history should be taken before proceeding for a chemical peeling. It is advisable to avoid excess sun exposure immediately after peel.[59]

CROSS TECHNIQUE OR DOT PEELING:

Ice pick and small boxcar scars can be treated effectively by this method. In this technique initially the skin is stretched and with the help of a wooden toothpick, 65-100% trichloroacetic acid (TCA) is applied to the base of the scar. Vaseline can be applied over the surrounding normal tissue to prevent damage. There will be mild burning sensation at the time of application.

Initially there will be destruction of epithelial tract, which is followed by collagen fibre formation during healing. Formation of collagen fibres takes about 2 to 3 weeks and can extend up to 4 to 6 weeks. The process can be repeated at an interval of 2-4 weeks for about two to three times.[5]

DERMABRASION/MICRODERMABRASION:

Dermabrasion was one of the first advanced treatment modality for acne scars.[44] Dermabrasion and microdermabrasion ablate the skin mechanically and induces re-epithelialization, thereby causing facial resurfacing.

Helps in remodelling the structural proteins in skin by penetrating till the papillary and reticular dermis. Dermabrasion causes complete removal of epidermis.

Microdermabrasion is a superficial form of dermabrasion. It removes only the outer layer of epidermis.[39]

RADIOFREQUENCY (RF):

Radiofrequency is an electromagnetic radiation which is nonionizing. Frequency varies from 3 to 300 GHz. In a fractional bipolar radiofrequency machine, the current will flow between the electrode pins through the skin. It induces skin injury by causing fractional deep dermal heating. Later wound healing and collagenization occurs as a response to the injury. The principle of sublative rejuvenation has been used which results in less destruction of epidermis and more dermal remodelling and has been found to decrease the side effect and increased the efficiency.[60]

SUBCISION:

Subcision is a procedure found to be effective in rolling scars. It is less effective for boxcar and icepick scars. Here a needle usually a Nokor needle is inserted under the skin, and moved in multiple directions (to and fro and in fanning movement). This procedure results in cutting of fibrotic strands beneath the scars. A dermal pocket is created and organization of blood occurs which is followed by formation of connective tissue.[61]

NEEDLING:

Needling is also known as collagen induction therapy.[62] Needle dermabrasion utilizes a technique in which a device made of needle studded barrel is rolled on the skin. This creates micropunctures till the level of papillary and mid dermis. Rolling is continued till there is bruising. This process commences the complex cascade of growth factor thereby resulting in collagen formation.[63] Results starts to appear by 6 weeks but takes up to 3 months for complete collagen production. [39]

LASER RESURFACING

The first MASER (Microwave Amplification by Stimulated Emission of Radiation) was built by Charles Townes and Arthur Schawlow in 1954.[64]

Gordon Gould coined the term LASER In 1957.[65]

Kumar Patel invented CO2 laser in 1960, and was first used by Leon Goldman (Father of laser medicine) in 1963.[66,67]

Different types of laser resurfacing methods are available (non-ablative and ablative). They are very helpful in treating scars other than deep ice pick scars.[62]

ABLATIVE LASERS:

CARBON DIOXIDE (CO2) LASER:

CO2 laser resurfacing utilizes energy at a wavelength of 10,600nm. This energy is absorbed by extracellular and intracellular water resulting in accelerated heating and vaporization of tissue. This procedure vaporizes tissue at 20 to 60 μm depth and creates thermal necrosis zone at a range of 20 to 50μm.[68] Wound healing and collagenization is induced by heating of dermis below the level of ablation. It also results in heat mediated contracture of tissue. Erythema may take months to disappear. Re-epithelialization occurs in 5 to 10 days. Hyper or hypopigmentation, persistence of demarcation line between treated and untreated area are the side effects. Can also result in scarring and infection.[5]

Erbium: Yttrium-Aluminium-Garnet (Er:YAG).

Er:YAG emits energy at a wavelength of 2940nm.[69] Due to its shorter wavelength, Er:YAG laser is ten times more precise for water than CO2 laser. It also decreases the residual thermal damage. Er:YAG laser can result in 5 to 10μm thermal necrosis zone and induces tissue vaporization at 20 to 25μm depth.[68] The epidermis and superficial papillary dermis absorb almost all the energy resulting in a much smaller area of damage beneath the level of ablation and in a more superficial ablation.[5] Side effects are lesser with reduced healing time.(70) Re-epithelialization occurs in 4 to 7 days.[68]

PLASMA SKIN RESURFACING:

Non laser device that produce plasma, from radiofrequency and nitrogen atoms. Releases heat energy to the skin directly by utilizing ionized nitrogen gas pulses. The epidermis sheds after the completion of healing. Elastin and collagen fibres form almost after 10 days of treatment. Side effects are uncommon. Side effects are erythema, edema, infection, temporary hyperpigmentation, epidermal de-epithelialization, and scarring. [5]

NONABLATIVE LASERS

Nonablative laser helps in reducing the side effects and the requirement for postoperative care.[39] They stimulate collagen formation in dermis while sparing the epidermis.[71]

Neodymium :yttrium aluminium garnet (Nd:YAG) laser:

Nd:YAG laser benefits individuals with dark and sensitive skin. These lasers emit light of infrared wavelengths. Light penetrates into the deep layers of skin (targeting underlying collagen and water) but it does not cause destruction of epidermal layer. [(5)]Thermal damage acts as a stimulus resulting in inflammatory mediator release, fibroblast activation, neocollagenesis, and dermal remodelling.[(72)]

This laser has the advantages of reduced recovery period with decreased risk of infections and other pigmentary complications.[(73)]

Diode laser:

The 1450nm diode laser in the infrared spectrum targets the water in the upper dermis, remodels the skin's underlying collagen, and promotes formation of new collagen. [(73)]Side effects are usually minimal and can include postoperative erythema, edema, and hyperpigmentation.[(69)]

Fractional carbon dioxide laser:

The CO2 laser targets both extracellular and intracellular water by emitting an invisible infrared light of 10,600 nm wavelength. Vaporization of skin occurs due to absorption of energy by water containing tissue. CO2 laser therapy has the advantage of quick recovery and minimal discomfort.[(8)]

Fractional laser technology always has an upper hand over the conventional ablative methods like ablative CO2 and erbium:YAG lasers as they have demand for a need of anesthesia, increase in downtime , requires good post-operative care, prolonged avoidance of sun exposure, risk of hyperpigmentation and scarring

especially in darker skin types. These drawbacks can be avoided by fractional laser technique.[7]

Principle of Fractional Photothermolysis:

The concept of fractional photothermolysis was introduced by Manstein and colleagues in the year 2004. The device emits light in a pixilated manner. This energy produce microscopic columns of thermal injury to the skin and creates microthermal zones (MTZs).[74] Compared to ablative techniques where uniform and confluent patch of epidermal/dermal injury is brought about, fractional resurfacing technique results in thermal ablation of microscopic epidermal columns and dermal tissue in a regularly spaced manner. Therefore, in fractional photolysis there will be thermal damage to a portion of skin, sparing the intervening areas unaffected.[75,76]

The machine type, laser wavelength, fluence of laser used and number of laser application affects the depth, size and number of vertical columns created by MTZs. In each treatment sittings about 15-25% of the skin surface make up the MTZs. MTZs ranges in diameter from 100 to 160μm and the density of MTZs can be adjusted.

The dermal-epidermal debris is then integrated into the columns of microscopic epidermal necrotic debris (MENDs) which is then by the process of transepidermal elimination is eliminated. Eventually re-epithelialization is stimulated which is mediated by adjacent normal tissue. After thermal ablation of tissue, production of epidermal stem cell reproduction and collagen fibres is initiated.

Similar principle is used in non-ablative fractional lasers but without epidermal ablation and elimination of dermal contents.

Downtime is reduced as there is a large portion of intervening normal skin and therefore healing occurs rapidly. Reduced downtime, minimal post procedure erythema and edema, favourability for darker skin individuals and decrease in dyschromic changes are the advantages of fractional lasers. [7]

INDICATIONS OF FRACTIONAL TREATMENT

Esthetic:

Acne scars, Striae distensae, wrinkling of skin, surgical and post burn scars, pigmentation due to minocycline, melasma, dyschromias, post-inflammatory hyperpigmentation, Nevus of Ota, hemangiomas, Poikiloderma of Civatte, telangiectatic matting.

Non-esthetic:

Colloid milium, Disseminated superficial actinic porokeratosis, Granuloma annulare. [7]

CONTRAINDICATIONS:

Active infections (bacterial and viral), tendency for keloid formation or hypertrophic scars, isotretinoin use within the previous six months, collagen vascular disorders, ongoing ultraviolet exposure history, previous history of radiation exposure to the treating area, body dysmorphic disorder, unrealistic expectations. [8]

COMPLICATIONS:

Fractional Carbon dioxide laser is followed by minimal complications which includes: post-inflammatory hyperpigmentation, exacerbation of acne and rosacea,

perioral dermatitis, formation of milia, contact dermatitis. Post inflammatory hyperpigmentation is more common in darker skin individuals. Hyperpigmentation gradually improves within 6 weeks.

Bacterial, viral and candidial infections, prolonged healing, hypopigmentation, persistence of erythema are the serious complications which is usually uncommon and other severe complications include disseminated infection and hypertrophic scar.

Immediate therapeutic measures should be initiated to prevent any severe consequences like dyspigmentation and permanent scarring. [8]

PUNCH EXCISION AND PUNCH ELEVATION:

Punch technique is mainly useful for boxcar and icepick scars.

Punch excision is a technique in which a punch instrument with the approximate size of scar is selected which is followed by excising the scar unto the subcutaneous layer. Later the defect is closed with the help of sutures.[11] A single nonabsorbable suture can be used for punch sizes of 2.5mm or more which will help in healing of wound and in minimizing the spread.[38] If the punch excision size is less, then it can be left for secondary intention healing.[77]

Punch elevation technique is useful for the treatment of box scars which are broad and those with no underlying fibrosis.[78] Here with the help of a punch instrument the scar is excised up to the subcutaneous plane, followed by tissue elevation and sutured slightly higher to the normal skin so as to compensate for the contraction which occurs during healing.[38]

FILLERS:

Injectable fillers are used to augment the soft tissue. It is best suited for soft atrophic scars and boxcar scars. Fanning, serial puncing, cross-hatching, linear threading, deep bolus and superficial microdroplet injections are the various modes of injection. Side effects include erythema, pain, swelling, infection, abscess.

Fillers can be temporary, semi-permanent, and permanent.

Temporary fillers last only for few months which necessitate repeated sessions thereby increasing the cost. Hyaluronic acid fillers triggers collagen production, augments the soft tissue and helps in improving the overlying the skin quality.[6,79]

Semi-permanent fillers are biostimulatory. It stimulates production of fibrous tissue and poly-ι-lactic acid. It typically lasts for two years.[80] Calcium hydroxylapatite (synthetic filler) has been proved helpful as a monotherapy in boxcar scars[81,82] and one week after subcision.[82]

Permanent fillers contain large particles therefore cannot be phagocytosed. They last for many years.[79] There can be permanent side effects hence necessitating complete removal. Silicone is cheaper and last for 10-20 years.[83] Polymethylmethacrylate (PMMA) is another permanent synthetic filler.[6]

FAT TRANSPLANTATION:

Fat can be used for augmentation. They are easily available, cheap, chances of allergy and other adverse effects are minimal.

Two phases of fat transplantation are Graft procurement and graft placement. Implantation of fat in multiple tunnels helps the graft to reach for maximal available blood supply. Maximum results can be seen after 3 months post procedure.[84]

PLATELET RICH PLASMA:

Platelets are cytoplasmic fragments of megakaryocytes. Platelets are about 2mm in diameter and they originate in bone marrow. The bioactive proteins present in platelet help in wound healing and hemostasis.

Wound healing is activated by the growth factors released by platelet. Seven major growth factors secreted by platelet are:

- Platelet derived growth factor (PDGF a and b),
- Transforming growth factor TGF (alpha and beta),
- Vascular endothelial growth factor (VEGF),
- Epidermal growth factor (EGF)
- Fibroblast growth factor (FGF)
- Connective tissue growth factor (CTGF),
- Insulin like growth factor –1 (IGF -1).[85]

Platelet-rich plasma (PRP) is an autologous concentration of human platelets in a small volume of plasma. Since it is a concentrate of platelets, it contains all the growth factors present in platelet.[86] In addition, PRP contains 3 proteins in blood: Fibrin, fibronectin and vitronectin which helps in cell adhesion.[85]

Activation of platelet causes degranulation, followed by bioactivation of secretory proteins (by adding carbohydrate and histones side chains).[86] The activated

proteins get released, binds to the transmembrane receptors of the target cells such as fibroblast, mesenchymal stem cells, epidermal cells, osteoblasts, endothelial cells. Binding with the transmembrane receptors triggers the intracellular signal protein to induce proliferation of cells, osteoid production, formation of matrix, collagen formation.[85]

Platelet secretes growth factors within 10 minutes of activation. Within one hour more than 95% of the pre-synthesized growth factor will be released.[87]

Classification

1. Pure Platelet-Rich Plasma (P-PRP) or leucocyte-poor PRP:

   Does not contain leucocytes and after activation contain low density fibrin network.
2. Leucocyte- and PRP (L-PRP):

   Contains leucocytes. After activation has a low density fibrin network.
3. Pure platelet-rich fibrin (P-PRF) or leucocyte-poor platelet-rich fibrin:

   These preparations do not contain leucocytes. Has a high density fibrin network. They exist only in activated gel form which makes them difficult to be injected.
4. Leucocyte and platelet rich fibrin (L-PRF) or second-generation PRP:

   Contain leucocytes and high density fibrin network. [88]

Preparation:

PRP is prepared by collecting patient's venous blood and centrifuging it at the time of treatment. Depending on the baseline platelet count, technique and device

used, approximately 3 to 5cc of PRP can be obtained from 30cc of patient's venous blood.[85]

Different anticoagulants can be used for collection of blood. Trisodium citrate is the most commonly used anticoagulant. Acid citrate dextrose (ACD) and Citrate Phosphate dextrose (CPD) with and without adenine (ACD-A and CPD-A) are the other anticoagulants.[89]

Differential centrifugation is used for PRP preparation. Depending on the specific gravity, acceleration force is adjusted.

