“EFFICACY OF PLATELET RICH PLASMA THERAPY IN PATIENTS OF MELASMA: A SINGLE-BLINDED PLACEBO CONTROLLED RANDOMIZED STUDY”

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(DERMATOLOGY, VENEREOLOGY AND LEPROLOGY)

AIIMS, JODHPUR **DR. NEELAM CHHAJED**

JUNE 2022

“EFFICACY OF PLATELET RICH PLASMA THERAPY IN PATIENTS OF MELASMA: A SINGLE-BLINDED PLACEBO CONTROLLED RANDOMIZED STUDY”

THESIS

Submitted to

All India Institute of Medical Sciences, Jodhpur

In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(DERMATOLOGY, VENEREOLOGY AND LEPROLOGY)

AIIMS, JODHPUR **DR. NEELAM CHHAJED**

JUNE 2022

DECLARATION

I hereby declare that thesis entitled "**EFFICACY OF PLATELET RICH PLASMA THERAPY IN PATIENTS OF MELASMA : A SINGLE-BLINDED PLACEBO CONTROLLED RANDOMIZED TRIAL"** embodies the original work carried out by the undersigned

Dr. Neelam Chhajed
Department of Dermatology,
Venereology and Leprology
AIIMS, Jodhpur

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled "**EFFICACY OF PLATELET RICH PLASMA THERAPY IN PATIENTS OF MELASMA : A SINGLE-BLINDED PLACEBO CONTROLLED RANDOMIZED TRIAL"** is the bonafide work of **Dr. NEELAM CHHAJED** carried out under our guidance and supervision, in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

Supervisor

DR. SAURABH SINGH
Associate Professor
Department of Dermatology, Venereology & Leprology

Co-Supervisors

DR. ABHISHEK BHARDWAJ
Additional Professor and Head
Department of Dermatology,
Venereology & Leprology

DR. ANIL BUDANIA
Associated Professor
Department of Dermatology,
Venereology & Leprology

DR. ANUPAMA BAINS
Assistant Professor
Department of Dermatology,
Venereology & Leprology

DR. SAPTARSHI MANDAL
Associated Professor
Department of Transfusion
Medicine

All India Institute of Medical Sciences, Jodhpur

CERTIFICATE

This is to certify that the thesis titled "**EFFICACY OF PLATELET RICH PLASMA THERAPY IN PATIENTS OF MELASMA : A SINGLE-BLINDED PLACEBO CONTROLLED RANDOMIZED TRIAL"** is the bonafide work of **Dr. NEELAM CHHAJED** carried out under our guidance and supervision, in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

Dr. Abhishek Bhardwaj

Additional Professor and Head,
Department of Dermatology,
Venereology and Leprology
AIIMS, Jodhpur

ACKNOWLEDGMENT

First and foremost, I would like to thank the Almighty for bestowing me the strength, knowledge, and ability to undertake this research study and to persevere and complete it satisfactorily. Without his blessings, this achievement would not have been possible.

It is my proud privilege to express my deep sense of gratitude and sincere thanks to my supervisor ***Dr. Saurabh Singh*** *who provided me an opportunity to work under his guidance. His guidance was paramount in providing a well-rounded experience and knowledge. He has always been a source of encouragement and inspiration. I wish to express my deep in debt gratitude for devoting his valuable time out of his busy schedule and being concerned for the completion of this project. I shall remain grateful to him forever.*

I express my sincere gratitude to my co-supervisors ***Dr. Abhishek Bhardwaj***, ***Dr. Anil Budania***, ***Dr. Anupama Bains, Dr. Saptarshi Mandal*** *for their constant support, encouragement, guidance and help throughout my work.*

I want to take moment to express my sincere thanks and gratitude to ***Dr. Suman Patra*** *for guiding me.*

Most importantly I would like to thank my parents, Mr. Vinod Kumar Chhajed and Mrs. Lalita Chhajed for their constant support and my loving sister Divya. Their support, encouragement and unconditional love were undeniably the bedrock upon which my life has been built.

I would like to acknowledge and thank Senior residents Dr. Varun, Dr. Maninder and Dr. Rinkal, for their presence and constant support. I am deeply grateful to Senior Resident, Dr. Utkrist Lahoria for his immense support and help during difficult times of my study period.

Thanking my senior Dr Afroz, my companion Dr Sahiba Rafi, my juniors Dr. Benetta, Dr. Monisha, Dr. Devesh, Dr. Nagbhusan, Dr. Shubham, Dr. Subhajit, Dr. Kriti, Dr. Nazneen, Dr. Shilpi, Dr. Yamini, Dr. Thoyyib, Dr. Priyanka, Dr.Akriti , Dr. Karthick for their help during my day to day work.

I wold also like to thank Dr Akhil Sir, Dr. Surjeet sir and Dr Sussender for their help.

Special thanks to Dr. Richa, Dr. Aakash, Dr. Isha, Dr. Netranand, Dr. Pankaj for their constant support.

I also thank our nursing officers Mr. Deepak, Mrs. Santosh and ward-lady Mrs. Ruby Yadav for their co-operation during injection procedures. I am grateful to our department clerk Mrs. Saroj, and other departmental staff Mr. Praveen, Mr. Rameez Raza, Mrs.Pooja, Mr. Poonaram for their co-operation.

Last but not the least I thank my patients, without their cooperation thesis would not have been possible.

DR. NEELAM CHHAJED

CONTENTS

SUMMARY

Background:

Melasma is a common acquired pigmentary disorder having multiple treatment options ranging from topical medications, oral therapies, peels and lasers and yet its treatment remains challenging due to its recurrent nature.

AIMS AND OBJECTIVE:

To evaluate the effectiveness of Platelet rich plasma (PRP) in treatment of melasma by comparing it with placebo (normal saline) and measuring its additive effect over and above Hydroquinone.

Methods :

This was an interventional, split face randomized control trial of 64 patients. PRP was injected intradermally over lesional skin on one half of face and placebo on other half by using insulin syringe. This treatment was administered 4 weekly for 4 sessions. Hydroquinone 2% cream was also applied over melasma site and sunscreen over full-face. Melasma was scored using Hemi-Modified Melasma area sevserity Index (Hemi-mMASI), Melasma Severity Index (MSI) and Investigators Global assessment scores (IGA) at each visit. Dermoscopy, patient's global assessment score and Melasma quality-of-life score were recorded at the start and end of the study.

Results:

Total 45 patients completed the study with female to male ratio 1.8:1 and mean age of 29 years. Hemi-mMASI at baseline in PRP group was 2.3 ± 1.47 which reduced to 1.56 ± 0.98 as compared to placebo group which reduced from 2.51 ± 1.79 to 1.59 ± 0.97 at the end of 16 weeks ($p > 0.05$). On comparing investigators global assessment between PRP and placebo group the difference was not statistically significant ($p<0.05$). Patients' global assessment was 50% improvement in 55% patients. Dermoscopy showed grade 1 improvement in pigmentation among 51% patients of PRP group and in 56% patients of saline group. MELASQOL score reduced by 26% between baseline and at 16 weeks. Side effects noted were mainly erythema, exacerbation of acne and pain during injection.

Conclusion:

Although PRP decreasing pigmentation is an interesting finding but on comparing PRP with saline, PRP had not provided any additional benefits over and above hydroquinone in our patients of melasma. More larger placebo controlled studies are required.

Limitation:

Limitation could be small sample size and limited number of PRP sittings.

LIST OF ABBREVIATIONS

UV	Ultraviolet
VEGF	Vascular endothelial growth factor
PIH	Post-inflammatory hyperpigmentation
DAGs	1,2-diacyglycerols
NO	Nitric oxide
ACTH	Adrenocorticotropic hormone
MSH	Melanocytic stimulating hormone
PDZK 1	PDZ domain kidney protein 1
WIF 1	WNT inhibitory factor
COC	Combined hormonal oral contraceptive pills
MASI	Melasma area and severity index
HQ	Hydroquinone
TA	Tranexamic acid
QNd:YAG	Q switched neodymium:yttrium-aluminium- garnet
Er:YAG	Erbium YAG laser
GA	Glycolic acid
FA	Fluocinolone acetonide
L-PRP	Leucocyte- and PRP
L-PRF	Leucocyte- platelet rich fibrin
PDGF	Platelet derived Growth Factor
TGFβ-1	Transforming Growth Factor- Beta1
FGF1	Fibroblast Growth Factor
ET 1	Endothelin 1
SCF	Stem cell factor
PKA	Protein kinase A
POMC	Proopiomelanocortin
CAMP	Cyclic adenosine monophosphate pathway

MITF	Melanocytic inducing transcription factor
ER	Estrogen receptor
CREB	CRE binding protein
NFAT 1	Nuclear factor of activated T cells
RCM	Reflectance confocal microscopy
MELASQOL	Melasma quality of life scale
TCC	Triple combination cream
QSRL	Q switched ruby laser
IPL	Intense pulse laser
TCA	Tricholoroacetic acid
RA	Retinoic acid
PRP	Platelet rich plasma
P-PRP	Pure platelet rich plasma
EGF	Epidermal Growth Factor
IGF-1	Insulin-like Growth Factor1
VEGF	Vascular Endothelial Growth Factor
mMASI	Mean modified Melasma area severity Index

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEXURES

INTRODUCTION

Melasma is a hyperpigmentary disorder characterized by irregular, brown pigmented macules over photo-exposed areas of face, such as forehead, cheeks, upper lips and nose. Though more common in females, it can also occur in men. It is more common in darker skin types, (Fitzpatrick skin types III to VI) compared to lighter skin types. Multiple etiological factors have been implicated in the pathogenesis of melasma including pregnancy, oral contraceptives, genetics, sun exposure, cosmetics and race [(1)].

Several agents are available for treatment of melasma which include various topical agents, chemical peels, lasers and oral therapy. Sunscreens and topical depigmenting creams remain mainstay of therapy. Traditional treatments for melasma includes hydroquinone, kojic acid, azelaic acid, retinoids, topical steroids and arbutin along with physical and chemical sun protection. Topical agents are generally used as the first line therapy followed by chemical peels as the second line followed by oral therapy and lasers, which are usually restricted to refractory cases not responding to other modalities[(2)]. The treatment of melasma still remains difficult and challenging inspite of the multiple therapeutic options due to recurrent nature and compliance with sun protective therapies which wane with time. Also, there are complications associated with these therapies such as erythema ,irritation and post-inflammatory hyperpigmentation (PIH). Platelet rich plasma (PRP) is an upcoming therapy for melasma as shown in existing literature.

PRP is a plasma rich in platelets and contains higher than normal platelet concentration, prepared by centrifugation of whole blood [(3)]. The alpha granules of platelets contain abundant cytokines and growth factors affecting wound healing, collagen production and control homeostasis[(4)].

Even though there are encouraging results in existing literature, effect of PRP on melasma has not been studied systematically in a placebo controlled trial. Therefore we planned to study the effects of PRP over and above standard therapy in patient of melasma in a split face manner.

REVIEW OF LITERATURE

Melasma is an acquired disorder of pigmentation disorder. It most commonly affects women with darker skin and is characterized by brown-coloured macules over sun-exposed areas of face such as forehead, cheeks, lips, and nose. Risk factors for melasma include genetic factors, UV light exposure, pregnancy, oral contraceptives, and drugs such as phenytoin [1,2,5,6].

EPIDEMIOLOGY

It has a prevalence of 1% in general population and 9 to 50% in higher-risk populations. It is particularly common among Hispanics, African Americans, Brazilians and Asians, where ultraviolet radiation is more intense[7]. In an Indian study incidence of 41% was found in paddy workers[8]. The age at onset on an average ranges between 20 and 30 years.

A study of 312 melasma patients in India showed that female to male ratio was 4:1[9]. In India, it was also discovered that males (33.5 and 3.5 years) and females (33.5 and 3.5 years) had similar average ages and disease durations (31.5 and 3.1 years)[7].

PATHOGENESIS OF MELASMA

Genetic factors, persistent ultraviolet (UV) exposure, and female hormones are main causes of melasma. Inflammation is also implicated in melasma development [10,11].

Genetic factors

Racial and positive family history are reported relating the melasma incidence[12]. For instance, Latin Americans and Hispanics and Asians with Fitzpatrick skin type III–V are likely to have pigmentary disorders such as melasma and post inflammatory hyperpigmentation (PIH)[10,13].

UV exposure

Ultraviolet exposure stimulates release of melanogenic factors from melanocytes and keratinocytes directly and indirectly. Direct effect of UV irradiation causes formation of 1,2- diacylglycerols' (DAGs) with protein kinase C-beta activation[14] and nitric

oxide (NO) production following cyclic guanosine monophosphate synthesis[15]. Keratinocyte-derived melanogenic factors such as Adrenocorticotropic hormone, Melanocyte-Stimulating hormone (MSH), Endothelin 1 (ET-1) indirectly affected by UV irradiation[16].

UV-induced melanogenesis is induced by the interaction of many paracrine secretion including Proopiomelanocortin (POMC) -derived peptides, ET-1 and stem cell factor (SCF). The binding of melanocortin to MC-1 receptor, leads to melanogenesis via cyclic adenosine monophosphate (AMP) pathway and then activates protein kinase A (PKA) and Melanocyte Inducing Transcription Factor (MITF). Keratinocytes also produce NO and cause melanogenic effect after the UV radiation. Additionally, dermal fibroblasts release Stem Cell Factor (SCF), on direct exposure to UV, suggesting the communication between melanocytes and fibroblasts in UV-induced hyperpigmentation[11,17,18].

Ortonne et al. reported that time spent outdoors during pregnancy was associated with risk of melasma onset. Post-pregnancy melasma is significantly related to darker skin type and most patients have family history of melasma. These factors often triggers melasma. The result showed a combination of genetic factor, Fitzpatrick skin type and UV exposure induce melasma[19].

Female sex hormones

Association of melasma and oral contraceptives during women's reproductive lifespan can also be an another important factor affecting the prognosis and severity of melasma development. Affected skin had increase in expression of estrogen receptor (ER). Significant immunohistochemical expression of ER- beta could only be found in dermal melasma. By binding of estrogen and ER, there is induction of melanogenesis.

PDZ domain protein kidney 1 (PDZK1) expression is upregulated in melasma affected skin. PDZK1 is overexpressed in melanocyte monocultures which results in overexpression of CRE-binding protein (CREB), MITF and tyrosinase. PDZ domain protein kidney 1(PDZK1) facilitates estrogen interactions with other proteins, thereby promoting melanogenesis and melanosome transfer in patients of melasma [20].

Local cutaneous factors

(1) Increased number of mast cell

Various studied showed even though the number of mast cells are increased in melasma lesions, mast cells do not have exact role in melanogenesis[10].

(2) Role of WIF-1 in fibroblasts in melanogenesis

Chronic sunlight exposure results in secretion of SCF from fibroblasts resulting in increased pigmentation[17,18]. Reduced Wnt inhibitory factor-1 (WIF-1) expression is involved in activation of melanogenesis and melanosome transfer.

WIF-1 is not expressed in melanocytes whereas it is present in cultured normal human keratinocytes and fibroblasts. The decreased expression of WIF-1 in keratinocytes and fibroblasts reduces the binding of WIF-1 to Wnts in melanocytes. This results in MITF upregulation and translocation of nuclear factor of activated T cells (NFAT) to nucleus Wnt/beta-catenin pathways[10,21].

(3) Increased vascularization

Altered dermal vasculature is found in melasma and UV-damaged skin. Blood vessels and vascular endothelial growth factor (VEGF) are increased in dermal melasma. However, VEGF role in melanogenesis is still not clear[22].

(4) Solar elastosis

In dermal melasma abnormalities of extracellular matrix are observed. Solar elastosis occurs due to prolonged sun exposure and is characterised by accumulation of abnormal elastic tissue. Photoaging plays an important role in development of melasma. Ultraviolet B exposure causes secretion of SCF, bFGF interleukin-1, endothelin-1, inducible nitric oxide synthase, MSH and PGE2 by keratinocytes. This leads to epidermal and dermal hyperpigmentation[10].

(5) Basement membrane disruption

Long time UV exposure leads to basement membrane disruption and eventually melasma. During chronic UV irradiation, the expression of collagen degrading proteins (MMP-2 and MMP-9) is upregulated which causes basement membrane disruption, causing melanocytes and melanin to drop in dermis[17].

MicroRNAs and their targets

MicroRNA-675 expression is reduced in lesional melasma skin. When miR-675 expression is increased tyrosinase and melanogenic factors are less activated. Thus microRNA play role in melasma[23,24].

CLINICAL FEATURES

It is characterised by patches of light brown to dark brown colour on sun-exposed skin. The lesions are generally symmetric and affect forehead, nose, cheeks, upper lip area, and chin. It is more common in females, with a male to female ratio being 1:4[9]. In an Indian study by Sarkar et al, major risk factors in men were - sun exposure (48.8%) and family history (39.0 percent) while in women were - Pregnancy (45.3%), sun exposure (23.9%) and combination oral contraceptive (19.4 percent). Pregnancy and use of oral contraceptives induces melasma in roughly 40-50 percent of female patients. After combined oral contraceptives about 8 to 34% women have been to shown to have melasma. It can also occur after hormone replacement therapy[12].

Classification of melasma

Common facial patterns of distribution for melasma include,

1) Centrofacial pattern affecting forehead, cheeks, nose, upper lip, and chin,
2) Malar pattern over lateral cheek areas and
3) Mandibular pattern over lower jawline in mandibular area.

Study by Yalamanchili et al[25], showed that the malar was the most common region affecting 68 percent, followed by the central face (25 percent) and mandible (7 percent). The most common pattern in women is the centrofacial pattern and amongst men, the malar pattern is predominant accounting for 44.1 to 61 percent of patients[26]. The second most common pattern in men is the centrofacial variant. Some patients may develop extrafacial melasma, which is far less common than the facial type. The study by Ritter et al on melasma patients in Brazil showed that 45 patients had extrafacial melasma. Sites involved were the most commonly arms (95 percent), followed by forearms (80 percent), chest (47 percent) and least commonly back (11 percent)[27]. However study by Tamega et al. on Brazilian women showed zygomatic to be most commonly involved area[13].

Melasma can also be classified according to the depth of pigmentation into

1) Epidermal – seen as light brown pigmentation
2) Dermal – seen as bluish grey pigmentation
3) Mixed - dark brown pigmentation.

   In the epidermal type, increased pigmentation is seen in all layers, whereas malanophages are present in papillary as well as reticular dermis in dermal type of melasma.

Melasma has a remitting and relapsing course. Spontaneous remission occurs rarely. Its relapse occurs with even with mild sun exposure. Also, because no effective medication exists to remove dermal pigment, dermal melasma may take longer to resolve than epidermal pigment. The epidermis is the source of dermal pigment, and if epidermal melanogenesis is blocked over an extended length of time, dermal pigment will not refill and will gradually fade.

Resistant cases and recurrences of melasma are common and unavoidable if sun protective regimes are inadequate[28].

DIAGNOSIS

Melasma is primarily diagnosed clinically.

Wood's lamp examination can help in identifying depth of pigment, especially in individuals with lighter complexions (Fitzpatrick phototypes I to III). Epidermal melanosis is characterized by well-circumscribed pigmentation with accentuated borders. Dermal melanosis is ill defined and has poorly circumscribed and is not accentuated under Wood's lamp illumination. Wood's lamp examination is however less reliable in Fitzpatrick phototypes IV, V, and VI. In these patients, the density of melanin can make variations between the skin layers difficult to differentiate[7].

Dermoscopy can be used for diagnosing melasma and assess intensity of pigmentation. Dermoscopy can reveal accentuation of the normal pseudoreticular pigmentary network, increased vascularity, telangiectasia and arcuate structures[8]. Dermoscopy also can distinguish melasma from ochronosis which shows attenuation of follicular openings by amorphous densely-pigmented structures[7,29].

Melasma is distinguished histologically by epidermal hyperpigmentation. There is no increase in the number of melanocytes. Melanocytes exhibit hypertrophy, with an increase in the number of dendrites and cytoplasmic organelles, indicating increased metabolic activity. The amount of melanin in all layers of the epidermis increases, as does the number of mature melanosomes, notably in the basal and suprabasal layers. There is a modest to moderate mononuclear infiltration of mast cells in the dermis, as well as enhanced vascularity and elastosis. Adjacent healthy skin and skin with melasma showed no differences in dermal pigmentation[7].

Reflectance confocal microscopy (RCM), a direct and non-invasive technique through which epidermis and upper dermis can be visualized can be used for melasma. At higher resolution we can see hypertrophied melanocytes with melanin in all layers of the epithelium and dermis. Thus this technique can be used in clinical trials for assessment of treatment response[7]. Morphological findings on RCM along with their histological correlates in patients with melasma described by Kang et al is shown in table 1[30].

Table 1: Histological and morphological correlation with reflectance confocal microscopy

Morphological criteria	RCM	Histological correlation
Epidermis	Hyper-refractile cobblestone cells	Hyperpigmented basal keratinocytes
	Dendritic cells	Activated melanocytes
Dermis	Plump bright cells	Melanophages
	Lacy structures	Solar elastosis
	Tubular structures	Blood vessels

ASSESSMENT OF SEVERITY :

Clinical scores :

The severity of facial melasma can be estimated by using various clinical scores like MASI score (Melasma Area and Severity Index) and Modified MASI, also tools like The most commonly used is MASI index, which was proposed by Kimbrough-Green et al, for clinical quantification of severity of facial melasma.

Melasma Area Severity Index score is calculated using 3 factors – melasma affected area (A), degree of hyperpigmentation (D) and its homogeneity (H). Face is divided into four quadrants - forehead (F), right malar region (MR), left malar region (ML) and chin (C).

Numerical score assigned corresponding to the percentage of area involved:

0= no involvement

1=< 10% involvement

2=10-29% involvement

3=30-49% involvement

4=50-69% involvement

5=70-89% involvement

6=90-100% involvement

The darkness of melasma(D) graded in comparison to normal skin as follows;

0=normal skin color without evidence of hyperpigmentation

1=barely visible hyperpigmentation

2=mild hyperpigmentation

3=moderate hyperpigmentation

4=severe hyperpigmentation.

Homogeneity of the hyperpigmentation (H) is graded on a scale of 0 to 4 as follows:

0=normal skin color without evidence of hyperpigmentation

1=specks of involvement

2=small patchy areas of involvement < 1.5 cm diameter

3=patches of involvement> 2 cm diameter

4=uniform skin involvement without any clear areas.

More the total score, more is the severity of disease.

In modified MASI, only hyperpigmentation and area is assessed. Modified MASI was proposed by Pandya et al which showed that assessing homogenicity in MASI was the most difficult part of calculation and that removing homogenecity from MASI score does not alter the validity and reliability of the score[31].

Melanin severity index (MSI) was proposed by Majid et al. in 2016 which claimed to have better Inter-rater reliability as compared to MASI, study has shown that both mMASI and MSI scores were fairly correlated with the objective data from mexameter and image analysis software. However, the validity of MSI was found to be superior as the Spearman's rho values were higher for MSI than those for mMASI.

For MSI, the formula used was $0.4\ (a \times p^2)\ l + 0.4\ (a \times p^2)\ r + 0.2\ (a \times p^2)\ n$ where "a" is the area, "p" is pigmentation, "l" is left face, "r" is right face, and "n" is nose [32].

