



Preliminary Evaluation of Calcium-Activated Platelet-Rich Plasma for the Treatment of Facial Melasma: A Single-Arm Pilot Study

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Abstract

Melasma is a chronic facial hyperpigmentation disorder influenced by genetic susceptibility, hormonal factors, and ultraviolet exposure. Growing evidence suggests that platelet-rich plasma (PRP) may be beneficial for the treatment of melasma through its reservoir of growth factors, particularly transforming growth factor- β (TGF- β), which regulates melanogenesis and promotes dermal repair. Calcium activation enhances platelet degranulation and accelerates the release of bioactive mediators, potentially augmenting the therapeutic efficacy of PRP. This prospective single-arm pilot study without a control group aimed to evaluate the clinical outcomes and safety of calcium-activated platelet-rich plasma (Ca-PRP) in Thai patients with facial melasma. A total of 10 participants meeting the inclusion criteria of Fitzpatrick skin types III–V, stable melasma involving the cheek area, and no prior melasma treatment for at least six months were enrolled. Participants received three sessions of intradermal Ca-PRP injections administered at four-week intervals. Clinical and instrumental assessments were conducted at baseline and at weeks 4, 8, and 12. Clinical severity was evaluated using the modified Melasma Area and Severity Index (mMASI), while objective pigmentation and erythema were assessed using the Mexameter-derived melanin index (MI) and erythema index (EI), respectively.

The mean mMASI score decreased from 8.5 ± 4.62 at baseline to 6.7 ± 3.06 at week 12, corresponding to a 15.8% reduction from baseline. The reduction was statistically significant at all follow-up visits ($p < 0.05$). Instrumental assessment demonstrated a gradual decrease in MI, reaching statistical significance at week 12, while EI showed a significant reduction at week 8. Ca-PRP treatment was well tolerated, and no serious adverse events were observed during the study period. These findings suggest that Ca-PRP may represent a promising regenerative therapeutic approach for the management of facial melasma. However, given the pilot nature of this single-arm study, further randomized controlled trials with larger sample sizes are required to confirm these preliminary findings and to clarify the underlying mechanisms.

Keywords: Calcium-activated PRP, Platelet-rich plasma, Melasma, Hyperpigmentation, Growth factors

1. Introduction

Melasma is a chronic hyperpigmentation disorder that primarily affects the face and often causes considerable psychosocial distress. Although traditionally regarded as a disorder of excessive melanin production, current understanding recognizes melasma as a multifaceted condition influenced by genetic predisposition, hormonal factors, and chronic ultraviolet exposure (Handel et al., 2014). Beyond melanocyte hyperactivity, alterations within the epidermal-dermal unit, including vascular changes, inflammatory processes, and photoaging-related damage, appear to play an important role in disease persistence and frequent relapse (Kang & Ortonne, 2010; Passeron, 2012; Passeron & Picardo, 2018; Kwon et al., 2016). Recent evidence further emphasizes the complex interplay between melanocytes, keratinocytes, and dermal factors in the pathogenesis of melasma (Lee, 2015).

Previous reviews and clinical investigations have explored regenerative approaches as adjunctive strategies for melasma management. PRP, an autologous concentration of platelets containing multiple bioactive growth factors and cytokines, has been increasingly used in dermatology for its roles in tissue repair, angiogenesis, and modulation of cellular communication. Several pilot studies and split-face trials have reported clinical improvement in melasma severity following intradermal PRP administration, as reflected by reductions in MASI scores and favorable safety outcomes (Sirithanabadeekul et al., 2019; Hofny et al., 2019; Tuknayyat et al., 2021; Abd Elraouf et al., 2023). Systematic reviews further suggest that PRP may offer



therapeutic benefit in melasma; however, the overall quality of evidence remains limited by heterogeneity in study design and treatment protocols (Sarkar & Gupta, 2022; Alshammari et al., 2024).

