Evaluation of platelet-rich plasma on hair regrowth and lesional T-cell cytokine expression in alopecia areata: A randomized observer-blinded, placebo-controlled, split-head pilot study

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Background: Platelet-rich plasma has shown some promise in the treatment of alopecia areata.

Objective: To evaluate the effect of platelet-rich plasma on hair regrowth and lesional T-cell cytokine expression in alopecia areata.

Methods: This was a randomized, placebo-controlled, split-head study involving 27 patients with alopecia areata (Severity of Alopecia Tool score ≥25%). Alopecia patches on either side of the scalp were randomized to receive 3 intradermal injections of platelet-rich plasma or normal saline at monthly intervals and evaluated 3 months after the last session. Lesional T-cell cytokine messenger RNA expression was compared pre- and posttreatment in the platelet-rich plasma–treated sites.

Results: The mean Severity of Alopecia Tool score did not change significantly compared with baseline with either platelet-rich plasma or placebo injections at any visit; however, the mean percentage reduction in the score in the platelet-rich plasma arm was more than in the placebo arm (9.05% ± 36.48% vs 4.99% ± 33.88%; $P = .049$) at final assessment. The mean interferon gamma ($P = .001$) and interleukin 17 cytokine ($P = .009$) messenger RNA expression decreased, whereas the mean interleukin 10 ($P = .049$) and FOXP3 ($P = .011$) messenger RNA expression increased significantly after platelet-rich plasma treatment.

Limitations: Small sample size and a relatively short follow-up.

Conclusion: Platelet-rich plasma was found to have limited efficacy in alopecia areata. However, it may play a role in restoring immune balance in the alopecic patches. (J Am Acad Dermatol 2021;84:1321-8.)

INTRODUCTION

Alopecia areata is a common cause of patchy nonscarring hair loss, with a lifetime risk of approximately 2%. Several treatment options are available for alopecia areata; however, no treatment is consistently effective, particularly for severe cases. Topical and intralesional corticosteroids are the first-line treatments for mild disease, whereas oral corticosteroids, topical immunotherapy, and other immunosuppressive agents are tried in severe cases. Emerging

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treatment options include Janus kinase inhibitors, apremilast, statins, 308 nm excimer laser, low-dose interleukin (IL) 2, and platelet-rich plasma.[1] There has been an increasing interest regarding platelet-rich plasma as a potential treatment in alopecia areata. Currently, there are only a few studies in the literature regarding its efficacy, most of which are low quality and show conflicting results.[2,3] Moreover, the exact mechanism of action of platelet-rich plasma in causing hair regrowth in alopecia areata is not known. We conducted this randomized controlled trial to evaluate the effect of autologous platelet-rich plasma on hair regrowth and lesional T-cell cytokine expression in alopecia areata.

CAPSULE SUMMARY

- Platelet-rich plasma has shown some promise in the treatment of alopecia areata, however, this randomized placebo-controlled split-head study suggests its limited efficacy as a stand-alone treatment for alopecia areata.
- Although better than placebo, platelet-rich plasma did not produce significant hair regrowth. Its effect on restoring local immune balance may warrant evaluation as adjuvant treatment.

METHODS

This was a randomized, observer-blinded, placebo-controlled, split-head, pilot study conducted in the Department of Dermatology and Venereology, All India Institute of Medical Sciences, New Delhi, India, after approval from the Institute Ethics committee. The trial was registered in the clinical trials registry of India.

Study population

Adult patients with alopecia areata with Severity of Alopecia Tool (SALT) score greater than or equal to 25% and duration 6 months or longer were included in the study after informed consent. A drug washout period of 2 and 4 weeks for topical and systemic therapies, respectively, was given to patients receiving these treatments.