Instrument based

Colorimetry and Mexameter can be used[7].

Reliability of the MASI score has been called into question by several authors, can thus newer techniques like colorimetry and mexameter have been developed for assessment of melasma.

Quality of life

Melasma although benign and asymptomatic can cause psychosocial as well as emotional discomfort, and thereby lowering patients' quality of life.

Balkrishnan et al. created and validated the MelasQoL (Melasma Quality of Life Scale) in 2003, which is a 10-question questionnaire designed to assess the impact of melasma on emotional state, social interactions, and everyday activities. The study showed that psychometric properties of the MELASQOL were comparable with the properties of the DLQI and the SKINDEX-16 (33). However in contrast to skindex-16 and general questionnaries DLQI, the English MelasQoL has higher internal consistency, validity and discriminatory power. Different countries all over the globe

have also validated MelasQoL (7). Hindi version of MELASQOL can be used Indian patients[34].

In Brazil, MelasQOL was recorded for 300 melasma patients. Among the responses that predominated, discomfort due to the spots was reported by 65% of patients, frustration by 55% whereas 57% were embarrassed about their skin[7].

TREATMENT :

Avoidance of exacerbating factors

This is very essential way to improve melasma. The patients should avoid exacerbating factors including oral contraceptive drugs like ethinyl estradiol, medroxy progesterone acetate, norethindrone, melasma-induced drugs like phenytoin and sun exposure[35].

Photoprotection

Strict photoprotection like avoiding sun exposure, protective clothing, and broad-spectrum sunscreens, is an essential for treating and preventing melasma. Broad spectrum sunscreens reduce melanocyte reactivation caused by sun exposure, therefore UV protection is necessary in addition to other forms of treatment[19]. Sunscreens are to be re-applied every 2-3 hourly and 1 teaspoon over face and neck each time.

In a Moroccan based study Lakhdar et al 85 pregnant women in their first trimester were taken, they applied sunscreen 2 hourly for 1 year. No darkening and even lightening of skin colour was seen in 79% women. New onset melasma developed in 5 women (2.7 percent), and the percentage of women who developed melasma decreased after application of SPF50 sunscreen every 2 hourly at day time[36].

Shorter wavelengths of visible light (415 nm) cause more pigmentation than longer wavelengths (630 nm)[37]. Another study found that more darker pigmentation was induced by visible light (VL) than that by UVA-1. The group that used UV + VL sunscreen showed 28% greater improvement compared to the other group using only UV sunscreen[38].

Sarkar et al studied the role of sunscreens alone in treatment of melasma and found that after 12 weeks of 3ml thrice daily application of broad spectrum sunscreen, MASI reduced from 12.38 to 9.15 ($P < 0.05$).

INTERVENTIONS FOR MELASMA

Following are the interventions used for melasma.

Table 2: Classification of interventions for Melasma

	A) WELL ESTABLISHED
	1. Hydroquinone
	2. Retinoids
	3. Triple combination
	4. Kojic acid
	5. Azelaic acid
	B) RELATIVELY NEWER MODALITIES
	1. Lignin peroxidase (LP)
	2. Magnolignan
	3. NAG
	4. Orchid extracts
	5. Dioic acid
	6. Octadienedicic acid
	7. Beta-Carotene
	8. Linoleic acid
	9. Silymarin
	10. 5% methimazole
	11. Pidobenzone
	12. Rucinol
	13. Licoroce extract
	14. Soymilk
	15. Mulberry extract

	16. Aloesin
	17. Flavonoids
	18. Ellagic acid (topical)
	19. Liquirtin (topical)
(ii)ORAL TREATMENT	**TRANEXEMIC ACID**
(iii)PROCEDURAL TREATMENTS	**A)CHEMICAL PEELS**
	1. Glycolic acid
	2. Trichloro acetic acid peel
	3. Salicylic acid
	4. Jessner's peel
	B) LASER
	1. CO_2 Laser
	2. ND:YAG Laser
	3. Q switch Ruby Laser
	C) MICRONEEDLING

Topical therapy

1) Hydroquinone

Hydroquinone is a hydroxyphenolic chemical inhibiting the conversion of DOPA to melanin by inhibiting tyrosinase. It also destroys melanosomes, but also causes necrosis of melanocytes by modifying the membrane structure[39]. It has been commonly used to treat melasma for decades. It is commonly used in 2-5% either alone or with other depigmenting agents. Ennes et al compared hydroquinone 4% cream with placebo in 48 patients. Group A was treated with hydroquinone 4% alone, Group B with placebo. Both groups applied sunscreen. The result showed 30% more improvement in group A > group B[40].

In a controlled study by Arndt et al, 1965 (*n* = 56), both 2% and 5% HQ creams were found to be equally efficaceous, with 80% patients showing marked improvement[41]. Inflammatory reactions were less with 2% Hydroquinone[42]. Adverse effects of hydroquinone include irritation, erythema, stinging, irritant or allergic contact dermatitis, milia, PIH and exogenous ochronosis. It is a blue-black discolouration of skin due to inhibition of oxidase activity by HQ, which results in accumulation of homogentisic acid, which polymerizes to form ochronotic pigment in dermis[43]. Histologically exogenous ochronosis is seen as banana shaped bodies in the dermis. It is more commonly reported by dark skinned patients especially when hydroquinone is used for prolonged duration.

2) Retinoids

Various topical retinoids are effective in melasma. Tretinoin increases epidermal turnover thus decreasing contact time between keratinocytes and melanocytes. It also decreases melanosome transfer, inhibits tyrosinase transcription, and interrupts synthesis of melanin. In a study by Griffifth et al. 19 patients each were treated with 0.1% tretinoin and with placebo, of which 68% patients with tretinoin had improvement and only 5% of placebo group showed improvement[44]. Side effects such as erythema and desquamation were noted in 88% of tretinoin group versus 29% of placebo group. Thus topical tretinoins are commonly used as combination therapy in order to reduce their side-effects.

3) Combinations

a) Kligman's Formula

The Kligman–Willis formula is 5% hydroquinone, 0.1% tretinoin and 0.1% dexamethasone combination. It was developed 30 years ago to reduce pigmentation in melasma, ephelides, and post inflammatory hyperpigmentation. The benefits of this regimen are irritancy reduction, effect lengthening, HQ oxidation prevention and penetration improvement[42].

b) Triple combination cream

Among the various triple Combination Cream (TCC), FDA approved contains 4% hydroquinone, 0.05% tretinoin, and 0.01% fluocinolone acetonide[5]. This triple combination cream was compared to HQ 4 percent and was found significantly better in a randomised trial on 260 Asian patients[45]. Cestari et al. assessed efficacy and safety of TCC, compared with topical hydroquinone in patients with moderate to severe facial melasma. For 8 weeks, subjects were randomly assigned to receive TCC applied once a day or HQ cream applied two times a day. Improvement of more than 75% of global severity score was present in 73% of TC cream patients and less than half of HQ cream patients. Erythema, a burning sensation, and desquamation were observed in both groups. For the treatment of moderate to severe facial melasma, TC is superior to HQ. The safety profiles of both drugs were comparable[46].

4) Kojic acid

Derived from fungus *Aspergillus orzyea*, it acts by inhibiting tyrosinase. It is used in concentrations of 2-4% to be applied daily over hyperpigmentation. In a study by Deo et al, kojic acid 1% alone, Kojic acid with 2% hydroquinone, Kojic acid with 0.1% betamethasone valerate and kojic acid with both hydroquinone 2% and Betamethasone was compared in 80 Indian patients of melasma. Highest efficacy was noted with kojic acid combined with hydroquinone[47].

5) Azelaic acid

Azelaic acid inhibits mitochondrial oxidoreducatase activity and thus decreasing hyperactive melanocytes' energy generation and DNA synthesis[48]. It also has antityrosinase activity. In study by Verallo et al. on 155 patients showed that Azelaic acid 20% was superior to Hydroquinone 2%[49]. Also in Study by Farshi et al. showed Azelaic acid being more efficacious than HQ 4%. However in study by Belinda et al showed HQ 4% to be more efficacious than Azelaic Acid 20%.

6) Arbutin

Arbutin is a synthetic compound where one molecule of D-glucose is bound to hydroquinone. It has anti-melanogenic action by anti-tyrosinase activity Arbutin was compared to a placebo in trial by Morag et al on 54 melasma patients for 8 weeks and a statistically significant difference was seen.

Table 3: Conventional topical drugs

Study	Type	Number of patients	Treatment given	Results	Adverse effects (AE)
Hydroquinone					
Arndt et al., 1965	A split face study.	20	Group 1: 5 HQ Group 2: 2% HQ X 12 weeks.	Equal improvement	Irritant reaction with 5% hydroquinone.
Ennes et al, 2000	A double-blind, comparative, placebo-controlled study	48	Group 1 : 4%HQ Group 2 Placebo x 12 weeks	40% patients – excellent improvement	-
Retinoids					
Grifith et al., 1993	A randomized, vehicle-controlled study	38	Group 1 : 0.1% tretinoin Group 2 : Placebo cream x 40 weeks	68% improvement in Tretinoin group versus 5% in placebo group.	Erythema and desquamation in 88% pts of group 1.
Triple combinations					
Taylor et al, 2003	Single blinded, randomised, multicentre trial.	641, Fitzpatrick types I to IV	TCC (tretinoin 0.05% + HQ 4% + FA 0.01%), versus DC (tretinoin+HQ, tretinoin + FA and HQ +FA) x 2 months	TC > DC (significant) in all the groups	Mild AE seen in all groups
Grimes et al, 2006	An open labelled, multicentre, community based, uncontrolled trial	1290 (1042 completed) Fitzpatrick skin types I to VI	0.05% tretinoin + HQ 4.% + FA 0.01% x 2 months	Significant improvement in MASI	---
Cestari et al , 2007	An open labelled, randomised trial.	120 patients	HQ 4% versus TCC (RA 0.05% + 4% HQ + FA 0.01%) x 2 months	Total clearance TCC (35%) > HQ (5.1%)	Mild AE incidence similar in both group
Chan et al 2008	A double blinded, randomised trial.	260 patients Asian	4% HQ + 0.05% RA+0.01% FA versus 4% HQ x 2 months	Significant reduction in MASI in TCC (64.2%) group than HQ (39.4%)	
Gong et al, 2015	A double blinded, randomised trial.	233 patients	4% HQ +0.05% RA+0.01% FA versus placebo x 2 months	TCC more effective	

Kojic Acid					
Monteiro et al., 2011	A double-blinded, randomised controlled trial.	60 Indian patients	KA 0.75% + 2.5% Vitamin vs Hydroquinone 4% x 3 months	HQ cream superior to KA	KA - 6.7% AE (erythema, burning sensation) HQ 3.3% AE (erythema)
Deo et al, 2013	A single-blinded, randomised controlled trial.	80 Indian patients	Comparison in 4 groups Group A: KA 1% Group B: KA 1% + HQ 2% Group C: KA 1% + BV 0.1% Group D: KA 1% + HQ 2% + BV 0.1% For 12 weeks	Efficacy of Group B highest B > D > A > C	NA
Azelaic Acid					
Verallo Rowell et al., 1989	A double-blinded, randomised trial.	155 patients	2% HQ versus 20% AA x 6 months	AA (73%) > HQ (19%)	Equal in both groups
Balina et al, 1991	A randomised clinical trial.	329 patients	4% HQ versus 20% AA x 6 months	Superior results with HQ (73%) as compared to AA (65%)	
Farsi et al,2011	An open labelled, randomised controlled trial	29 patients	AA 20% twice daily versus 4% HQ x 2 months	AA significantly better than HQ	AE with HQ > AA
Arbutin					
Morag et al,	A double-blind, controlled randomized control trial	54 patients	Arbutin (Leaf extract) versus placebo Duration: 8 weeks	Statistically significant lightening	NA
KEY : HQ – hydroquinone, TCC –triple combination, DC- double combination, FA- flucinolone acetonide, KA- kojic acid, RA- retinoic acid, AA- azelaic acid, BV- Betamethasone valerate, MASI – Melasma area severity index.					

LESS WELL ESTABLISHED THERAPIES: [40]

There are a number of molecules that have been tried in treatment of melasma with variable results and the same have been summarized in table 4.

Table 4 : Less well established topical agents and topical agents and their clinical trials.

Drug	Mechanism	Study	Treatment given.	Number of patients	Result	Side effect(s)
Lignin peroxidase (LP)	oxidizing and breaking down melarin	Mauricio et al, 2011	LP versus 2% HQ cream	51 patients	LP cream provided significant skin-lightening as compared HQ	Minimal adverse effects
		Draelos et al, 2015	Group 1: LP versus HQ Group 2 : LP versus placebo	60 women	Parity between LP and HQ, LP superior the placebo LP superior to HQ : better skin texture	Minimal adverse effects
Magnolignan	Inhibits the maturation of typosinase	Takeda et al, 2006	0.5% Magnolignan x 6 months	51 female	Significant improvement	No side effects
NAG	Inhibit conversion of protyrosinase to tyrosinase	Iraji et al, 2009	4% NAG + 2% nicotinamide vs 4% HQ on each side of face x 12 weeks	30 females	Efficacy NAG + nicotinamide slightly more than HQ Statistically insignificant.	Side efforts of NAG+nicotinamide slightly less than HQ
Orchid extracts	Antioxidant	Tadokoro et al, 2010	Plant extracts including orchid extracts vs 3% Vitamin C derivative	48 Japanese females	Significant improvement with plant extracts (orchid extracts) formulation, parity with Vitamin C	Minimal adverse effects
Dioic acid	Affects tyrosinase transcriptional and melanosome transfer	Tirado-Sanchez et al, 2009	1% dioic acid versus 2% HQ x 12 weeks	96 female patients	Significant reduction in pigmentation, parity with HQ	Lesser side effects than HO
B-Carotene	Saturates melanocyte receptors and reduce melanin production	Kar, 2002	B-carotene lotion on melasma	31 adults	Moderate benefit in melasma	Minimal advesse effects
Linoleic acid(LA)	Accelerate tyrosinase degradation	Lee et al, 2002	Group A- Placebo Goup B : 2% Lincomycin (LM) + 0.05% Betamethasone Valerate (BV) Group C: 2% LM , 0.05% (BV) and 2% LA once at night	20 in each group	2% mixed with 0.05% BV and 2% LA caused significant improvement in melasma as compared vehicle LM with BV	Minimal adverse effects
Silymarin	Inhibits melanogenesis	Elfar et al, 2015	IXA injection versus silymarin versus 50% peels Group A (intradermal TXA injection),	60 female patients	Topical silymarin – moderate improvement, comparable with peel, superior to intradernal	Minimal adverse effects

			Group B (topical alymarin cream) and Group C (GA peeling 50%)		TXA	
5% methemazole	Peroxidase inhibitor	Malek et al, 2013	5% sethimazole on melasma x 8 weeks	3 HQ-resistant melasma patients	Significant improvement of melasma	Minimal adverse effects
Pidobenzone		Zanieri- et al, 2008	pédobenzone gel 4% twice daily x 16 weeks.	20 patients	Significant benefit in melasma	Minimal advesse effects
Rucinol	Inhibition of tyrosinase and TRP-1	Khemis et al,	0.3% rucinol versus placebo x 12 weeks	32 patients	Lower pigmentation score the rucinol treated side benefits were maintained for amother weeks	Mild stinging, burning, erythema, peeling, dayness, deesquamation
		Huh et al., 2010	0.1% liposome-encapsulated rucinol versus vehicle x 8 weeks	23 patients	Significantly lower pigmentation scotes with the liposome encapsulated rucinol	No adverse effects reported
Licorice extract	Tyrosinase inhibition and anti-inflammatory	Costa et at	Emblica, licorice, and belides 7% versus 2% HQ Divided into 2 groups of 28 patients: Group A - cream with Emblica licorice, and belides 7% used twice Group B – HQ 2% at night x 8 weeks	56 patients	Moderate improvement in melasma.	Burning sensation and increase of number of previous acne lesions in patients; but less than HQ
Soymilk	Restoratio of skin barrier. inhibit melanosome phagocytosis by keratinocytes via inhibition of PAR-2	Mao-Qiang et al	double-blind, study of a soy-containing moisturizer with a broad-spectrum sunscreen	in 68 patients,	significant improvements in fine wrinkles and pigmentary changes were demonstrated after 3 months of usage	Minimal advesse effects
Mulberry extract	Tyrosinase inhibitor, Free radical scavenger	Sarkar et al, 2013	Plant etract compared to topical 3% vitamin C derivative in Japanese female patients with melasma.	50 patients	markedly improved the Melasma Area and Severity Index (MASI) score in an RCT in	Minimal adverse effects
Aloesin	competitively inhibits the conversion of tyrosine to DOPA and DOPA to dopachrome.	Choi et al,	randomized comparative subjects were UV irradiated (210 mJ) on volar forearm and were divided into 4 groups: vehicle control, aloesin-treated, arbutin-treated and aloesin and arbutin-treated. Aloesin and/or arbutin were administered 4 times a day for 15 days.	--	Aloesin suppressed pigmentation by 34%, arbutin by 43.5%, and the co-treatment by 63.3%, compared with the control ($N = 15$; $P < 0.05$)	---
Flavonoids	anti-inflammatory and antioxidant properties and are competitive inhibitors of tyrosinase	Altaei et al, 2012	3 groups treatment was applied twice daily x 4 weeks.	96 patients	excellent improvement and lesion size reduction	No adverse effects
Ellagic acid (topical)	Inhibit tyrosinase and melanocyte proliferation.	Ertam et al,	Synthetic ellagic acid + arbutin vs plant derived ellagic acid	10 patients	Both groups showed improvement, similar efficacy.	---
Liquirtin (topical)	dispersion of melanin.	Amer et al, 2000	liquiritin cream on one side of face and with vehicle cream on other side twice daily x 4 weeks.	20 females	75% patients has excellent response.	Minimal – mild irritation

Level of evidence

Treatment of melasma has variable level of evidence and strength of recommendations, which has been shown in the following table 7 [40].

Table 5: Level of evidence

Level of Evidence	
I	One large randomized controlled trial (RCT)
II	Small RCTs
III	Prospective cohort studies
IV	Retrospective cohort studies or case-control studies
V	Case reports, experts' opinion

Table 6 : Strength of recommendation

Strength of recommendation	
A	Strong evidence, strongly recommended
B	Strong or moderate evidence, generally recommended
C	Insufficient evidence
D	Moderate evidence against efficacy, generally not recommended
E	Strong evidence against efficacy, never recommended

Table 7: Melasma drugs level and strength of recommendation.

Drug	Level of evidence	Strength of recommendation
2% HQ	II	C
4% HQ	I	B
2% HA, 0.05%RA, 0.1% dexamethasone	III	B
4% HQ, 0.05% RA, 0.1% FA	I	A
Hydro-oryanisol	II-III	C
Lignin peroxidase	I	B
Magnolignan	II-II	C
NAG	I	B
Orchid extracts	II-II	C
Dioic acid	II-II	C
Octadienedicic acid	II-II	C
B-Carotene	II-II	C
Linoleic acid	I	B
Silymarin	II-II	C
5% methemazole	III	C
Pidobenzone	II-III	C
Rucinol	I	B
Licorice extract	I	B

Oral drug - Tranexamic acid (TXA)

Tranexamic acid is an anti-plasmin drug, reduces arachidonic acid production, resulting in a decrease in melanocyte-stimulating hormone (MSH) and thereby reduces pigment production. It is used in 500–1500 mg/day in a divided dose. While no direct comparisons of tranexamic acid formulations have been conducted, current studies reveal that over 90% of those treated orally improved after 2–6 months, compared to approximately 95% of patients treated with topical 2% preparations who improved after 3 months. However, screening for thrombosis risk factors is required before beginning treatment because it can lead to deep vein thrombosis.. Patients on oral contraceptive pills and those having deranged coagulation profile should not be given oral tranexamic acid. Other minor side effects include – abdominal bloating, nausea and menstrual irregularity.

In a 3-month research by Rosario et al. comparing the efficacy of TXA 250 mg twice daily to placebo on 39 patients showed that the MASI score was reduced by 49 percent in TXA group after three months, compared to 18 percent in the placebo group.

Table 8: Table showing studies using tranexamic acid

Study	Type	No. of patients	Treatment given	Results	Adverse effects
Karn et al, 2012	A randomised controlled trial.	260	TXA 250 mg + HQ cream Placebo + HQ cream twice daily, for 12 weeks	Significant decrease of the MASI scores in group A at weeks 8 and 12 and in group B at week 8.	Group A: oligomenorrhea, belching, abdominal cramps, palpitation, urticarial rash with angioedema. Group B: exogenous ochronosis.
Padhi et al, 2015	An open labeled randomized comparative trial.	40	oral TXA 250 mg + TC Cream Placebo + TC cream TXA 250 mg twice daily, TC cream daily, for 12 weeks	Significant decrease in mean MASI score in group A compared to B.	Erythema and burning sensation in both groups, hypopigmentation and oligomenorrhoea in group A.
Khurana et al, 2019.	A prospective, randomized, open-label study	64	Group A - microneedling + TXA 0.4% Group B - oral TXA 250 mg A: monthly, B: twice daily, for 3 months	Significant decrease in MASI score in Group B > A	Group A - mild pain and erythema. Group B: gastritis, oligomenorrhea.

Procedural therapies[46]

1) Lasers

A variety of lasers and light devices have been tried in melasma.

a) Pigmentary lasers - Q-switched ruby laser (QSRL) and Q-switched neodymium:yttrium–aluminium-garnet (QNd:YAG) lasers , Intense pulsed light (IPL)
b) Vascular lasers - pulsed dye, Copper bromide
c) Ablative lasers - carbon dioxide (CO_2) and erbium:YAG (Er:YAG)

- Neodynium:Yttrium Laser (Nd: YAG):

  The chromophore melanin is selectively targeted by non-ablative Q-switched lasers, such as the Q-switched ruby laser (QSRL) and Q-switched neodymium:yttrium–aluminium–garnet (QNd:YAG) lasers. Long-term benefits have not been provided by QSRL nor QNd:YAG. Low fluence QNd:YAG laser is the most commonly used laser in melasma trials. Study by Moubasher *et al*. showed that NDYAG laser is inferior as compared to TCA 25% peel.

- Alexandrite laser:

  Alexandrite lasers, being more specific causes less post inflammatory hyperpigmentation than Q switched Nd:YAG lasers. Fabi et al. found that the LFQS Nd:YAG laser performed better than the Low Fluence Q Switched alexandrite laser, but the results were statistically insignificant.

- Copper bromide:

  In Thailand, a nonrandomized trial on copper bromide laser found no significant improvement among 24 patients. Copper bromide laser was not found better than Triple Combination in a split face Randomised control trial of 20 patients.

- Combinations

Lasers have been combined with Triple combinations, tranexamic acid, peels and also with two different lasers have been combined. Efficacy of combination therapy is better than monotherapy.