Despite encouraging results, important challenges remain. Reported clinical responses to PRP vary substantially, which may be partly explained by differences in PRP preparation methods, platelet concentration, delivery techniques, and activation protocols. Experimental studies indicate that platelet activation is a critical determinant of growth factor release kinetics and biological activity, with calcium-based activation promoting a more sustained release of bioactive molecules compared with non-activated preparations (Landesberg et al., 2000; Cavallo et al., 2016; Borghese et al., 2016). However, clinical data specifically examining calcium-activated PRP (Ca-PRP) in melasma are scarce. Addressing this gap may provide clearer insight into whether optimization of PRP activation can enhance clinical outcomes and improve the consistency of therapeutic responses in melasma treatment.

Calcium gluconate is commonly used as an activating agent in PRP preparation. The addition of calcium ions initiates the coagulation cascade and triggers platelet activation, leading to the degranulation of platelet α -granules and the subsequent release of growth factors such as transforming growth factor- β (TGF- β), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). These mediators may influence melanogenesis, dermal remodeling, and inflammatory pathways implicated in melasma pathophysiology. In contrast, non-activated PRP relies primarily on physiological activation within the tissue following injection, which may result in slower and less controlled release of these bioactive factors (Margono et al., 2022).

However, clinical data specifically evaluating the role of calcium-activated PRP (Ca-PRP) in melasma remain limited. Therefore, the present study aimed to evaluate the longitudinal clinical outcomes and safety of Ca-PRP in patients with facial melasma by assessing progressive changes in the modified Melasma Area and Severity Index (mMASI) scores, as well as objective pigmentation parameters, over a 12-week follow-up period. We hypothesized that Ca-PRP treatment would result in progressive improvement in melasma severity through modulation of melanogenesis and dermal repair mechanisms.

2. Objectives

1. To evaluate the longitudinal therapeutic efficacy of Ca-PRP for facial melasma affecting the cheek area by assessing progressive changes in mMASI scores at 4-week intervals from baseline through week 12.
2. To quantify objective changes in skin pigmentation and erythema following Ca-PRP treatment using Mexameter-derived melanin index (MI) and erythema index (EI) at baseline and at weeks 4, 8, and 12.
3. To evaluate the safety and tolerability of Ca-PRP treatment in patients with facial melasma throughout the study period.

3. Materials and Methods

3.1 Study Design

This study was designed as a prospective single-arm pilot clinical study to evaluate the clinical outcomes of calcium-activated platelet-rich plasma (Ca-PRP) in patients with facial melasma. Participants received Ca-PRP injections across the entire affected area without comparison to non-activated PRP or other treatment modalities. Clinical assessments were performed at baseline and at each follow-up visit.

3.2 Participants

Ten participants aged 18–60 years, both male and female, with clinically diagnosed epidermal or mixed-type melasma were enrolled. As this investigation was designed as a pilot study to explore preliminary clinical outcomes and feasibility, a formal sample size calculation was not performed. A sample size of 10 participants was considered adequate for this exploratory pilot investigation.

Inclusion criteria were as follows:

- (1) Fitzpatrick skin type III–V.
- (2) Stable melasma at the cheek area; and
- (3) No prior treatment for at least 6 months.



The exclusion criteria were as follow:

- (1) Pregnancy or lactation.
- (2) Active facial infection or inflammatory dermatoses.
- (3) Bleeding disorders or anticoagulant use.
- (4) History of keloid formation.
- (5) Other severe systemic disease.
- (6) Use of hormonal contraceptives within 12 months prior to enrollment or during the study period.
- (7) Receipt of any laser- or energy-based treatment for melasma within 6 months prior to enrollment;

and

(8) Use of topical agents or injectable therapies aimed at reducing pigmentation within 3 months prior to enrollment. Written informed consent was obtained before enrollment.

3.3 PRP Preparation

Ten milliliters of venous blood were processed using a standardized double-spin centrifugation protocol to concentrate platelets, consisting of sequential soft and hard spins as previously described (Marx et al., 1998; Ehrenfest et al., 2009). After centrifugation, approximately 2 mL of PRP was obtained. All preparation procedures were performed under sterile conditions to minimize the risk of contamination.