Intervention

Alopecia patches on either side of the scalp vertex were allocated with block randomization to receive autologous platelet-rich plasma or normal saline (placebo) injections. Platelet-rich plasma was prepared with a manual double-spin protocol with a platelet concentration approximately 4 times higher than that of the whole blood. Briefly, whole venous blood (40 mL) was centrifuged in a standard laboratory centrifuge at 20°C. The first spin was conducted for 10 minutes at 160*g*. The resulting supernatant plasma was then centrifuged for 10 minutes at 400*g*. The upper two-thirds of the cell-poor plasma supernatant was removed and the platelet pellet was suspended in the lower third of the plasma by gently shaking the tube.[4] Platelet-rich plasma was activated with calcium gluconate (1:9 ratio) before injection. Three sessions of intradermal injections of 4 to 5 mL of platelet-rich plasma or placebo (0.1 mL/cm^2) were given with a 1-mL insulin syringe at 0, 4, and 8 weeks, with follow-up at 12, 16, and 20 weeks. Because the platelet-rich plasma and placebo differed from each other in color and consistency, the dermatologist administering them and patients were not blinded to the treatment. No topical or systemic treatment for alopecia areata was allowed during the study period.

Assessment

SALT score and percentage reduction in markers for dystrophic hair (broken hair, black dots, exclamation mark hair, and yellow dots) at every visit were evaluated by examination of serial clinical photographs and dermoscopic images, respectively, by 2 independent observers blinded to the treatment modalities and not involved in the study. The dermatologist injecting the treatment was not involved in the evaluation of treatment response. The degree of percentage of SALT score change and percentage reduction in dystrophic hair markers was categorized on a 6-point Likert scale as −1 (worsening), 0 (no change), 1 (<25% improvement), 2 (25%-49% improvement), 3 (50%-74% improvement), and 4 (≥75% improvement). Similarly, patients also rated the degree of hair regrowth on the 6-point Likert scale as just stated as a Patient Global Assessment score.

A 3-mm punch biopsy was performed from the platelet-rich plasma–treated alopecic patch for comparison of T helper and T regulatory cell cytokine expression at baseline and 12 weeks. T helper cell type (Th) 1 (IL-2 and interferon gamma), Th2 (IL-4 and IL-10), Th17 (IL-17 and IL-22), and T regulatory (FOXP3) cytokine messenger RNA (mRNA) expression in the platelet-rich plasma–treated lesional skin was estimated with real-time polymerase chain reaction, as described elsewhere.[5]

Outcome measures

The primary outcome measure was the percentage reduction in SALT score, whereas secondary outcome measures included Patient Global Assessment score, percentage reduction in

Abbreviations used:

IL: interleukin
mRNA: messenger RNA
SALT: Severity of Alopecia Tool
Th: T helper cell type

dystrophic hair markers, and change in lesional T helper and T regulatory cytokine mRNA expression.

Statistical analysis

Continuous variables are presented as mean ± standard deviation or median (range) and categoric variables as frequency (%). Intraclass correlation coefficient was calculated to test the inter-rater reliability of the SALT scores between the 2 observers. Paired *t* test or Wilcoxon's signed rank test was used for comparing paired continuous variables; Fisher's exact test, for categoric variables. Statistical significance was considered at $P \leq .05$. Data were analyzed in accordance with an intention-to-treat protocol. Statistical analysis was performed with Stata/SE (version 14.2, StataCorp).

RESULTS

Our study included 27 patients. All except 2 patients had previously failed 1 or more systemic therapies; 2 patients had tried only topical corticosteroids before. The baseline clinical and demographic characteristics of the patients are summarized in Table I. Sixteen patients (59.3%) completed the study; 1 patient was lost to follow-up after week 4, 2 patients after week 8, 2 after week 12, and 6 after week 16.

The mean baseline SALT score on scalp vertex was comparable between the platelet-rich plasma and placebo arms (14.94 ± 5.01, median 16 vs 15.03 ± 5.32, median 17.75; $P = .79$). The intraclass correlation coefficient between the 2 observers for SALT scores was 0.948 (95% confidence interval 0.933-0.959; $P < .001$).

SALT score reduction

The mean SALT scores at 20 weeks did not differ statistically significantly from baseline in both the platelet-rich plasma (14.94 ± 5.01 vs 13.79 ± 6.43; $P = .09$) and placebo (15.03 ± 5.32 vs 14.34 ± 6.32; $P = .23$) arms. The mean percentage reduction in SALT score in the platelet-rich plasma and placebo arms was 9.05% ± 36.48% (95% confidence interval −5.38% to 23.48%; median 0%) and 4.99% ± 33.88% (95% confidence interval −8.41% to 18.39%; median 0.625%), respectively, at 20 weeks, with the difference between the 2 arms being statistically significant ($P = .049$). Fig 1 shows the mean percentage SALT score reduction at every visit in the platelet-rich plasma and placebo arms, with the difference between them becoming statistically significant at 16 weeks ($P = .04$) and at 20 weeks ($P = .05$).