2) Chemical peels

Chemical peels commonly used in melasma include glycolic acid, salicylic acid, Jessner's peel, and trichloroacetic acid. The peeling agent has superficial effects, allowing for the elimination of epidermal melanin as well as melanin from keratinocytes, as well as preventing melanosome transfer to keratinocytes. Peels are done every 2 to 4 weekly intervals for 5-6 sessions.

a) Glycolic Acid:

It is used in concentration ranging from 20 to 70%. In Trial of Dayal et al, glycolic Acid peel with Azelaic acid was compared with Azelaic acid alone. It showed glycolic acid with azelaic acid showed better results than azelaic acid alone. One of these research evaluated the efficacy of a GA (20–70%) peel to a triple Combination (Hydroquinone 2 % , RA 0.05 % , Flucinolone 0.01 %). GA peel (cwith azelaic acid cream) and triple combination showed similar efficacy in terms of MASI improvement.

b) Trichloroacetic acid (TCA) peel

Trichloroacetic acid is used in concentration of 10-20%.TCA peel was compared to GA peel by Puri et al. and concluded that both had similar efficacy.

c) Jessners solution

Ejaz *et al.* compared Jessner's solution and 30% salicylic acid and found that both the peels have similar efficacy in reducing melasma.

3) Microneedling

Most of the study shows that microneedling is not effective in melasma[50].

Table 9: Procedural therapies in melasma

Study		No. of patients	Treatment given	Results	SIDE EFFECTS
Lasers in Melasma					
Moubasher *et al*., 2014	A randomized, controlled clinical trial.	65 patients	Group A: TCA 20% Group B: TCA 25% Group C: TCA 30% every 2 weeks up to 8 sessions Group D: QS-Nd: YAG 532 nm every month up to 6 sessions	TCA group B showed significantly greater reduction in MASI	Q-switched Nd: YAG 532 nm showed higher incidence of postinflammatory hyperpigmentation
Fabi *et al*., 2014	A randomized, split-face clinical trial	20 patients	Group A : LFQS Nd: YAG laser Group B: LFQS alexandrite laser (755 nm)	Improvement in mMASI- Group A - 27% Group B – 19% statistically insignificant	No significant adverse effects
Yun et al 2015	Split-face, randomized study	26, Asian	Group 1- Fractionated IPL weekly x 6 sessions, Conventional IPL biweekly, 3 sessions	In group A, the modified MASI score decreased continuously, while in group B the MASI score initially decreased then increased during the treatment course.	Marked darkening of melasma in one patient in group B after the third treatment.

Shakeeb et al 2018	A randomised controlled trial.	96	Group A : Triple combination cream Group B : IPL Group C : IPL + triple combination cream cream nightly, IPL biweekly, for 2 months	Significantly better MASI score reduction in group C than A or B.	N/A
Peels in Melasma					
Glycolic Acid Peel					
Mahajan et al, 2011	A Randomized Controlled Study	40	Group A: GA peel (sequential) and AA 20% Group B : TC (HQ2%, tret 0.05%, fluocino 0.01%)	No significant Difference between the two groups	
Dayal et. Al,2017	An open-label, prospective, randomized interindividual clinical trial.	60	Group A :GA peel + 20% AA cream every 3 weeks, 8 peeling sessions Group B :20% AA cream twice daily, for 24 weeks	Significant decrease in MASI and MelasQoL scores after 12 weeks. Group A > B	Erythema, burning sensation in both groups.

Garg et al 2019	A Randomized Controlled trial.	30, Indians	35% GA full-face peel 35% GA full-face peel followed by 10% TCA spot peel 35% GA full-face peel followed by 20% TCA spot peel every 2 weeks, 4 sessions	Significant reduction of MASI scores in all groups.	Erythema, pruritus, burning sensation, transient hyperpigmentation.
Trichloroacetic Acid Peel					
Puri et al., 2012	A non randomised clinical trial	30 patients	Gr A: 15% TCA Gr B: 35% GA peel (every 3 weeks) Duration: 15 weeks	Both TCA and GA were equally effective	
Abdel-Meguid et al, 2017	A single-blinded split face randomised controlled trial.	24	Group A: TCA 20%– 25% + Jessner's solution Group B : TCA 20%– 25% Biweekly, 6 sessions	Significant decrease in MASI score in both groups, with significantly better results in group A.	Erythema, burning sensation, discomfort, pruritus, hyperpigmentation, crustation in both groups.
Sahu P et	An open	60	20% TCA peel	MASI,	Post peeling

al, 2017	labelled prospective randomized study	Indians	+ 5% ascorbic acid cream 20% TCA peel Peels -every two weeks, cream nightly, for 12 weeks	MelasQoL, Percentage decrease in MASI, in group A > group B.	erythema, burning and stinging sensation, inflammatory hyperpigmentation in both groups. Pruritus in group B.
Ejaz *et al.,2008*	A double blind, randomized, interventional comparative study.	60	Jessner's solution every 2 weeks versus 30% salicylic acid peel every 2 weeks	Both groups show efficacy, difference statistically insignificant.	
Microneedling					
Balevi et al, 2017	A comparative study	41	GROUP A : 30 % SA peel GROUP B : SA 30% with microneedling with vitamic C. Every 2 weeks for 2 months	No significant difference between two groups	Burning sensation in Group B during injections.
Kaleem et al, 2020	A split-face non-randomized clinical trial	50 patients	Group A : microneedling with TXA 0.4% on the left side of the face Group B : microneedling with saline 0.9% on the right side of the face Every 2 weeks for 12 weeks	Hemi-mMASI score showed significant reduction in group A compared to group B.	Erythema, swelling, and burning on both cheeks.

Key : TCA - Trichloroacetic acid, LF QS Nd: YAG – Low fluence Q switched Neodymium:yttrium–aluminium-garnet laser, IPL- Intense Pulse Light laser, GA – glycolic acid, AA – Azelaic acid, TXA – Tranexamic acid, MASI – Melasma Area Severity Index, mMASI – modified Melasma Area Severity Index, MELASQOL – Melasma quality of life index.

Platelet-Rich Plasma (PRP)

PRP is a plasma containing higher-than-normal platelet concentrations. It is prepared from small amount of patients own blood. PRP is used in oral and maxillofacial surgery, orthopedics and sports medicine, plastic surgery and cosmetic surgery for over 30 years. In dermatology it is used in androgenic alopecia, for faster wound healing, atrophic scar and striae , for skin rejuvenation and lately for hyperpigmented lesions[51–54].

Platelets are formed in marrow from megakaryocytes. They contain α-granules, dense granules, lysosomes and mitochondria [54].

Contents of α-granules are Insulin-like Growth Factor-1(IGF-1), Platelet derived growth factors (PDGF), Transforming Growth Factor- Beta (TGFβ), platelet factor 4 and clotting proteins (such as thrombospondin, fibronectin, factor V, and von Willebrand factor). Dense granules contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), ionized calcium, histamine and serotonin.

Platelet-Rich Plasma when comes in contact with collagen, granules fuse to cell membrane to release growth factors such as PDGF, EGF, TGF-beta. These secretory proteins get active and upon activation they bind to transmembrane receptors affecting many cell types. This leads to gene expression controlling - cellular proliferation, collagen synthesis and tissue regeneration[55].

These growth factors are secreted by platelets within 10 min after activation and hence PRP should be used within 1 hr[4].

It is prepared by centrifugation of whole blood. More than 1 million/ μL platelet concentration is believed to be enough therapeutic effect [55].

Table 10: Classification of PRP [55]

	Pure Platelet- Rich Plasma (P-PRP)	Leucocyte PRP (L-PRP)	Pure Platelet-Rich Fibrin (P-PRF)	Leucocyte Platelet-Rich Fibrin (L-PRF)
With WBC	-	√	-	√
High density fibrin network	-	-	√	√

a) Pure Platelet-Rich Plasma (P-PRP) or leucocyte-poor PRP products: They are PRP without WBC and fibrin network. This type of PRP helps wound repair.

b) Leucocyte- and PRP (L-PRP) products: They are PRP with WBC and without fibrin network.

c) Pure platelet-rich fibrin (P-PRF): It is also known as leucocyte-poor platelet-rich fibrin PRP. It is PRP without WBC and with a high-density fibrin network. It is formed only in gel form.

d) Leucocyte- and platelet-rich fibrin(L-PRF) or second-generation PRP products: They are PRP with WBC and fibrin network.

In this study, we have used P-PRP, free of WBC and fibrin, as WBC can promote inflammation in melasma, and fibrin has no role in melasma treatment.

Table 11: Growth factors found in alpha-granule [56–58]

Growth Factor	Biological Activity
EGF (Epidermal Growth Factor)	Enhance wound healing Accelerate epidermal regeneration Cell differentiation and mitosis
PDGF (Platelet derived Growth Factor)	Cellular migration enhance motility of fibroblasts, endothelial cells and neurons
IGF-1 (Insulin-like Growth Factor1)	Angiogenesis Cell differentiation
TGFβ-1 (Transforming Growth Factor- Beta1)	Mitogen for fibroblasts, smooth muscle cells, and osteoblasts Promote angiogenesis and extracellular matrix production
VEGF (Vascular Endothelial Growth Factor) A, B and C	Induce angiogenesis Alter vascular physiology and permeability
FGF1 (Fibroblast Growth Factor)	Induce fibroblast proliferation and angiogenesis

2) PRP in Clinical Practice

a. PRP in androgenic alopecia

Parul Singhal et al. injected PRP 2 weekly for 4 sittings and observed that there was clinical improvement in hair counts, thickness, and hair root strength[59].

Betsi E. E. et al. showed that in early stage of alopecia PRP injections had better results and improvement lasted longer[60]. Growth factors from PRP lead to hair regrowth by inducing proliferation of dermal papilla cells.

b. PRP in peri-orbital hypermelanosis

Salah Hashin Al-shami injected PRP intradermally monthly in whole face for 3 months[61]. There was 38% improvement which is moderate in 6 months. Another study by Mehryan et al. showed improvement in hyperpigmentation. In Single sitting, 1.5ml PRP was injected in 10 patients and significant improvement was found ($P = 0.010$)[62].

c. PRP in melasma

In a randomized, placebo-controlled trial of PRP by Sirithanabadeekul et al, for treatment of melasma ($n = 10$) , found that 2 weekly PRP injections significantly improved melasma in 6 weeks of treatment, which was shown by decrease in mMASI scores, and increased patient satisfaction, however assessment with Mexameter (erythema and melanin index) did not show significant difference [3]. Another study by Tuknayat et al also showed improvement in melasma on 54 Indian patients. Similarly few case reports are also present on PRP, stating that PRP has efficacy in melasma.

Case studies have demonstrated its efficacy in reducing hyperpigmention in melasma, which was not compared with standard treatment in a controlled trial. Hence, we aimed to investigate the effectiveness of PRP injection for melasma treatment in a randomized, split-face, single-blinded prospective trial.

Table 12: PRP STUDIES IN MELASMA[3,63,64]

Study	Study type	No. of patients	PRP Sittings	Single/ double spin PRP	1st spin rate and time	2nd spin rate and time	Response
Garg et al., 2014	Case report	1	6 sittings at irregular interval in 32 weeks.	Double	1200 RPM X 12 min	4800 RPM x 18 mins	MASI improved from 64 to 34.
Çayirli M et al., 2014	Case report	1	3, every 15 days	Single	3500 rpm x 8 mins	N/A	>80% Improvement in melasma
Sirithanabadeekul et al, 2019	Pilot randomised control trial	10	2 weekly x 3 sittings	Single	3200 RPM X 4 minutes	N/A	28.9% change in mMASI.
Hofny et al, 2019	Split face trial Intradermal injections on the left side and microneedling before and after PRP application on the right side	23	3 sitting 4 weekly interval	Double	1600 RPM x 10 mins	4000 RPM x 10 min	>50% reduction in mMASI IN 48% CASES
Gamea et al., 2020	RCT 2 groups Group A-Topical TA 5 % BD 12 weeks Group B-Additional intradermal PRP injections at	40 total, Group A = 20 Group B = 20	4 sittings 3 weeklyX 12 weeks	Double	2000 RPM x 3 mins	5000 RPM x 5 min	Group A- 58.9 % reduction in mMASI 2. Group B - 70.2 % reduction in mMASI improvement GROUP B > A, statistically significant
Tukyanat el al, 2021	Open label prospective trial	40	3 sitting 4 weekly interval	Double	1600 RPM X 10 min	4000 RPM X 10 min	Average 54.5% reduction in mMASI

MATERIALS & METHODS

1) STUDY SETTING:

This study was conducted on consecutive patients of melasma attending the Dermatology, Venereology and Leprology Out Patient Department at AIIMS Jodhpur.

2) STUDY DESIGN:

Split face single blinded placebo controlled randomized study.

Group A : Topical Hydroquinone 2% + intradermal Platelet rich plasma (PRP)

Group B : Topical Hydroquinone 2% + intradermal Normal saline (NS)

3) STUDY PARTICIPANTS:

A) Inclusion criteria:

Patients fulfilling all the following criteria were included in study, after informed written consent.

1. Patients with facial melasma with bilateral involvement.
2. Age $\geq$ 18 yrs

B) Exclusion criteria:

Patient having any of the following criteria were excluded from study.

1. Pregnancy and Lactation
2. Bleeding tendencies
3. On treatment with any topical or oral or procedural treatment of melasma at the time of study or in last two weeks
4. Obvious infection at skin site
5. Hormone contraception or hormone replacement therapy

4) SAMPLING:

SAMPLE SIZE : 64 patients

Sirithanabadeekul P et al have reported Melanin index values at 10 weeks as 238.63 ± 16.4 in PRP group and of 249.47 ± 21.36 in the control group. Considering this for sample size calculation, we estimated that 64 patients will be

needed for the split face study at 95% confidence interval, 80% power and 30% contingency.

Sample size calculation was done using this formula

$$n_1 = \frac{(\sigma_1^2 + \sigma_2^2 / \kappa)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

$$n_2 = \frac{(\kappa * \sigma_1^2 + \sigma_2^2)(z_{1-\alpha/2} + z_{1-\beta})^2}{\Delta^2}$$

The notation for the formulae are:

n_1 = sample size of Group 1

n_2 = sample size of Group 2

σ_1 = standard deviation of Group 1

σ_2 = standard deviation of Group 2

Δ = difference in group means

κ = ratio = n_2/n_1

$Z_{1-\alpha/2}$ = two-sided Z value (eg. Z=1.96 for 95% confidence interval).

$Z_{1-\beta}$ = power

Reference:
Bernard Rosner. Fundamentals of Biostatistics (5th edition). (based on equation 8.27)

5) STUDY DURATION:

Jan 2020 to June 2021 (18 months)

6) ETHICAL APPROVAL:

Thesis proposal was approved by the Institutional Ethics Committee of All India Institute of Medical Sciences, Jodhpur vide certificate reference no. AIIMS/IEC/2020/2055 dated 1st January 2020 and CTRI registration no. CTRI/2020/07/026311 dated 2nd July 2020.

7) PRIMARY EFFICACY PARAMETERS

1) Modified Melasma Area Severity Index (Annexure 2) – at each visit
2) Melasma Severity Index was calculated (Annexure 3) - at each visit
3) Investigators global assessment (0 to 100%) - at each visit
4) Patients global assessment score (0 to 100%) at baseline and end of study

8) SECONDARY EFFICACY PARAMETERS

1) Dermoscopy: At baseline and end of study (Grade 1 – Poor response, Grade 2 – fair response, Grade 3 – good response, Grade 4 – excellent response)
2) MELASQOL (Annexure 4) at baseline and end of study
3) Side effects – as reported by patients at any time during the study

9) STUDY PROCEDURE:

All the patients were aware of the intervention, but were unaware of the side of face where the intervention was given. Also the co-assessor were unaware of the interventional and the placebo sides.

Patients applied hydroquinone 2 % on both cheeks daily at night from baseline to week 8, then 5 days per week from week 9 to week 12 thereafter 3 days per week till total 16 weeks. However hydroquinone application was not tapered in patients who did not achieve at least 50% response from baseline. Clinical photography was done using 7T model of Oneplus (OnePlus technology, Shenzhen, China). The lighting condition and position of patients was kept uniform as far as possible.

10) PLATELET RICH PLASMA PREPARATION

Whole blood was withdrawn in a 20 ml vial from the patients into Vacuette® trisodium citrate tube.

Draw whole blood (20 ml) by venipuncture

↓

Collect in 20 ml conical tubes containing anticoagulant (Trisodium citrate)

↓

Centrifuge at 160 g for 10 minutes

↓

Aspirate plasma + buffy coat

↓

Centrifuge at 400 g for 10 minutes

↓

Platelet-rich plasma (PRP)

11) **PATIENT PREPARATION AND INTRADERMAL INJECTION**:

Pre-procedure topical analgesic cream (2.5% lidocaine and 2.5% prilocaine, EMLA) was applied on the lesions and kept for one hour before treatment and subsequently removed before administering the injection.

Platelet rich plasma was activated using calcium gluconate and 0.1 ml was injected intradermally into melasma-affected areas on one side of the face in a random manner, and 0.1 ml of normal saline was injected intradermally on the other (placebo group) using insulin syringe keeping inter-pucture gap of 0.5 to 1 cm of face. Volume was decided according to the area involved.

Post procedure patients were asked to apply broad- spectrum sunscreens.

Patient were advised to avoid sun exposure, and not to use any other topical preparations on the lesions during the study period.

12) STATISTICAL ANALYSIS

The presentation of the Categorical variables was done in the form of number and percentage (%). On the other hand, the quantitative data were presented as the means ± SD and as median with 25th and 75th percentiles (interquartile range).
The following statistical tests were applied for the results:

1. The comparison of the variables which were quantitative were analysed using Paired t test across follow up and independent t test between two groups.
2. The comparison of the variables which were qualitative in nature were analysed using Chi-Square test. If any cell had an expected value of less than 5 then Fisher's exact test was used.

The data entry was done in the Microsoft EXCEL spreadsheet and the final analysis was done with the use of Statistical Package for Social Sciences (SPSS) software, IBM manufacturer, Chicago, USA, ver 21.0.

For statistical significance, p value of less than 0.05 was considered statistically significant.

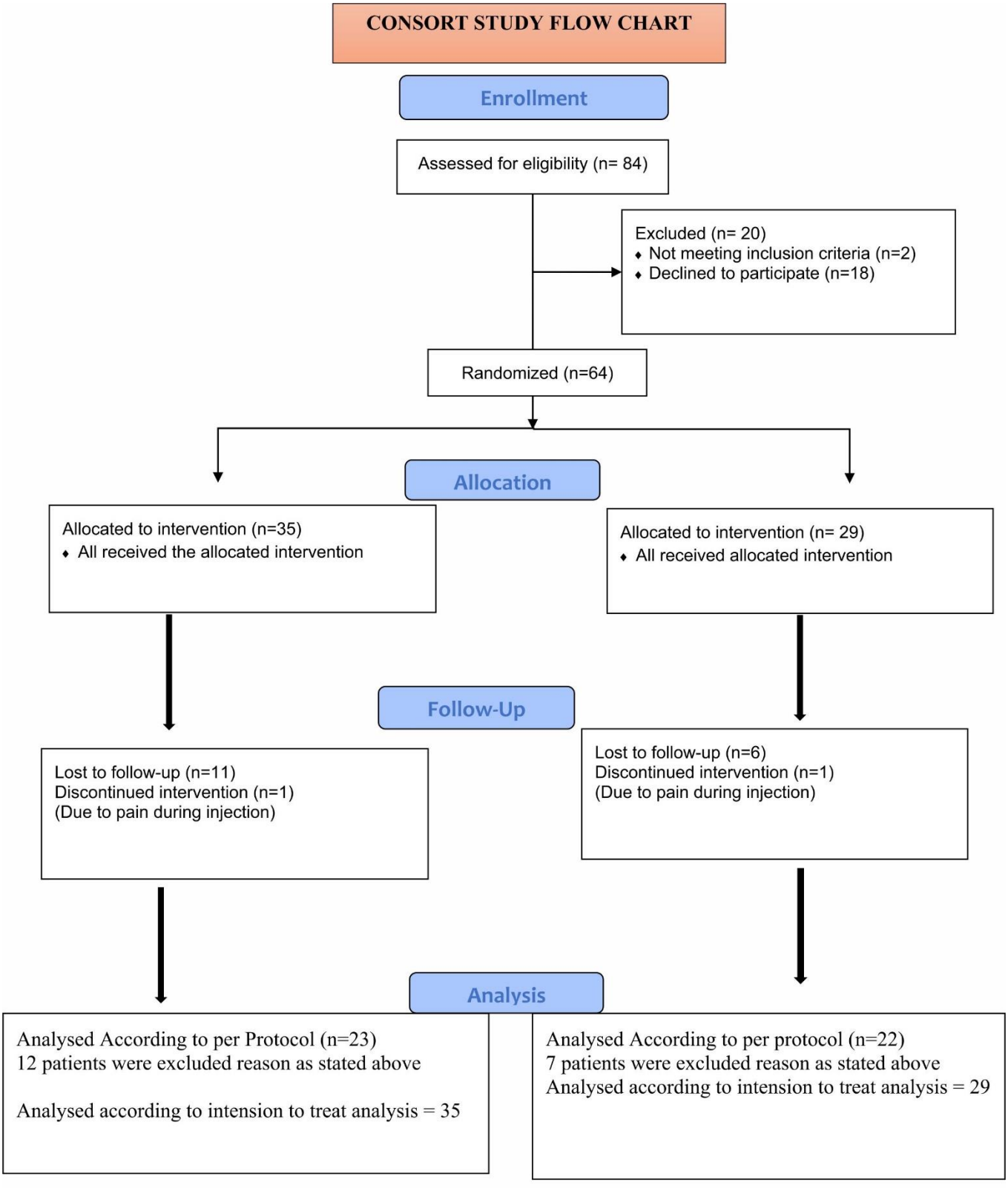


FIG 1: STUDY FLOW CHART

RESULTS

Total 64 patients were recruited of which 45 patients completed follow up and results of these patients are presented according to per protocol (PP) analysis, unless specified otherwise.

1) Demographic Data

Demographic data in our study is shown in table 13.

In our study 29 (64.44%) patients were females and 16 (35.56%) patients were males (figure 2).

Overall age group of study subjects is shown in figure 3. Mean age (in years) of study subjects was 29.49 ± 8.7 with median (25th-75th percentile) of 27 (22-38)

Education wise, the biggest group was of graduates at 25 (55.56%) patients as shown in table 13.

Table 13:-Distribution of demographic characteristics of study subjects.

Demographic characteristics	**Frequency**	**Percentage (%)**
Gender		
Female	29	64.44%
Male	16	35.56%
Age(years)		
Mean age (in years)	29.49 ± 8.7	
Education		
Primary school	1	2.22%
Middle school	3	6.67%
High school	3	6.67%
Intermediate	6	13.33%
Graduate	25	55.56%
Post graduates	7	15.56%
Total	**45**	**100%**

Figure 2:-Distribution of gender of study subjects.

