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Research Article

A NOVEL APPROACH TO INTRALESIONAL AUTOLOGOUS SERUM THERAPY IN MELASMA: AN OPEN-LABEL, PROSPECTIVE STUDY

RAKESH KUMAR MEENA[1]*, VIVEK SAGAR[2], SUDHANSHU SHARMA[1], DHRUVENDRA PANDEY[3]

[1]Department of Dermatology, Venereology, and Leprosy, Government Autonomous Medical College, Ratlam; Madhya Pradesh, India.
[2]Department of Dermatology, Venereology, and Leprosy, Dr. Baba Saheb Ambedkar Medical College and Hospital, Rohini, New Delhi, India.
[3]Department of Community Medicine, Government Autonomous Medical College, Ratlam; Madhya Pradesh, India.
*Corresponding author: Rakesh Kumar Meena; Email: rakesh.meena003@gmail.com

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ABSTRACT

Objective: The study aimed to assess the effectiveness of intralesional autologous serum therapy in Melasma.

Methods: A total of 30 patients over 18 years of age were enrolled in the study with prior approval from the institute's ethical committee. Serum was injected intradermally into the melasma lesions of the patient every 2 weeks with a 30G insulin syringe. Patients were called for follow-up every 15 days until 90 days. On each visit, clinical response to treatment was calculated using the modified Melasma Area and Severity Index (mMASI) score, and side effects were also noted, if any.

Results: The reduction in mean mMASI score from baseline was not significant till the second visit (day 15) (p=0.317), while it was statistically significant from the third visit onwards (day 30) (p=0.024). The reduction in mean mMASI score from baseline 3.95±3.23 to 2.31±2.16 (41.51% improvement) is suggestive of a good response, which was also statistically significant (p≤0.001).

Conclusion: Study patients perceived a significant improvement in pigmentation during the therapy period without any considerable side effects. Thus, autologous serum therapy may be a viable alternative to the existing treatment modalities for Melasma.

Keywords: Melasma, Autologous serum therapy, Intralesional, modified Melasma Area and Severity Index.

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INTRODUCTION

Melasma is the most common acquired cause of facial hyperpigmentation among Asians. Melasma is seen predominantly among females, especially in their reproductive years, with significant psychosocial impact. About 10% of cases occur in men. As per the study by Sarkar *et al.*, the prevalence of melasma in men was seen in 20.5% of patients [1]. Melasma is characterized by irregular dark to brown macules and patches commonly involving the cheeks, forehead, nose, upper lip, and chin and more often recalcitrant to treatment.

Different therapeutic modalities, especially the gold standard Kligman's regimen, have been used to treat melasma. At present, there are various treatment options available, including depigmenting agents, chemical peels, laser therapy, and dermabrasion, but none of them is universally effective. All the above treatment options have side effects when used for a longer duration, also they have limited efficacy, and melasma relapses on discontinuation of therapy. As there are no satisfactory treatment options available to treat melasma, we should try to develop newer, safer, and more innovative treatments for treating this psychosocial disorder, which causes profound cosmetic disfigurement, significant stress, and embarrassment to the patient [2,3].

Medical science is constantly changing, and the changes are driven by research. Progression in the field of biological treatments has led to the emergence of autologous products based on the patient's bioactive proteins, known as growth factors. Injection of a patient's self blood or serum, known as auto-hemotherapy, was a standard dermatologic treatment in the early 1900s. Dermatologists shun autohemotherapy due to a lack of supporting evidence. Ravaut [4] and Spiethoff [5] (1913) described their use of autohemotherapy for various dermatologic conditions. After that, autohemotherapy became a standard treatment for many dermatologic disorders worldwide.

Studies have reported statistically significant improvements in Melasma Area and severity index score with platelet-rich plasma treatment and highlighted the role of various growth factors, such as transforming growth factor (TGF); epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), in reducing pigmentation. Serum components include various growth factors, vitamins, minerals, and fibronectin (Table 1), and thus can be a more powerful weapon for recalcitrant, frustrating, and recurrent melasma. The rationale for using intralesional autologous serum therapy for melasma is that it is cost-effective, easy to use, and relatively free from systemic side effects.

