Original Article

Combination of Plasma Rich in Growth Factors With Topical 4% Hydroquinone Compared With Topical 4% Hydroquinone Alone in the Treatment of Dermal Type of Melasma: A Single-Blinded Randomized Split-Face Study

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Abstract

Background: Response to the current available treatments of melasma, dermal type, in particular, is usually gradual and can result in possible side effects. **Aim and Objectives:** In this study, we aim to evaluate the efficacy of the combination of plasma rich in growth factors (PRGF) and topical 4% hydroquinone (HQ) in comparison with monotherapy using topical 4% HQ alone in the treatment of dermal type of melasma. **Materials and Methods:** This is a single-blinded, randomized, split-face clinical trial on twenty female patients with dermal type of melasma. Patients were asked to apply topical 4% HQ on both sides of their face at night for 6 months. In each participant, one side of the face was randomly chosen to receive monthly intradermal injections of PRGF for 3 sessions. Efficacy of the treatment was assessed using hemi melasma area and severity index (MASI) score, physician's global assessment (PGA), and patients' global assessment (PtGA). **Results:** Both groups revealed significant improvement in hemi-MASI score during the treatment course. Mean percentage of improvement at the end of study was 40.38 ± 6.04% and 33.42 ± 3.23% in the combination therapy and monotherapy groups, respectively (P = 0.31). PGA demonstrated excellent-to-marked improvement in melasma in 25% and 5% of patients in the combination therapy and monotherapy groups, respectively (P = 0.31). PtGA showed high levels of satisfaction in 15% of patients in the combination therapy group (vs. 0% in the monotherapy group) (P = 0.05). **Conclusion:** Differences between the two treatment groups in terms of hemi-MASI and PGA scores were not statistically significant; however, patients demonstrated higher satisfaction with combination of PRGF and topical 4% HQ compared with topical HQ alone. Thereby, combination of PRGF and topical 4% HQ can be suggested as a safe alternative therapeutic approach and may hold promise in the development of future therapeutic options for dermal type of melasma.

Keywords: *Autologous-based materials, biomaterial, hydroquinone, melasma, plasma rich in growth factors*

Introduction

Melasma is a disease characterized by the development of brown-to-gray patches in sun-exposed areas of the skin. Current evidence shows that subjects with melasma have hyperactive melanocytes with large dendritic processes that transfer melanin to nearby keratinocytes. Exposure to sunlight stimulates fibroblasts to produce various cytokines, such as stem cell factor (SCF) and hepatocyte growth factor (HGF). Sun exposure can also inhibit production of transforming growth factor-beta (TGF-β) in keratinocytes and fibroblasts and consequently may cause skin hyperpigmentation. Moreover, sun exposure may upregulate matrix metalloproteinase (MMP) that leads to the disruption and vacuolization of basal layer resulting in melanin incontinence and dermal pigmentation. In addition, increased mast cells and degranulation of histamines can stimulate melanocytes to proliferate, migrate, and produce more melanin.[1]

Platelet-rich plasma (PRP) is an autologous platelet-based material that contains high concentrations of platelets (3-7 times higher than their concentration in the blood circulation). PRP can release various types of growth factors through α-granules

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of platelets including epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-I), platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF-β).[2-5] Plasma rich in growth factors (PRGF) is another plasma-derived product that differs from PRP in several aspects. First, in the process of obtaining PRGF, calcium chloride is used as an anticoagulant instead of thrombin, thus forming a fibrinous mesh with gelatinous consistency. This property can lead to a more gradual release of growth factors in PRGF compared with PRP. PRGF may also contain regenerative cells as well as coagulative and bioactive proteins that will help skin regeneration and rejuvenation. In addition, in contrast to PRP, PRGF does not contain leukocytes, and as a result, it lacks the proinflammatory enzymes (such as proteases and hydrolases).[2,6]

Nowadays, there are various types of therapeutic modalities for the treatment of melasma including different types of lightening agents and energy-based devices. Nonetheless, response to the treatment is usually gradual and may have possible side effects.[7-9] Currently, hydroquinone (HQ) is the standard lightening agent for the treatment of melasma. HQ inhibits melanization through suppression of the tyrosinase enzyme; however, its long-term application can lead to several side effects including ochronosis, contact dermatitis, and guttate hypopigmentation.[10,11]

According to the advantages of PRGF mentioned, in this study, we decided to evaluate the efficacy of combination of PRGF and topical 4% HQ compared with monotherapy with topical 4% HQ in the treatment of dermal type of melasma.