Can be prepare by PRP method or buffy coat method.

PRP method:

Here two centrifugations are done to get final platelet concentrate. After first centrifugation, red blood cells are collected and second centrifugation will yield the platelet concentrate. Whole blood is collected in anticoagulant containing tubes. In the first spin, a constant acceleration is used which separate RBCs from rest of whole blood volume. First step separates whole blood into 3 layers- upper layer rich in platelets and WBCs, intermediate layer (known as buffy coat) which contains mostly WBCs, and the bottle layer containing RBCs.

For pure PRP (P-PRP), upper layer and superficial buffy coat are separately transferred to an empty tube. For obtaining leucocyte rich PRP (L-PRP), the entire layer of buffy coat and few RBCs are separated and transferred. This is followed by second spin. The upper portion is platelet poor plasma which is removed. The last one third portion (last 5ml of plasma) contain pellets which is homogenized to create Platelet rich plasma.

Buffy coat method:

Buffy coat is rich in leucocytes. They are obtained by high speed centrifugation of the whole blood. Separating the thin buffy coat from the lower layer containing RBCs is a difficult task.[85]

PROCEDURE

PRP method:

Whole blood collected in Citrate Dextrose (ACD) tubes (whole blood should not be cooled before or during the process)

↓

Collected blood is centrifuged by soft spin

↓

Supernatant plasma rich in platelets is transferred to a sterile tube which does not contain an anticoagulant.

↓

The tube into which the supernatant plasma is transferred undergoes hard spin

↓

After the second spin the upper two third portion is platelet poor plasma and the lower one third is the platelet rich plasma.

↓

Pellets rich in platelets are formed at the bottom of the tube.

↓

Platelet poor plasma in the upper part is removed and pellets rich in platelets is suspended in minimum plasma by shaking the tube gently.

Buffy coat method:

Whole blood is stored at 20-24°C before centrifugation.

↓

Blood is centrifuged at high speed

↓

Three layers are formed at the end of centrifugation (the upper part is platelet poor plasma, intermediate layer is rich in platelet and WBCs and the lower part contains RBCs.

↓

The upper layer is removed and the buffy coat is transferred to an empty sterile tube

↓

Second centrifugation is done at a low speed so as to separate WBCs or can use leucocyte filtration pressure. (90)

After the preparation of PRP, it remains stable for 8 hours or more in the anticoagulated state. Activation of platelets is required for the secretion of granule contents. Bovine thrombin was used as an activator previously. It is no longer recommended. Calcium chloride is a safer alternative. Calcium chloride is slower. Takes about 20 minutes for platelet activation. Moreover, PRP without an activator also results in direct platelet activation once injected into collagen tissue.(89)

FACTORS INFLUENCING PRP YIELD

Drawing of blood

Using small bore needles to draw blood can lead to unintentional platelet activation which is avoided by using large bore needle (more than 22G).(91)

Centrifugation:

To augment the earth's gravitational forces a centrifuge can be used to provide a centrifugal force which is several thousand times the force of gravity.

Differential Centrifugation is used for separating the cells. RCF (relative centrifugal field) is the force which separates the two phases. RCF is expressed as multiples of the earth's gravitational field (g). Quicker sedimentation can be achieved by accelerating the 'g' which is the force exerted on the contents. This results in separating of the aqueous solution.

Revolutions per minute (rpm) can be obtained by the formula

Formula g = (1.118 × 10-5) R S2

(g is the RCF, R is the radius of the rotor (from centre of rotor to sample) in centimetres and S is the speed of the centrifuge in revolutions per minute).

g depends on the radius of the centrifuge motor. Acceleration forces differs if the same machine with different motor is used.[92]

Temperature

Temperature is a major factor in preventing platelet activation during processing. For PRP 21°C–24°C is the recommended temperature for centrifugation of blood.[85]

Anticoagulants

Recommended anticoagulants are those with anticoagulants with citrate and dextrose of sodium citrate. EDTA is not preferred.[93]

Activation of PRP:

Common activators are calcium chloride, thrombin and mechanical trauma. If PRP is injected in soft tissue it does not require any exogenous activator as collagen acts like a natural activator.[91]

USES OF PRP IN DERMATOLOGY:

Alopecia areata

Striae distensae

Acne and traumatic scar

Skin rejuvenation

Dermal augmentation

Vitiligo

Lichen sclerosus

Chronic ulcer

Melasma

Lipodermatosclerosis

Refractory nail disorders

Apart from its uses in dermatology, it was used previously in hematology, cardiac surgery. It has been proved useful in ophthalmology, dentistry because of its anti-inflammatory and immunomodulatory action. Due to its regenerative and wound healing property it can be used in orthopaedic and sports medicine. [9,94]

SAFETY OF PRP:

True PRP being an autologous preparation does not have any major side effects. It does not have the risk of Hepatitis B, C or HIV transmission. Occasionally it may cause local injection site reactions and secondary infections.[95]

MATERIALS AND METHODS

SOURCE OF DATA:

Patients attending Dermatology Outpatient Department in tertiary care hospital, Belagavi.

DURATION OF STUDY:

The study was conducted over a period of one year from 1st January 2020 to 31st December 2020.

ETHICAL CLEARANCE:

Clearance was obtained from institutional ethical committee.

STUDY DESIGN:

Split-face comparative interventional study.

SAMPLE SIZE: 32

Calculated with the help of previous similar studies.

The formula used for sample size calculation is,

$$n = \frac{2\left(Z\alpha_{/2} + Z\beta\right)^2}{\left(\frac{|\mu_1 - \mu_2|}{\sigma}\right)^2}$$

Where μ_1 is the mean of the first group, μ_2 is the mean of the second group and σ^2 is the common error variance, for 95% level of significance $Z_{\alpha/2}$ value is 1.96 and for 80% power Z_β value is 0.84.

INCLUSION CRITERIA:

i. 18 to 40 years
ii. Moderate to severe atrophic acne scars (according to Goodman and Baron's qualitative score)

EXCLUSION CRITERIA:

i. Age < 18 years and > 40 years
ii. Patients with active acne, active herpes infection, keloid tendency, pregnant and lactating mothers, HIV and HBV infection, bleeding diathesis, patients with unrealistic expectations.

DATA COLLECTION:

1) Data was collected by a single examiner and recorded in case record proforma.
2) Detailed history regarding the scars, duration of scars, occupation of the patients, use of cosmetics, exposure to sunlight, past, family and treatment history were taken.
3) Detailed general, physical, systemic and dermatological examination to look for the type and site of scars performed.
4) High resolution photographs were taken with the help of a digital camera (Sony DSC-H300/BC) with identical camera settings at all visits.
5) Photographs were taken with the help of an instrument with 2 parts (fixed and movable part). Chin of the patient was placed over the fixed part and camera was placed over the mobile part, and photographs taken at an angle of 0^{o} and 45^{o}.
6) Procedure was performed after taking written informed consent from all participating patients.

7) Patients were followed up 72 hours after procedure to assess the side effects and was recorded regularly.

METHODS:

1) Local anaesthetic agent was applied over the face and left for 45 min, followed by cleansing of the face with acetone.
2) Both eyes of the patients were protected with the help of an eye mask.
3) Right side of patient's face was treated with fractional non-ablative carbon dioxide laser followed by intradermal platelet rich plasma injections.
4) Left side of the face was treated with fractional non-ablative carbon dioxide laser.
5) Patients were prescribed topical antibiotics, sunscreens and emollients post procedure.
6) A total of 3 sessions were performed with an interval of 6 weeks between every session.
7) At the end of study, assessment was done with the help of-
    i. Goodman and Baron's Qualitative and Quantitative score
    ii. Visual Analog Scale of patient satisfaction
    iii. Physician assessment of improvement (with the help of photographs and done by a physician who was not involved in the study).
8) Records were maintained and analysed statistically.
9) Fractional CO2 laser settings -
    - Derma India
    - Scan mode
    - Power - 12 W

- Distance- 0.5 mm
- Duration- 0.5 ms
- Number of passes – 2

10) Platelet Rich Plasma settings-

- Remi 8C centrifugation machine
- 1st spin - 1000 rpm for 10 min, 2nd spin – 2000 rpm for 5 min
- Anticoagulant - Citrate Phosphate Dextrose
- Amount of blood collected- 18 ml
- Amount of plasma injected- 0.1 ml with a gap of 1 cm between points.

VISUAL ANALOG SCALE OF PATIENT SATISFACTION:

- 0- Not satisfied
- 1- Slightly satisfied
- 2- Very satisfied
- 3- Extremely satisfied

PHYSICIAN ASSESSMENT USING QUARTILE GRADING:

- GRADE 1- 0-25% improvement
- GRADE 2- 26-50% improvement
- GRADE 3- 51-75% improvement
- GRADE 4- 76-100% improvement

STATISTICAL ANALYSIS:

- Data was analysed using software R Version 4.0.2 and Microsoft Excel.
- Continuous variables were represented by mean ± standard deviation/median (range).
- Categorical variable represented by frequency and percentage.
- Association between categorical variable calculated by Chi square test.
- Mean between groups calculated with the help of two sample t-test.
- Symmetry test used to compare distribution of categorical variables.
- Paired t test was used to compare mean over time point
- P value ≤ 0.05 considered statistically significant.

PHOTOGRAPHS OF INSTRUMENTS USED

Photograph 1: Instrument used for taking photographs of patient's face at 0^{o} and 45^{o} angle

Photograph 2: REMI R-8C machine used for PRP centrifugation

Photograph 3: Fractional Carbon dioxide laser (non-ablative)

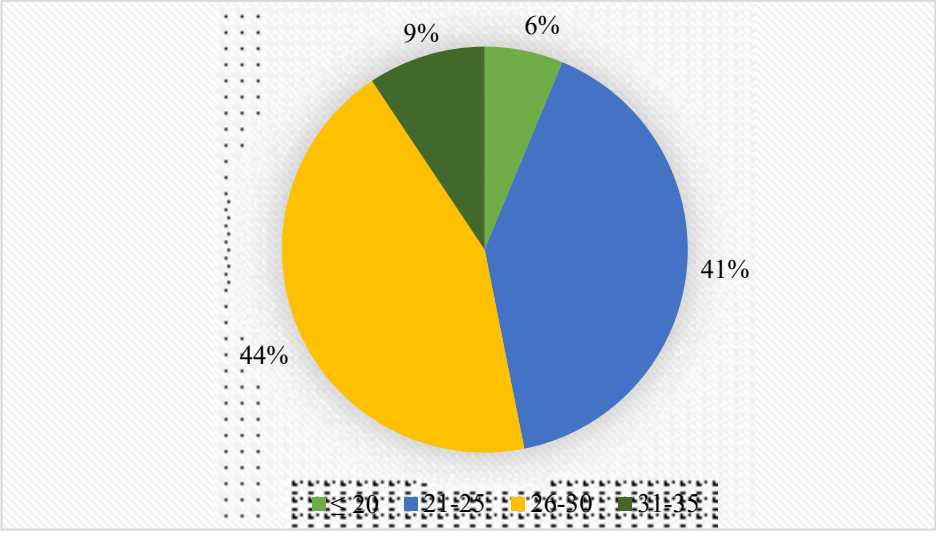
RESULTS

A total of 32 patients were included in the study.

Table 5: Distribution of patients by age (years)

AGE GROUP (YEARS)	NUMBER OF SUBJECTS (%)
≤20	2 (6.25%)
21-25	13 (40.63%)
26-30	14 (43.75%)
31-35	3 (9.38%)
Mean age (years)	**25.75 ± 3.61**

Chart 1: Distribution of patients by age (years)

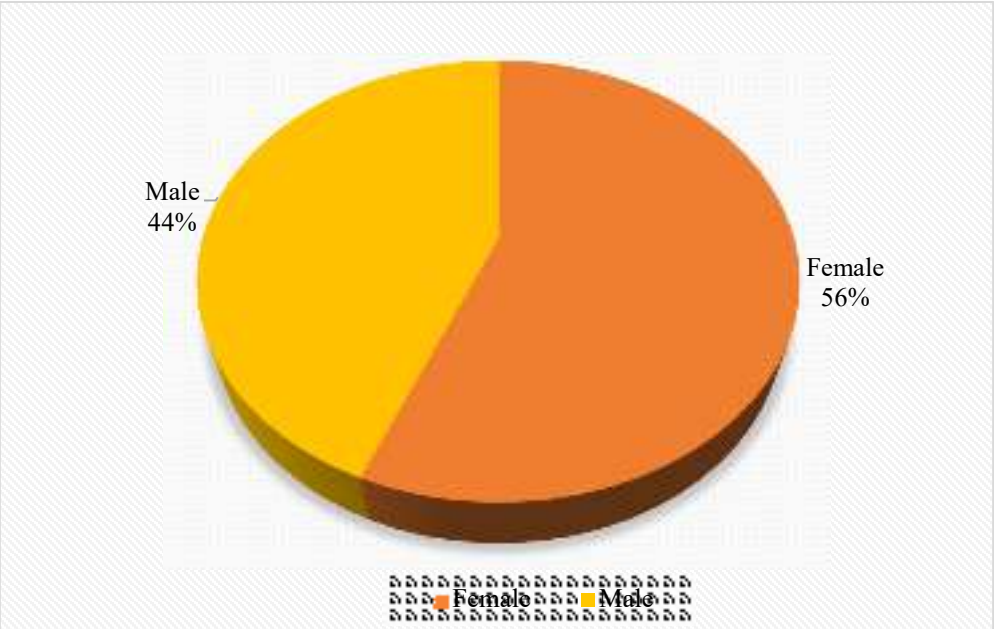


The mean age group of patients was 25.75 ± 3.61, with most of the patients belonging to age group of 26-30 years (43.75%), followed by 21-25 years (40.63%), 9.38% between 31-35 years and 6.25% of patients had an age less than 20 years.