MATERIALS AND METHODS

It is an open-label, prospective, and experimental study. All the subjects over 18 years of age with facial melasma and not taking any treatment for the past 4 weeks attending the dermatology outpatient department (OPD) of the institute were enrolled. Females with pregnancy and lactation, patients with any other concomitant facial dermatoses, and on treatment, including oral contraceptive pills or immunosuppressive medications, were excluded from the study. Participants were informed about anticipated side effects at the injection site, like erythema, burning sensation, and pain, and informed consent was obtained from every subject. All the patients were also advised not to use their usual facial formulations during serum therapy. Subjects who refused to participate in the trial or lost on follow-up were instructed to use standard alternative treatments.

Five milliliters (5 ml) of blood was collected from the patient in a vial without anticoagulant. The blood sample was allowed to clot for half an hour and then centrifuged at 2000 rpm for 15 min to prepare serum. The supernatant was aspirated and injected intradermally into the Melasma lesions of the patient every 15 days. All the participants were

called for follow-up every 15 days until 90 days. On each visit, clinical response to therapy was calculated using the modified Melasma Area and severity index (mMASI) score, and side effects were also noted, if any.

Data were analyzed using SPSS Statistics software (version 21.0, IBM; Co., Armonk, NY, USA). Friedman's analysis of variance was used for the overall descriptive analysis of quantitative data, and the Wilcoxon signed-rank test was used to compare the pre- and post-treatment mMASI scores of successive visits. Results were depicted as the mean±standard deviation (SD). $p<0.05$ was considered significant.

RESULTS

A total of 30 subjects were enrolled in this open-label, prospective study. The mean age of patients was 36.43±7.65 (mean±SD). Out of the enrolled subjects, 22 patients completed the stipulated 90-day therapy period, while eight subjects were lost on follow-up: Five after the first visit and three from the second visit onwards. Loss of follow-up might be due to an inadequate response or other unspecified reasons. Final data analysis was done on 22 subjects. The mMASI score at baseline and day-90 of all the subjects is depicted in Fig. 1. Out of the 22 subjects who completed therapy, excellent improvement (>75% reduction) was observed in 2 subjects (9.09%); good response (51–75% reduction) in 1 patient (5.54%); moderate response (25–50% reduction) in 14 patients (63.63%); and poor to no response (<25% reduction) in 5 patients (22.72%) (Fig. 2).

Table 1: Serum components and their concentrations

Serum components	Serum concentration
Proteins	
Total protein	66–81 mg/mL
Lysozyme	5.0–10.2 µg/mL
Lactoferrin	0.17–0.28 mg/mL
Albumin	41–51 mg/mL
IgA	0.93–3.93 mg/mL
IgD	0.03 mg/mL
IgE	0.4 µg/mL
IgG	8.61–17.47 mg/mL
IgM	0.33–1.83 mg/mL
Growth factors	
EGF	0.72 ng/mL
TGF-α, male	147 pg/mL
TGF-α, female	147 pg/mL
TGF-β1	140.3 ng/mL
Vitamins	
Vitamin A	200–500 ng/mL
Vitamin C	5–9 µg/mL
Antioxidants	
Tyrosine	77 µM
Carbohydrate	
Glucose	0.6–1.2 g/L
Electrolytes	
Na^+	138–145 mM
K^+	3.6–4.8 mM
Ca^{++}	8.8–10.1 mM
Cl^-	101–108 mM
HCO_3^-	21–29 mM
NO^-	0.19 mM
PO_4^{---}	1.42 mM
SO_4^+	0.53 mM

The reduction in mean mMASI score from baseline day 0 (3.95±3.23; Mean±SD) to successive visits is as follows: Day 15, (3.87±3.27; p=0.317), day 30, (3.54±3.02; p=0.024), day 45 (2.98±2.27; p=0.001), day 60 (2.79±2.20; p=0.001), day 75 (2.46±2.25; p=<0.001), and day 90 (2.31±2.16; p≤0.001) (Tables 2 and 3). Concerning the duration of therapy, the reduction in mean mMASI score from baseline was not significant at the second visit (day 15) (p=0.317), while it was statistically significant from the third visit onward (day 30) (p=0.024). At the end of therapy, the mean mMASI reduced from baseline 3.95±3.23 to 2.31±2.16 (41.51%) (Fig. 3), which was statistically significant (p≤0.001) (Table 2).