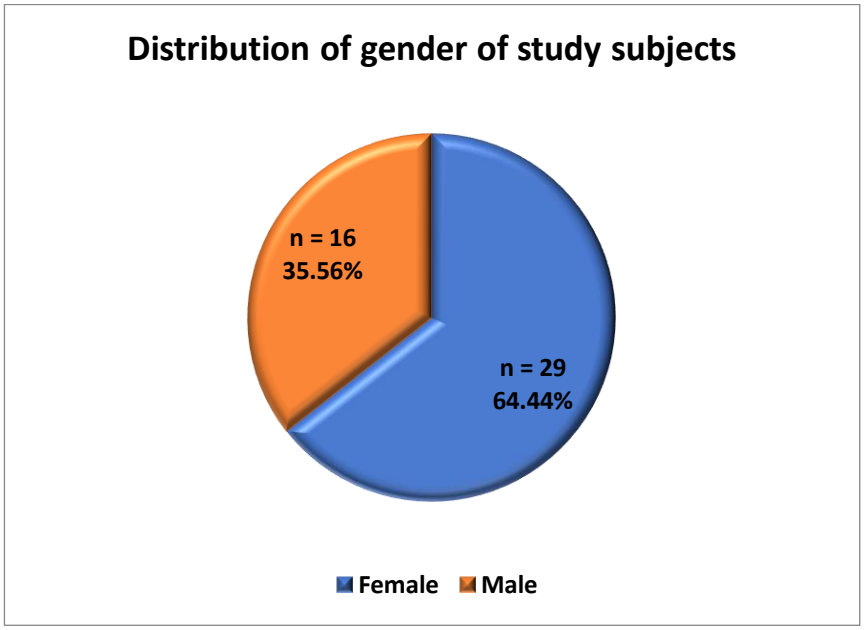
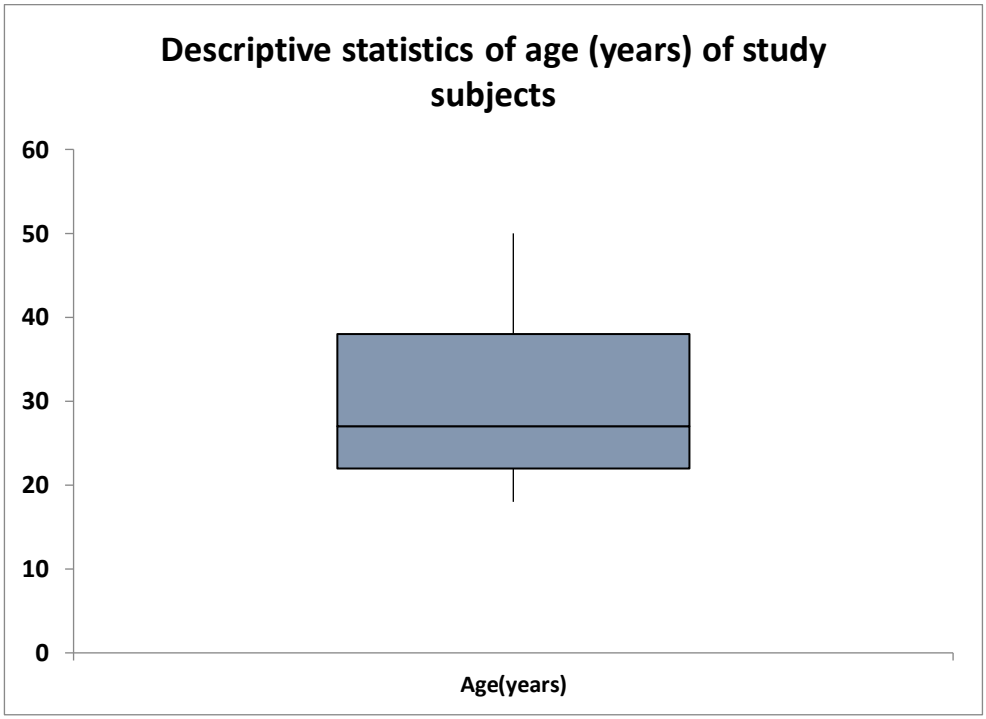


Figure 3:-Box and Whisker Plot of age (years) of study subjects.



2) Basic clinical parameters

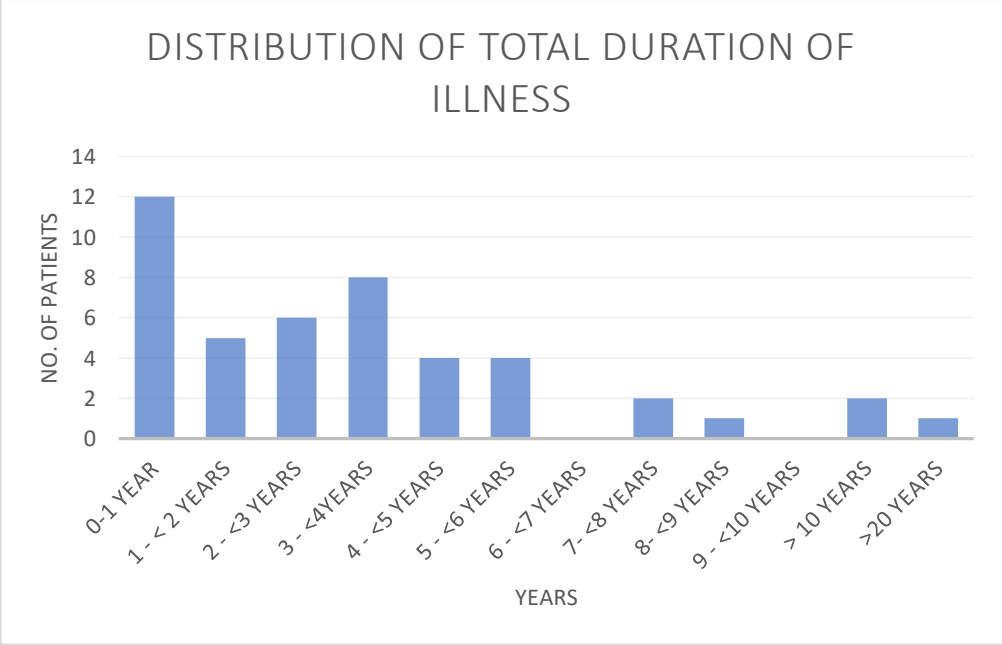
A) Total duration of illness

In 12 patients (26.6%), total duration of illness was less than 1 year, followed by 3-4 years of duration in 8 (17.78%) patients (Table 14 and figure 4).

Table 14 :-Distribution of total duration of illness (years) of study subjects.

Total duration in years	**No. Of patients**	**Percentage (%)**
0 to <1	12	26
1 to <2	5	11
2 to <3	6	13
3 to <4	8	17
4 to <5	4	8
5 to <6	4	8
6 to <7	0	0
7 to <8	2	4
8 to <9	1	2
9 to <10	0	0
>10	2	4
>20	1	2
Total	45	100

Figure 4:- Bar Graph showing total duration of illness (years) of study subjects.



B) *Site of Platelet Rich Plasma injection (PRP)*: In 23 (51.11%) of patients, PRP injection was given on right side of face and in remaining 22 patients (48.89%) PRP was given on left side of face.

C) *Distribution of co-morbidity among study subjects* **:** Majority that is 39 (86.67%) patients did not have any significant co-morbidity in the past (Table 15).

Table 15 : Distribution of co-morbidities among the study subjects

Co-morbidities	Frequency	Percentage
No Relavant co-morbidity	39	86.67%
Hypertension	1	2.22%
Tuberculosis	2	4.44%
Thyroid	1	2.22%
other co-morbidity	3	6.67%

D) Distribution of menstrual and Obstetric history in study subjects:

Out of the 29 female patients, 17 (58.62%) of patients had positive obstetric history, and 4 (13.79%) had irregular menstrual history.

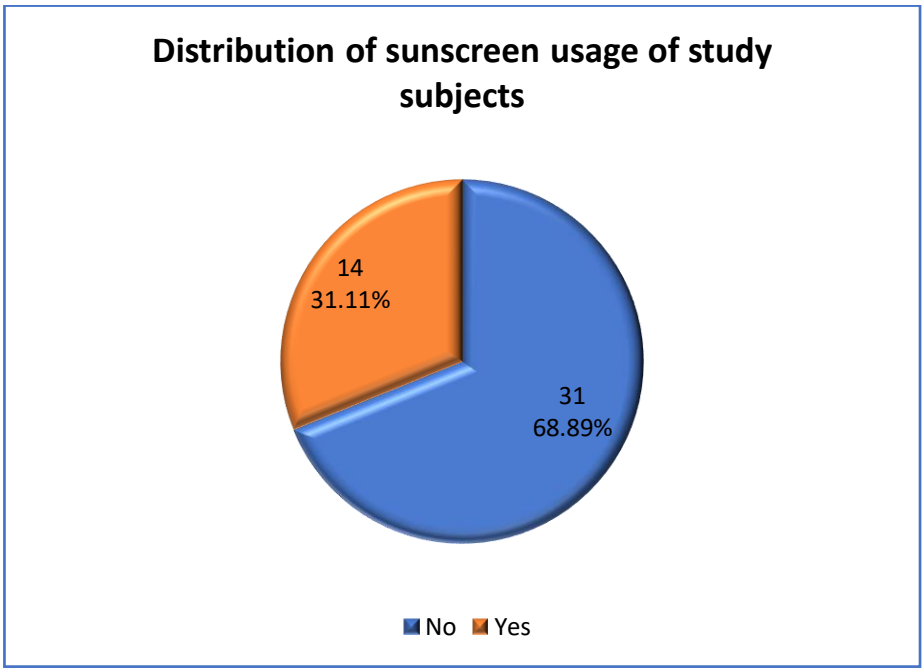
E) Use of sunscreen among study subjects

Majority of the patients 31 (68.89%) did not use sunscreen. Only 14 out of 45 patients (31.11%) used sunscreen (Table 16 and figure 5).

Table 16:-Distribution of sunscreen use among the study subjects.

Sunscreen usage	Frequency	Percentage
No	31	68.89%
Yes	14	31.11%
Total	45	100.00%

Figure 5:-Distribution of sunscreen usage among study subjects.



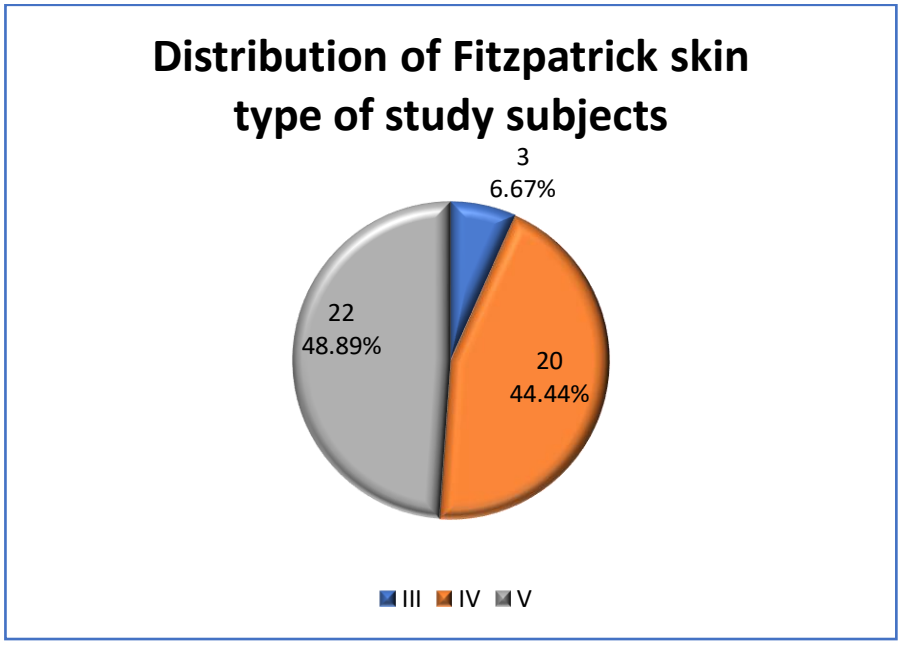
F) *Fitzpatrick skin type* **:**

In majority 22 (48.89%) patients, Fitzpatrick skin type was V followed by IV in 20 (44.44%) patients. This is shown in table 17 and fig 6.

Table 17:-Distribution of Fitzpatrick skin type of study subjects.

Fitzpatrick skin type	Frequency	Percentage
III	3	6.67%
IV	20	44.44%
V	22	48.89%
Total	45	100.00%

Figure 6 :-Distribution of Fitzpatrick skin type of study subjects.



G) Distribution of colour of melasma

In majority, 26 (57.78%) patients, colour of melasma was dark brown. Colour was light brown in the rest 19 (42.22%).

H) Distribution of pattern of melasma

In 28 (62.22%) patients, pattern of involvement was malar and in the rest 17 (37.78%) the pattern was centrofacial. (Table 18).

Table 18 : Distribution of pattern of melasma:

Pattern of involvement	Frequency	Percentage
Centrofacial	17	37.78%
Malar	28	62.22%

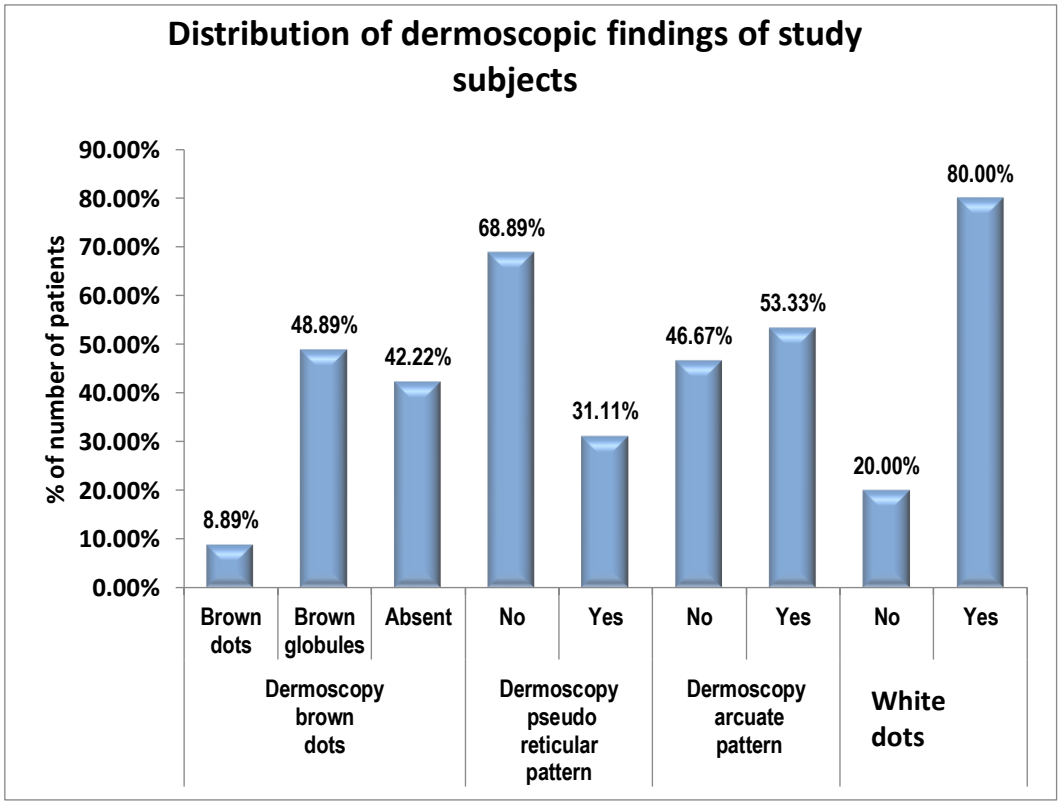
I) Distribution of Baseline dermoscopic finding

In a majority of patients (24, 53.3%) arcuate pattern was seen, followed by predominantly brown globules in 22 (48.9%) cases. The rest is shown in table 19 and figure 7.

Table 19 :-Distribution of baseline dermoscopic patterns among study subjects:

Dermoscopic findings	Frequency	Percentage
Brown dots		
Predominantly Brown dots	4	8.89%
Predominantly Brown globules	22	48.89%
Pseudoreticular pattern		
Yes	14	31.11%
Arcuate pattern		
Yes	24	53.33%
White dots		
Yes	36	80.00%

Figure 7:-Distribution of baseline dermoscopic findings among study subjects



3) Efficacy parameters

A) Modified Melasma Area Severity Index (mMASI) and Hemi-Modified Melasma Area Severity Index (Hemi-mMASI)

a) Comparison of modified melasma area severity index {Full face} between baseline and follow up of study subjects**.**

Mean value of mMASI of study at baseline and 16 weeks was 4.85 ± 3.24 and 3.17 ± 1.96 respectively (table 20 and figure 8). Significant reduction was seen at at visits 4, 8, 12 and 16 weeks. The ITT analysis is shown in table 21 wherein the significant difference was also noted at all visits.

Table 20: Comparison of modified melasma area severity index {Full face} between baseline and follow up of study subjects (PP analysis).

Visit	mMASI (Full face) Mean ± SD	P value
Baseline	4.85 ± 3.24	-
4 weeks	4.6 ± 3.37	0.047
8 weeks	4.09 ± 2.93	0.002
12 weeks	3.62 ± 2.22	0.003*
16 weeks	3.17 ± 1.96	0.001*

Paired t test

Figure 8:-Comparison of modified melasma area severity index {Full face} between baseline and follow up of study subjects.

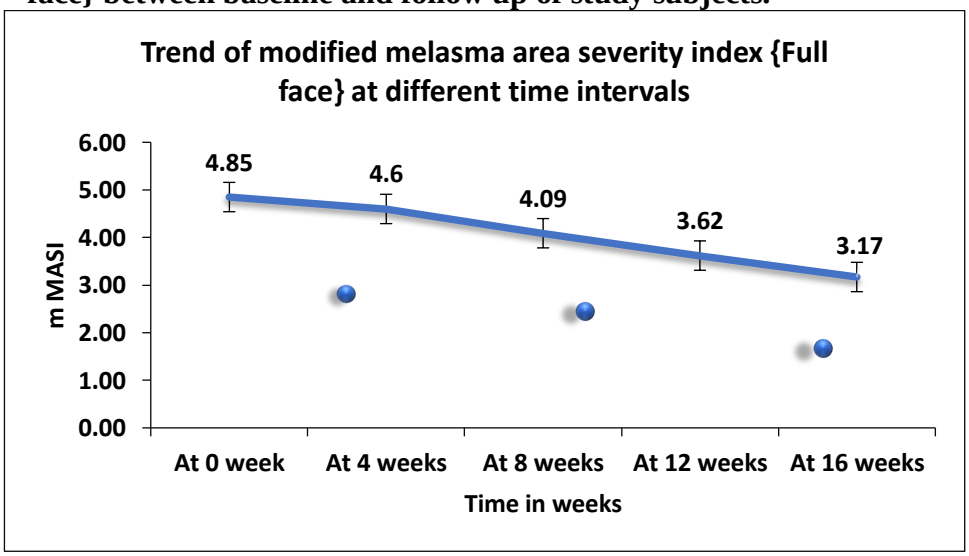


Table 21 : Comparison of modified melasma area severity index {Full face} between baseline and follow up of study subjects (ITT analysis)

Visit	mMASI (full face) Mean ± SD	P value
Baseline	4.59 ± 2.95	-
4 weeks	4.35 ± 3.07	0.010
8 weeks	4.17 ± 3	0.011
12 weeks	3.62 ± 2.18	0.0002
16 weeks	3.09 ± 1.97	0.0001*

Paired t test

b) Comparison of Hemi-mMASI in Group A(intragroup analysis)

Mean value of Hemi-mMASI {Group A} of study subjects at baseline and 16 weeks was 2.3 ± 1.47 and 1.56 ± 0.98 respectively (table 22). Significant reduction seen at 8,12 and 16 weeks. The ITT analysis is shown in table 23 wherein significant difference is notes at 8, 12 and 16 weeks..

Table 22:- Comparison of Hemi-mMASI in Group A (intragroup PP analysis)

Hemi-modified melasma area severity index {Group A}	Mean ± SD	P value
At 0 week	2.3 ± 1.47	-
At 4 weeks	2.21 ± 1.57	0.181
At 8 weeks	1.99 ± 1.35	0.004
At 12 weeks	1.79 ± 1.13	0.001
At 16 weeks	1.56 ± 0.98	0.0001*

Paired t test

Table 23:-Comparison of H-mMASI in Group A (intragroup ITT analysis)

Hemi-modified melasma area severity index {Group A}	Mean ± SD	P value
At 0 week	2.2 ± 1.37	-
At 4 weeks	2.1 ± 1.45	0.065
At 8 weeks	2.03 ± 1.4	0.018
At 12 weeks	1.8 ± 1.11	0.0004
At 16 weeks	1.52 ± 0.98	0.0001*

Paired t test

c) Comparison of **Hemi-mMASI** in Group B (intragroup analysis)

Mean value of hemi-modified melasma area severity index {Group B} at baseline and at 16 weeks of study subjects was 2.51 ± 1.79 and 1.59 ± 0.97 respectively (table 24). P values were significant at 4, 8, 12 and 16 weeks. The ITT analysis in table 25 shows significant difference at 8, 12 and 16 weeks.

Table 24:- Comparison of Hemi-mMASI in Group B (intragroup - PP analysis)

Hemi-modified melasma area severity index {Group B}	Mean ± SD	P value from mean
At 0 week	2.51 ± 1.79	-
At 4 weeks	2.37 ± 1.84	0.027
At 8 weeks	2.08 ± 1.61	0.001
At 12 weeks	1.81 ± 1.1	0.0002*
At 16 weeks	1.59 ± 0.97	0.0001*

Paired t test

Table 25:- Comparison of Hemi-mMASI in Group B (intragroup-ITT analysis)

Hemi-modified melasma area severity index {Group B}	Mean ± SD	P value
At 0 week	2.36 ± 1.61	-
At 4 weeks	2.24 ± 1.66	0.005
At 8 weeks	2.12 ± 1.63	0.009
At 12 weeks	1.8 ± 1.07	0.0001*
At 16 weeks	1.55 ± 0.97	0.0001*

Paired t test

d) Comparison of **Hemi-mMASI** between groups A and B (intergroup analysis)

Mean of hemi-modified melasma area severity index at baseline, and at 16 weeks in group A was 2.3 ± 1.47 and 1.56 ± 0.98 respectively and in group B was 2.51 ± 1.79 and 1.59 ± 0.97 respectively with no significant difference between them (table 26 and figure 9). The ITT analysis is shown in table 27 wherein no significant difference is noted.

Table 26 : Comparison of hemi-mMASI between group A and B (intergroup PP analysis).

Hemi-modified melasma area severity index	**Group A(n=45)** (Mean ± SD)	**Group B(n=45)** (Mean ± SD)	**P value**
At 0 week	2.3 ± 1.47	2.51 ± 1.79	0.553
At 4 weeks	2.21 ± 1.57	2.37 ± 1.84	0.65
At 8 weeks	1.99 ± 1.35	2.08 ± 1.61	0.77
At 12 weeks	1.79 ± 1.13	1.81 ± 1.1	0.93
At 16 weeks	1.56 ± 0.98	1.59 ± 0.97	0.88

Independent t test

Figure 9:- Trend of hemi-mMASI at different time intervals in groups A and B.