Table 6: Distribution of patients by gender

GENDER	NUMBER OF PATIENTS (%)
Female	18 (56.25%)
Male	14 (43.75%)

Chart 2: Distribution of patients by gender

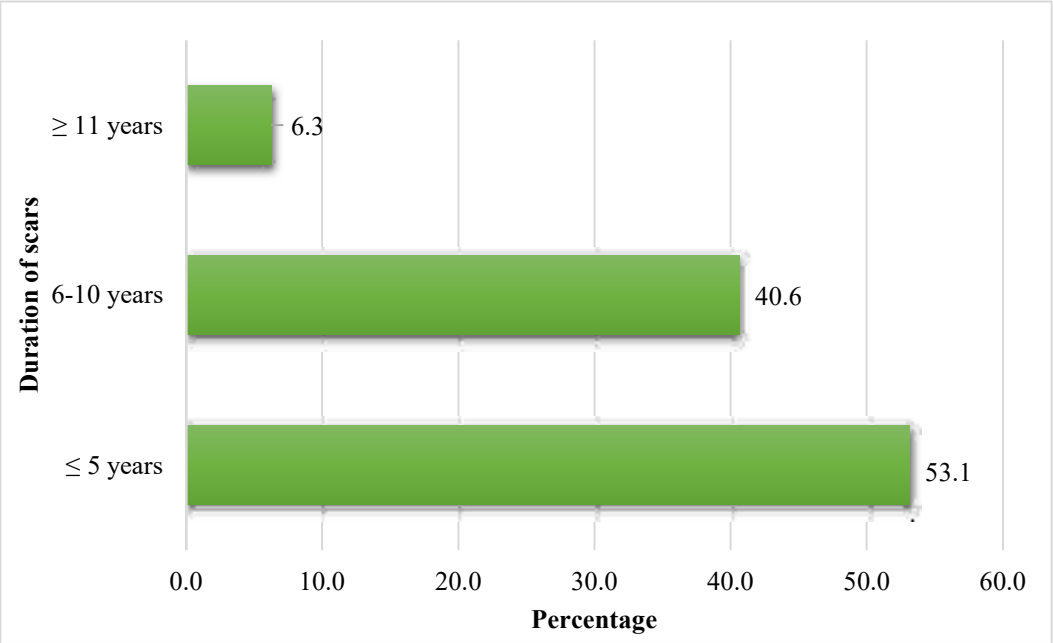


Majority of the patients were females (56%) with 44% belonging to male gender.

Table 7: Distribution of patients by duration of scars (years)

DURATION OF SCARS (YEARS)	NUMBER OF PATIENTS (%)
≤ 5 years	17 (53.13%)
6-10 years	13 (40.63%)
≥ 11 years	2 (6.25%)
Mean duration of scars (years)	**5.94 ± 2.73**

Chart 3: Distribution of patients by duration of scars (years)

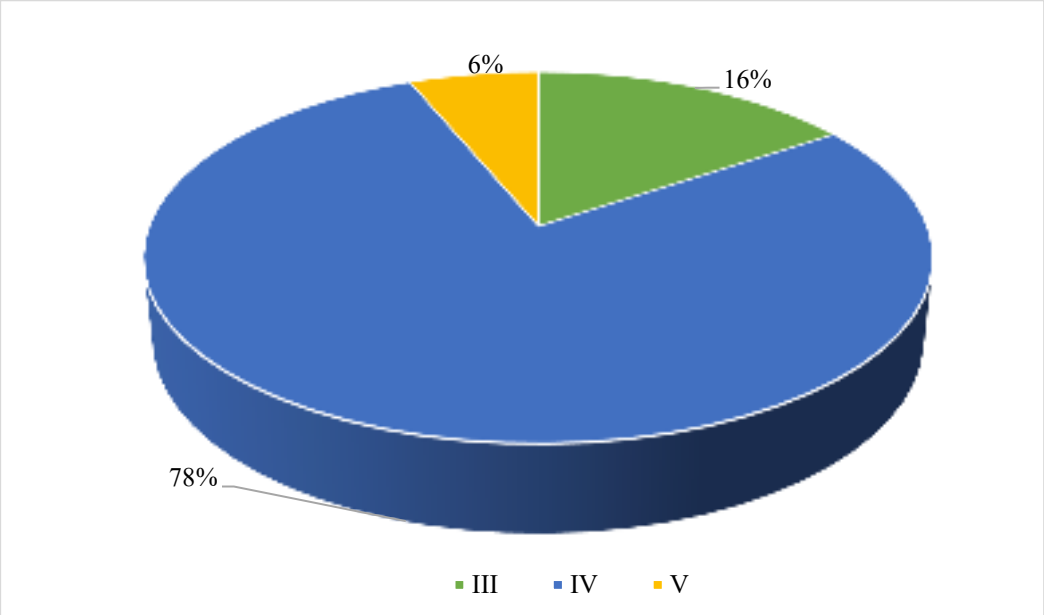


The mean duration of acne scars was 5.94 ± 2.73. 53.1% of patients had a duration ≤ 5 years. 40.6% of patients had scar duration of 6-10 years and 6.3% had scars for ≥ 11 years.

Table 8: Distribution of patients by Fitzpatrick skin type

FITZPATRICK SKIN TYPE	NUMBER OF PATIENTS (%)
III	5 (15.63%)
IV	25 (78.13%)
V	2 (6.25%)

Chart 4: Distribution of patients by Fitzpatrick Skin Type

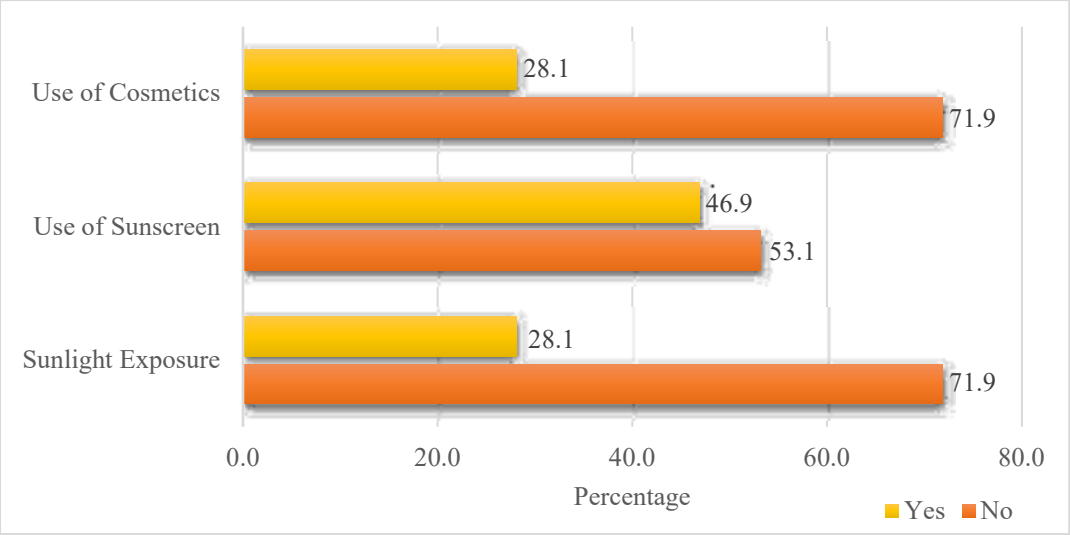


Almost 78.13% belonged to Fitzpatrick skin type IV and 15.63% and 6.25% of patients belonged to type III and V respectively.

Table 9: Distribution of patients by use of Cosmetics, Sunscreens and Exposure to sunlight

USE OF COSMETICS	NUMBER OF PATIENTS (%)
No	23 (71.88%)
Yes	9 (28.13%)
USE OF SUNSCREEN	
No	17 (53.13%)
Yes	15 (46.88%)
SUNLIGHT EXPOSURE	
No	23 (71.88%)
Yes	9 (28.13%)

Chart 5: Distribution of patients by use of Cosmetics, Sunscreens and Exposure to sunlight

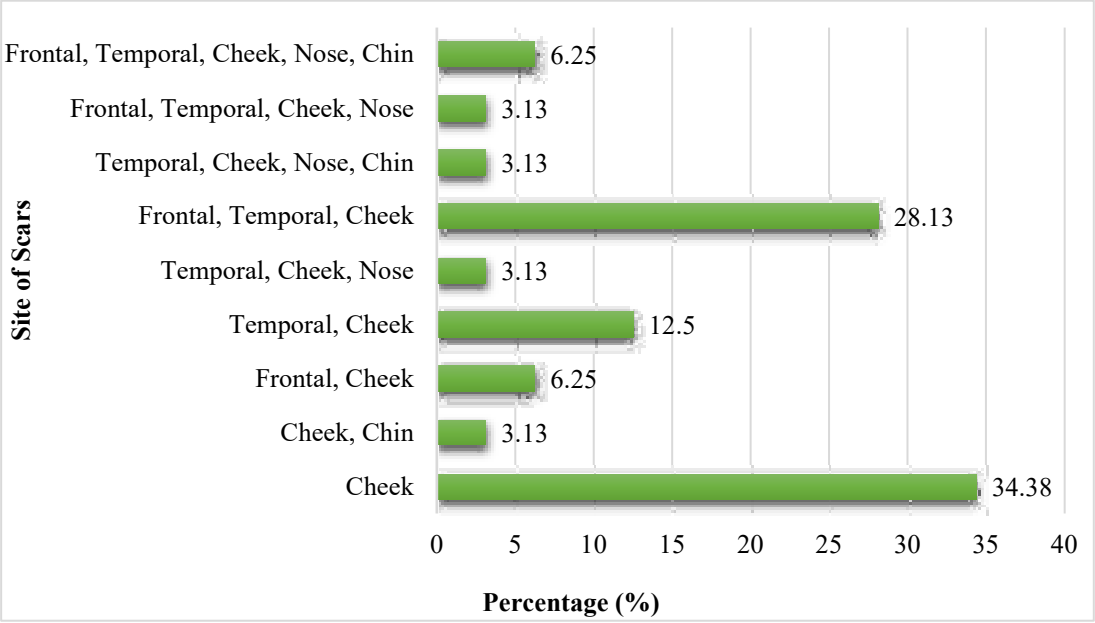


Majority of the patients had no history of use of any cosmetics (71.88%) or sunscreen (53.13%). While 28.13% and 46.88% of patients had history of use of cosmetics and sunscreen. History of sunlight exposure was observed in 28.13% of patients.

Table 10: Distribution of patients by the site of scars

SITE OF SCARS	NUMBER OF PATIENTS (%)
Cheek	11 (43.38%)
Cheek, Chin	1 (3.13%)
Frontal, Cheek	2 (6.25%)
Temporal, Cheek	4 (12.5%)
Temporal, Cheek, Nose	1 (3.13%)
Frontal, Temporal, Cheek	9 (28.13%)
Temporal, Cheek, Nose, Chin	1 (3.13%)
Frontal, Temporal, Cheek, Nose	1 (3.13%)
Frontal, Temporal, Cheek, Nose, Chin	2 (6.25%)

Chart 6: Distribution of patients by Site of Scars

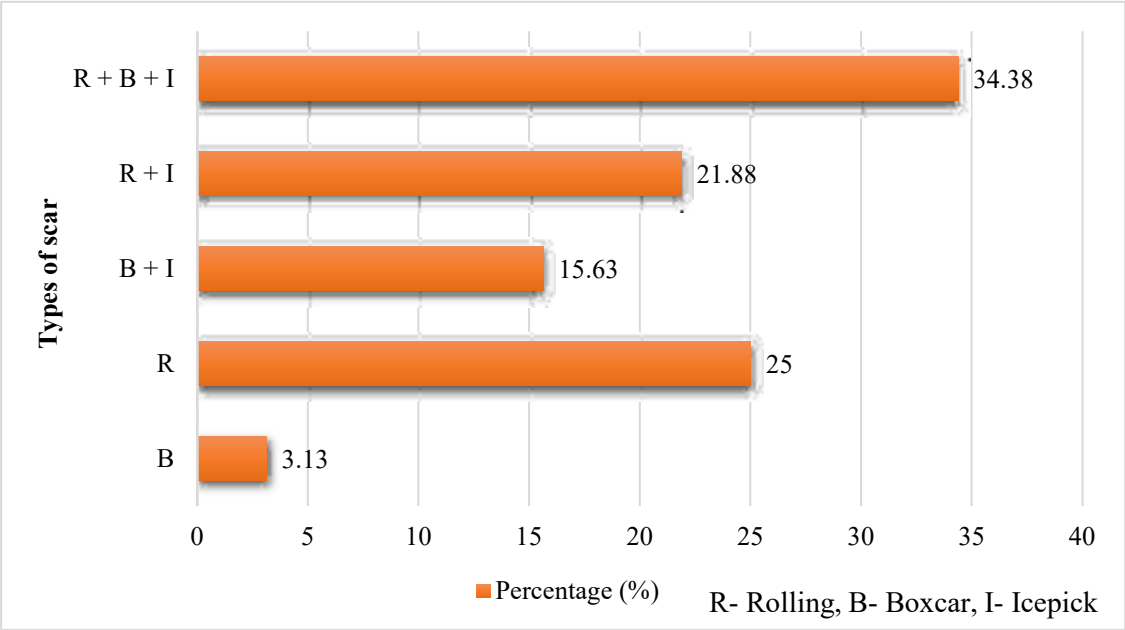


Most of the participants had scars over cheeks, but involvement of cheeks alone was found in 43.38% of patients. However, combination of frontal, temporal and cheek region was seen in 28.13%