According to the Likert categories of percentage SALT score change, 6 patients (22.2%) had comparable hair regrowth on the platelet-rich plasma–treated and placebo-treated sites, 9 (33.3%) had a better hair regrowth on the platelet-rich plasma–treated sites (Fig 2), 3 (11.1%) had a better response on the placebo-treated sites, 4 (14.8%) had no hair regrowth on either side, and 5 (18.5%) experienced worsening on both sides. Overall, a 50% reduction in SALT score was achieved by 4 (14.8%) platelet-rich plasma–treated scalp sites and 2 (7.4%) placebo-treated scalp sites ($P = .67$) (Fig 3, *A*).

Table I. Baseline characteristics of patients with alopecia areata

Characteristic	Patients (n = 27)
Sex, no. (%)	
Men	13 (48.2)
Women	14 (51.8)
Age, mean ± SD, y	23.89 ± 4.64 (range 18–35)
Disease duration, mean ± SD, y	6.05 ± 4.82 (range 0.5–19)
Patterns of alopecia areata, no. (%)	
Patchy	5 (18.5)
Patchy with ophiasis	7 (37.8)
Subtotalis/totalis	6 (22.2)
Universalis	9 (33.3)
Extensive (SALT score ≥50%) alopecia areata, no. (%)	22 (81.5)
SALT score, mean ± SD, %	74.33 ± 24.42 (median 80; range 27–100)
Extrascalp involvement, no. (%)	24 (88.9)
Nail changes, no. (%)	17 (62.9)
History of atopy, no. (%)	5 (18.5)
Previous therapies, no. (%)	
Oral betamethasone minipulse	7 (25.9)
Daily oral corticosteroids	17 (62.9)
Topical corticosteroids	18 (66.6)
Intralesional triamcinolone acetonide	10 (37)
Other oral immunosuppressives (methotrexate, azathioprine, cyclosporine)	10 (37)
Oral PUVAsol	4 (14.8)
Sulfasalazine	2 (7.4)
Topical minoxidil	4 (14.8)

PUVA, Psoralen ultraviolet A solar therapy; *SALT*, severity of Alopecia Tool; *SD*, standard deviation; y, years.