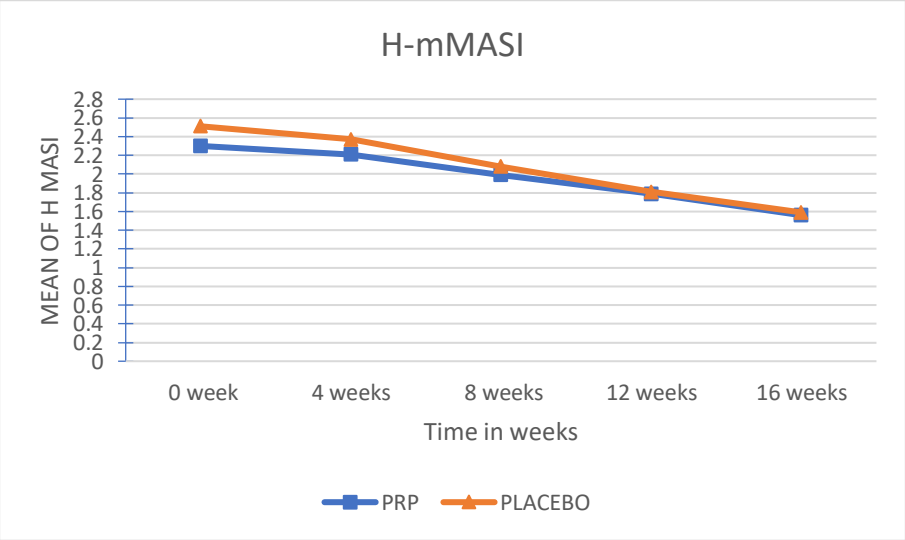


Table 27 : Comparison of hemi-mMASI between group A and B (intergroup ITT analysis)

Hemi-modified melasma area severity index	Group A(n=45) Mean ± SD	Group B(n=45) Mean ± SD	P value
At 0 week	2.2 ± 1.37	2.36 ± 1.61	0.54
At 4 weeks	2.1 ± 1.45	2.24 ± 1.66	0.62
At 8 weeks	2.03 ± 1.4	2.12 ± 1.63	0.78
At 12 weeks	1.8 ± 1.11	1.8 ± 1.07	0.99
At 16 weeks	1.52 ± 0.98	1.55 ± 0.97	0.88

Independent t test

e) Comparison of **change in hemi-mMASI** between groups A and B-

No significant difference was seen in change in hemi-mMASI between group A and B. Mean change in hemi-mMASI at baseline, and at 16 weeks in group A was 0.12 ± 0.37 and 0.76 ± 0.98 respectively and the corresponding values in group B were 0.15 ± 0.4 and 0.93 ± 1.1 respectively (table 28 and fig 10). The ITT analysis is shown in table 29 wherein no significant difference is noted.

Table 28 :- Comparison of change in hemi-mMASI between group A and B (PP analysis).

Change in Hemi-modified melasma area severity index	Group A(n=45) Mean ± SD	Group B(n=45) Mean ± SD	P value
At 4 weeks	0.12 ± 0.37	0.15 ± 0.4	0.71
At 8 weeks	0.32 ± 0.73	0.46 ± 0.86	0.40
At 12 weeks	0.52 ± 0.95	0.71 ± 1.11	0.38
At 16 weeks	0.76 ± 0.98	0.93 ± 1.1	0.44

Independent t test

Figure 10:- Trend of change in Hemi-mMASI in groups A and B.

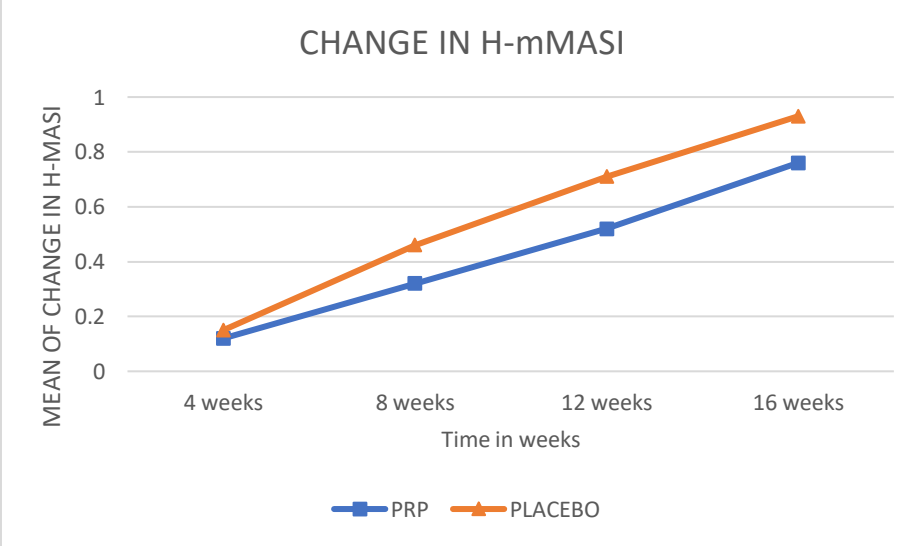


Table 29:-Comparison of change in hemi-mMASI between group A and B (ITT analysis).

Change in Hemi-modified melasma area severity index	Group A(n=45) Mean ± SD	Group B(n=45) Mean ± SD	P value
At 4 weeks	0.12 ± 0.33	0.14 ± 0.36	0.78
At 8 weeks	0.27 ± 0.78	0.39 ± 0.95	0.514
At 12 weeks	0.51 ± 0.93	0.71 ± 1.09	0.315
At 16 weeks	0.77 ± 0.98	0.97 ± 1.11	0.367

Independent t test

B) *Comparison of Melasma severity index {Full face} between baseline and follow up of study subjects.*

Mean value of melasma severity index of study subjects at baseline and 16 weeks was 18.28 ± 13.54 and 10.53 ± 8.52, respectively (p value 0.0001) as shown in table 30.

Table 30:-Comparison of Melasma severity index {Full face} between baseline and follow up of study subjects

Melasma severity index {Full face}	Mean ± SD	P value from mean
At 0 week	18.28 ± 13.54	-
At 4 weeks	17.17 ± 14	0.12
At 8 weeks	14.48 ± 12.41	0.003
At 12 weeks	12.11 ± 9.23	0.0001*
At 16 weeks	10.53 ± 8.52	0.0001*

Paired t test

C) *Comparison of investigators global assessment (IGA) between group A and B*

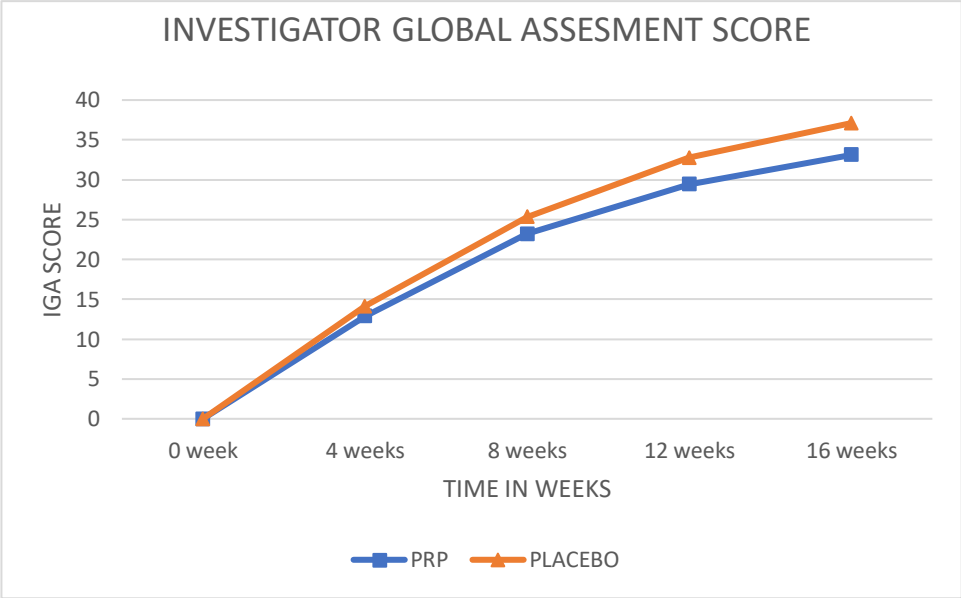
No significant difference was seen in investigators global assessment between groups A and B (p value <0.05). Mean IGA at 4 weeks; and at 16 weeks in group A was 12.89 ± 13.55 and 33.11 ± 19.75 respectively and in group B was 14.11 ± 13.07 and 37.11 ± 18.07 respectively (table 31 and fig 11).

Table 31:- Comparison of IGA between group A and B (PP analysis)

Investigators global assessment	**Group A(n=45)** Mean ± SD	**Group B(n=45)** Mean ± SD	**P value**
At 0 week	0 ± 0	0 ± 0	1*
At 4 weeks	12.89 ± 13.55	14.11 ± 13.07	0.664*
At 8 weeks	23.22 ± 14.89	25.33 ± 16.32	0.52
At 12 weeks	29.44 ± 17.56	32.78 ± 16.08	0.34
At 16 weeks	33.11 ± 19.75	37.11 ± 18.07	0.31

Independent t test

Figure 11 : Trend of IGA at different time intervals in groups A and B.



D) *Comparison of patient's global assessment (PGA) between group A and B*

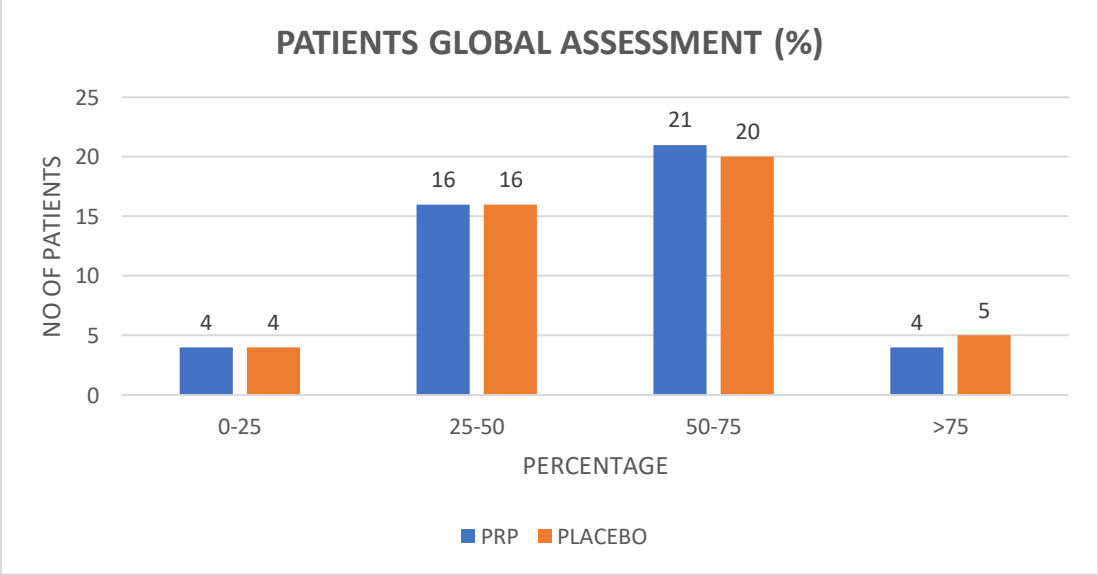
Distribution of PGA at 16 weeks was comparable in groups A and B (p value= 0.999). Maximum patients were in range of 50 to < 75% group. This is shown in table 32 and fig 12.

Table 32 :-Comparison of PGA between group A and B

PGA (%)	Group A(n=45)	Group B(n=45)	P value
0-<25	4 (8%)	4 (8%)	0.999‡
25-<50	16(35%)	16(35%)	
50-<75	21(46%)	20 (44%)	
>75	4(8%)	5(11%)	
Total	45 (100%)	45 (100%)	

‡ **Fisher's exact test**

Figure 12:- Bar graph showing comparison of PGA between groups A and B



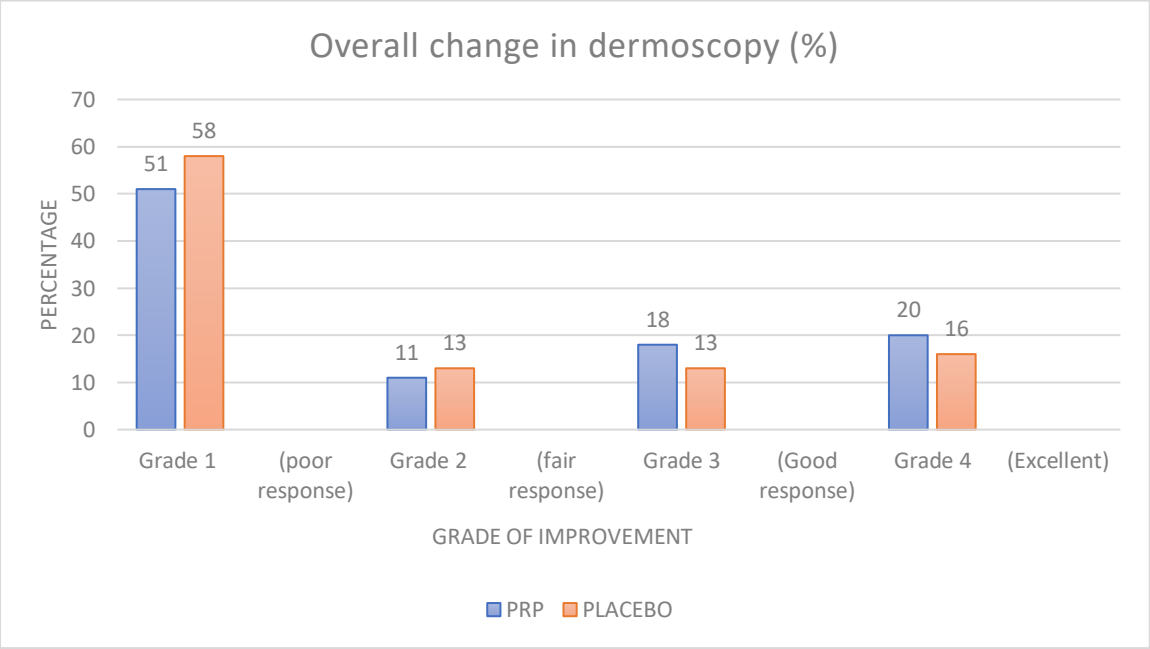
E) *Post-treatment dermoscopic improvement*

Dermoscopic analysis showed Grade1 response in 51.1% of patients in PRP group and 57.7% in placebo group as showing in table 33605 and figure 13.

Table 33: Post treatment dermoscopic improvement in groups A and B

Overall change in dermoscopy	PRP N (% age)	**PLACEBO** N (% age)
Grade 1 (poor response)	23 (51.1)	26(57.7)
Grade 2 (fair response)	5 (11.1)	6(13.3)
Grade 3 (Good response)	8 (17.7)	6(13.3)
Grade 4 (Excellent)	9 (20.0)	7(15.5)

Figure 13 : Bar graph showing post-treatment dermoscopic improvement in groups A and B at 16 weeks.



F) *Comparison of Melasma quality of life(MELASQOL) scale between baseline and follow up of study subjects*

Mean value of MELASQOL scale of study subject at baseline and 16 weeks was 41.82 ± 17.27 and 30.93 ± 16.65, respectively (Table 33).

Table 33:-Comparison of Melasma quality of life scale between baseline and follow up of study subjects

Melasma quality of life scale	Mean ± SD	P value
At 0 week	41.82 ± 17.27	0.0031*
At 16 weeks	30.93 ± 16.65	

G) *Comparison of adverse effects between group A and B.*

Distribution of adverse effects was comparable between group A and B (p value=1). As represented in table 34.

Table 34 : Comparison of adverse effects between group A and B.

Adverse effects	Group A(n=45)	Group B(n=45)	P value
Pain on injection	45 (100%)	45 (100%)	No p value
Erythema	5 (11.11%)	5 (11.11%)	1†
Burning	2 (4.44%)	1 (2.22%)	1
Acne	1 (2.22%)	1 (2.22%)	1

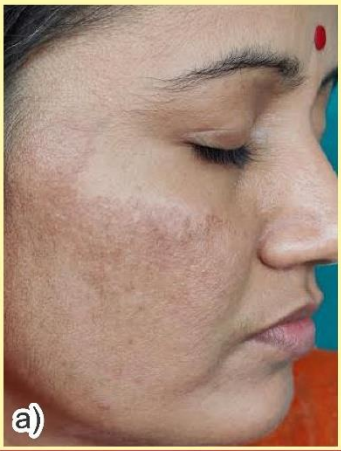
† **Chi square test**

H) *Comparison of treatment induced additional effects in groups A and B*

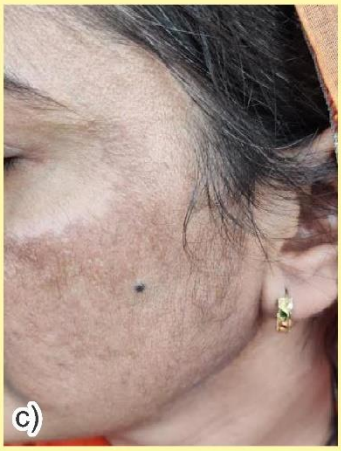
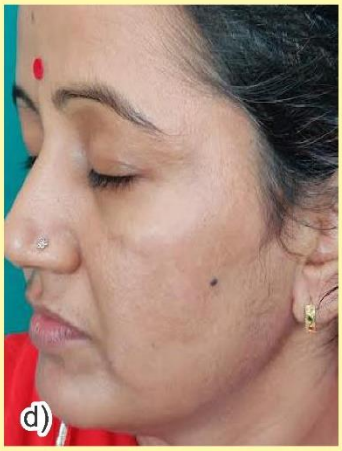
Distribution of additional effects was comparable between groups A and B with improvement in shine over skin was seen in 60% vs 68.89% respectively (p value=0.378); Improvement in scar pigmentation was seen in 2.22% cases in each group(p value=1).

PATIENT NO. 26 figure 14

HYDROQUINONE + PRP (RT SIDE)

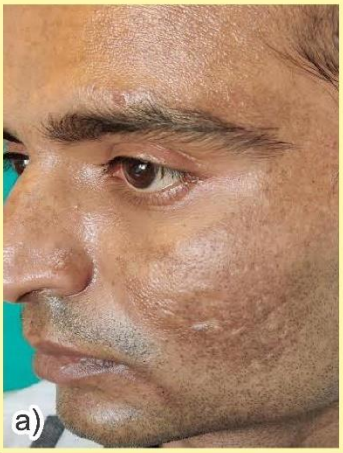
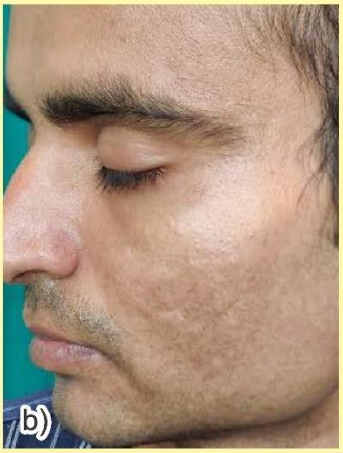
AT BASELINE	AT 16 WEEKS
	
H-mMASI – 2.7	**H-mMASI – 2.4** **IGA – 75%** **PGA – 50%**

HYDROQUINONE + NS (LT SIDE)

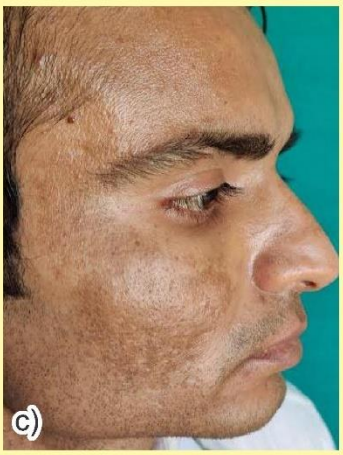
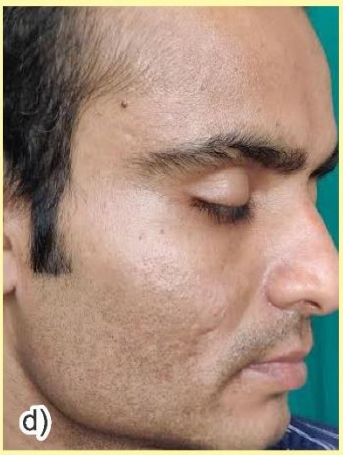
	
H-mMASI – 2.7	**H-mMASI – 2.4** **IGA – 85%** **PGA – 50%**

PATIENT NO. 35 figure 15

HYDROQUINONE + PRP (LT SIDE)

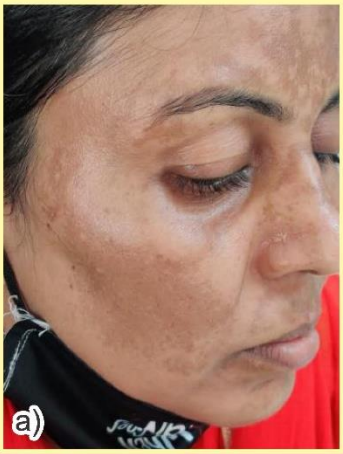
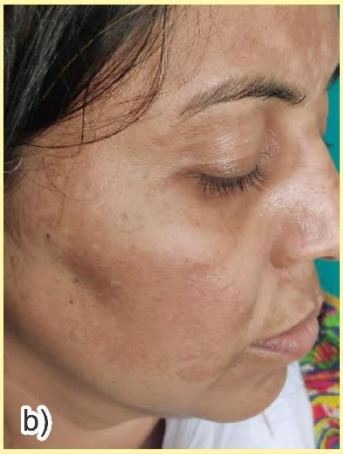
AT BASELINE	AT 16 WEEKS
	
H-mMASI – 2.7	**H-mMASI – 0.6** **IGA – 60%** **PGA – 90%**

HYDROQUINONE + NS (RT SIDE)

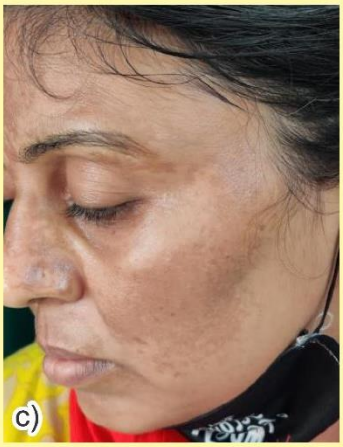
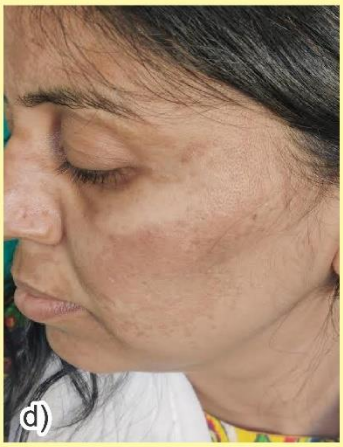
	
H-mMASI – 3.15	**H-mMASI – 10.5** **IGA – 65%** **PGA - 90%**

PATIENT NO. 3 figure 16

HYDROQUINONE + PRP (RT SIDE)