Table 11: Distribution of patients by Type of Scars

TYPE OF SCARS	NUMBER OF PATIENTS (%)
Boxcar	1 (3.13%)
Rolling	8 (25%)
Boxcar, Icepick	5 (15.63%)
Rolling, Icepick	7 (21.88%)
Rolling, Boxcar, Icepick	11 (34.38%)

Chart 7: Distribution of patients by types of Scars

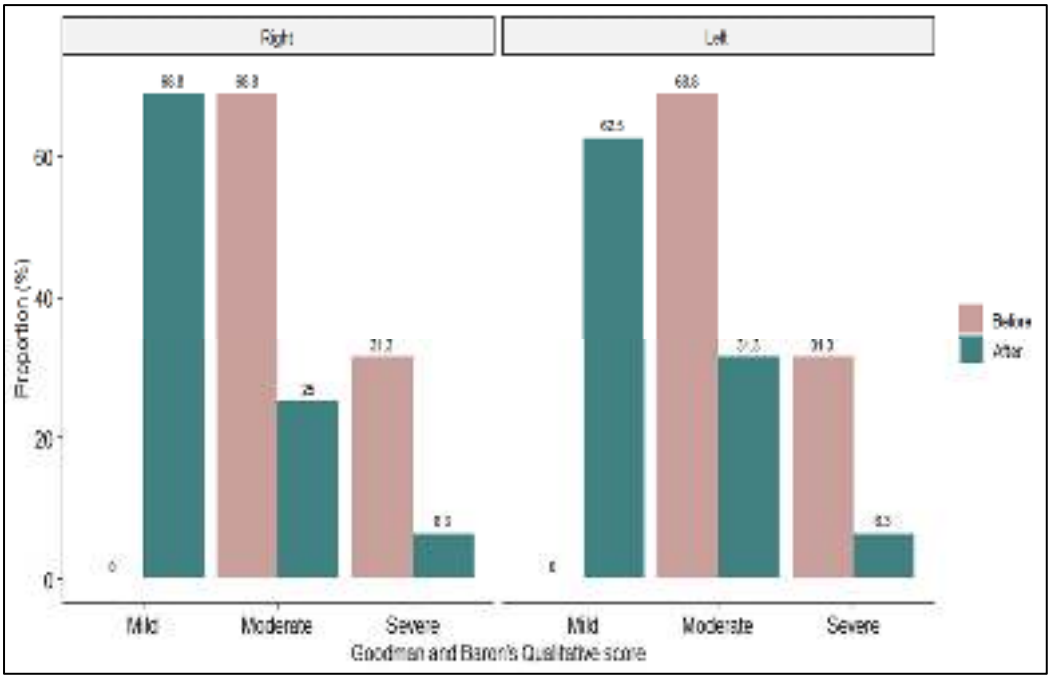


Rolling scar was the most common individual scar observed (25%). Most of the patients had combination of scars, among which, rolling, boxcar and icepick scar (34.38%) constituted major portion.

Table 12: Comparison of Goodman and Baron's qualitative score within and between both sides of the face

TIME POINT	GOODMAN AND BARON'S QUALITATIVE SCORE	SIDE OF THE FACE		P VALUE
		RIGHT SIDE	LEFT SIDE	
BEFORE	Moderate	22 (68.75%)	22 (68.75%)	1
	Severe	10 (31.25%)	10 (31.25%)	
AFTER	Mild	22 (68.75%)	20 (62.5%)	**0.9115**
	Moderate	8 (25%)	10 (31.25%)	
	Severe	2 (6.25%)	2 (6.25%)	
P VALUE		< 0.0001	< 0.0001	-

Chart 8: Comparison of Goodman and Baron's qualitative score within and between both sides of the face



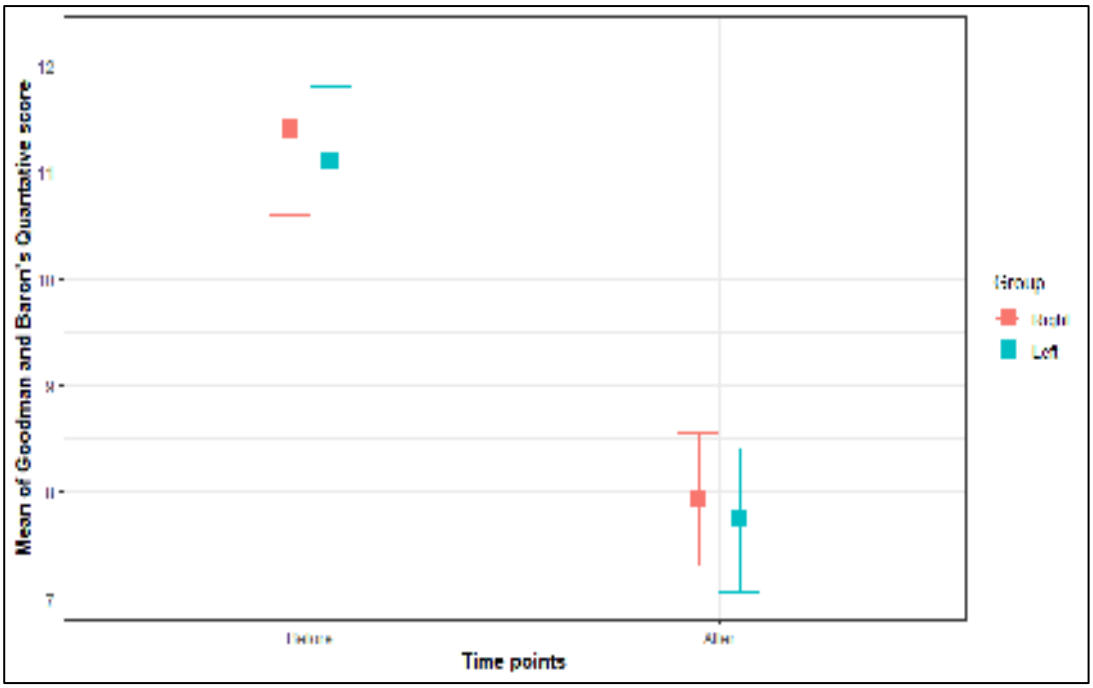
In the qualitative score by Goodman and Baron, 68.75% of patients had moderate scars and 31.25% had severe scars at baseline. At the end of study, 2 patients (6.15%) remained to be severe. Majority of the patients found significant improvement over both the sides. At the end of study, on right side, there were 68.75% with mild scars, 25% had moderate scars and 6.25% had severe scars. Over left side of the face, 62.5% had mild scars, 31.25% had moderate scars, and 6.15% had severe scars.

There was no statistically significant improvement in the quality of scars over right side of the face as compared to left side of the face (p- 0.9115).

Table 13: Comparison of Goodman and Baron Quantitative score between and within both the sides of face

TIME POINT	GOODMAN AND BARON'S QUANTITATIVE SCORE		P VALUE
	RIGHT SIDE	LEFT SIDE	
BEFORE	11.38 ± 2.17	11.12 ± 1.98	0.6316
AFTER	7.94 ± 1.85	7.75 ± 1.97	0.6957
P VALUE	< 0.00001	< 0.00001	-
Change in score	3.44 ± 1.13	3.38 ± 1.07	**0.8214**

Chart 9: Comparison of Goodman and Baron Quantitative score between and within both the sides of face



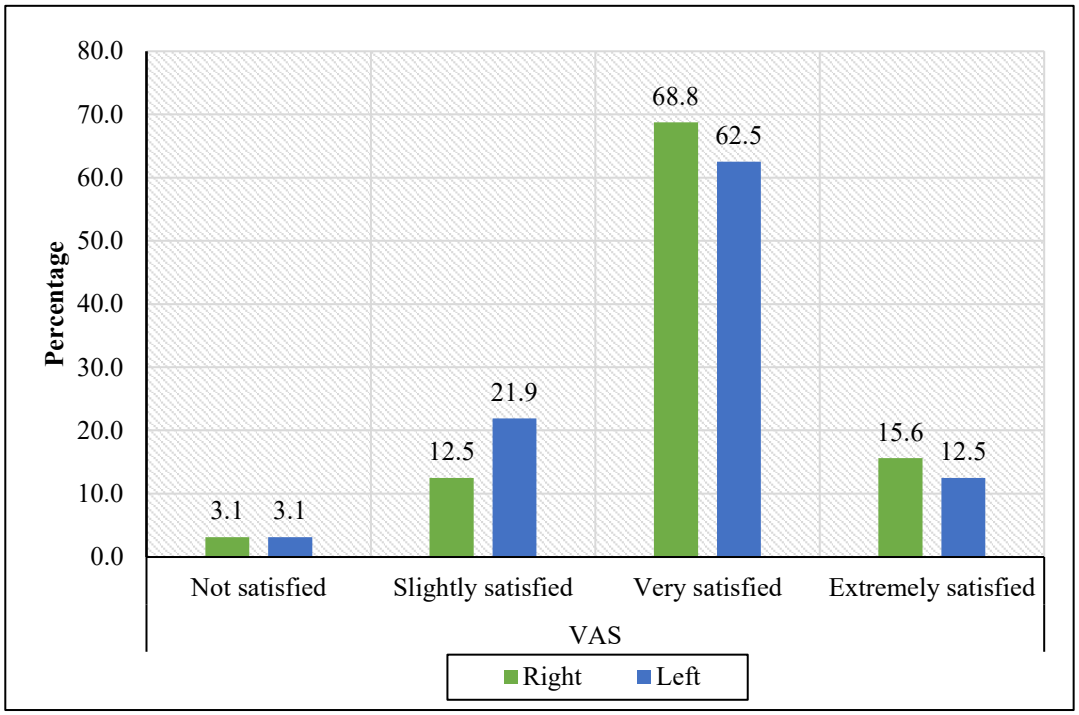
The mean of quantitative score was calculated at baseline and at end of the study over both sides of face and a change in score analysed. Over right side, an improvement of 3.44 ± 1.13 was found and over left side, points improved was 3.38 ± 1.07.

There was statistically significant improvement in scars, but the results were comparable over both sides with a statistically non significant result between both sides of face (p-0.8214).

Table 14: Comparison of Visual Analog Scale between and within both the sides of face

VAS	SIDE OF THE FACE		P VALUE
	RIGHT SIDE	LEFT SIDE	
Not satisfied	1 (3.13%)	1 (3.13%)	**0.8571**
Slightly satisfied	4 (12.5%)	7 (21.88%)	
Very satisfied	22 (68.75%)	20 (62.5%)	
Extremely satisfied	5 (15.93%)	4 (12.5%)	

Chart10: Comparison of Visual Analog Scale between and within both the sides of face



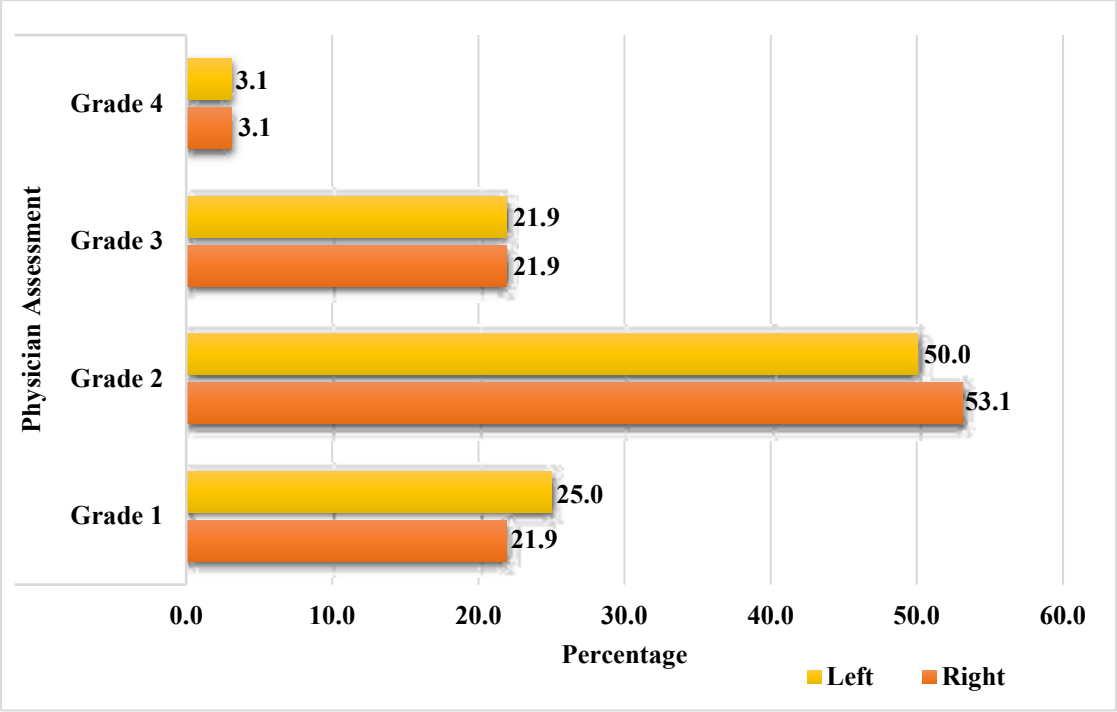
On analysing VAS Score for patient satisfaction, majority of patients were very satisfied with the treatment over both sides (68.75% over right side and 62.5% over left side). 15.93% and 12.5% of patients were extremely satisfied, 12.5% and 21.88% was slightly satisfied and 3.13% was not satisfied over right and left side of the face respectively.