Materials and Methods

Study design

This is a single-blinded randomized split-face clinical trial on twenty female patients with melasma. All participants signed the informed consent form before the study enrolment. The study was approved by the ethical committee of our medical center, and the study protocol was registered in the Iranian Registry of Clinical Trials. All procedures in the study were in line with the Declaration of Helsinki.

Participants

Adult (18 to 50 years old) female patients with symmetrical patches of melasma on malar areas of their face were recruited. Only patients who had dermal type of melasma confirmed by Wood's lamp examination were enrolled in the study. Exclusion criteria were epidermal or mixed type of melasma, receiving oral contraceptive pills or any hormone therapy up to one year before beginning of the study, or patients who used any lightening agents or had undergone any laser therapy during the 6 months before the study enrolment. Other exclusion criteria were pregnancy, lactation, history of sensitivity to hydroquinone, photosensitivity, suffering from any serious systemic diseases (including anemia and coagulative or bleeding disorders), dermatologic diseases with positive Koebner phenomenon, active infection at the injection site of the lesions, and thrombocytopenia (platelets less than 50000). Demographic features (including age and skin type) and the duration of skin lesions were recorded.

PRGF preparation

Preparation of PRGF was performed according to the manufacturer's instructions (BD vacutainer tube, Mainolab Co., Iran). In this process, 20 cc venous blood was collected in 10 cc tubes containing 2 ml of 3.8% sodium citrate. The tubes were then centrifuged (RooyaGen kit, Tehran, Iran) at 580 g for 8 minutes. Fraction 2 (F2), exactly above the buffy coat layer, was separated from bottom (including red blood cells and buffy coat) and upper (including platelet-poor plasma) layers. F2 was then stored in a separate tube at room temperature with 100 µl 10% calcium chloride for 60 minutes; the resultant liquid was centrifuged for the second time at 460 g for 15 minutes and was used as PRGF for injections using 1 cc insulin syringes.

Treatment protocol

Patients were asked to apply topical 4% HQ (Sobhan Daru Drug Industries) on both sides of their face once at night for 6 months. In each patient, one side of the face was randomly selected to receive PRGF injections in monthly intervals at the baseline visit and the first and second months after commencing the treatment.

For each cm^2 of the lesions, 0.1 ml of PRGF was injected. Before injections, topical anesthetic cream (lidocaine 2.5% and prilocaine 2.5%) was used under occlusion for 45 minutes. Then, patients were asked to wash their face with water after which the skin was disinfected with 70% ethanol. Injections were performed on the first day of the beginning of the treatment and at monthly intervals for a total of three sessions. Participants were asked to apply sun block cream with sun protective factor (SPF) 50 every two hours during the day.

Assessment of treatment

Photographs were taken from participants by a digital camera (SX410IS, Canon, Tokyo, Japan) at baseline (W0), first month (W4), second month (W8), and 6 months (W24) after baseline. Efficacy of treatment was assessed based on hemi melasma area and severity index (hemi-MASI) score.[12] In addition, physician's global assessment (PGA) was performed by two dermatologists who were not aware of the treatment group assignments. PGA was classified based on the percentage of reduction in pigmentation as excellent (more than 75%), marked (50%-75%),

good (25%-49%), fair (less than 25%) improvements, and no response (no improvement). Furthermore, patients were asked about their satisfaction of the treatment at W24. Patients' global assessment (PtGA) was categorized as highly satisfied, moderately satisfied, partially satisfied, and not satisfied.

Statistical analysis

Data were analyzed by SPSS software (IBM SPSS Statistics for Windows, Version 22 Armonk, NY: IBM.). Quantitative data were described by mean and standard deviation. Frequency and percentage were used for description of qualitative data. Efficacy of treatments (based on hemi-MASI score) was compared between the two groups using the Wilcoxon test and the Friedman test. The Chi-square test was used for evaluation of the results of PGA and PtGA. In addition, a Chi-square test was used for comparison of adverse effects between the two groups. *P* value less than 0.05 was considered statistically significant.