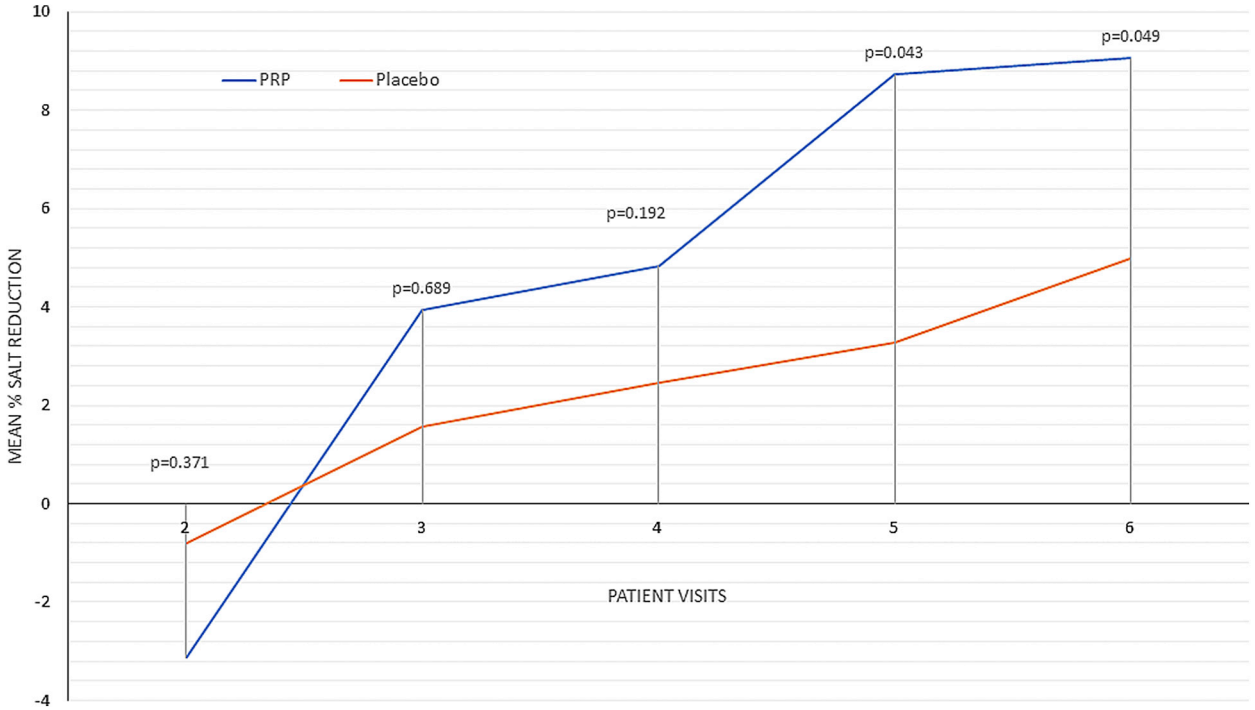

Fig 1. Comparison of mean percentage Severity of Alopecia Tool score reduction between the scalp sites treated with platelet-rich plasma and placebo at all follow-up visits. *PRP*, Platelet-rich plasma; *SALT*, Severity of Alopecia Tool.

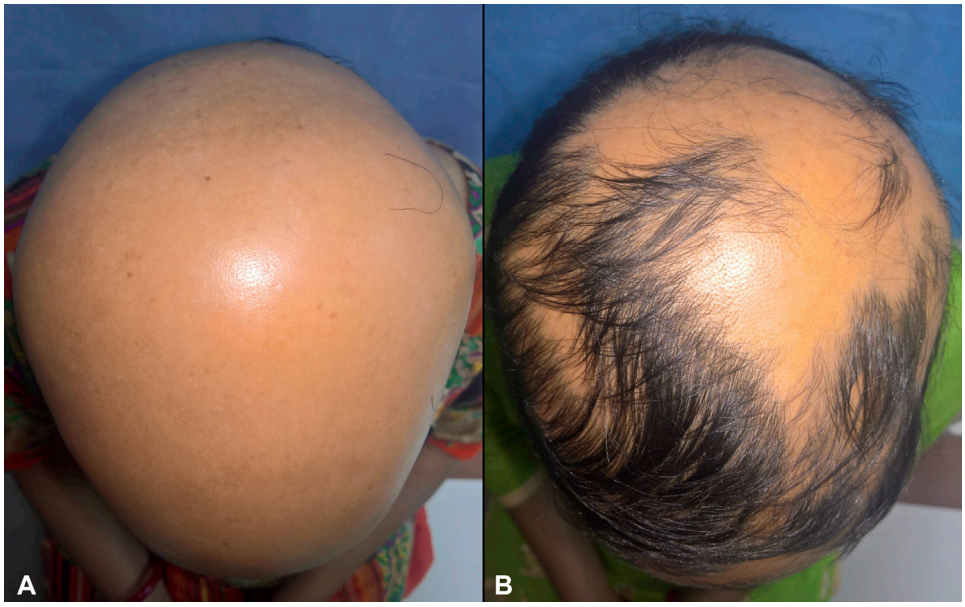

Fig 2. Alopecia areata. Clinical photographs of the scalp showing better hair regrowth on platelet-rich plasma–treated side (patient's right side percentage Severity of Alopecia Tool score reduction 50%-74%) than placebo-treated side (patient's left side percentage Severity of Alopecia Tool score reduction 25%-49%) at 20 weeks.