From the results, it is observed that, most of the patients were more satisfied with the right side of face compared to left. But the values were not statistically significant (p-0.8571)

Table 15: Comparison of Physician assessement grading between and within both the sides of face

PHYSICIAN ASSESSMENT	SIDES OF FACE		P VALUE
	RIGHT SIDE	LEFT SIDE	
Grade 1	7 (21.88%)	8 (25%)	1
Grade 2	17 (53.13%)	16 (50%)	
Grade 3	7 (21.88%)	7 (21.88%)	
Grade 4	1 (3.13%)	1 (3.13%)	

Chart11: Comparison of Physician assessement grading between and within both the sides of face



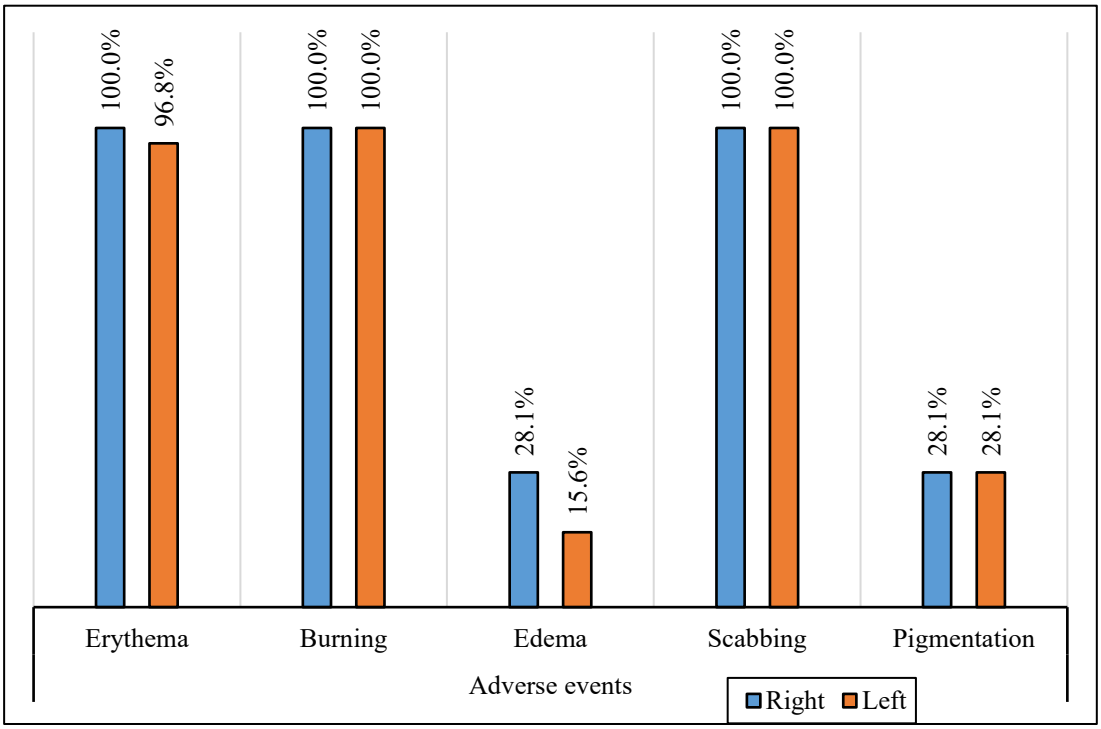
About 53.13% and 50% of patients had grade 2 improvement (26-50%) over right and left side of face respectively. Followed by grade 1 improvement (0-25%) in 21.88% over right side and 25% over left side. Grade 3 improvement (51-75%) was seen in 21.88% over both side and grade 4 (76-100%) in 3.13% of patients over both sides.

There was significant improvement after the treatment. But there was no difference between both sides of the face (p- 1)

Table 16: Comparison of adverse effects over side of face

SIDE EFFECTS	SIDE OF FACE		P VALUE
	RIGHT SIDE	LEFT SIDE	
ERYTHEMA	32 (100%)	31 (96.88%)	1
BURNING	32 (100%)	32 (100%)	1
EDEMA	9 (28.13%)	5 (15.63%)	**0.2265**
SCABBING	32 (100%)	32 (100%)	1
PIGMENTATION	9 (28.13%)	9 (28.13%)	1

Chart 12: Comparison of adverse effects over side of face



Erythema, sensation of burning, edema, scabbing and post inflammatory pigmentation are the common side effects observed. Immediate side effects like erythema, burning sensation and scabbing were seen in almost all patients (100%). Edema as seen only in 28.13% over right side and 15.63% over left side. Pigmentation was the only long term side effects observed which was seen in 28.13% over both sides.

There was no difference in adverse effects between both sides of face. Even though edema was more over right side of the face, the values were not statistically significant (p-0.2265)

DISCUSSION

The present study is a split face comparative prospective study conducted over a period of 1 year from January 2020 to December 2020 in the Department of Dermatology, Venereology and Leprosy, KLE'S Dr Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

32 patients completed the study. Patients of both the gender with age group between 18 to 40 years with moderate to severe atrophic acne scars according to Goodman and Baron's Qualitative Score were included in the study. At the end of study, the results were compiled and analyzed with the help of Goodman and Baron's Qualitative and Quantitative Score, Visual Analog Scale of patient satisfaction and Physician Assessment which was performed by a physician who was not involved in the study.

Our study when compared to other similar studies had few findings in common and many were contrary.

In our study, there were 32 subjects with mean age group of 25 ± 3.61 years. The age group was comparable to study performed by Kar et al and Sharma et al, wherein the mean age group was 25.06 ± 4.44 years and 26.93 ± 4.77 years respectively.[96,97]

The minimum and maximum age observed in our study was 19 years and 33 years respectively and the findings were similar to a study done by Gawdat et al, which showed a minimum age of 19 years and a maximum of 35 years.[98] However,

in a study by Galal et al, the minimum and maximum age was 20 and 42 years respectively.[99]

At final analysis there were 18 females and 14 males in our study with a male to female ratio of 1:1.28. The sex distribution in our study showed female predominance as opposed to studies conducted by Kar et al[96]and Sharma et al,[97] which showed a male predominance with a male to female ratio of 2:1 and 1.3:1 respectively. This contradictory finding in our study was likely due to the irregular follow up by men in our study as compared to women who completed the study with regular follow up visits.

The mean duration of scars in our study was 5.94 ± 2.74 years. Whereas the mean duration of scars in a study by Galal et al [99] was 10.4 ± 3.1 years and in a study by Sharma et al it was 2.13 ± 1.00 years.[97]

Our study showed a large portion of patients with Fitzpatrick skin type IV in 78.13%, type III in 15.63% and with 6.25% of patients with type V skin type. The study findings varied with the study performed by Abdel et al, where majority of the patients had a type III skin (70%) with 16.6% and 13.3% of type V and type IV skin respectively. This difference can be explained by the fact that skin types exhibit regional variations.[100]

In our study, most of the patients had involvement of cheeks alone (34.38%), followed by frontal, temporal and cheek (28.13%). On the other hand, in a study by Pooja et al, involvement of cheeks was found in 66.6%, forehead in 28.3%, and cheeks and chin together in 5% of cases.[101]

In type of scars, a combination of rolling, boxcar and icepick scars was seen in 34.38% of patients in our study, rolling and icepick scars in 21.88%, boxcar and icepick in 15.63%, rolling and boxcar alone was seen in 25% and 3.13% respectively.

Study by Abdel et al, showed that rolling scars were found in 36.67%, icepick and boxcar scars in 33.33% and 30% of cases respectively. However, combination of scars was not mentioned.[100] Whereas in a study by Pooja et al, 26.6% of patients had a mix of boxcar, rolling and icepick scars, 23.3% had linear, rolling and boxcar scars and predominant boxcar scars in 20% of patients.[101] This is in contrary to our study where majority of the individual scars belonged to rolling type. A similar finding was observed in a study by Arsiwala et al, wherein most of the patients had rolling scars (42.4%) with boxcar scars and icepick scars seen in 39.4% and 18.2% respectively.[102]

According to Goodman and Baron's qualitative score, 68.75% of patients had moderate acne scars and 31.25% with severe scars over both sides of face at baseline in our study. By the end of study, the scores improved with majority of patients exhibiting mild scars followed by moderate scars, while 2 patients remained with severe scars over both sides of the face. Most of the patient's scores reduced by one grade, few with 2 grades and very few with no change in grade of acne scars. The improvement in the quality of scar over both sides of the face was comparable.

The results in our study were corresponding to a split face study conducted by Kar et al, where topical PRP was used instead of intradermal PRP. The improvement in grade over the side treated with combined therapy and fractional CO2 laser monotherapy was 0.66 ± 0.05. There was no difference between both sides of the face

in terms of quality of scars even though there was significant improvement of scars over both sides at the end of study.[96]

Sharma et al in their split face study with fractional CO2 laser and combination of fractional CO2 laser and intradermal PRP, concluded that there was significant improvement in acne scars over the side treated with combination therapy, with a change in mean of 1.37 ± 0.36 as compared to fractional CO2 laser alone with a mean change of 1.04 ± 0.09.[97]

Abdel aal et al in their study, reached a conclusion that the side of face treated with ablative CO2 laser and PRP injection had more improvement in the quality of scars as compared to the side treated with ablative CO2 laser alone. At baseline, 60% of patients had moderate scars and 10% patients had severe scars over both sides of the face. But at the end of study, macular scars were observed in 50% and 23.3%, mild scars in 33.33% and 43.3%, moderate scars in 13.3% and 26.6%, severe scars were seen in 3.33% and 6.67% over the side treated with combination therapy and ablative CO2 laser monotherapy respectively. According to their study, side of the face treated with ablative CO2 laser and PRP injection showed more reduction in severity of scars compared to left side of the face.[100]

A study by Babu AM et al compared fractional CO2 laser and PRP injections for acne scar treatment, where patients were separated into 2 groups with 10 patients each. Group A received PRP and group B received fractional CO2 laser. At the end of 3 sessions, the change in mean qualitative score over group A patients was 0.4 ± 0.09 and in group B was 0.39 ± 0.05. The improvement of both treatment modality in their study was comparable.[103]

In our study the mean of Goodman and Baron's quantitative score was calculated over right and left side of the face at baseline and at the end of study and score change was calculated. Over right side of the face, the change in score was 3.44 ± 1.13 and over left side of the face it was 3.38 ± 1.07 with a p value of 0.6957. Even though there was significant improvement in mean score before and after the treatment over both sides of the face, there were no statistically significant improvement over right side of the face as compared to left side.

Kar et al in their study calculated the change in mean of quantitative score over right side of the face treated with fractional C02 laser alone and over left side of the face treated with fractional CO2 laser and intradermal PRP was 4.5 ± 1.5 and 4.6 ± 2.324 respectively. There was no significant improvement in the scores over right side of the face even though there was improvement in score before and after the study over both the sides of the face.[96]

Our study did not agree with study performed by Arsiwala et al, where the change in score at the end of study was 4.17 ± 1.528 over group A treated with fractional CO2 laser with topical PRP and 3.15 ± 1.676 over group B treated with fractional CO2 laser alone.[102]

Shah et al, in a similar split face study, attained a change in mean quantitative score of 10.62 ± 1.71 over the side of face treated with ablative CO2 laser and PRP and a score change of 7.73 ± 0.96 over the side treated with fractional ablative CO2 laser and normal saline injections, thereby inferring that there was statistically significant improvement of score over the side treated with PRP combination.[104] This result was in contrast to our study.

In a study conducted by Pooja et al, comparing Fractional CO2 laser with PRP in the treatment of acne scars, 20 patients received fractional CO2 laser and 20 patients received PRP. At the end of 4 sittings, they concluded that fractional CO2 laser was more efficacious then PRP with a p value of 0.00.[100]

Patient satisfaction in our study was assessed with the help of Visual Analog Scale (VAS). 68.75% of patients were very satisfied, 15.63% were extremely satisfied, 12.5% were slightly satisfied and only 3.13% were not satisfied with the treatment over right side of the face. Over left side of the face 62.5% were very satisfied, 21.88% were slightly satisfied, 12.5% were extremely satisfied and 3.13% were not satisfied. Results showed that even though patients found more improvement of acne scars over the right side of face, the values were not statistically significant (p value - 0.8571)

The findings in our study agreed with the study by Faghini et al, wherein 56.2% and 43.8% of patients were satisfied or very satisfied over side of the face treated by combination of fractional ablative CO2 laser with PRP and fractional ablative CO2 laser alone respectively. Though the side treated with combined modality showed more satisfaction rate, the difference was not statistically significant.[105]

Abdel aal et al compared the patient satisfaction in a split face study, where 13.3% were highly satisfied, 40% were satisfied and 23.3% very neutral over right side of the face treated with fractional ablative CO2 laser and PRP. Over left side of the face treated with fractional ablative CO2, 20% of patients were satisfied, 50% were neutral and 30% were dissatisfied at the end of study. There was statistically significant value of satisfaction over right side of the face as compared to left side.[100]

In a study by Galal et al, majority of the patients were more satisfied by combined treatment modality with fractional CO2 laser and PRP. 50% of patients were very satisfied with the combination treatment modality as compared to 3.3% with the laser alone.[99]

Physician assessment in our study showed that majority of the patients had an improvement of 26-50% (Grade 2) over both sides of the face. Grade 1 improvement was found in 21.88% and 25%, grade 2 improvement in 53.13% and 50%, grade 3 in 21.88% and 21.88%, grade 4 in 3.13% and 3.13% over right and left side of the face respectively with a p value of 1. There was no difference in improvement of scars between both sides of face.