Results

Twenty patients with a mean age of 40.35 ± 4.94 (ranging from 34 to 50) years old were enrolled in this study. All participants completed the study protocol [Figure 1]. Most of the patients had skin type III (70%), and the remaining had skin type IV. Mean duration of the disease was 9.65 ± 4.65 (ranging from 3 to 20) months.

Both groups showed significant improvement in hemi-MASI score at W8 [Table 1, Figures 2 and 3]. Mean percentage of reduction in hemi-MASI score at W8 was 40.74 ± 5.61% and 30.92 ± 2.82% in the combination therapy and monotherapy groups, respectively. Moreover, mean percentage of reduction in hemi-MASI score at W24 was 40.38 ± 6.04% and 33.42 ± 3.23% in combination and monotherapy groups, respectively. In terms of hemi-MASI score reduction, the differences between the two groups were not statistically significant at W8 (*P* = 0.65) and W24 (*P* = 0.32) time points. PGA revealed excellent-to-marked improvement in melasma in 25% and 5% of patients in the combination therapy and monotherapy groups, respectively, but the difference between the two groups was not statistically significant [*P* = 0.31, Table 2].

PtGA showed high and moderate satisfaction levels in 15% and 85% of the patients in the combination therapy group, respectively, whereas patients in the monotherapy group reported only moderate and partial satisfaction levels (85% and 15%, respectively). The difference between two treatment groups was not statistically significant.

Table 1: Efficacy of treatment based on hemi-MASI score in both groups

Treatment sessions	Monotherapy group (Mean±SD)	Combination therapy group (Mean±SD)	*P*
Baseline	4.53±0.67	4.11±0.53	0.21
Week 4	4.06±0.68	3.75±0.57	0.26
Week 8	3.09±0.56	2.97±0.47	0.65
Week 24	3.12±0.57	2.91±0.47	0.32
P	0.001	0.007	

MASI: Melasma area and severity index; SD: standard deviation

Table 2: Degree of improvement based on physician's global assessment

Treatment sessions		Monotherapy group *n* (%)	Combination therapy group *n* (%)	*P*
Week 4	Fair	9 (45)	9 (45)	1
	No response	11 (55)	11 (55)	
Week 8	Fair	20 (100)	20 (100)	1
Week 24	Excellent	0 (0)	2 (10)	0.31
	Marked	1 (5)	3 (15)	
	Good	12 (60)	10 (50)	
	Fair	7 (35)	5 (25)	
P		0.001	0.005	

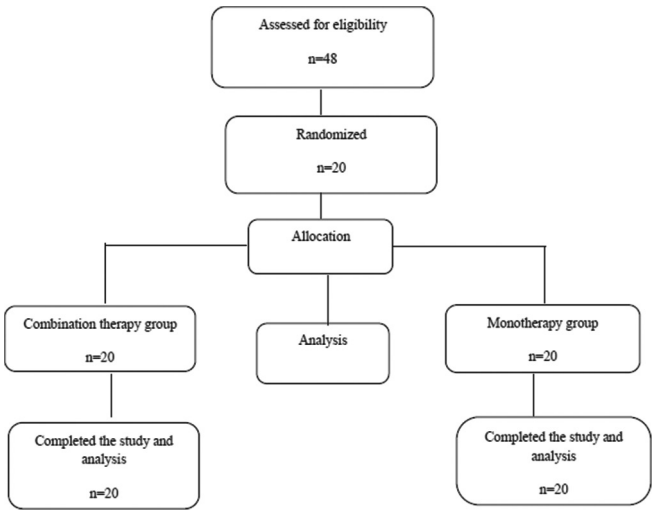

Figure 1: Flowchart of the study

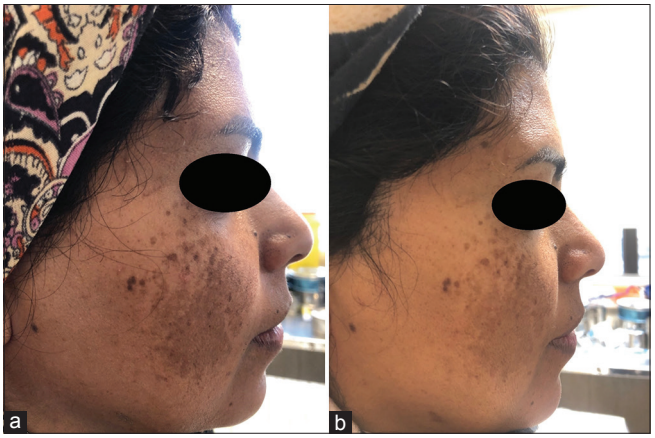

Figure 2: (a) Pretreatment. (b) Post-treatment; significant improvement after 24-week treatment with combination of plasma rich in growth factors (PRGF) with topical 4% hydroquinone