Dermoscopic evaluation

Dermoscopic markers of dystrophic hair were observed on dermoscopic examination in 14 (51.6%) platelet-rich plasma–treated sites and 15 (55.6%) placebo-treated sites at baseline. Overall, 9 (64.3%) platelet-rich plasma–treated and 10 (66.7%)

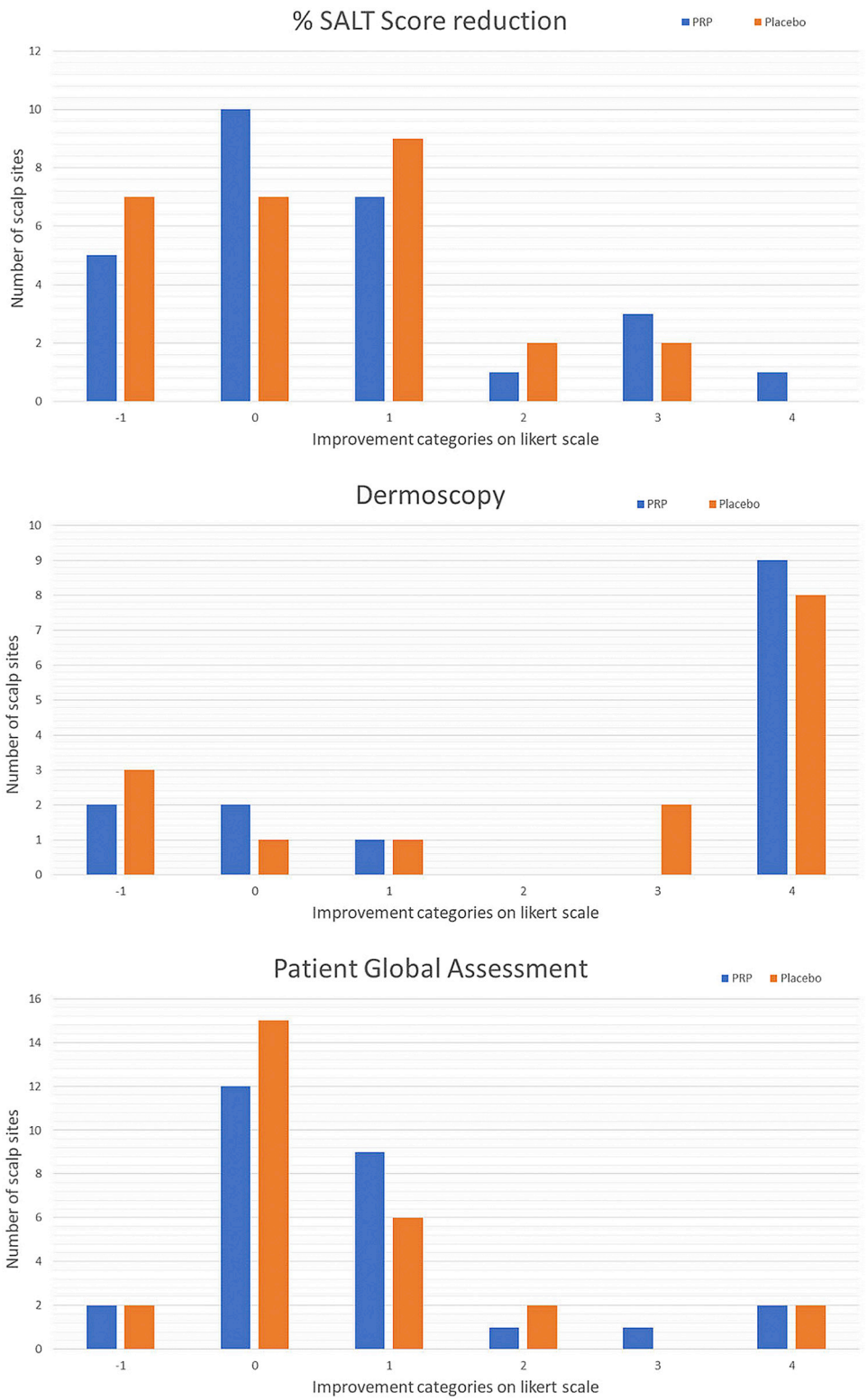

Fig 3. Improvement according to Likert scale categories (−1, worsening; 0, no change; 1, <25% improvement; 2, 25%-49% improvement; 3, 50%-74% improvement; and 4, ≥75% improvement) in platelet-rich plasma–treated and placebo-treated scalp sites at 6 months in percentage Severity of Alopecia Tool score reduction, dermoscopic evaluation, and Patient Global Assessment score. *PRP*, Platelet-rich plasma; *SALT*, Severity of Alopecia Tool.