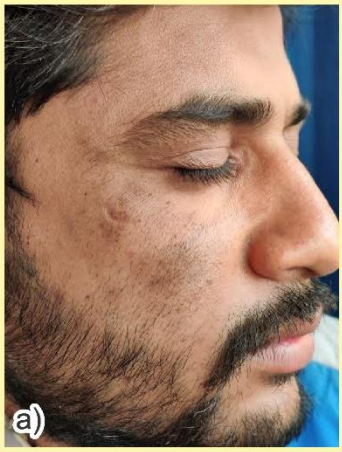
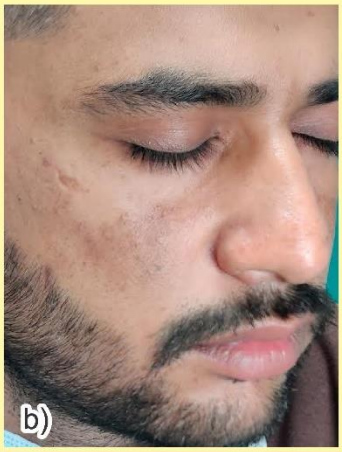
AT BASELINE	AT 16 WEEKS
	
H-mMASI – 8.1	**H-mMASI – 3.1** **IGA – 40%** **PGA – 30%**

HYDROQUINONE + NS (LT SIDE)

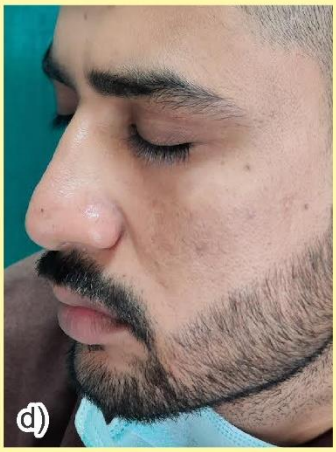
	
H-mMASI – 8.1	**H-mMASI – 3.1** **IGA –30%** **PGA – 30%**

PATIENT NO. 18 figure 17

HYDROQUINONE + PRP (RT SIDE)

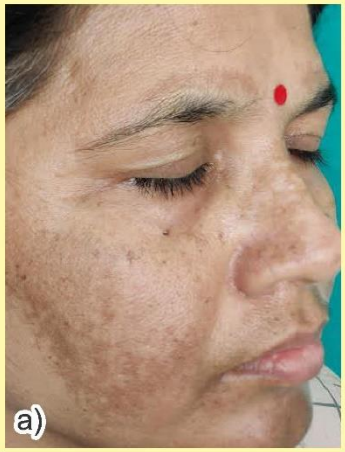
AT BASELINE	AT 16 WEEKS
	
H-mMASI – 0.9	**H-mMASI – 0.6** **IGA –50%** **PGA – 40%**

HYDROQUINONE + NS (LT SIDE)

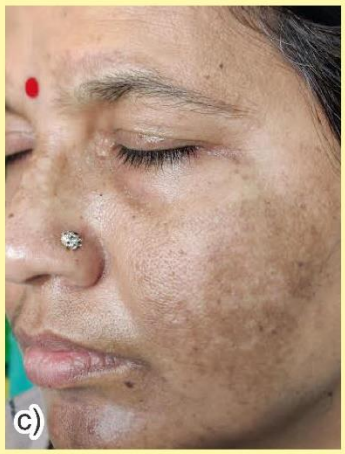
	
H-mMASI – 0.9	**H-mMASI – 0.6** **IGA – 45%** **PGA – 50%**

PATIENT NO. 33 figure 18

HYDROQUINONE + PRP (RT SIDE)

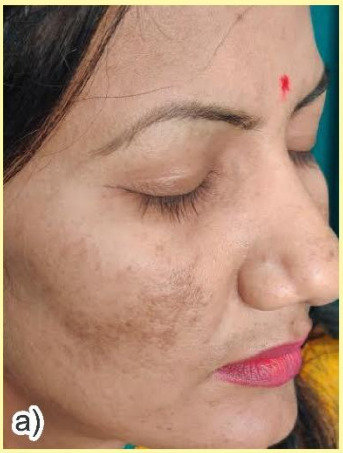
AT BASELINE	AT 16 WEEKS
	
a)	b)
H-mMASI – 4.05	**H-mMASI – 3.9** **IGA – 50%** **PGA – 50%**

HYDROQUINONE + NS (LT SIDE)

	
c)	d)
H-mMASI – 5.55	**H-mMASI – 3.9** **IGA – 45%** **PGA - 50**

PATIENT NO. 34 figure 19

HYDROQUINONE + PRP (RT SIDE)

AT BASELINE	AT 16 WEEKS
	
a)	b)
H-mMASI – 3	**H-mMASI – 2.1** **IGA – 25%** **PGA – 20%**

CLINICAL PICTURE – HYDROQUINONE + NS (LT SIDE)

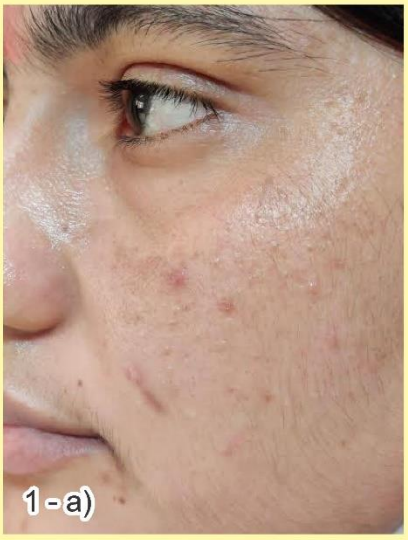
	
c)	d)
H-mMASI – 3	**H-mMASI – 2.1** **IGA – 25%** **PGA – 20%**

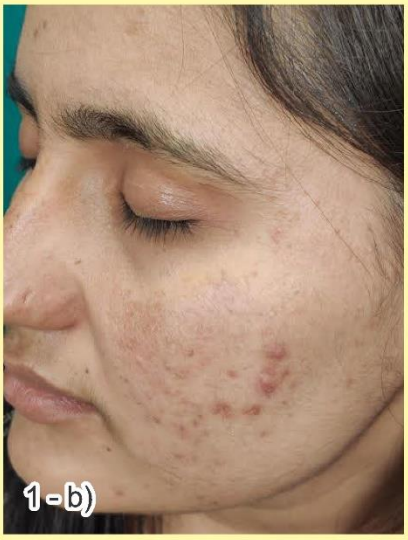
SIDE EFFECTS figure 20

1. EXACERBATION OF ACNE (CASE 5)

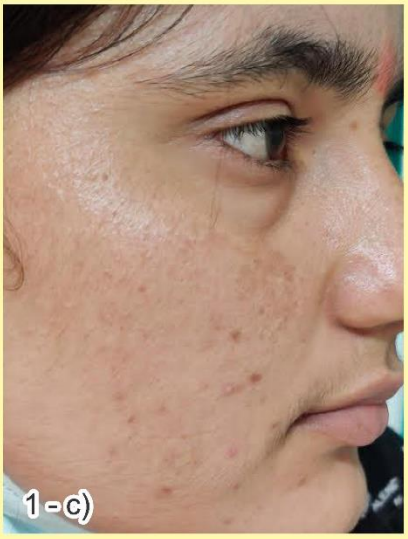
1.A NORMAL SALINE LEFT SIDE

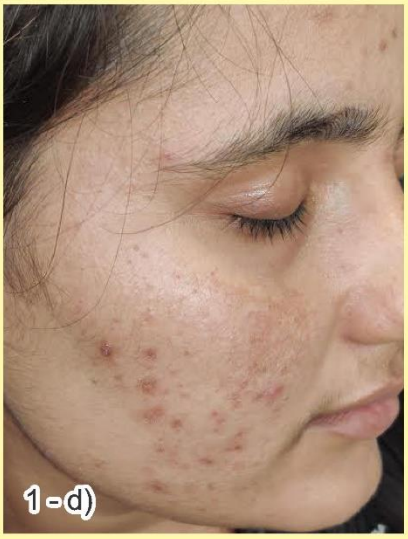
AT BASELINE	AT 16 WEEKS





1.B PRP RIGHT SIDE

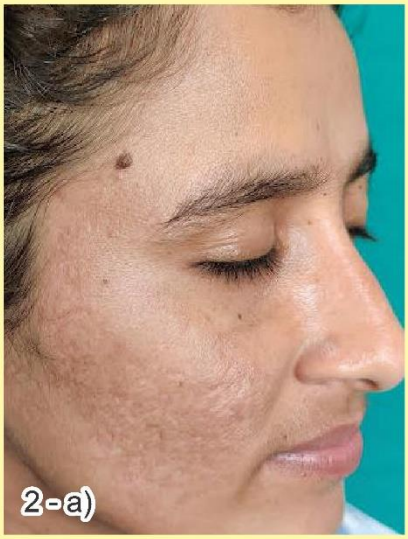




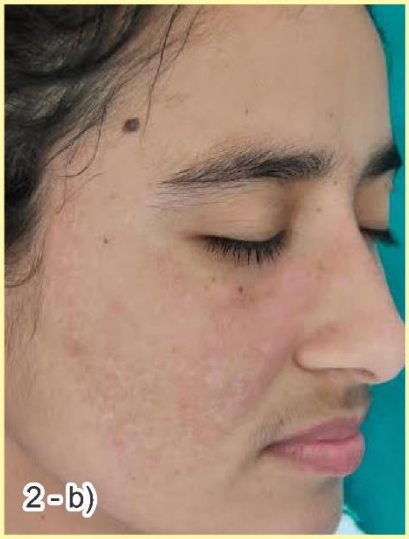
2. ERYTHEMA (CASE 54)

2.A NORMAL SALINE RIGHT SIDE

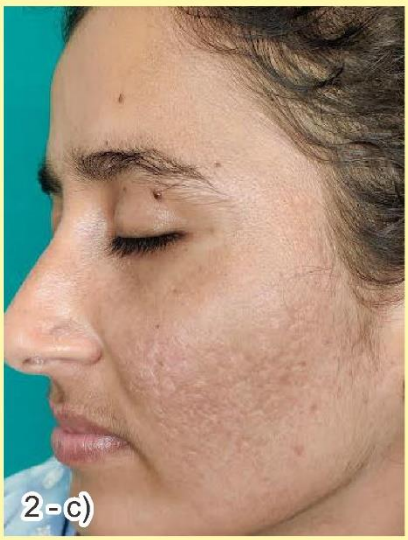
AT BASELINE

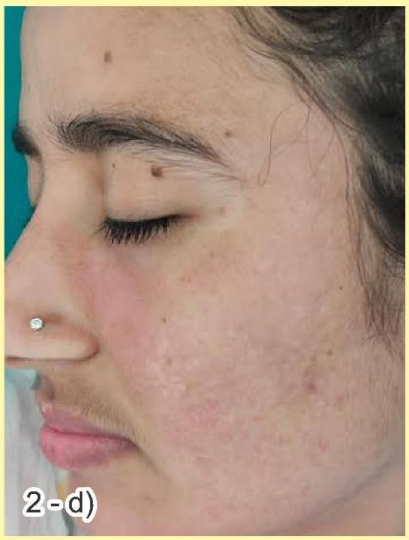


AT 16 WEEKS



2.B PRP LEFT SIDE



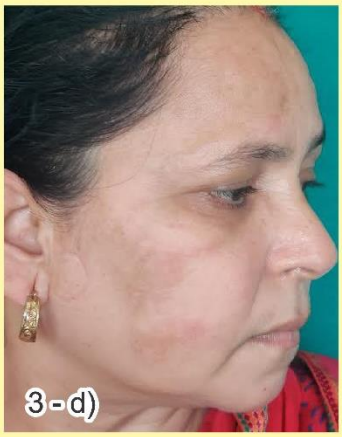


3. TEXTURE CHANGE OF SKIN

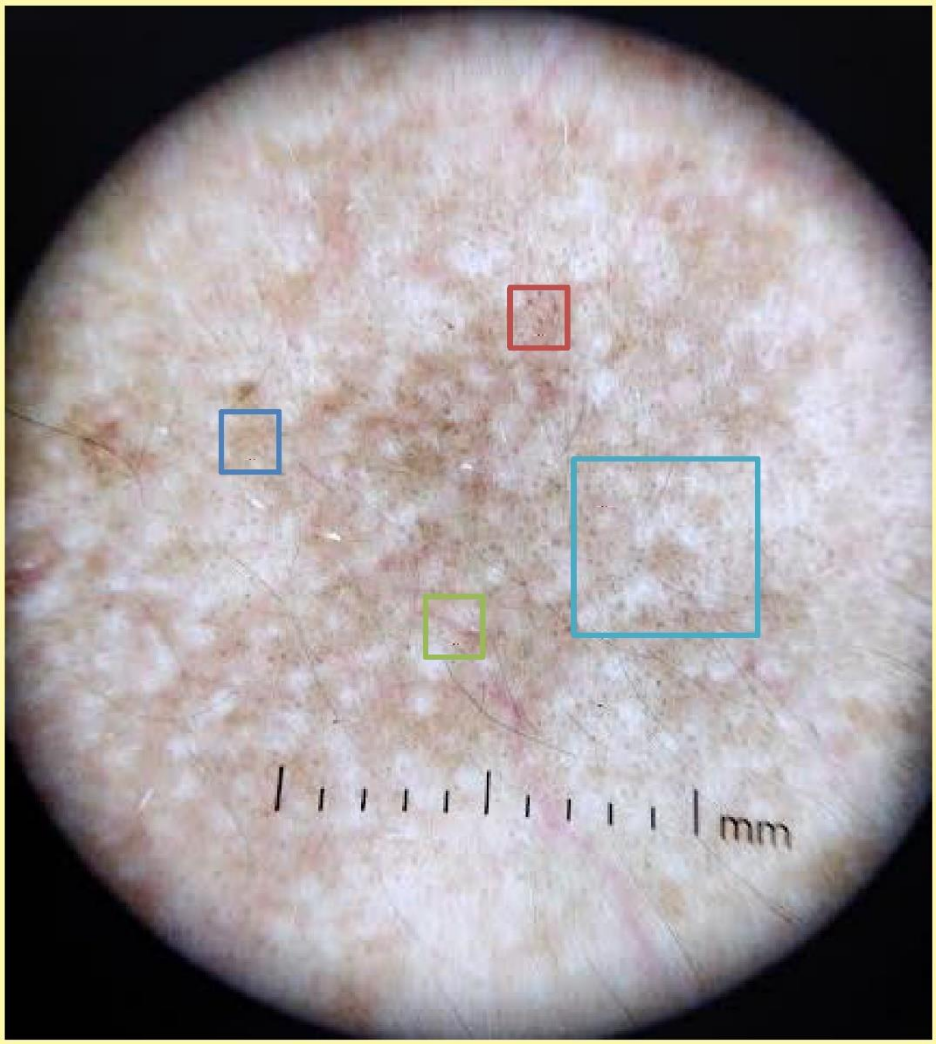
3.A NORMAL SALINE LEFT SIDE

AT BASELINE	AT 16 WEEKS
	
3-a)	3-b)
	Skin became more smoother

3.B PRP RIGHT SIDE

	
3-c)	3-d)
	Skin became more smoother

DERMOSCOPY OF MELASMA (Figure 21)



BROWN DOTS

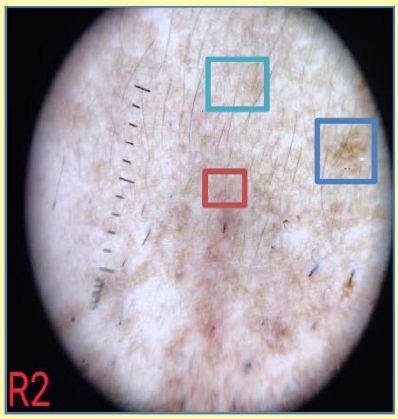
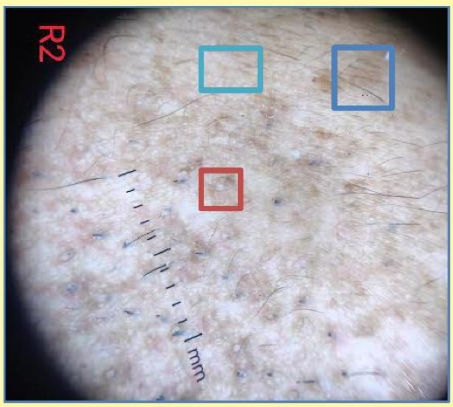
GLOBULES

PSEUDORETICULAR

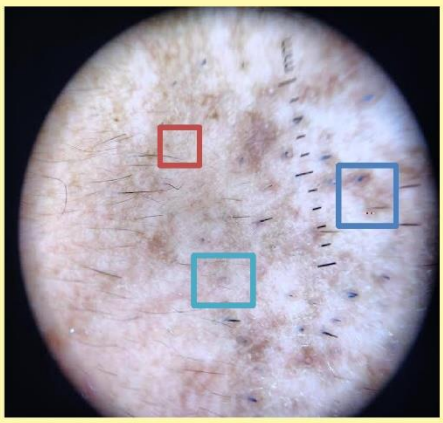
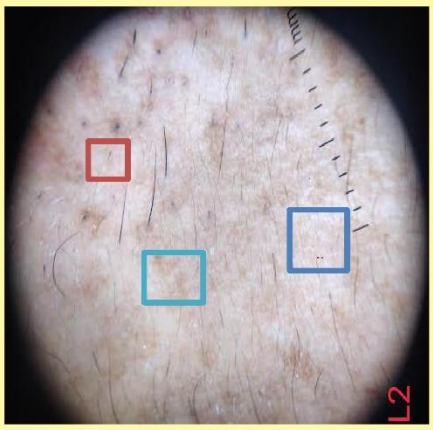
TELENGECTASIA

DERMOSCOPY CASE 43 (figure 22)

DERMOSCOPY – HYDROQUINONE + NS

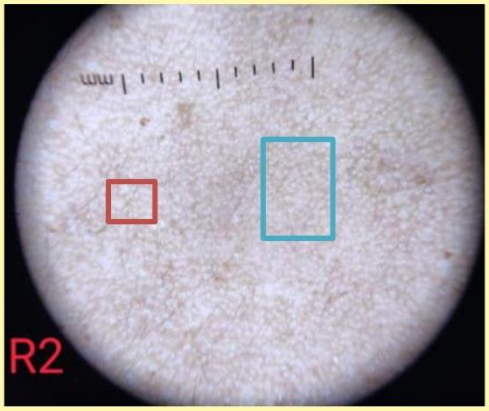
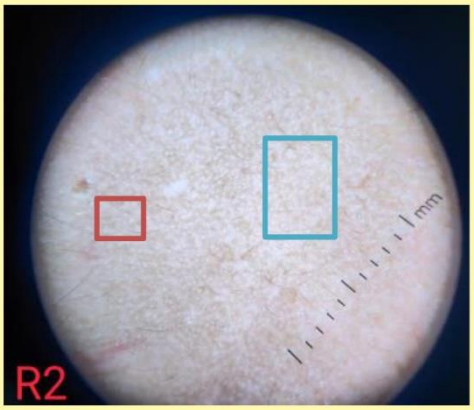
AT BASELINE	AT 16 WEEKS
	
BROWN DOTS AND GLOBULES WITH RETICULAR PIGMENTATION	**IMPROVEMENT – GRADE 1**

DERMOSCOPY – HYDROQUINONE + PRP

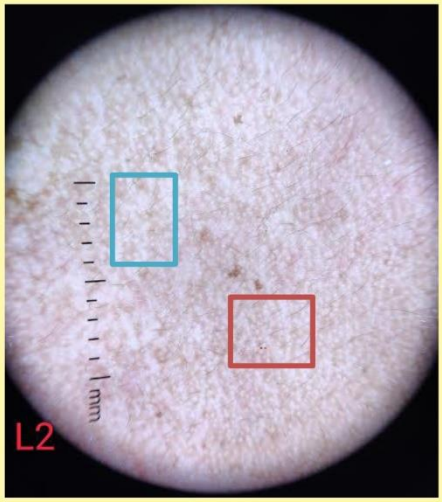
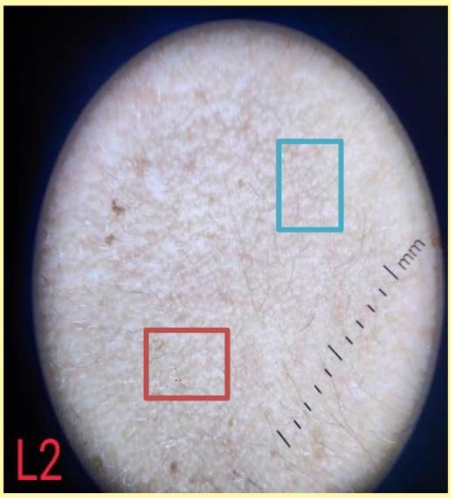
	
BROWN DOTS AND GLOBULES WITH RETICULAR PIGMENTATION	**IMPROVEMENT: GRADE 4**

DERMOSCOPY CASE 38 (figure 23)

DERMOSCOPY – HYDROQUINONE + NS

AT BASELINE	AT 16 WEEKS
	
BROWN DOTS AND RETICULAR PIGMENTATION	**IMPROVEMENT - GRADE 3**

DERMOSCOPY – HYDROQUINONE + PRP

	
BROWN DOTS AND RETICULAR PIGMENTATION	**IMPROVEMENT – GRADE 3**

DISCUSSION

Based on our inclusion criteria, 64 consenting patients with melasma were recruited from Dermatology, Venereology and Leprology Out patient department (OPD) at All India Institute of Medical Sciences, Jodhpur between Jan 2020 and June 2021 out of which 45 patients completed the follow up.

Most of the patients in our study were young with mean age was 29.5 ± 8.7 years (18 to 50 years) which was similar with Achar et al[9] 29.9 years (14 to 54 years). However in study by Yalamanchili et al[25] had relatively older cohort with mean age of 37.1 years.

Female to male ratio in our study was 1.8:1 which is similar with most of the studies like Yalamanchili at al[25] having female to male ratio of 2.1:1. A much higher female preponderance is shown by other studies like Achar et al[9] showing female to male ratio of 4:1, Satish et al[65] showing 4.2:1 and Kumar et al.[66] with 6.4:1. This might be because females are involved in traditional Rajasthani household chores and hence may not be able to visit hospital for dermatological condition which might be considered trivial by the family. Also since they are restricted to house they may be less exposed to sun and hence might have lesser melasma. Sun exposure is the main risk factor for melasma and intensity of sunlight is probably higher in Rajasthan. Men are traditionally outdoor workers in Rajasthan and this could possibly be the reason for the greater proportion of men in our study group.

Education-wise in our study had more of graduates (55.5%) and post graduates (15.5%). Not many studies have looked at the educational status of patients. Similarly, Coban et al and Sarkar et al have reported a significant proportion of patients to be graduates at 54.9% and 30%, respectively [67,68]. Increase in education may have lead them to increased awareness about their skin condition, possibly explaining higher representation among our study participants.

Duration of melasma ranged from 2 months to 20 years, with a mean of 3.4 ± 4.1 years, which is similar to that of Achar et al[9] and different from Sarkar et al[12].

In our study, majority of the patients (31, 68.9%) did not use sunscreen. Only 14 out of 45 patients (31.1%) used sunscreen which is similar to the study done by Sarkar et al[68] which showed that 30.3% of the patients in northern India were using sunscreens regularly. Similarly, Hexsel et al [69] found that about 29.9% patients were using sunscreen at melasma onset. Poor use of sunscreen may be due to the ignorance, on part of patients about the role of sunlight in their disease. The fact remains that sunscreen usage is both costly and inconvenient.