Our study results go in hand with a study conducted by Faghini et al, where fair (25-50%) or good (50-75%) improvement was noted in 87.5% of patients on side treated with combination of fractional CO2 laser and PRP and in 68.8% of patients on other side of the face treated with fractional CO2 laser and injections of normal saline at the end of 4 months of second session with a p value of 0.23 which was not statistically significant.[105]

In a study conducted by Gawat et al, Grade 4 (> 75 %) improvement was seen in 66.7% of patients treated with fractional CO2 laser and PRP injections, 60% of patients treated with fractional CO2 laser and topical PRP and only in 26.7% of patients treated with fractional CO2 laser alone.[98] These findings did not agree with our study findings.

Shah et al in their split face study, showed a better improvement on the fractional CO2 laser and intradermal PRP treated side as compared to fractional CO2

laser and normal saline injection side. In the study 14 sites (23.33%) obtained excellent (>75%) response at the end of study, out of which 10 sites belonged to PRP side and 4 sites to normal saline side. 19 treatment sites (31.67%) had good improvement (50-75%) of which, 12 and 7 sites were treated with PRP and normal saline respectively. Moderate improvement (25-50%) were noted in 21 treatment sides (35%) of which most of the sites belonged to normal saline side. Poor improvement (0-25%) was seen in only 1 in PRP side, with the remaining belonging to normal saline treated side. [104]

Our study did not agree with another study by Abdel et al, where right side of the face was treated with ablative carbon dioxide laser and PRP. Results showed excellent improvement in 13.3% of patients, 40% with marked improvement, 26.6% and 20% of patients showed moderate and mild improvement respectively. On left side of the face treated with fractional ablative CO2 laser alone, 30% showed marked improvement, 30% and 40% showed moderate and mild improvement respectively and none with excellent improvement. The result concluded that there was statistically significant improvement over right side of the face as compared to left side.[100]

Our study was supported by a randomized study conducted by Godara et al, where group I was treated with fractional CO2 laser and group II was treated with fractional CO2 laser and PRP. The results were assessed by Patient and Observer Scar Assessment Scale at the end of 4 sessions. In their study even though there were statistically significant difference in the scar quality before and after the study ($P < 0.001$), there was no notable difference between both the groups in terms of improvement of scars.[106]

In the present study, erythema, burning sensation, edema, scabbing and pigmentation were the common side effects observed. Erythema, edema and scabbing were present in almost all the patients. While edema and pigmentation were seen in less than 30 % of the patients. All the side effects resolved with a downtime of 4-5 days except pigmentation which took 6 to 8 weeks to resolve. There was no difference in adverse effects between both the sides of face (p value =1) expect for edema which was more over right side of the face, but the values were not statistically significant (p value = 0.2265)

Similar side effects were observed by Shah et al study, wherein they found persistent erythema, edema, hyperpigmentation and in addition there was acneiform eruptions. However, in their study, the above-mentioned adverse effects were statistically more over the side treated with fractional CO2 laser and normal saline injections as compared to the fractional CO2 laser and intradermal PRP treated side.[104]

Kar et al in their study, noted side effects which included redness, pain and swelling. The adverse effects were found to be lesser over the side treated with fractional CO2 laser and PRP as compared to side treated with fractional CO2 laser alone (p value < 0.05) and values were statistically significant.[96] The results were contradictory to our study.

In a study conducted by Arsiwala et al, erythema, edema, pain, hyperpigmentation, aggravation of acne and secondary infection were the adverse effects observed. The side effects were more over group B treated with fractional CO2 laser monotherapy but the values were not statistically significant.[103]

The common side effects observed in a study by Sharma et al were erythema, edema, pain and others being dryness, scabbing, persistent erythema, and post inflammatory pigmentation. Erythema and burning sensation were seen in almost all patients post procedure over both sides of the face, which was in support to our study. Whereas edema (90%), persistent erythema (26.7%), scabbing (80%), dryness (90%), aggravation of acne (26.7%) and post inflammatory hyperpigmentation (33.3%) were more over the side treated with fractional CO2 laser monotherapy.[97]

Gawdat et al observed side effects including erythema, edema, crusting, pain, hyperpigmentation, and acneiform eruptions. Results showed that the adverse effects were lesser over areas treated with fractional CO2 laser and PRP with a shorter downtime (p value- 0.02). But pain was more over the side treated with intradermal PRP (mean - 7.1± 1.2) as compared to other side of the face treated with laser alone (mean - 3.0 + 0.7).[98]

As opposed to other similar studies, a study by Faghini et al reached a conclusion that the side treated with fractional CO2 laser and PRP had more side effects compared to the side treated with fractional CO2 laser alone. Erythema and edema were the major side effects. On days 0,2 and 4, the erythema over side treated with combination therapy was more than the other side of the face (p - 0.003, p - 0,007, p - 0.003). In terms of edema, on day 0, 2, and 8, fractional CO2 laser and PRP treated side had more edema than the other side (p - 0.003, p - 0.004, p - 0.004).[105]

A study by Godara et al also concluded that the side effects were more over the side treated with combination of fractional CO2 laser and PRP. In the study, pain and erythema were the common side effects noticed. They explained the aggravation

of redness and pain as a possible result of platelet initiating and propagating inflammatory process.[106]

The variation in our study findings with other studies can be attributed to the difference in duration of study and the interval between every session. Moreover, in all the above mentioned studies including our study, results were assessed with the help of subjective methods, which in itself can lead to disparity in result evaluation.

Limitations of our study include small sample size, short follow up period and lack of objective methods for result assessment.

CONCLUSION

The study was conducted to evaluate the efficacy of intradermal platelet rich plasma when combined with fractional carbon dioxide laser in the treatment of post acne scars and also to study the various side effects associated with the therapy.

At the end of study, majority of the patients found significant improvement in the scars. Most of the patients were very satisfied with the treatment and obtained an improvement of 26-50%. Adverse events belonged to immediate side effects with only pigmentation as the long-term effect seen in less than 30% of patients. Although all the side effects subsided with a downtime of 4 to 5 days, pigmentation resolved only after 6 to 8 weeks.

The satisfaction of patients and improvement of scars following three sessions of treatment was comparable over both sides of face and there was no statistically significant difference over right side of the face where intradermal platelet rich plasma was combined as compared to left side of the face. The adverse effects also did not vary between the sides.

Therefore, addition of platelet rich plasma did not provide any synergistic effects in the treatment outcome. There was no improvement or worsening of side effects and there was no decrease or increase in the duration of side effects following inclusion of intradermal platelet rich plasma

Hence to conclude, by avoiding platelet rich plasma, we can cut down on an extra interventional therapy, thereby reducing the cost and increasing patient compliance.

SUMMARY

The study was a split-face comparative interventional study conducted over a period of one year from January 2020 to December 2020 in the dermatology department of KLE'S Dr. Prabhakar Kore Hospital, Belagavi. The study was initiated after obtaining institutional ethical committee clearance and a written consent from the participants.

All patients in the age group of 18 to 40 years with moderate to severe atrophic acne scars according to Goodman and Baron's Qualitative score was included in the study. Patients with active acne, active herpes infection, keloid tendency, bleeding diathesis, collagen vascular disorders, pregnant and lactating mothers and patients with unrealistic expectations were excluded from the study.

The objective of the study was to evaluate and compare the efficacy and safety of fractional carbon dioxide laser and combined use of fractional carbon dioxide laser and platelet rich plasma (PRP) in the treatment of acne scars.

The sample size of the study was 32 patients. A detailed history was taken from the patient before the start of study, which included details of scars, duration, past and family history, treatment history, history of usage of cosmetics, sunscreen, exposure to sunlight and history of endocrine disorders.

Clinical photographs of the patients were taken with the help of a digital camera (Sony DSC-H300/BC E32 digital camera) at baseline and at 6 weeks after each session. Photographs were taken with the help of an instrument which had 2

parts (fixed and movable part). Chin of the patient was placed over the fixed part and camera over the movable part. The photographs were taken at $0^0$and 45^0 angle.

In this split face study, right side of the face was treated with fractional carbon dioxide laser (non-ablative) and intradermal platelet rich plasma. Left side of the face was treated with fractional carbon dioxide laser only. A total of 3 sessions were performed with an interval of 6 weeks between every session. The patients were advised topical antibiotics, emollients and sunscreen post procedure. The patients were followed up on third day of procedure to look for side effects and the same was documented regularly.

The results were compiled and assessed with the help of three parameters (Goodman and Baron's qualitative and quantitative score, visual analog scale of patient satisfaction and physician assessment).

Majority of participants in the study belonged to the age group of 22-30 years of age with a female predominance. Most common individual scar observed was rolling scar with maximum involvement of cheeks.

At the end of study, even though there was improvement in scars over both sides of the face, there was no statistically significant difference between both sides of the face according to Goodman and Baron's qualitative and quantitative score.

Visual Analog Scale measuring patient satisfaction showed that majority of patients were very satisfied with the treatment. Even though patients were more satisfied with right side of the face, the values were not statistically significant.

Physician assessment grading of the scars done at the end of study showed that most of the patients belonged to grade 2 (26-50%) improvement over both the

sides of face. There was no difference between both the sides in terms of improvement of scars.

Most of the side effects observed in the study were immediate effects, which was present in almost all patients on both sides of face. Only long-term side effect observed was post inflammatory hyperpigmentation. There was no predilection for any side of the face in terms of side effects. Edema was observed more over right side of the face, but the values were not significant.

Hence, use of fractional carbon dioxide laser leads to improvement of acne scars with less long-term side effects. However, use of PRP along with laser as an adjuvant does not provide an increase in efficiency of fractional carbon dioxide laser in the treatment of acne scars.

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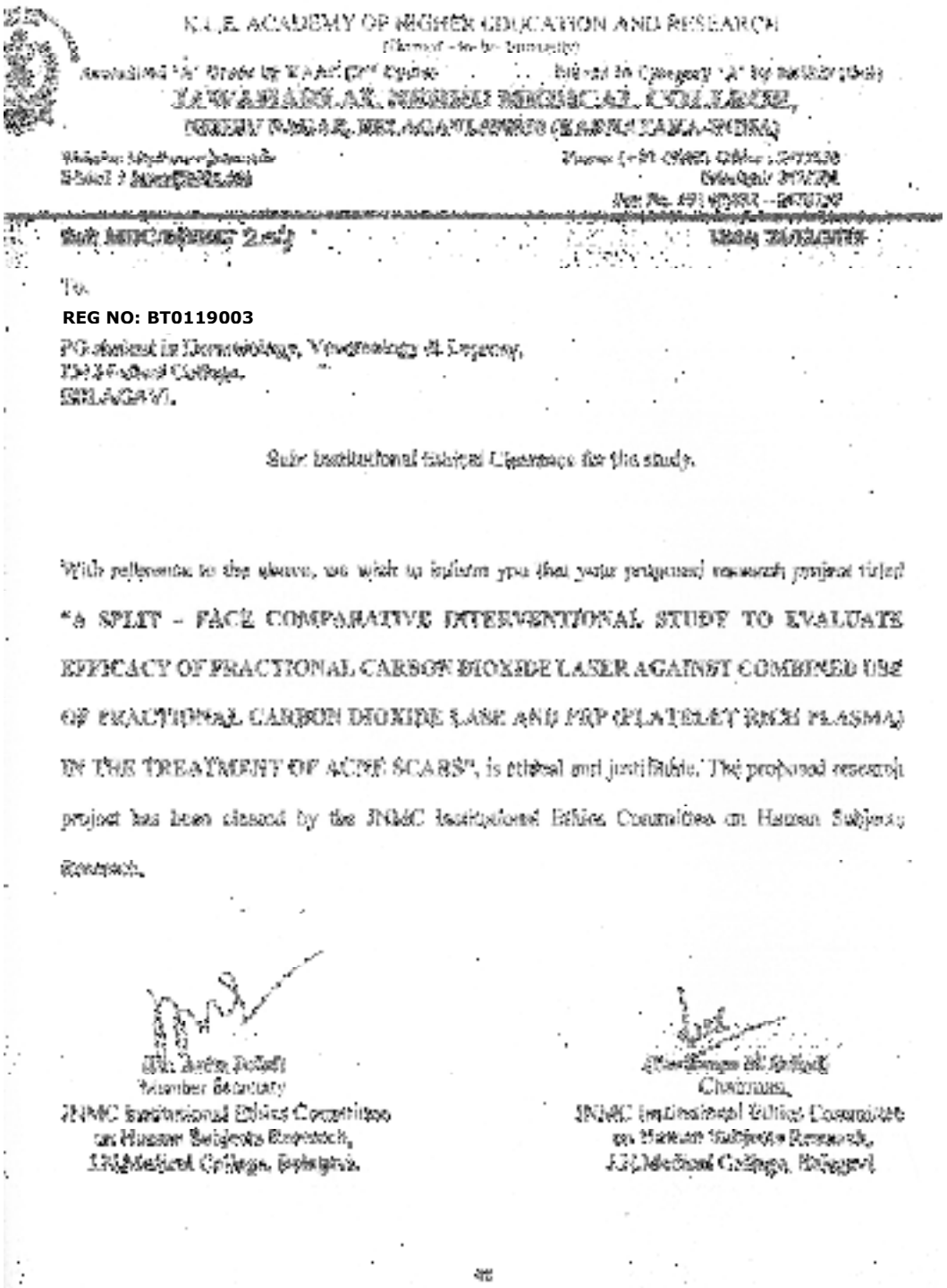
ANNEXURE-I- ETHICAL CLEARANCE LETTER



K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH

To,

REG NO: BT0119003

PG student in Dermatology, Venereology & Leprosy,
JN Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled "A SPLIT – FACE COMPARATIVE INTERVENTIONAL STUDY TO EVALUATE EFFICACY OF FRACTIONAL CARBON DIOXIDE LASER AGAINST COMBINED USE OF FRACTIONAL CARBON DIOXIDE LASER AND PRP (PLATELET RICH PLASMA) IN THE TREATMENT OF ACNE SCARS", is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE -II

INFORMED CONSENT FORM

ID NO:

Title of the study: **"A SPLIT-FACE COMPARATIVE INTERVENTIONAL STUDY TO EVALUATE EFFICACY OF FRACTIONAL CARBON DIOXIDE LASER AGAINST COMBINED USE OF FRACTIONAL CARBON DIOXIDE LASER AND PLATELET RICH PLASMA IN THE TREATMENT OF ACNE SCARS"**

The study is conducted by _____ Post Graduate (M. D) student in Dermatology under the guidance of _____ Associate Professor, Department of Dermatology, Venereology and Leprosy, JNMC, Belagavi.