Platelet activation was achieved by adding 0.2 mL of calcium gluconate to the PRP, followed by gentle mixing and an activation period of five minutes at room temperature (approximately 25 °C). The activated PRP was injected intradermally at approximately 1-2 mm depth using a 32-gauge needle immediately after activation. A fixed total volume of 2 mL of Ca-PRP was administered per treatment session for all participants.

The final platelet concentration relative to baseline whole blood platelet count was not quantified in this study. However, PRP preparation was performed using a standardized double-centrifugation protocol to ensure procedural consistency across all samples.

3.4 Injection Technique and Study Protocol

After applying topical anesthesia for 30 minutes and using a 32-gauge needle, a total volume of 2 mL of Ca-PRP per session was administered intradermally at approximately 1-cm intervals throughout the melasma-affected regions. Each participant underwent three treatment sessions at 4-week intervals and was instructed to avoid excessive sun exposure throughout the study period by using daily sunscreen with an SPF ≥ 50 .

3.5 Outcome Measures

Outcome evaluation included both clinical and instrumental assessments. The primary outcome was the change in mMASI scores from baseline to each follow-up visit. Secondary outcomes included changes in MI and EI measured using the Mexameter device, as well as treatment safety assessed by procedure-related adverse events.

Clinical severity was assessed using the mMASI at baseline and at each follow-up visit to evaluate changes in pigmentation intensity and lesion extent the mMASI assessment was performed using a site-specific, unweighted scoring method. Specifically, only the area (A) and darkness (D) components of a single, preselected cheek were evaluated. Scores from other facial regions, including the forehead, contralateral cheek, and chin, were not included, and regional weighting factors were intentionally omitted, as they are designed for whole-face severity assessment rather than localized lesion evaluation. This approach was selected to enable consistent longitudinal assessment at a single anatomical site and to maintain methodological alignment with objective instrumental measurements. All mMASI assessments were performed by an independent dermatologist who was not involved in the treatment procedures to minimize potential assessment bias. The evaluator was blinded to the previous scores during follow-up visits.

Objective pigmentation and erythema were further assessed using the Mexameter-derived MI and EI, which provide quantitative measures of melanin content and vascular-related skin erythema at



standardized cheek sites using a Mexameter device (Mexameter MX18, Courage + Khazaka, Cologne, Germany).

Safety was monitored throughout the study by recording procedure-related adverse events, including pain, erythema, edema, bruising, post-inflammatory hyperpigmentation, and signs of infection. All outcome measures, including mMASI, MI, and EI, were assessed longitudinally at the participant level, with each participant contributing a single dataset based on the cheek-area evaluation.

3.6 Statistical Analysis

The authors hypothesized that treatment with calcium-activated platelet-rich plasma (Ca-PRP) would result in clinical improvement in melasma over time. Descriptive statistics were used to summarize demographic characteristics. Longitudinal analyses were performed to evaluate changes in MI, EI, and mMASI at different follow-up time points compared with baseline.

All statistical analyses were conducted at the participant level, with each participant contributing a single dataset based on the cheek-area assessment. As the outcome variables were continuous, repeated-measures analysis of variance (ANOVA) was applied, using baseline values as the reference. Mean differences and corresponding 95% confidence intervals were calculated for each follow-up visit. Statistical analyses were performed using SPSS software, with a two-sided p-value of less than 0.05 considered statistically significant.

4. Results and Discussion

4.1 Results

Baseline Demographic Characteristics

Baseline demographic and clinical characteristics of the study participants are summarized in Table 1. The study included ten patients with melasma, predominantly female (70%), with a mean age of 46.7 ± 6.57 years (range, 35–55 years). Most participants reported no underlying disease and no regular medication use (both 80%). The mean duration of melasma was 7.9 ± 3.67 years, ranging from 3 to 15 years. A family history of melasma was reported in 60% of participants. Regular sunscreen use was reported by the majority of patients (90%), with only one participant reporting no routine use. Regarding skin phototype, most participants had Fitzpatrick skin phototype IV (50%), followed by phototype III (30%) and phototype V (20%). All participants were classified as having mixed-type melasma.