Majority of our patients (22, 48.9%) had Fitzpatrick skin type V followed by IV 20 (44.4%), and Fitzpatrick skin type III only in 3 patients (6.7%). These results are consistent with review by Sarkar et al in which majority of the patients belonged to skin types IV (48.3%) and V (40.7%) and Guinot et al (70) having 45% patients of type IV and 40% type V skin. However, Hexsel et al [69] found 36% patients with Type III Fitzpatrick skin type. In western Indian population, we have more people with Fitzpatrick skin type IV and V, so is the case in our study. Regional and racial differences maybe accounting for these differences.

In our study majority of the patients (28, 62.2%) had malar pattern, whereas centrofacial was only seen in 17 patients (37.8%). This was consistent with studies by Satish et al (65), Yalamanchili et al [25] and with Sarkar et el[68] all of which had malar pattern being predominant in 65.9%, 68% and 42% respectively. These findings are different from the study by Achar et al[9] which found centrofacial as the most common pattern (55.4%). A study found centrofacial pattern to be predominant in women whereas malar pattern was the most common in men[26]. However in our study females also had malar area as the most common site of involvement, while males had equal involvement of both malar area and centrofacial area. As malar area is prominent area of face so more sun exposure occurs over that area thus leading to more predilection for this site.

In majority of our patients (26, 57.8%) colour was dark brown which was in disagreement with Charupalli et al(71) in which light brown in 52.8% patients and dark brown/black in 20.8% patients.

An ancillary aim of our study was to look at dermoscopic characteristics to better standardise assessment for melasma. In majority of patients, brown globules were seen 48.9%, arcuate pattern was present 24 (53.3%) and white dots indicating peri-

follicular sparing was seen 80%. This is different from the results of Yalamanchili et al where reticulate pattern was seen in 95% cases and peri-follicular sparing in 97% patients. In a study by Abdel et al[72], pseudoreticular pattern was seen in 45% patients, globules in 93.5% cases , arcuate pattern in 9.7% cases and perifollicular sparing in 67.7% cases.

Mean modified Melasma Area Severity Index (mMASI) at start of treatment of full face was 4.85 which reduced to 3.17 at the end of 16 weeks, which is 34.6% improvement. This is lower than Tuknayat et al which has shown improvement of 54% (13.7 baseline to 6.2 at 16 weeks) in mMASI using PRP therapy[73]. This could due difference the method of making PRP, as neither methods nor the total number of sittings required for improvement is standardized.

Mean Hemi-mMASI at start of study in PRP group was 2.3 ± 1.5 which reduced upto 1.5 ± 0.9 as compared to that in the placebo group where it reduced from 2.5 ± 1.8 to 1.59 ± 0.9 at the end of 16 weeks (p=0.44). Results of both is not statistically significant. Sirithanabadeekul (3) et al showed that mean Hemi-mMASI scores in the PRP group reduced from 4.92 ± 0.96 to 3.5 ± 0.6 which were significant on comparing with placebo whose mean Hemi-mMASI reduced from 4.9 ± 0.8 at baseline to 4.5 ± 0.9 (P = 0.042). The authors advised only sun screen in addition to intra dermal injection, whereas our subjects used HQ 2% as well. Our study did not reveal any difference in the efficacy of PRP when compared with saline over and above the standard treatment of HQ 2%. The possible reasons maybe that PRP is not effective or it may be due to less sittings of PRP therapy.

Melasma severity Index (MSI) of full face was 18.3 ± 13.5 at baseline which improved upto 10.5 ± 8.5 at 16 weeks. It is a relatively newer score and has not been used in any study to measure clinical outcomes. We tried using this score in our study as it gives distinct value to pigmentation more than that for area as suggested by Majid et al reducing the interobserver bias[32]. As per MSI, % reduction seen in our study is 42.4% which is more than mMASI.

In terms of patient global assessment, approximately 54 to 55% patients reported more than 50% reduction in melasma indicating good response in majority of patients which is similar to study by Tuknayat et al reporting 47.5% patients to be very pleased with PRP therapy in melasma[73]. Investigators global assessment improved

by 33% in PRP group and 37% in placebo group (p= 0.31) indicating moderate improvement.

Post therapy lightening in dermoscopy was Grade 1 (poor improvement) in 51% patients of PRP and 58% patients of Saline. This is in contrast to Hay et al which showed good improvement in 45% patients after QS ND: YAG laser. Additional effects such as improvement in texture were also comparable between the two groups which is similar to Tuknayat et al which showed improvement in skin quality and wrinkles[73].

Side effects and complications reported after PRP injections were minimal including pain during injection[73] and mild erythema and the ones after hydroquinone included erythema, burning sensation, skin irritation, acneiform lesions. In our study pain was reported by all patients, erythema was seen in 11.1% in both groups, burning sensation was seen in 4.4]4% in group A and 2.2% in group B and acne eruption in 2.2% in both groups. The latter is in agreement with Brozina et al, in which 1 patient developed acne on application of hydroquinone[74]. However we cannot be sure whether side effects were due to HQ or saline or PRP, but seeing older studies, some of these side effects may be due to HQ.

Melasma treatment should not only strive to reduce the objective scores, but also to improve the patient's sense of well-being and quality of life. To assess this we used English and Hindi MELASQOL. It was however used for overall entire face, as the questionnaire did not report questions for opposite sides of face. Melasma quality of life index at baseline was 42 out of 70 and at 16 weeks was 31 out of 70, showing a reduction of 26%. In a study done by Sarkar et al, MELASQOL decreased from 47 to 38, showing a reduction of 19% which was lower than that of our study. The possible reason could be sunscreen alone usage by Sarkar et al whereas we used sunscreen with HQ 2% with intradermal saline or PRP injection.

The intragroup analysis on both cheeks show that there is a decreasing trend on mMASI at each visit, and the decrease is significant at each visit. Thus we can infer that hydroquinone 2% is moderately effective in management of melasma with minimal side effects. It is also a possibility that addition of PRP or normal saline has lead to decrease in the existing adverse effects of hydroquinone like burning sensation or irritation of skin or erythema.

Hydroquinone with PRP was not better then hydroquinone with saline, it might be due to the fact that PRP decreases pigmentation by increasing volume and hence saline also might be effective by the same mechanism, thus providing similar efficacy[63]. In our study lesser number of PRP sittings done which is similar to study done by Sirithanabadeekul et al[3], who had given 4 sittings of PRP, while it is in contrast with Garg et al which showed improvement in resistant melasma case after 6 sittings of PRP injection. As PRP is a relatively newer therapy and there are various methods of its preparation like single spin or double spin, with different number of rotations, and there is no standardization regarding it. Moreover the frequency at which PRP injections are to be given is also not fixed, we have given 4 weekly injections in our study while Sirithanabadeekul et al[3] had given 2 weekly injections. Another case report by Çayırlı et al also showed 80% improvement with 3 PRP injection given at 2 weekly interval[63]. Thus total number of injection, area and amount, technique and handling of PRP is not fixed and may cause decrease in its effect. Small size bore of needle may cause inhibition of growth factor. So also increased concentration of white blood cell in PRP may have inhibitory effect leading to its less response[75]. In our study mainly patients were of Fitzpatrick skin type V followed by IV and had dark brown melasma, and more severe mixed type melasma with telangiectasia in darker skin types is associated with less response[76,77].

PRP also has potency of increasing pigmentation with its effects on growth factors and cytokines as shown by Usyal et al[78].

Also Hemi-mMASI was not easy to calculate and there was an element of subjectivity despite using scales. Thus right vs left was not easy and a minor change in area or colour of melasma was not easily detected, leading to almost similar change in Hemi-mMASI in both the groups. More qualitative scales or automated programs are therefore required for better quantification. Sun protection behaviour may be not proper and may have caused increase in pigmentation and decreased the effects of PRP.

Limitation of this study could be attributed to small sample size and limited number of PRP sittings.

CONCLUSION

The study was Interventional, prospective, placebo controlled randomized control trial, recruited the patients from the Department of Dermatology, Venereology and Leprology OPD, AIIMS, Jodhpur. The study included 64 patients with melasma on bilateral cheeks. They were divided randomly, Platelet rich plasma (PRP) was injected on right side in 35 patients and on left side in 29 patients. On the other half normal saline was injected. Therapy was given every 4-weekly for 4 sessions. Follow up was done at 4, 8, 12 and 16 weeks with Hemi-mMASI and investigators global assessment for each cheek separately. Patients global assessment, dermoscopy, MELASQOl was done at baseline and at 16 weeks.

Overall the mean age of patients in our study was 24.5 ± 8.7 years (13-60 years) and female to male ratio was 1.8:1. The mean duration of illness was mean 3.4 ± 4.1 years (2 months – 15 years). Majority of the patients did not use sunscreen prior coming to hospital (69%). Among the skin types, darker skin type was more common than the lighter ones, V > IV > III.

Most common pattern of distribution was malar followed by centrofacial. Dark brown colour of melasma was more than the light brown.

Among the efficacy parameters, there was significant reduction in mMASI of entire face at each visit with overall 34.6% reduction.

Hemi-mMASI in both groups (Hydroquinone with PRP and hydroquinone with NS) independently showed decreasing trend from baseline to last visit (16 weeks), 33.3% in PRP group and 36.6% in the saline group.

Melasma severity index showed 42.4% reduction and MELASQOL score showed 26% reduction which was calculated for the entire face.

In terms of patient global assessment, approximately 54 to 55% patients reported more than 50% reduction in melasma indicating good response in majority of patients.

Investigators global assessment which was 33% in PRP and 37% in the placebo group (P=0.31). This indicates that though there was reduction in melasma overall, there was no difference between the two sides.

Dermoscopic characteristics in majority of our patients showed brown globules 48.9%, arcuate pattern 24(53.3%) and white dots indicating peri-follicular sparing in 80% of patients.

Most common side effects and complications reported in our study was pain reported by all patients, erythema was seen in 11.1% in both groups, burning sensation was seen in 4.4% in PRP and 2.2% in placebo and acne eruption in 2.2% in both groups.

Thus we can infer that addition of PRP injection to 2% hydroquinone has not provided any increased benefit on comparing with normal saline injection. Also there lies a possibility that PRP/saline might have decreased the side effects of hydroquinone.

Platelet rich plasma therapy for melasma is relatively novel concept, and it was not possible to reach a definitive conclusion. Thus more and better randomized controlled clinical studies are needed to verify our conclusions.

REFERENCES

1. Sarkar R, Arora P, Garg VK, Sonthalia S, Gokhale N. Melasma update. *Indian Dermatol Online J*. 2014;5(4):426-435

2. Rodrigues M, Pandya AG. Melasma: clinical diagnosis and management options. *Australas J Dermatol*. 2015;56(3):151-163.

3. Sirithanabadeekul P, Dannarongchai A, Suwanchinda A. Platelet-rich plasma treatment for melasma: A pilot study. *J Cosmet Dermatol*. 2020;19(6):1321-1327.

4. Marx RE. Platelet-rich plasma (PRP): what is PRP and what is not PRP? *Implant Dent*. 2001;10(4):225–8.

5. Sehgal VN, Verma P, Srivastava G, Aggarwal AK, Verma S. Melasma: treatment strategy. *J Cosmet Laser Ther*. 2011;13(6):265-279.

6. Rivas S, Pandya AG. Treatment of melasma with topical agents, peels and lasers: an evidence-based review. *Am J Clin Dermatol*. 2013;14(5):359–76.

7. Handel AC, Miot LDB, Miot HA. Melasma: a clinical and epidemiological review. *An Bras Dermatol*. 2014;89:771–82.

8. Shenoi SD, Davis SV, Rao S, Rao G, Nair S. Dermatoses among paddy field workers--a descriptive, cross-sectional pilot study. *Indian J Dermatol Venereol Leprol*. 2005;71(4):254–8.

9. Achar A, Rathi SK. Melasma: a clinico-epidemiological study of 312 cases. *Indian J Dermatol*. 2011;56(4):380-382.

10. Lee A-Y. Recent progress in melasma pathogenesis. *Pigment Cell Melanoma Res*. 2015;28(6):648–60.

11. Kwon SH, Hwang YJ, Lee SK, Park KC. Heterogeneous Pathology of Melasma and Its Clinical Implications. *Int J Mol Sci.* 2016;17(6):824.

12. Sarkar R, Puri P, Jain RK, Singh A, Desai A. Melasma in men: a clinical, aetiological and histological study. *J Eur Acad Dermatol Venereol*. 2010;24(7):768–72.

13. Tamega A de A, Miot LDB, Bonfietti C, Gige TC, Marques MEA, Miot HA. Clinical patterns and epidemiological characteristics of facial melasma in Brazilian women. *J Eur Acad Dermatol Venereol*. 2013;27(2):151–6.

14. Carsberg CJ, Ohanian J, Friedmann PS. Ultraviolet radiation stimulates a biphasic pattern of 1,2-diacylglycerol formation in cultured human melanocytes and keratinocytes by activation of phospholipases C and D. *Biochem J*. 1995;305 (Pt 2)(Pt 2):471-477.

15. Roméro-Graillet C, Aberdam E, Biagoli N, Massabni W, Ortonne JP, Ballotti R. Ultraviolet B radiation acts through the nitric oxide and cGMP signal transduction pathway to stimulate melanogenesis in human melanocytes. *J Biol Chem*. 1996;271(45):28052-28056.

16. Miot LD, Miot HA, Polettini J, Silva MG, Marques ME. Morphologic changes and the expression of alpha-melanocyte stimulating hormone and melanocortin-1 receptor in melasma lesions: a comparative study. *Am J Dermatopathol*. 2010;32(7):676–82.

17. Torres-Álvarez B, Mesa-Garza IG, Castanedo-Cázares JP, Fuentes-Ahumada C, Oros-Ovalle C, Navarrete-Solis J, et al. Histochemical and immunohistochemical study in melasma: evidence of damage in the basal membrane. *Am J Dermatopathol*. 2011;33(3):291–5.

18. Kang HY, Hwang JS, Lee JY, Ahn JH, Kim J-Y, Lee E-S, et al. The dermal stem cell factor and c-kit are overexpressed in melasma. *Br J Dermatol*. 2006;154(6):1094–9.

19. Ortonne JP, Arellano I, Berneburg M, Cestari T, Chan H, Grimes P, et al. A global survey of the role of ultraviolet radiation and hormonal influences in the development of melasma. *J Eur Acad Dermatol Venereol*. 2009;23(11):1254–62.

20. Kim N-H, Cheong KA, Lee TR, Lee A-Y. PDZK1 upregulation in estrogen-related hyperpigmentation in melasma. *J Invest Dermatol*. 2012;132(11):2622–31.

21. Kim J-Y, Lee T-R, Lee A-Y. Reduced WIF-1 expression stimulates skin hyperpigmentation in patients with melasma. *J Invest Dermatol*. 2013;133(1):191–200.

22. Kim EH, Kim YC, Lee E-S, Kang HY. The vascular characteristics of melasma. J *Dermatol Sci*. 2007;46(2):111–6.

23. Kim N-H, Choi S-H, Kim C-H, Lee CH, Lee TR, Lee A-Y. Reduced MiR-675 in exosome in H19 RNA-related melanogenesis via MITF as a direct target. *J Invest Dermatol*. 2014;134(4):1075–82.

24. Kim K-H, Bin B-H, Kim J, Dong SE, Park PJ, Choi H, et al. Novel inhibitory function of miR-125b in melanogenesis. *Pigment Cell Melanoma Res*. 2014;27(1):140–4.

25. Yalamanchili R, Shastry V, Betkerur J. Clinico-epidemiological Study and Quality of Life Assessment in Melasma. *Indian J Dermatol*. 2015;60(5):519.

26. Sarkar R, Ailawadi P, Garg S. Melasma in Men: A Review of Clinical, Etiological, and Management Issues. *J Clin Aesthet Dermatol*. 2018;11(2):53.

27. Ritter CG, Fiss DVC, Borges da Costa J a. T, de Carvalho RR, Bauermann G, Cestari TF. Extra-facial melasma: clinical, histopathological, and immunohistochemical case-control study. *J Eur Acad Dermatol Venereol*. 2013;27(9):1088–94.

28. Basit H, Godse KV, Al Aboud AM. Melasma. In: *StatPearls*. Treasure Island (FL): StatPearls Publishing; 2021.

29. Mishra SN, Dhurat RS, Deshpande DJ, Nayak CS. Diagnostic utility of dermatoscopy in hydroquinone-induced exogenous ochronosis. *Int J Dermatol*. 2013;52(4):413–7.

30. Kang HY, Bahadoran P, Suzuki I, Zugaj D, Khemis A, Passeron T, et al. In vivo reflectance confocal microscopy detects pigmentary changes in melasma at a cellular level resolution. *Exp Dermatol.* 2010;19(8):e228-233.

31. Pandya AG, Hynan LS, Bhore R, Riley FC, Guevara IL, Grimes P, et al. Reliability assessment and validation of the Melasma Area and Severity Index (MASI) and a new modified MASI scoring method. *J Am Acad Dermatol*. 2011;64(1):78–83, 83.e1-2.

32. Majid I, Haq I, Imran S, Keen A, Aziz K, Arif T. Proposing Melasma Severity Index: A New, More Practical, Office-based Scoring System for Assessing the Severity of Melasma. *Indian J Dermatol*. 2016;61(1):39–44.

33. Balkrishnan R, Mcmichael AJ, Camacho FT, Saltzberg F, Housman TS, Grummer S, et al. Development and validation of a health-related quality of life instrument for women with melasma. *Br J Dermatol*. 2003;149(3):572–7.

34. Sarkar R, Garg S, Dominguez A, Balkrishnan R, Jain RK, Pandya AG. Development and validation of a Hindi language health-related quality of life questionnaire for melasma in Indian patients. *Indian J Dermatol Venereol Leprol*. 2016;82(1):16–22.

35. Resnik S. Melasma induced by oral contraceptive drugs. *J Am Med Assoc*. 1967;199(9):601-605.

36. Lakhdar H, Zouhair K, Khadir K, et al. Evaluation of the effectiveness of a broad-spectrum sunscreen in the prevention of chloasma in pregnant women. *J Eur Acad Dermatol Venereol*. 2007;21(6):738-742.

37. Duteil L, Cardot-Leccia N, Queille-Roussel C, Maubert Y, Harmelin Y, Boukari F, et al. Differences in visible light-induced pigmentation according to wavelengths: a clinical and histological study in comparison with UVB exposure. *Pigment Cell Melanoma Res.* 2014;27(5):822–6.

38. Castanedo-Cazares JP, Hernandez-Blanco D, Carlos-Ortega B, Fuentes-Ahumada C, Torres-Álvarez B. Near-visible light and UV photoprotection in the

treatment of melasma: a double-blind randomized trial. *Photodermatol Photoimmunol Photomed*.

39. Boo YC. Arbutin as a Skin Depigmenting Agent with Antimelanogenic and Antioxidant Properties. *Antioxidants (Basel)*. 2021;10(7):1129.

40. Sarkar R, Gokhale N, Godse K, et al. Medical Management of Melasma: A Review with Consensus Recommendations by Indian Pigmentary Expert Group. *Indian J Dermatol*. 2017;62(6):558-577.

41. Arndt KA, Fitzpatrick TB. Topical Use of Hydroquinone as a Depigmenting Agent. *J Am Med Assoc*. 1965;194(9):965–7.

42. Obagi S, Gandhi RK. Chapter 85 - Skin Rejuvenation and Resurfacing. In: Brennan PA, Schliephake H, Ghali GE, Cascarini L. *Maxillofacial Surgery*. 3rd ed. Elsevier; 2017:1286–1301.

43. Ferreira Cestari T, Hassun K, Sittart A, de Lourdes Viegas M. A comparison of triple combination cream and hydroquinone 4% cream for the treatment of moderate to severe facial melasma. *J Cosmet Dermatol*. 2007;6(1):36-39.

44. Griffiths CEM, Finkel LJ, Ditre CM, Hamilton TA, Ellis CN, Voorhees J j. Topical tretinoin (retinoic acid) improves melasma. A vehicle-controlled, clinical trial. *Br J Dermatol*. 1993;129(4):415–21.

45. Chan R, Park KC, Lee MH, et al. A randomized controlled trial of the efficacy and safety of a fixed triple combination (fluocinolone acetonide 0.01%, hydroquinone 4%, tretinoin 0.05%) compared with hydroquinone 4% cream in Asian patients with moderate to severe melasma. *Br J Dermatol*. 2008;159(3):697-703.

46. Neagu N, Conforti C, Agozzino M, et al. Melasma treatment: a systematic review. *J Dermatolog Treat*. 2021;1-39.

47. Deo KS, Dash KN, Sharma YK, Virmani NC, Oberai C. Kojic Acid vis-a-vis its Combinations with Hydroquinone and Betamethasone Valerate in Melasma: A

Randomized, Single Blind, Comparative Study of Efficacy and Safety. *Indian J Dermatol*. 2013;58(4):281–5.

48. Fitton A, Goa KL. Azelaic acid. A review of its pharmacological properties and therapeutic efficacy in acne and hyperpigmentary skin disorders. *Drugs*. 1991;41(5):780–98.

49. Sarma N, Chakraborty S, Poojary SA, Rathi S, Kumaran S, Nirmal B, et al. Evidence-based Review, Grade of Recommendation, and Suggested Treatment Recommendations for Melasma. *Indian Dermatol Online J*. 2017;8(6):406–42.

50. Hofny ERM, Abdel-Motaleb AA, Ghazally A, Ahmed AM, Hussein MRA. Platelet-rich plasma is a useful therapeutic option in melasma. *J Dermatolog Treat*. 2019;30(4):396-401.

51. Nurden AT. Platelets, inflammation and tissue regeneration. *Thromb Haemost*. 2011;105 Suppl 1:S13-S33.

52. Mourão CF de AB, Valiense H, Melo ER, Mourão NBMF, Maia MD-C. Obtention of injectable platelets rich-fibrin (i-PRF) and its polymerization with bone graft: technical note. *Rev Col Bras Cir*. 2015;42(6):421–3.

53. Fitch-Tewfik JL, Flaumenhaft R. Platelet granule exocytosis: a comparison with chromaffin cells. *Front Endocrinol (Lausanne)*. 2013;4:77.

54. Kahr WH. Granules and thrombus formation. *Blood*. 2009;114(5):932-933.

55. Arshdeep, Kumaran MS. Platelet-rich plasma in dermatology: boon or a bane? *Indian J Dermatol Venereol Leprol*. 2014;80(1):5–14.

56. Foster TE, Puskas BL, Mandelbaum BR, Gerhardt MB, Rodeo SA. Platelet-rich plasma: from basic science to clinical applications. *Am J Sports Med*. 2009;37(11):2259-2272.

57. Arnoczky SP, Shebani-Rad S. The Basic Science of Platelet-rich Plasma (PRP): What Clinicians Need to Know. *Sports Med Arthrosc Rev*. 2013;21(4):180–5.

58. Sánchez-González DJ, Méndez-Bolaina E, Trejo-Bahena NI. Platelet-rich plasma peptides: key for regeneration. *Int J Pept.* 2012;2012:532519.

59. Singhal P, Agarwal S, Dhot PS, Sayal SK. Efficacy of platelet-rich plasma in treatment of androgenic alopecia. *Asian J Transfus Sci*. 2015;9(2):159–62.