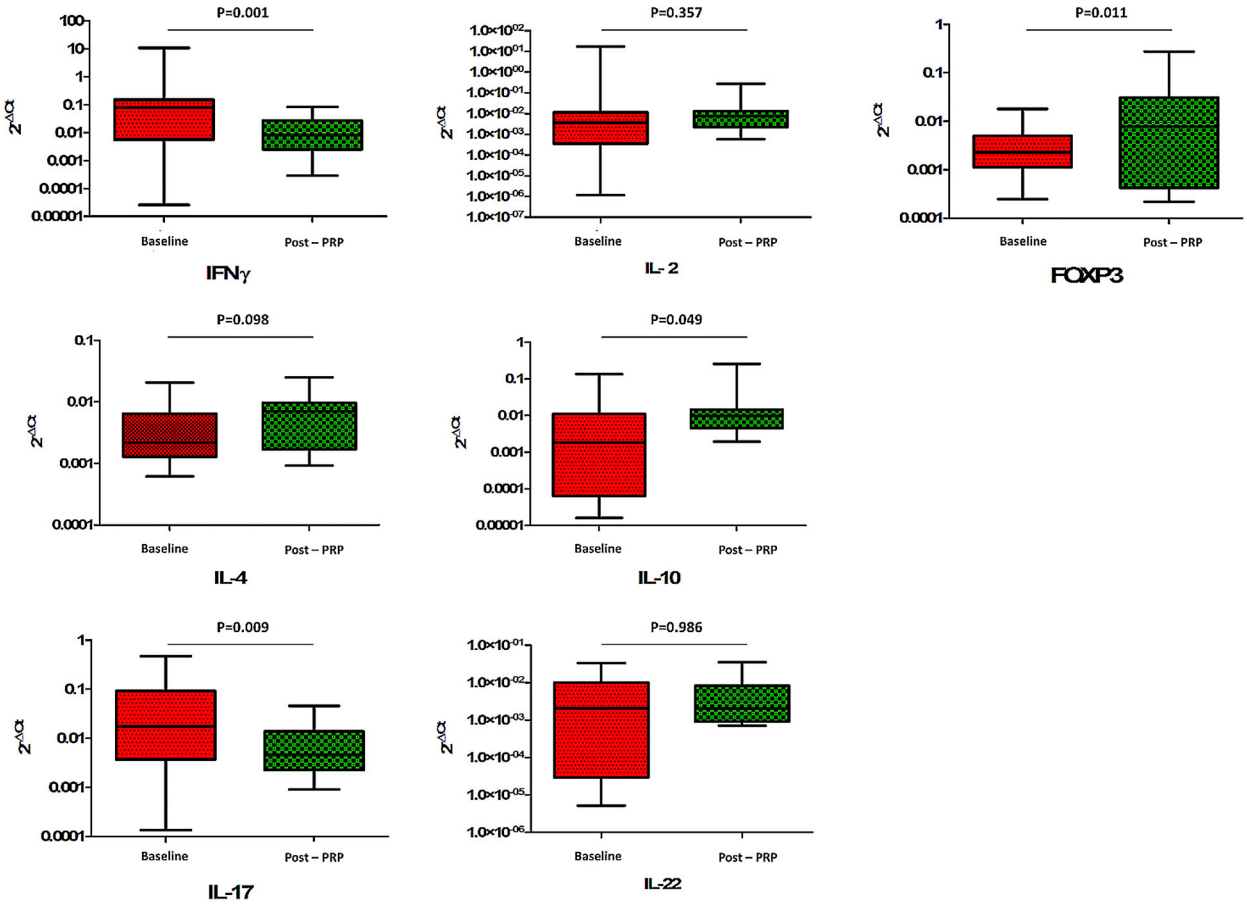

Fig 4. Comparison of various T-cell cytokine messenger RNA expression profiles in the platelet-rich plasma–treated lesional skin (baseline vs 12 weeks). *IFN*, Interferon; *IL*, interleukin; *PRP*, platelet-rich plasma.

placebo-treated scalp sites showed greater than or equal to 50% reduction in dystrophic hair markers at 20 weeks. The percentage reduction in dystrophic hair markers was comparable between the 2 treatment arms at 20 weeks (P = .86) (Fig 3, *B*).