RESPECTED SIR / MADAM

We invite you to participate in our study, as you are eligible for the same. During the study you will be asked questions in detail regarding your present complaints. Fractional CO2 laser alone will be done on one side of face and combination of CO2 laser and PRP (Platelet rich plasma) will be done on the other side of face. Digital photographs will be taken before and after the procedure. Side effect of this treatment are redness, scarring, post inflammatory hyper/hypopigmentation, infection.

PURPOSE OF THE STUDY

To compare the efficacy and safety of both the treatment methods

PROCEDURE

You will undergo CO2 laser on one half of the face (left side) and combination of CO2 laser and PRP on other half of the face (right side).

RISKS AND BENEFITS

The result of the study will help in identifying a better treatment option for acne scars

ALTERNATIVES

If you decide not to participate in this study, you will still be receiving the usual standard care for your disease

PRIVACY AND CONFIDENTIALITY

Your privacy will be respected and all information collected about you during the course of this study will be kept confidential. Your identity will remain undisclosed.

RELATIONS WITH THE INSTITUTIONAL POLICY

The J N Medical College will provide, within the limitations of the laws of the state of Karnataka, facilities and medical attention to patients who suffer injuries as a result of participating in this project.

FINANCIAL INCENTIVES

You shall not be receiving any payment or any financial incentives for participating in this study.

AUTHORIZATION TO PUBLISH RESULTS

The results of this study may be published for scientific purpose or presented to a scientific group. Your identity, however will be maintained confidential at all times.

VOLUNTARY PARTICIPATION

In case you need further information regarding your right as a study participant, you may please contact **Dr ROOPA M BELLAD**, Chairman of the ethical Committee, J N Medical College, Belagavi.

STATEMENT OF CONSENT

I.D. NO

I, Mr / Ms/ Mrs ……..., volunteer and consent to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I accept to participate in the study. All the information regarding this study, and side effect of the treatment is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers.

PARTICIPANT`S NAME:

Signature of left thumb print of the participant:

WITNESS NAME:

Signature of witness:

SIGNATURE OF THE INVESTIGATOR:

Date:

STATEMENT OF ASSENT

I.D.NO:

I Mr / Ms/ Mrs …….

Parent /guardian of …… consent to enrol my daughter /son to participate in this study. I have read the consent document or it has been read to me in my vernacular language. I give my acceptance on behalf of my daughter/ son for her participation in the study. All the information regarding this study and its side effects is provided to me and I have understood the same. I have been given the opportunity to ask questions and obtain appropriate answers

PARTICIPANT`S NAME:

PARTICIPANT`S PARENT / GUARDIAN NAME:

Signature or left thumb print of parent / guardian of participant:

WITNESS NAME:

Signature of witness:

SIGNATURE OF THE INVESTIGATOR:

Date:

ANNEXURE III

PROFORMA

TITLE: "A SPLIT-FACE COMPARATIVE INTERVENTIONAL STUDY TO EVALUATE EFFICACY OF FRACTIONAL CARBON DIOXIDE LASER AGAINST COMBINED USE OF FRACTIONAL CARBON DIOXIDE LASER AND PRP (PLATELET RICH PLASMA) IN THE TREATMENT OF ACNE SCARS"

Name: Case No:

Age:

Sex: Date: 1st sitting:

2nd sitting:

Address with phone number: 3rd sitting:

Email ID:

Education:

Occupation:

Chief Complaint:

Duration:

1) Exposure to sunlight: Present ☐ Absent ☐

2) Use of sunscreen: Present ☐ Absent ☐

3) Use of cosmetics: Present ☐ Absent ☐

4) Skin (Fitzpatrick skin type):

5) Site of scar:

6) Type of scar: Rolling ☐ Icepick ☐ Boxcar ☐

7) Other findings (if any):

H/o Treatment taken for acne:

1) Systemic Present ☐ Absent ☐ Mention (If any)

2) Topical Present ☐ Absent ☐ Mention (If any)

3) Laser Present ☐ Absent ☐ Details (If any)

4)Others Present ☐ Absent ☐ Mention (If any)

Past History:

1) Application of steroids Present ☐ Absent ☐

2) Herpes infection Present ☐ Absent ☐

3) Keloidal tendency Present ☐ Absent ☐

4) Bleeding disorders Present ☐ Absent ☐

5) H/o Diabetes Mellitus Present ☐ Absent ☐

6) H/o Hypertension Present ☐ Absent ☐

7) Any other co-morbidities Present ☐ Absent ☐

Mention (if any)

Family History of Acne: Present ☐ Absent ☐

Personal History:

Diet Mixed ☐ Veg ☐

Sleep Adequate ☐ Disturbed ☐

Menstrual History Regular ☐ Irregular ☐

If irregular, details

H/o PCOS: Present ☐ Absent ☐

Obstetric History:

General Physical Examination:

1) PR: bpm BP: mm/Hg

2) Weight: Kg

3) Height: cm

4) BMI:

5) Pallor Present ☐ Absent ☐

Systemic Examination:

Procedure:

CO2 Laser:-

Wavelenght: 10,600 nm

Device name: Dermaindia

Mode: Scan mode

Intensity of 1st, 2nd, 3rd sittings: 12 W

Distance: 0.5mm

Repeat: Single

Duration: 0.5 ms

No of passes: 2

PRP:-

10 ml of blood drawn in a Sodium Citrate tube and subjected to two centrifugation steps

1st centrifugation (Soft Spin): 1000 rpm for 10 min

2nd Centrifugation (Hard Spin): 2000 rpm for 5 min

CO2 laser + PRP (Platelet rich plasma) done on Right Side of face

CO2 laser alone done on Left side of face

GOODMAN AND BARON'S SCALE

RIGHT SIDE OF FACE (CO2 LASER + PRP):

GRADE	Before treatment	1ST Sitting	2nd Sitting	After 3rd Sitting
Qualitative grade				
Quantitative grade				

LEFT SIDE OF FACE (CO2 LASER ALONE)

GRADE	Before treatment	1ST Sitting	2nd Sitting	After 3rd Sitting
Qualitative grade				
Quantitative grade				

VISUAL ANALOG SCALE (PATIENT SATISFACTION)

SCORE	First session		Second session		Third session	
	Right	Left	Right	Left	Right	Left
0- Not satisfied						
1- Slightly satisfied						
2- Very satisfied						
3- Extremely satisfied						

SIDE EFFECTS :(IF ANY)

PHYSICIAN'S ASSESSMENT GRADING

GRADE	% improvement after 3 sessions
Grade 1	0-25%
Grade 2	26-50%
Grade 3	51-75%
Grade 4	76-100%

GRADE (RIGHT SIDE): GRADE (LEFT SIDE):

REMARKS:

ANNEXURE IV – PHOTOGRAPHS

PATIENT 1

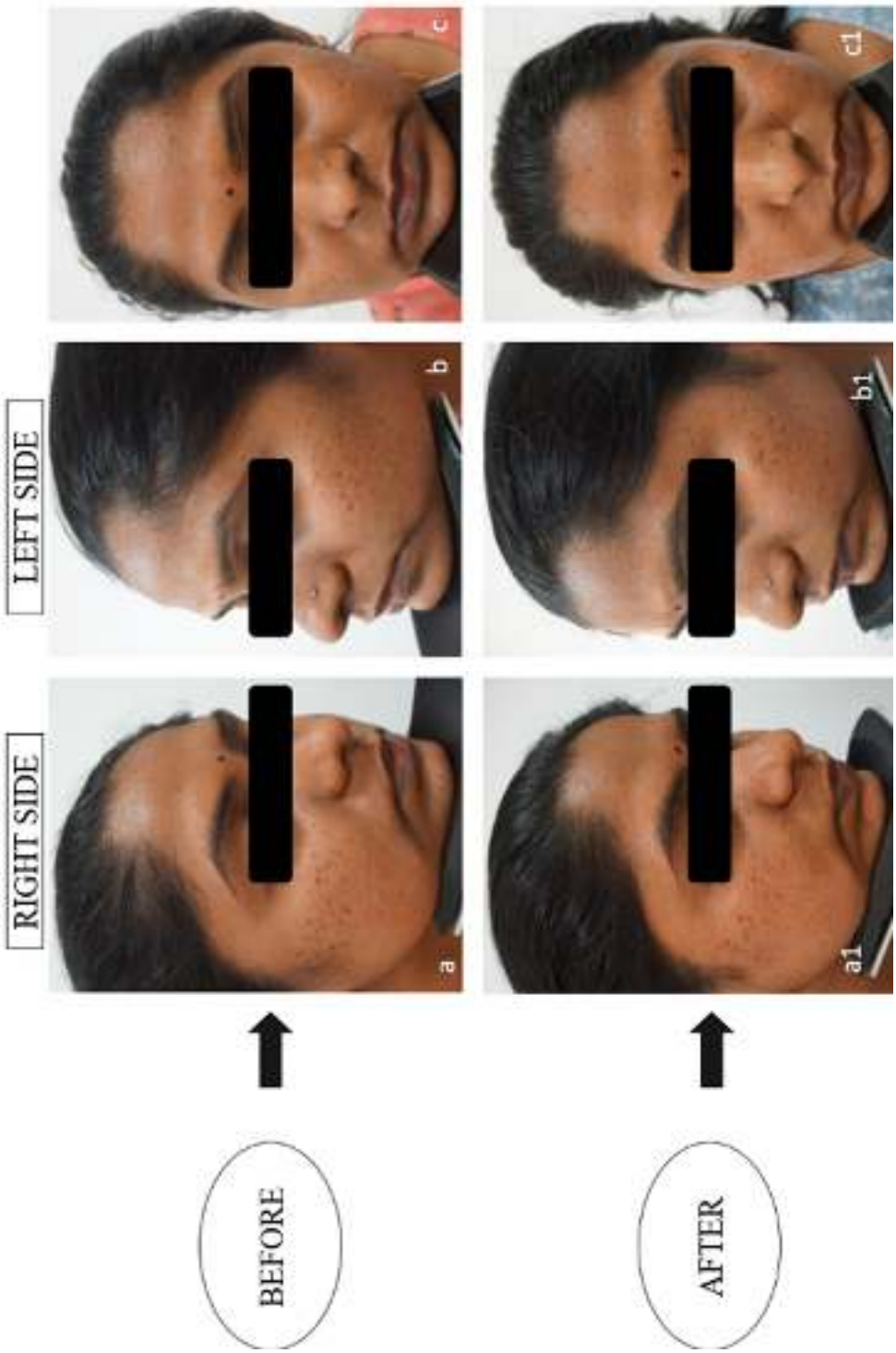


Figure 4: a: image of right side of face at baseline, a1: 6 weeks after 3rd session: b: image of left side of face at baseline, b1: 6 weeks after 3rd session: c: baseline image of face at 0°, c1: 6 weeks after 3rd session.