DISCUSSION

In the past few years, the concept of endogenous regenerative medicine has gained the attention of dermatologists. In Indian medicine, autohemotherapy has been used for years in the treatment of several diseases, such as chronic inflammation, immunodeficiency, vascular conditions, osteoarthritis, allergies, atopic dermatitis [6], and various other skin disorders [7]. Recently, several investigators have evaluated autohemotherapy as a treatment for urticaria and eczema. Preparations such as platelet-rich plasma (PRP), serum, and its derivatives have emerged as intriguing modalities to promote skin regeneration [8]. Several findings suggest that these preparations may elicit significant anti-inflammatory and immune-modulatory effects for managing different dermatological conditions [9-11].

The available evidence indicates that autohemotherapy does not have major side effects. The serum is defined as "blood plasma without the clotting factors or as blood with all cells and clotting factors removed." Serum includes all proteins without clotting factors, several growth factors, all electrolytes, antibodies, antigens, hormones, and exogenous substances like drugs or microorganisms. The concentration of several serum components is summarized in Table 1 [12].

Like autohemotherapy, autologous serum therapy has been used for the successful treatment of various ailments. Bajaj *et al.* [13] showed the effectiveness of autologous serum therapy in both auto-reactive and non-auto-reactive patients with chronic urticaria. Higuchi A [12] (2018) concluded that autologous serum eye drops are a widely used treatment for dry eye syndrome both in Japan and internationally. Datta *et al.* [14] summarized that subcutaneous autologous serum therapy is as effective as conventional intramuscular autologous serum

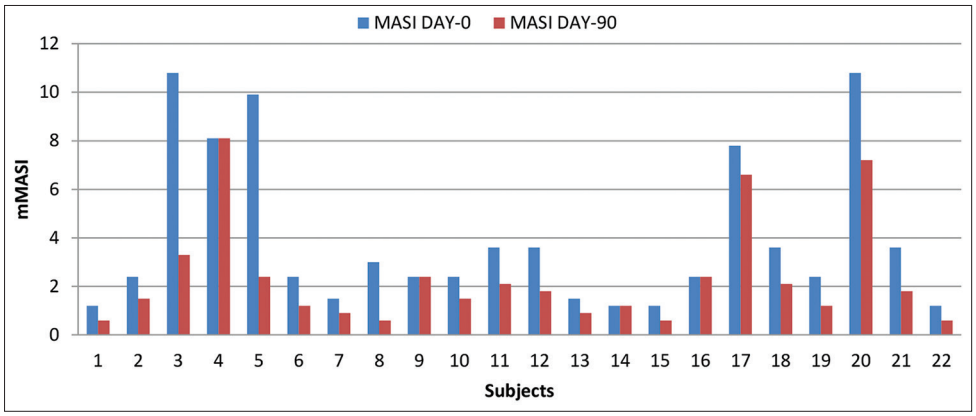

Fig. 1: The modified Melasma Area and severity index score at baseline and day 90 of all the subjects

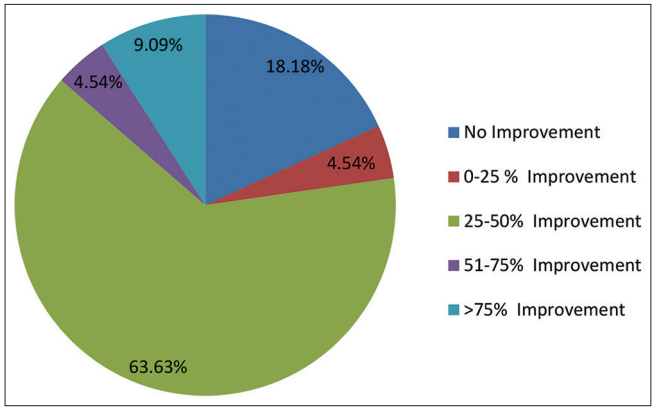

Fig. 2: Improvement in the modified Melasma Area and severity index scores of patients