Table 1 Baseline demographic and clinical characteristics of the study participants (N = 10)

Characteristic	Category	Value (%) N = 10
Sex	Female	7 (70)
	Male	3 (30)
Age (years)	Mean \pm SD	46.7 ± 6.57
	Range	35–55
Underlying disease	No	8 (80)
	Yes	2 (20)
Current medication	No	8 (80)
	Yes	2 (20)
Duration of melasma (years)	Mean \pm SD	7.9 ± 3.67
	Range	3–15
Family history of melasma	No	4 (40)
	Yes	6 (60)
Sunscreen use	No	1 (10)
	Daily use	9 (90)
Fitzpatrick skin phototype	III	3 (30)
	IV	5 (50)
	V	2 (20)
Melasma type	Mixed	10 (100)

Data are presented as mean \pm standard deviation or number (percentage), as appropriate.



Measurement Reliability

The intra-observer reliability of instrumental measurements was summarized in Table 2. The assessment demonstrated excellent reliability for both parameters. The intraclass correlation coefficient (ICC) for the melanin index (MI) was 0.980 (95% CI, 0.966–0.989), while the ICC for the erythema index (EI) was 0.963 (95% CI, 0.938–0.979). Both measures showed statistically significant reliability ($p < 0.001$), indicating high consistency and reproducibility of Mexameter-based assessments across repeated evaluations by the same observer.

Table 2 Intra-observer reliability of Melanin Index (MI) and Erythema Index (EI)

Parameter	Intraclass Correlation Coefficient (ICC)	95% Confidence Interval		p-value
Melanin Index (MI)	0.980	0.966	0.989	< 0.001*
Erythema Index (EI)	0.963	0.938	0.979	< 0.001*

Table 3 Longitudinal changes in mMASI, MI, and EI during 12-week follow-up after treatment (N = 10)

Outcome measure	Time point	Mean \pm SD	Mean differences (95%CI)	p-value
Melanin Index (MI)	Baseline	297.77 \pm 64.92	Reference	–
	4 weeks	293.7 \pm 45.33	-4.07 (-21.29, 13.15)	0.643
	8 weeks	288.03 \pm 50.42	-9.73 (-23.27, 3.8)	0.159
	12 weeks	284.7 \pm 53.6	-13.07 (-25.84, -0.3)	0.045*
Erythema Index (EI)	Baseline	378.37 \pm 71.71	Reference	–
	4 weeks	353.67 \pm 68.29	-24.7 (-50.49, 1.09)	0.06
	8 weeks	355.87 \pm 72.09	-22.5 (-35.19, -9.81)	0.001*
	12 weeks	369.5 \pm 74.74	-8.87 (-36.31, 18.58)	0.527
mMASI score	Baseline	8.5 \pm 4.62	Reference	–
	4 weeks	7.1 \pm 3.78	-1.4 (-2.54, -0.26)	0.016*
	8 weeks	7 \pm 3.3	-1.5 (-2.75, -0.25)	0.019*
	12 weeks	6.7 \pm 3.06	-1.8 (-3.07, -0.53)	0.005*

Data are presented as mean \pm standard deviation. Mean differences and 95% confidence intervals were derived from repeated-measures ANOVA with baseline as the reference.

* $p < 0.05$ indicates statistical significance.

Longitudinal Clinical and Instrumental Outcomes

Longitudinal analysis of instrumental and clinical outcomes over the 12-week follow-up period was summarized in Table 3.

Melanin index (MI) demonstrated a gradual decrease following treatment. No statistically significant changes were observed at weeks 4 and 8 compared with baseline. However, a statistically significant reduction in MI was observed at week 12 (mean difference -13.07 ; 95% CI -25.84 to -0.30 ; $p = 0.045$).

Erythema index (EI) showed a statistically significant reduction from baseline at week 8 (mean difference -22.50 ; 95% CI -35.19 to -9.81 ; $p = 0.001$), whereas changes at weeks 4 and 12 were not statistically significant.