60. Betsi E-E, Germain E, Kalbermatten DF, Tremp M, Emmenegger V. Platelet-rich plasma injection is effective and safe for the treatment of alopecia. *Eur J Plast Surg* 2013;36(7):407-12.

61. Al-Shami SH. Treatment of Periorbital Hyperpigmentation Using Platelet-Rich Plasma Injections. *Am J Dermatol Venereol*. 2014;3(5):87–94.

62. Mehryan P, Zartab H, Rajabi A, Pazhoohi N, Firooz A. Assessment of efficacy of platelet-rich plasma (PRP) on infraorbital dark circles and crow's feet wrinkles. *J Cosmet Dermatol*. 2014;13(1):72-78.

63. Çayırlı M, Çalışkan E, Açıkgöz G, Erbil AH, Ertürk G. Regression of Melasma with Platelet-Rich Plasma Treatment. *Ann Dermatol*. 2014;26(3):401–2.

64. Garg S, Khillan K, Bharija SC. Platelet-Rich Plasma Therapy in the Treatment of Recalcitrant Melasma. *Dermatol Surg*. 2019;45(3):482-484.

65. S Satish DA, Aparna AD, Radhika VK. A clinico-epidemiological study of melasma in 402 patients in an office-based practice. *Clin Dermatol Rev*. 2019;3(2):154

66. Kumar S, Mahajan BB, Kamra N. Melasma in North Indians: A clinical, epidemiological, and etiological study. *Pigment International*. 2014;1(2):95.

67. Coban M. Quality of Life of the Patients with Melisma. *J Cosmo Trichol.* 2018;4(1000133):2.

68. Sarkar R, Jagadeesan S, Basavapura Madegowda S, et al. Clinical and epidemiologic features of melasma: a multicentric cross-sectional study from India. *Int J Dermatol*. 2019;58(11):1305-1310.

69. Hexsel D, Lacerda DA, Cavalcante AS, Machado Filho CAS, Kalil CLPV, Ayres EL, et al. Epidemiology of melasma in Brazilian patients: a multicenter study. *Int J Dermatol*. 2014;53(4):440–4.

70. Guinot C, Cheffai S, Latreille J, Dhaoui MA, Youssef S, Jaber K, et al. Aggravating factors for melasma: a prospective study in 197 Tunisian patients. *J Eur Acad Dermatol Venereol*. 2010;24(9):1060–9.

71. Charupalli K, Rajasekhar TS, Mukkara M. Clinico-epidemiological study of melasma in men. *J Clin Sci Res*. 2018;7(1):19.

72. Abdel Hay R, Mohammed FN, Sayed KS, Abd El Fattah NA, Ibrahim S. Dermoscopy as a useful tool for evaluating melasma and assessing the response to 1064-nm Q-switched Nd:YAG laser. *Dermatol Ther*. 2020;33(4):e13629.

73. Tuknayat A, Thami GP, Bhalla M, Sandhu JK. Autologous intralesional platelet rich plasma improves melasma. *Dermatol Ther*. 2021;34(2):e14881.

74. Bronzina E, Clement A, Marie B, Fook Chong KT, Faure P, Passeron T. Efficacy and tolerability on melasma of a topical cosmetic product acting on melanocytes, fibroblasts and endothelial cells: a randomized comparative trial against 4% hydroquinone. *J Eur Acad Dermatol Venereol*. 2020;34(4):897–903.

75. Pavlovic V, Ciric M, Jovanovic V, Stojanovic P. Platelet Rich Plasma: a short overview of certain bioactive components. *Open Med (Wars)*. 2016;11(1):242–7.

76. Amini F, Ramasamy T.S., Yew CH, Response to intradermal autologous platelet rich plasma injection in refractory dermal melasma: report of two cases. *J Health Trans med*. 2015;18,1-6

77. Chan NPY, Ho SGY, Shek SYN, Yeung CK, Chan HH. A case series of facial depigmentation associated with low fluence Q-switched 1,064 nm Nd:YAG laser for skin rejuvenation and melasma. *Lasers Surg Med*. 2010;42(8):712–9.

78. Uysal CA, Ertas NM. Platelet-Rich Plasma Increases Pigmentation. *J Craniofac Surg*. 2017;28(8):e793.

ANNEXURE – I

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2020/2055 Date: 01/01/2020

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2019-20/965**

Project title: **"Efficacy of platelet rich plasma therapy in patients of melasma: A single-blinded placebo controlled randomized study"**

Nature of Project:	**Research Project**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Neelam Chhajed**
Guide:	**Dr.Saurabh Singh**
Co-Guide:	**Dr.Abhishek Bhardwaj, Dr. Anil Budania, Dr.Anupama Bains & Dr.Saptarshi Mandal**

This is to inform that members of Institutional Ethics Committee (Annexure attached) met on **23-12-2019** and after through consideration accorded its approval on above project. Further, should any other methodology be used, would require separate authorization.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.
- In case of any issue related to compensation, the responsibility lies with the Investigator and Co-Investigators.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma

Member Secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Enclose:
1. Annexure 1

Page 1 of 2

ANNEXURE – II

Modified MASI Calculation

Modifed MASI total score = 0.3 × A (forehead) × D (forehead) + 0.3×A (left malar)×D (left malar) +

0.3×A (right malar)×D (right malar) + 0.1×A (chin)×D (chin)

The severity of the melasma in each of the four regions (forehead, right malar region, left malar region and chin) is assessed based on two variables: percentage of the total area involved (A) and darkness (D)

The darkness of the melasma (D) is compared to the normal skin and graded on a scale of 0 to 4 as follows:

Scoring of darkness of melasma (D)	**Scoring of area involvement**
Score 0 : Normal skin colour, no evidence of hyperpigmentation Score 1 : Barely visible hyperpigmentation, Score 2 : Mild hyperpigmentation Score 3 : Moderate hyperpigmentation Score 4 : Severe hyperpigmentation	Score 1 : < 10% area involved Score 2 : 10-29% involved Score 3 : 30-49% involved Score 4 : 50-69% involved Score 5 : 70-89% involved Score 6 : 90-100% involved

ANNEXURE – III

MELASMA SEVERITY INDEX CALCULATION (MSI)[19]

Scoring of Pigmentation	**Scoring of area involvement**
Score 0 : No visible pigmentation Score 1 : Barely visible pigmentation, Score 2 : Mild Pigmentation Score 3 : Moderate Pigmentation Score 4 : Severe Pigmentation	Score 1 : < 10% area involved Score 2 : 11-30% involved Score 3 : 31-60% involved Score 4 : > 60% involved

MSI = 0.4 (a x p^2) l + 0.4 (a x p^2) r + 0.42(a x p^2) n

'a' stands for 'area of involvement' , 'p' for 'severity of pigmentation', 'l' for left half of face, 'r' for right half of face and 'n' for nose.

ANNEXURE – IV

MELASQOL

English Melasma quality of life scale (MELASQOL) *	Hindi Melasma quality of life scale (MELASQOL) †	Hindi Melasma quality of life scale (Hi-MELASQOL) †
The appearance of your skin condition	आपकी झाइया / चेहरे के दाग दिखने में कैसे लगते हैं	Aapki jhaaiyan/chehre ke daag dikhne mein kaise lagte hain
Frustration about your skin condition	क्या आप अपनी झाइया / चेहरे के दाग के बारे में कठिनाई अनुभव करते हैं	Kya aap apni jhaaiyan/chehre ke daag ke bare mein kathinaai anubhav karte hain
Embarrassment about your skin condition	क्या आप अपनी झाइयों से लज्जित अनुभव करते हैं	Kya aap apni jhaaiyon se lajjit anubhav karte hain
Feeling depressed about your skin condition	क्या आप अपनी झाइयों के बारे में उदासी महसूस करते हैं	Kya aap apni jhaaiyon ke baarey mein udaasi mahsoos karte hain
The effects of your skin condition on your interaction with other people (e.g. interaction with family, friends, close relationship etc.)	क्या आपकी झाइया लोगों से (जैसे परिवार, मित्रों, सगे-सम्बंधियों, पारस्परिक प्रक्रिया, इत्यादि) व्यवहार करते समय प्रभाव डालती हैं	Kya aapki jhaaiyan logon se (jaisey parivaar, mitron, sagey-sambandhiyon, paarasparik prakriya, ityaadi) vyavahar karte samay prabhaav daalti hain
The effects of your condition on your desire to be with people	क्या आपकी झाइया लोगों के साथ मेलजोल पर प्रभाव डालती हैं	Kya aapki jhaaiyan logon ke saath meljol par prabhaav daalta hain
Your skin condition making it hard to show affection	क्या आपकी झाइया प्यार प्रदर्शन करते समय मुश्किल बना देती हैं	Kya aapki jhaaiyan pyaar pradarshan karte samay mushkil banadeti hain
Skin discoloration making you feel unattractive to others	बदरंग त्वचा के कारण आप लोगों से स्वयं को अनाकर्षक महसूस करते हैं	Badrang twacha ke kaaran aap logon se swayamko anaakarshak mahsoos karte hain
Skin discoloration making you feel less vital or productive	बदरंग त्वचा के कारण आप अपने को कम उत्पादक महसूस करते हैं	Badrang twacha ke kaaran aap apneko kam utpaadak mahsoos karte hain
Skin discoloration affecting your sense of freedom	बदरंग त्वचा आप की आज़ादी और आत्मविश्वास की भावना को प्रभावित करता है	Badrang twacha aap ki aazaadi aur aatmavishwas ki bhaavna ko prabhaavit karta hai

*Each question was graded by the patient on a Likert scale of 1 (not bothered at all) to 7 (bothered all the time).The score of MELASQOL ranges from 7 to 70. A higher score indicates worse melasma-related health-related quality of life.

† मापक्रम १ (में बिल्कुल भी चिंता नहीं करते) से ७ के मापक्रम (हमेशा चिंता करते हैं) को विषय दर, बारे में कैसा महसूस करते हैं? १ -बिल्कुल भी चिंता नहीं करते, २ - बहुत कम, ३ - कुछ अक्सर, ४ - कभी कभी, ५ -थोड़ा कुछ, ६ - बहुत कुछ, ७ - हमेशा चिंता करते हैं. मेलास्कोल के ७ से ७० तक आंके

ANNEXURE –V
Proforma

Serial No: AIIMS ID:

Patient Name: Phone no :

Age/ Sex: Education:

Father's name:

Occupation:

Chief Complains:

History:

Drug History :

Past History:

DM/ HTN/ TB/Epilepsy/Asthma :

K/C/O thyroid disease ?

Any co-morbidities :

Menstrual History :

Obstretric History:

Occupational History:

1. Exposure to sunlight: Yes/No
2. Duration of Exposure
3. Type of exposure: intermittent/continuous/seasonal
4. Usage of sunscreen : Yes/No

Systemic Symptoms : If Any

General Examination:

Pallor – Icterus – Cyanosis – Clubbing – Lymphadenopathy – Pedal oedema –

Cutaneous Examination:

Fitzpatrick skin type:

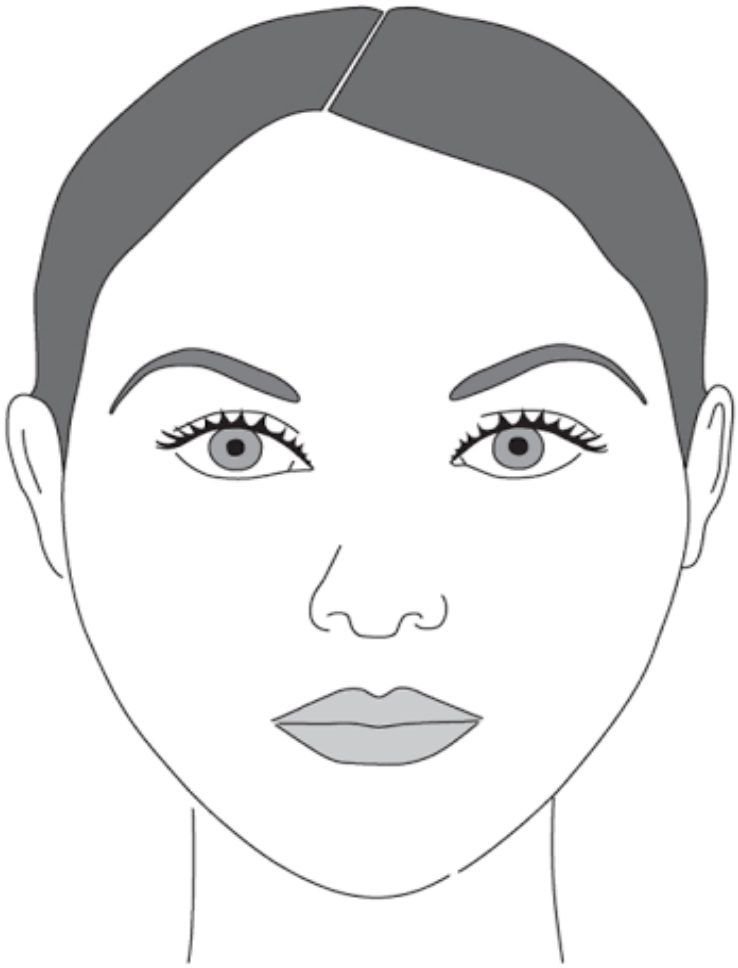
Colour of Melasma pigmentation:

1. Pattern of involvement
   Centrofacial (cheeks, forehead, upper lips, nose and chin)
   Malar (cheeks and nose)
   Mandibular(ramus of mandile)

2. Colour
   Light brown
   Bluish grey
   Dark Brown

3. Wood's lamp examination
   Enhancement seen
   No enhancement seen
   Patchy enhancement seen

4. Dermoscopic findings:



Diagnosis:

Treatment:

FOLLOW UP

Weeks	**0**	**2**	**4**	**8**	**12**	**16**
mMASI						
MSI						
IGA						
MELASQOL						
WOODS LAMP						
DERMOSCOPY						

MELASQOL

English Melasma quality of life scale (MELASQOL) *	Hindi Melasma quality of life scale (MELASQOL) †	Hindi Melasma quality of life scale (Hi-MELASQOL) †
The appearance of your skin condition	आपकी झाइया / चेहरे के दाग दिखने में कैसे लगते हैं	Aapki jhaaiyan/chehre ke daag dikhne mein kaise lagte hain
Frustration about your skin condition	क्या आप अपनी झाइया / चेहरे के दाग के बारे में कठिनाई अनुभव करते हैं	Kya aap apni jhaaiyan/chehre ke daag ke bare mein kathinaai anubhav karte hain
Embarrassment about your skin condition	क्या आप अपनी झाइयों से लज्जित अनुभव करते हैं	Kya aap apni jhaaiyon se lajjit anubhav karte hain
Feeling depressed about your skin condition	क्या आप अपनी झाइयों के बारे में उदासी महसूस करते हैं	Kya aap apni jhaaiyon ke baarey mein udaasi mahsoos karte hain
The effects of your skin condition on your interaction with other people (e.g. interaction with family, friends, close relationship etc.)	क्या आपकी झाइया लोगों से (जैसे परिवार, मित्रों, सगे-सम्बंधियों, पारस्परिक प्रक्रिया, इत्यादि) व्यवहार करते समय प्रभाव डालती हैं	Kya aapki jhaaiyan logon se (jaisey parivaar, mitron, sagey-sambandhiyon, paarasparik prakriya, ityaadi) vyavahar karte samay prabhaav daalti hain
The effects of your condition on your desire to be with people	क्या आपकी झाइया लोगों के साथ मेलजोल पर प्रभाव डालती हैं	Kya aapki jhaaiyan logon ke saath meljol par prabhaav daalta hain
Your skin condition making it hard to show affection	क्या आपकी झाइया प्यार प्रदर्शन करते समय मुश्किल बना देती हैं	Kya aapki jhaaiyan pyaar pradarshan karte samay mushkil banadeti hain
Skin discoloration making you feel unattractive to others	बदरंग त्वचा के कारण आप लोगों से स्वयं को अनाकर्षक महसूस करते हैं	Badrang twacha ke kaaran aap logon se swayamko anaakarshak mahsoos karte hain
Skin discoloration making you feel less vital or productive	बदरंग त्वचा के कारण आप अपने को कम उत्पादक महसूस करते हैं	Badrang twacha ke kaaran aap apneko kam utpaadak mahsoos karte hain
Skin discoloration affecting your sense of freedom	बदरंग त्वचा आप की आज़ादी और आत्मविश्वास की भावना को प्रभावित करता है	Badrang twacha aap ki aazaadi aur aatmavishwas ki bhaavna ko prabhaavit karta hai

*Each question was graded by the patient on a Likert scale of 1 (not bothered at all) to 7 (bothered all the time).The score of MELASQOL ranges from 7 to 70. A higher score indicates worse melasma-related health-related quality of life.

† मापक्रम १ (में बिल्कुल भी चिंता नहीं करते) से ७ के मापक्रम (हमेशा चिंता करते हैं) को विषय दर, बारे में कैसा महसूस करते हैं? १ -बिल्कुल भी चिंता नहीं करते, २ - बहुत कम, ३ - कुछ अक्सर, ४ - कभी कभी, ५ -थोड़ा कुछ, ६ - बहुत कुछ, ७ - हमेशा चिंता करते हैं. मेलास्कोल के ७ से ७० तक आंके

ANNEXURE – VI

Informed Consent Form

Title of Thesis/ Dissertation: **Efficacy of Platelet Rich Plasma Therapy in patients of Melasma : A single-blinded placebo controlled randomized study**

Name of PG Student: **Dr. Neelam Chhajed** Tel. No. **9819093184**

Patient/ Volunteer Identification No.: _____

I, _____ S/o or D/o _____

R/o _____

give my full, free, voluntary consent to be a part of the study "**Efficacy of Platelet Rich Plasma Therapy in patients of Melasma : single-blinded placebo Randomized control study"**, the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and I am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individuals or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression

(If minor, Parent/Guardian signature)

This to certify that the above consent has been obtained in my presence.

Date: _____ _____

Place: _____ Signature of PG Student

1. Witness 1

_____

Signature

Name: _____

Address: _____

_____

2. Witness 2

_____

Signature

Name: _____

Address: _____

_____

ANNEXURE – VII

अखिल भारतीय चिकित्सा विज्ञान संस्थान

जोधपुर, राजस्थान

सूचित सहमति प्रपत्र

थीसिस का शीर्षक: **"मेल्स्मा के रोगियों में प्लेटलेट रिच प्लाज्मा थेरेपी की प्रभावकारिता : अ सिंगल ब्लायंडेड प्लसीबो कंट्रोल्ड रैंडमायज़्ड स्टडी"**

पीजी छात्र का नाम: डॉ. नीलम छाजेड़ मोबाइल नंबर: **9819093184**

रोगी / स्वयंसेवी पहचान संख्या:_____

मैं, _____ एस / ओयाडी / ओ _____

आर / ओ _____

उपरोक्त अध्ययन **"मेल्स्मा के रोगियों में प्लेटलेट रिच प्लाज्मा थेरेपी की प्रभावकारिता : अ सिंगल ब्लायंडेड प्लसीबो कंट्रोल्ड रैंडमायज़्ड स्टडी"** का एक हिस्सा बनने के लिए मेरी पूर्ण स्वतंत्र, स्वैच्छिक सहमति देता हूँ।

जिस प्रक्रियाऔर प्रकृति को मुझेअपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाया गया है मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसी भी कारण दिए बिना किसी भी समय अध्ययन से बाहर निकलनेका मेराअधिकार है।

मैं समझता हूं कि मेरेऔर मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी को याविनियामक प्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखा जा सकता है।मैं इनलोगों के लिए मेरे रिकॉर्डों तक पहुंचकी अनुमति देता हूं।

तारीख : _____ _____

जगह: _____ हस्ताक्षर / बाएंअंगूठेकाछाप

(नाबालिगकि, माता-पिता / अभिभावक हस्ताक्षर)

यह प्रमाणित करने के लिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई है

तारीख : _____

जगह: _____ पीजी छात्र के हस्ताक्षर

1. गवाह 1 2. साक्षी 2

_____ _____

हस्ताक्षर हस्ताक्षर

नाम: _____ नाम: _____

पता: _____ पता: _____

ANNEXURE – VIII

All India Institute of Medical Sciences Jodhpur, Rajasthan

PATIENT INFORMATION SHEET (PIS)

This document has been given to provide more information about the disease and this research related to melasma

The current research project is titled: **"Efficacy of Platelet Rich Plasma Therapy in patients of Melasma : Single-blinded placebo control study "**

Melasma is an acquired disease of sun- exposed skin. It presents as irregular hyperpigmented patches most commonly seen on cheeks, upper lips, the chin, and the forehead. It is the most common pigmentary disorder among Indians. Main risk factor is sun-light and hormones. Various treatment modalities are available like topical creams, oral drugs, chemical peels, injections etc . But the main concern of this disease is recurrence.

The basic goal of this research is to search for a better modality to treat and prolong recurrence free period and thereby playing a role in guiding medication.

The patient is also informed that all the information given by him will be kept confidential. The patient also reserves the right that during this research, patient can withdraw the consent & can be out of this research without explaining the reasons.

Principle investigator: Dr. Neelam Chhajed Contact number: 9819093184

ANNEXURE – IX

अखिल भारतीय चिकित्सा विज्ञान संस्थान
जोधपुर, राजस्थान

रोगी सूचना पत्रक

यह दस्तावेज मेलास्मा रोग के बारे में अधिक जानकारी प्रदान करने के लिए दिया गया है और से संबंधित यह शोध प्रदान किया गया है।

वर्तमान शोध परियोजना का शीर्षक है: **"मेल्स्मा के रोगियों में प्लेटलेट रिच प्लाज्मा थेरेपी की प्रभावकारिता : अ सिंगल ब्लायंडेड प्लसीबो कंट्रोल्ड रैंडमायज़्ड स्टडी"**

मेलास्मा सूर्य के संपर्क में आने वाली त्वचा की एक बीमारी है। यह अनियमित हाइपरपिगमेंटेड पैच के रूप में प्रस्तुत करता है जो आमतौर पर गाल, ऊपरी होंठ, ठोड़ी और माथे पर देखा जाता है।यह भारतीयों में सबसे आम वर्णक बीमारी है। मुख्य जोखिम कारक सूर्य प्रकाश और हार्मोन है। विभिन्न उपचार तौर-तरीके सामयिक क्रीम, मौखिक दवाएं, रासायनिक छिलके, इंजेक्शन जैसे उपलब्ध है। लेकिन इस बीमारी की मुख्य चिंता पुनरावृत्ति है।

इस शोध का मूल लक्ष्य उपचार और पुनरावृत्ति मुक्त अवधि को लम्बा खींचने के लिए बेहतर तरीके से खोज करना है और इस तरह से मार्गदर्शक दवा की भूमिका निभानी है।

रोगी को यह भी सूचित किया जाता है कि उसके द्वारा दी गई सारी जानकारी गोपनीय रखी जाएगी। रोगी यह भी अधिकार सुरक्षित रखता है कि इस शोध के दौरान, रोगी सहमति वापस ले सकता है और कारणों को बताने के बिना इस शोध से बाहर हो सकता है।

प्रमुख जांचकर्ता: डॉ नीलम छाजेड़ संपर्क संख्या: 9819093184