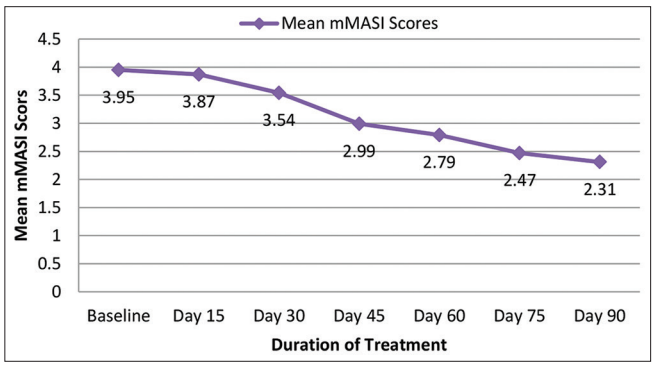

Fig. 3: Reduction in mean modified Melasma Area and severity index scores of patients

Table 2: Descriptive analysis of Friedman's test showing mean and interquartile range

mMASI	Mean±SD*	Minimum	Maximum	Percentiles		
				25th	50th	75th
Day 0	3.95±3.23	1.2	10.8	1.50	2.40	4.65
Day 15	3.87±3.27	1.2	10.8	1.42	2.40	4.65
Day 30	3.54±3.02	1.2	10.8	1.42	2.40	3.82
Day 45	2.98±2.27	0.6	8.1	1.20	2.40	3.22
Day 60	2.79±2.20	0.6	8.1	1.20	2.40	3.22
Day 75	2.46±2.25	0.6	8.1	0.90	1.65	2.70
Day 90	2.31±2.16	0.6	8.1	0.90	1.65	2.40

*p<0.001 for within-group comparison of mMASI using Friedman's test

Table 3: Comparison of the pre-and post-treatment mMASI scores

Comparison in between	Wilcoxon signed-rank test p-value
mMASI DAY 0 – mMASI DAY 15	0.317
mMASI DAY 0 – mMASI DAY 30	0.024
mMASI DAY 0 – mMASI DAY 45	0.001
mMASI DAY 0 – mMASI DAY 60	0.001
mMASI DAY 0 – mMASI DAY 75	<0.001
mMASI DAY 0 – mMASI DAY 90	<0.001

therapy, and patient compliance is an additional advantage without compromising the therapeutic efficacy in chronic urticaria. Similarly, Millan [15] also suggested that autologous serum may help manage clinical symptoms derived from sensitive skin alterations. In Melasma, Panda *et al.* [16] concluded that the combination of micro-needling with the application of topical autologous PRP was found more efficacious and had better patient satisfaction in comparison to treatment with micro-needling alone.

Limitations

The efficacy of autologous serum therapy could not be compared with a placebo or a standard treatment because of a single-arm study. However, controlled, randomized, blinded, and prospective studies are required to correctly establish the efficacy. Any possible investigator bias could not be eliminated because of open-label treatment allocation. Despite these limitations, we believe this work serves to document the safety and efficacy of autologous serum therapy as an adjuvant treatment for melasma.

CONCLUSION

Concerning the duration of therapy, the reduction in mean mMASI score from baseline was not significant at the second visit (day 15) (p=0.317), while it was statistically significant from the third visit onward (day 30) (p=0.024). At the end of therapy, the reduction in mean mMASI score from baseline 3.95±3.23 to 2.31±2.16 (41.51% improvement) is suggestive of a good response, which was also statistically significant (p≤0.001). Subjects also perceived a significant improvement in pigmentation during the therapy period. No adverse effects were observed with the therapy except for tolerable pain at the injection site. Autologous serum therapy may thus be a viable alternative to the existing treatment modalities for melasma.

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AUTHOR'S CONTRIBUTION

The first three authors equally contribute to manuscript concept, design, data acquisition, and manuscript preparation. The fourth author contributes to the statistical analysis.

CONFLICTS OF INTEREST

None.

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