Patients with lighter skin complexion significantly showed a higher response rate as compared with those with darker skin complexion in both treatment groups. In the combination therapy group, patients with shorter duration of pigmentation demonstrated significantly better outcomes compared with participants with longer duration of lesions [Table 3].

Side effects were transient and slight in both treatment groups. No significant difference was found between the two groups regarding the side effects of treatments [Table 4].

Discussion

Recently, there have been advancements in the application of autologous biomaterials in medicine and cosmetic fields. These therapeutic agents have various advantages including their ease of preparation, affordability, low immunogenicity, and their high safety profile. PRP and PRGF are among these biomaterials that contain various types of growth factors. Such factors may stimulate synthesis of collagen fibers and promote proliferation of blood vessels, thus leading to regeneration of extracellular matrix, soft tissue augmentation, and skin glowing. Transforming growth factor-B (TGF-B) has suppressing effects on melanocytes and melanogenesis through inhibitory effects on kinase, tyrosinase enzyme, microphthalmia transcription factor (MITF), and paired-box homeotic gene (PAX). In addition, TGF-β can restore basement membrane through stimulating synthesis of laminin, collagen IV, and tenascin. PDGF, another growth factor available in PRP, has been shown to stimulate fibroblasts to synthesize more collagen and extracellular matrix proteins and can lead to skin volumization and skin glowing. Furthermore, IGF-1, FGF, EGF, and VEGF bind to specific receptors on the surface of epithelial cells, fibroblasts, endothelial cells, and mesenchymal cells and may result in overexpression of genes and proteins involved in suppressing melanogenesis and tissue regeneration.[3,13,14]

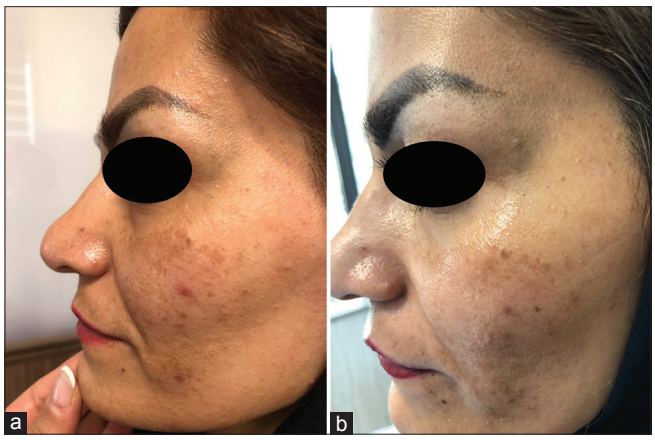

Figure 3: (a) Pretreatment. (b) Post-treatment; noticeable improvement after 24-week treatment with topical 4% hydroquinone

To date, there are a few studies that evaluate efficacy of PRP injection on melasma lesions as a monotherapy or in combination with other therapeutic lightening methods (such as topical tranexamic acid (TXA), microneedling, or Q-switched laser).[13] Mumtaz *et al*.[14] demonstrated significantly superior efficacy of three monthly sessions of intradermal PRP compared with mesotherapy with TXA (4 mg/cc, three monthly sessions). Hofny *et al*.[15] showed significant reduction in melasma lesions with application of PRP with either mesotherapy method or after microneedling during three months of treatment. Excellent-to-marked improvement has been reported in 39.1% and 30.4% of patients in microneedling and mesotherapy groups, respectively. In their study, most of the patients had epidermal type of melasma (78.3%), which generally responds better to treatment than mixed or dermal types of the disease.