Clinical severity assessed using the modified Melasma Area and Severity Index (mMASI) decreased over time. Statistically significant reductions in mMASI scores were observed at weeks 4, 8, and 12 compared with baseline ($p = 0.016$, 0.019 , and 0.005 , respectively).

No serious adverse events were observed during the study period. No cases of persistent erythema or swelling were reported. Mild transient ecchymosis at the injection site was observed in 1 participant and resolved spontaneously without intervention.



Figure 1 Clinical photographs of a patient with Fitzpatrick skin type V showing changes in melasma area and pigmentation severity on the cheek at baseline, week 4, week 8, and week 12 following treatment with Ca-PRP.

4.2 Discussion

This prospective pilot study evaluated the clinical outcomes and safety of Ca-PRP in Thai patients with facial melasma using both clinical and instrumental assessments. The findings demonstrated a progressive reduction in clinical severity as assessed by the mMASI, accompanied by favorable trends in objective instrumental parameters during the 12-week follow-up period. The statistically significant reduction in mMASI scores observed at weeks 4, 8, and 12 indicated progressive improvement in clinical severity following Ca-PRP treatment. In contrast, objective pigmentation assessed by MI showed a more gradual response, reaching statistical significance only at week 12. This temporal difference may reflect the biological processes underlying melasma improvement, as modulation of melanocyte activity and dermal remodeling may require time before measurable reductions in melanin content become apparent. EI demonstrated a significant reduction at week 8, suggesting a potential early effect of Ca-PRP on the vascular or inflammatory components of melasma. However, this change was not sustained at the final follow-up visit, which may reflect the transient nature of vascular modulation or interindividual variability, particularly in mixed-type melasma. The differing temporal responses between clinical and instrumental outcomes highlight the multifactorial pathophysiology of melasma. Clinical improvement may occur before detectable changes in objective pigmentation measurements, reflecting combined effects on epidermal pigmentation, dermal remodeling, and inflammatory pathways.

These findings are broadly consistent with previous studies investigating platelet-rich plasma in melasma treatment. Abd Elraouf et al. (2023) reported significant improvement in MASI scores following intradermal PRP injections. In the present study, improvement in mMASI scores was observed as early as week 4, suggesting a potential early clinical response following Ca-PRP treatment. Calcium activation may enhance platelet degranulation and facilitate the release of growth factors stored within platelet α -granules, including transforming growth factor- β , platelet-derived growth factor, and vascular endothelial growth factor, which may contribute to modulation of melanogenesis and dermal repair.

Although statistically significant improvement was observed, the findings should be interpreted cautiously. Melasma severity may fluctuate over time and can also be influenced by external factors such as ultraviolet exposure and photoprotection practices. Therefore, the observed improvement cannot be attributed solely to the intervention in the absence of a comparator group.

Several limitations should be acknowledged. The study was conducted with a small sample size, a single-arm design, and a relatively short follow-up period. In addition, the absence of a control group limits the ability to determine whether the observed improvement was attributable specifically to Ca-PRP treatment.

Further randomized controlled trials with larger sample sizes and longer follow-up durations are warranted to confirm these findings. In particular, comparative studies evaluating calcium-activated PRP versus standard conventional PRP may help clarify whether calcium activation provides additional therapeutic benefit in the management of melasma.



5. Conclusion

In this pilot study, calcium-activated platelet-rich plasma (Ca-PRP) demonstrated favorable clinical outcomes in patients with facial melasma. Significant and progressive improvement in clinical severity was observed as assessed using mMASI throughout the follow-up period. Objective instrumental assessment revealed a delayed but statistically significant reduction in melanin index, while erythema index showed an earlier transient improvement.

Ca-PRP was well tolerated, with no serious adverse events reported, supporting its short-term safety profile. These findings suggest that Ca-PRP may offer a potential regenerative-based therapeutic option for melasma management. However, given the limitations of this single-arm pilot study, including the small sample size and short follow-up duration, further randomized controlled trials with larger sample sizes and extended follow-up are required to confirm efficacy and to optimize treatment protocols.

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7. References

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