PATIENT 2

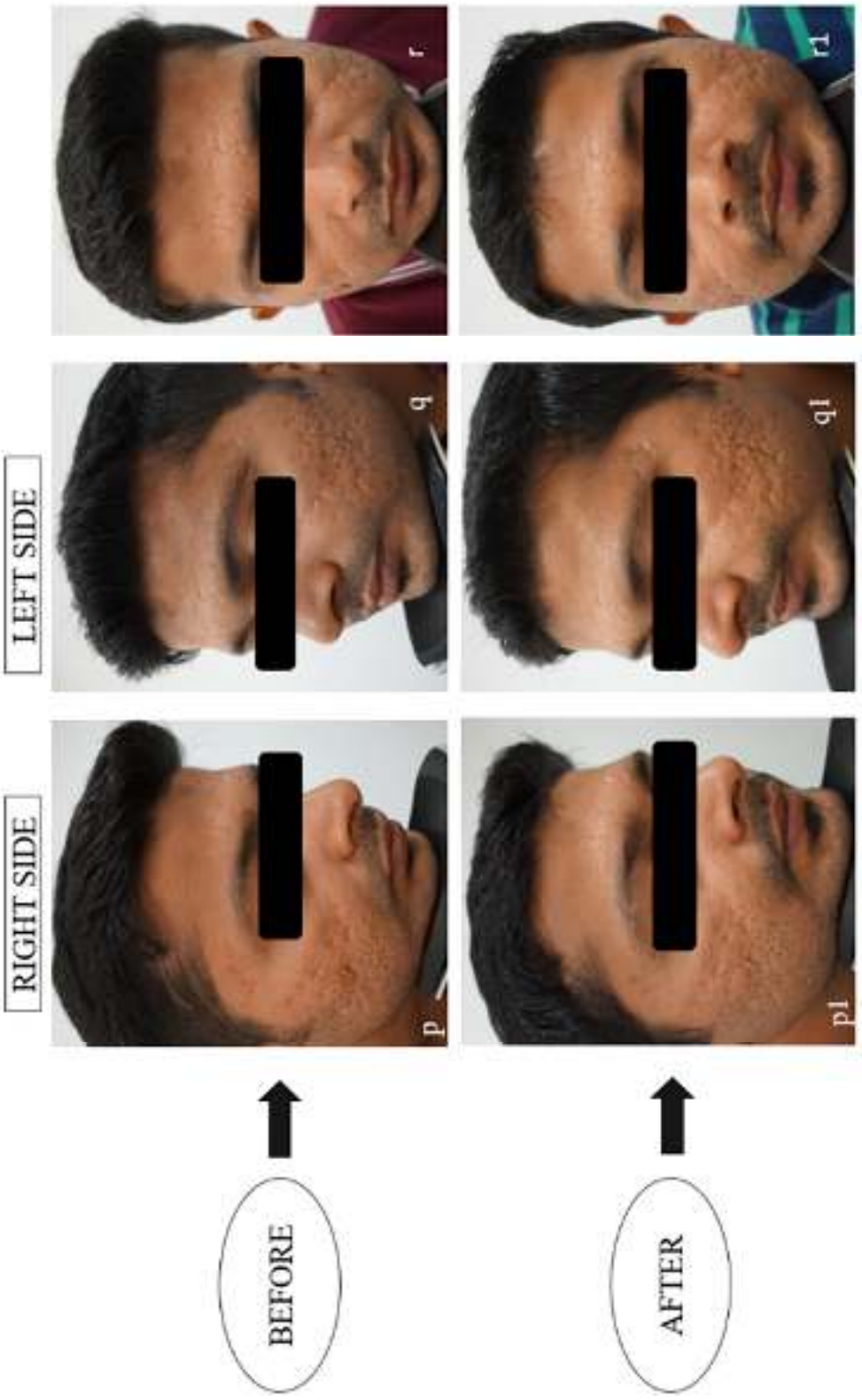


Figure 5: p-image of right side of face at baseline, p1- 6 weeks after 3rd session: q- image of left side of face at baseline, q1- 6 weeks after 3rd session: r- baseline image of face at 0º angle, r1- 6 weeks after 3rd session.

ANNEXURES V - MASTER CHART

Serial No	Name	Age	Sex	Occupation	Duration of scars(years)	Sunlight Exposure	Use of Sunscreen	Use of Cosmetics	Fitzpatrick Skin type	Site of Scar					Type of scar			Treatment history	H/o Steroid use	H/o Endocrine disorders	Family h/o acne	GOODMAN AND BARON SCORE							
										Frontal	Temporal	Cheek	Nose	Chin	Rolling	Boxcar	Icepick					Qualitative				Quantitative			
																						Right		Left		Right		Left	
																						Before	After	Before	After	Before	After	Before	After
1	Saddhamhusain	28	M	Engineer	8	yes	yes	no	IV	no	no	es	no	no	no	yes	yes	yes	no	no	no	no	4	3	4	3	13	10	12	10
2	Vaibhav	29	M	Business	7	yes	no	no	IV	yes	yes	yes	no	no	no	yes	yes	no	no	no	yes	3	2	3	2	12	9	14	10
3	Kaveri	23	F	Student	3	no	no	no	V	no	no	yes	no	no	yes	no	yes	yes	yes	no	no	3	2	3	2	7	4	7	4
4	Hemavati	23	F	Accounter	4	no	yes	no	IV	no	yes	yes	yes	no	yes	no	no	yes	no	no	yes	3	2	3	2	12	6	11	5
5	Gazala	22	F	Student	10	no	no	yes	III	no	no	yes	no	no	yes	no	yes	no	no	yes(PCOS)	no	3	2	3	2	9	5	8	4
6	Shivaprasad	23	M	Student	5	no	no	no	IV	no	yes	yes	no	no	yes	no	no	no	no	no	no	3	2	3	2	12	9	12	9
7	Madhumati	23	F	Student	6	yes	yes	yes	IV	yes	yes	yes	no	no	yes	yes	yes	yes	no	yes(PCOS)	yes	4	4	4	4	15	10	14	10
8	Vamsha	30	F	Doctor	10	no	yes	yes	IV	yes	yes	yes	yes	yes	yes	yes	yes	yes	no	no	yes	4	4	4	4	16	11	14	10
9	Krutika	31	F	Engineer	8	no	yes	no	IV	no	no	yes	no	no	yes	yes	yes	no	no	no	no	3	2	3	2	9	5	8	5
10	Manjunath	27	M	Electrician	8	yes	no	no	V	yes	yes	yes	yes	yes	no	yes	yes	no	yes	no	no	4	3	4	3	14	11	12	9
11	Kunal	29	M	Engineer	4	yes	yes	no	IV	yes	yes	yes	no	no	yes	yes	yes	no	no	no	no	3	2	3	3	11	9	11	8
12	Nikita	20	F	Student	3	no	yes	no	III	no	no	yes	no	yes	yes	no	yes	no	no	no	no	3	2	3	2	12	10	11	9
13	Sushma	27	F	NIL	13	no	no	no	IV	yes	no	yes	no	no	yes	yes	yes	yes	no	no	yes	3	2	3	2	8	6	9	6
14	Maryln	28	F	NIL	3	no	no	no	IV	yes	yes	yes	no	no	no	yes	yes	no	no	no	yes	3	2	3	2	11	8	12	9
15	Aditi	21	F	Student	4	no	yes	no	III	no	no	yes	no	no	yes	no	no	no	no	no	no	3	2	3	2	12	8	10	7
16	Hallavva	24	F	Engineer	5	no	yes	yes	IV	no	no	yes	no	no	yes	no	yes	no	no	no	no	3	2	3	2	11	7	12	7
17	Rukaiyya	23	F	Student	3	no	no	yes	III	yes	no	yes	no	no	yes	no	yes	no	no	no	no	3	2	3	2	9	5	10	5
18	Nivetha	26	F	Doctor	7	no	yes	yes	IV	yes	yes	yes	no	no	yes	yes	yes	yes	no	no	yes	4	2	4	2	14	10	13	10
19	Sreedevi	28	F	Nurse	4	no	no	no	IV	no	no	yes	no	no	yes	no	yes	no	no	no	no	3	2	3	2	9	7	9	6
20	Shivashankar	24	M	Student	6	yes	no	no	IV	no	yes	yes	no	no	yes	yes	yes	no	yes	no	yes	3	2	3	2	10	8	11	9
21	Akshata	23	F	Student	3	no	yes	yes	IV	no	yes	yes	no	no	yes	no	no	no	no	no	no	4	3	4	3	14	9	14	10
22	Amol	26	M	Engineer	7	yes	yes	no	IV	no	yes	yes	no	no	yes	yes	yes	no	no	no	no	3	2	3	3	12	10	13	11
23	Trupti	28	F	NIL	4	no	yes	yes	III	yes	yes	yes	no	no	yes	yes	yes	yes	no	no	no	3	2	3	2	9	6	9	7
24	Amrutha	27	F	Doctor	7	no	yes	yes	IV	no	yes	yes	yes	yes	yes	no	yes	yes	no	no	yes	4	3	4	3	13	9	14	9
25	Kishan	25	M	Doctor	5	no	no	no	IV	yes	yes	yes	no	no	no	yes	yes	no	no	no	no	3	3	3	3	9	7	9	6
26	Dhanaraj	28	M	Engineer	9	no	no	no	IV	yes	yes	yes	yes	no	yes	yes	yes	yes	no	no	no	4	3	4	3	13	8	12	7
27	Nagaraj	32	M	Professional	12	no	no	no	IV	yes	yes	yes	no	no	yes	no	no	no	no	no	yes	3	2	3	2	9	6	8	6
28	Prakash	30	M	Pharmacist	6	yes	no	no	IV	yes	yes	yes	no	no	yes	no	no	no	yes	no	no	4	3	4	3	13	8	12	8
29	Amar	19	M	Student	2	no	no	no	IV	no	no	yes	no	no	yes	no	no	no	no	no	no	3	2	3	2	12	8	11	7
30	Subham	21	M	Student	4	no	yes	no	IV	no	no	yes	no	no	yes	yes	yes	no	no	no	yes	3	2	3	2	11	8	12	9
31	Vikas	23	M	GYM Trainer	5	yes	no	no	IV	no	no	yes	no	no	yes	no	no	no	no	no	no	4	3	4	3	13	9	11	9
32	Shanta	33	F	Nurse	5	no	no	no	IV	no	no	yes	no	no	no	yes	no	no	no	no	no	3	2	3	2	10	8	11	7

VISUAL ANALOG SCALE		PHYSICIAN ASSESSMENT		ADVERSE EFFECTS											
Right	Left	Right	Left	RIGHT						LEFT					
				Erythema	Burning	Edema	Scabbing	Pigmentation	Others	Erythema	Burning	Edema	Scabbing	Pigmentation	Others
1	1	1	1	yes	yes	no	yes	yes	no	yes	yes	no	yes	yes	no
1	1	1	1	yes	yes	yes	yes	no	no	yes	yes	yes	yes	no	no
2	2	2	2	yes	yes	no	yes	yes	no	yes	yes	no	yes	yes	dryness
3	3	4	4	yes	yes	no	yes	no	pain,aggravation of acne	yes	yes	no	yes	no	no, aggravation of cane
2	1	2	2	yes	yes	no	yes	no	no	no	yes	no	yes	no	no
2	2	2	2	yes	yes	yes	yes	no	no	yes	yes	no	yes	no	no
2	2	2	2	yes	yes	yes	yes	no	no	yes	yes	no	yes	no	no
2	2	3	3	yes	yes	yes	yes	yes	no	yes	yes	yes	yes	yes	no
2	1	3	3	yes	yes	no	yes	yes	no	yes	yes	no	yes	yes	no
0	0	1	1	yes	yes	yes	yes	yes	dryness, pain	yes	yes	yes	yes	yes	dryness
2	2	2	2	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
2	2	2	2	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
2	2	2	2	yes	yes	no	yes	yes	no	yes	yes	no	yes	yes	dryness
2	1	2	1	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
2	2	3	3	yes	yes	no	yes	no	no	yes	yes	no	yes	no	dryness
2	2	2	2	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
3	3	3	3	yes	yes	no	yes	no	pain	yes	yes	no	yes	no	pain
2	2	2	2	yes	yes	yes	yes	no	pain	yes	yes	yes	yes	no	no
2	2	2	2	yes	yes	no	yes	yes	no	yes	yes	no	yes	yes	no
2	2	2	2	yes	yes	yes	yes	no	pain	yes	yes	no	yes	no	no
2	2	1	1	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
2	2	2	2	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
1	1	1	1	yes	yes	yes	yes	no	no	yes	yes	yes	yes	no	no
3	3	2	2	yes	yes	no	yes	no	pain	yes	yes	no	yes	no	pain
2	2	1	1	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
2	2	2	2	yes	yes	no	yes	no	pain	yes	yes	no	yes	no	no
2	2	3	3	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
3	2	2	2	yes	yes	no	yes	no	dryness, pain	yes	yes	no	yes	no	dryness
2	2	3	3	yes	yes	no	yes	no	no	yes	yes	no	yes	no	no
2	2	2	2	yes	yes	no	yes	no	no	yes	yes	no	yes	no	dryness
3	3	3	3	yes	yes	no	yes	yes	no	yes	yes	no	yes	yes	no
1	1	1	1	yes	yes	yes	yes	yes	pain	yes	yes	no	yes	yes	no

ANNEXURE-VI

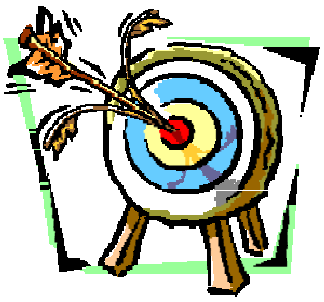
KEY TO MASTER CHART

M – MALE

F – FEMALE

PCOS – POLYCYSTIC OVARY SYNDROME

Introduction



Aims & Objectives

Review of Literature

Methodology

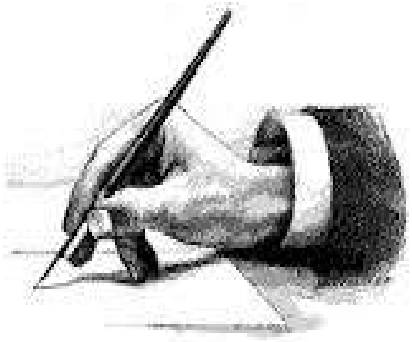
Results

Discussion

Conclusion

Summary

Bibliography



Annexure-I

Annexure-II

Annexure-IV

Annexure-V

Annexure-VI