Tuknayat *et al*.[16] evaluated the efficacy of three monthly sessions of PRP on melasma lesions. They showed 54.5% reduction in modified MASI (mMASI) score. The majority of the patients (90%) were satisfied or very satisfied with the treatment results. No relapse was reported at 3-month follow-up after the treatment. No serious side effects were observed; only xerosis and pruritus were reported in 35% and 25% of the subjects, respectively. Most of the patients in their study had epidermal type of melasma (69.5%) similar to the study by Hofny *et al*.[15,16]

Gamea *et al*.[17] showed that adding intradermal PRP (four sessions, every three weeks) to topical 5% liposomal

Table 3: Efficacy of treatment based on skin types and duration of disease in both treatment groups

Variables			Degree of improvement based on physician's global assessment *n* (%)				*P*
			<25%	25%-50%	51%-75%	≥75%	
Combination therapy group	Skin types	III	0 (0)	9 (90)	3 (100)	2 (100)	0.001
		IV	5 (100)	1 (10)	0 (0)	0 (0)	
	Duration	<6 months	0 (0)	0 (0)	1 (33.3)	2 (100)	0.002
		≥6 months	5 (100)	10 (100)	2 (66.7)	0 (0)	
Monotherapy group	Skin types	III	2 (28.6)	11 (91.7)	1 (100)	0 (0)	0.012
		IV	5 (71.4)	1 (8.3)	0 (0)	0 (0)	
	Duration	<6 months	0 (0)	3 (25)	0 (0)	0 (0)	0.3
		≥6 months	7 (100)	9 (75)	1 (100)	0 (0)	

Table 4: Side effects of two treatment groups

Variables	Monotherapy group *n* (%) *n*=20	Combination therapy group *n* (%) *n*=20	*P*
Pain and burning	4 (20)	3 (15)	0.67
Erythema	1 (5)	1 (5)	1
Pruritus	5 (25)	6 (30)	0.72

TXA cream (twice a day for 12 weeks) can significantly boost the lightening effects of TXA. In their study, more than 50% improvement in mMASI score was observed in 35% and 20% of the patients in the combination therapy and monotherapy groups, respectively (P = 0.026). In addition, 25% and 5% of the patients in the combination therapy and monotherapy groups were highly satisfied with the results, respectively. In our study, excellent-to-marked improvement (more than 50% improvement) in melasma was observed in 25% and 5% of female patients in the combination therapy and monotherapy groups, respectively. Our study through PtGA also showed that 15% of the patients in the combination therapy group were highly satisfied with the treatment (vs. 0% in monotherapy group). Our study was only performed on patients with dermal type of melasma, whereas half of the patients in Gamea *et al*.[17] study had epidermal type of melasma; this can explain the lower response rate in the current study compared with Gamea *et al*. study.[17]

Another randomized split-face study by Adel *et al*.[4] on twenty female patients with melasma (30% epidermal type and 70% mixed type), compared the efficacy of combination of PRP injections and intense pulse light (IPL) with PRP injections alone (four sessions, every other week). They showed that the mean percentage of reduction in mMASI score after treatment was 23.85% and 22.86% in the combination therapy and monotherapy groups, respectively. There was no significant difference between the two groups regarding PGA, patients' global assessment, and the improvement in mMSAI score. Likewise, in the current study, the mean percentage of reduction in MASI score at the end of study was not statistically significant between the two groups. However, in our study, despite including merely dermal type of melasma that is more resistant to treatment, percentage of reduction in the MASI score was higher than the results in Adel's study, which could be due to the application of topical 4% HQ in both treatment groups and using PRGF instead of PRP in our study.

Limitations

In the current study, we were not able to evaluate efficacy of the treatments based on objective methods such as spectrophotometry, histological, or immunohistochemical evaluations. Moreover, our study only included female subjects and lacks information about treatment response in male patients. Further studies with a larger number of participants are recommended. In addition, future studies aiming to investigate the efficacy of PRGF compared with PRP are suggested.

Conclusions

Although there was no significant difference between two treatment groups regarding hemi-MASI and PGA, patients demonstrated higher satisfaction with the combination of PRGF and topical 4% HQ compared with topical HQ alone. Therefore, the combination of PRGF and topical 4% HQ can be recommended as a safe alternative therapeutic approach, especially in dermal type of melasma.

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Conflicts of interest

There are no conflicts of interest.

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