Patient Global Assessment score

Overall, a Patient Global Assessment score of greater than or equal to 3 (≥50% improvement) was reported for 3 (11.1%) platelet-rich plasma–treated sites and 2 (7.4%) placebo-treated sites. The scores were comparable between the platelet-rich plasma and placebo arms at all visits (P = .81 at 20 weeks) (Fig 3, *C*).

Lesional T helper and T regulatory cytokine mRNA expression

The change in mean lesional cytokine mRNA expression before and after platelet-rich plasma treatment (n = 21) is shown in Fig 4. The mean interferon gamma (P = .001) and IL-17 cytokine (P = .009) mRNA expression decreased significantly, whereas the mean IL-10 (P = .049) and FOXP3 (P = .011) mRNA expression increased significantly after platelet-rich plasma treatment. There was no statistically significant change in the mean mRNA expression of IL-2, IL-4, and IL-22 after platelet-rich plasma treatment.

Adverse effects

Pain or sensitivity on the injected scalp sites was reported by a majority of the patients (85.2%; n = 23/27) after the procedure, which resolved in 2 to 5 days with oral analgesics. No serious adverse effects were noted in the platelet-rich plasma–treated or placebo-treated sites.

DISCUSSION

In our study, the hair regrowth on platelet-rich plasma–treated sites was on average twice that of placebo-treated sites. However, overall platelet-rich

plasma was not found to be particularly effective in the treatment of alopecia areata in our cohort of patients. A case series including 25 patients with chronic severe alopecia areata also reported limited efficacy of platelet-rich plasma, with none of the 9 patients who completed the 1-year follow-up achieving noticeable cosmetic results.[6] Another case series of 10 patients with alopecia totalis reported no significant hair regrowth after a single intradermal injection of platelet-rich plasma.[7] This is in contrast to the earlier preliminary encouraging results published in the literature.[8,9] A randomized controlled trial in which patients with patchy alopecia areata (n = 45) were treated with 3 monthly sessions of platelet-rich plasma, intralesional corticosteroid, or placebo reported complete remission in 60% of those treated with platelet-rich plasma compared with complete remission in 26.6% of those treated with intralesional corticosteroids at 12 months. Platelet-rich plasma also led to a greater reduction in dystrophic hair count on dermoscopy than intralesional corticosteroids.[10] In a nonrandomized comparative study on patients with limited patchy alopecia areata (SALT score <25%; n = 74), platelet-rich plasma had an earlier clinical response than intralesional corticosteroids but it was statistically insignificant (53% vs 35% at 6 weeks; $P = .60$), whereas all patients in both the treatment groups had complete resolution at 9 weeks and 3 months.[11] Another recent randomized controlled study involving patients with limited alopecia areata (n = 40) found platelet-rich plasma to be less effective than intralesional corticosteroids.[12] However, it is difficult to make a fair comparison between different studies because of the different patient profiles, platelet-rich plasma preparations, and treatment protocols.

Despite the relative lack of clinical efficacy as a stand-alone treatment, our study provides some evidence that platelet-rich plasma may restore the immune balance in alopecia areata patches. The changes in Th1, Th17, and T regulatory cytokine expression after platelet-rich plasma treatment appear corrective, in accordance with the previously reported upregulation of Th1 and Th17 cytokines and downregulation of T regulatory cytokines in alopecia areata.[13-15] Transforming growth factor-beta, a potent immunosuppressive cytokine, is present in platelet-rich plasma and may be responsible for this effect.[16] Platelet-rich plasma may also exert an immunomodulatory effect by stimulating the dermal mesenchymal and hair bulge stem cells.[17] The mechanism of action of platelet-rich plasma in alopecia areata is not yet clear, with some studies suggesting a growth-promoting effect on the hair follicles and dermal papillae through its growth factors and activation of Wnt/catenin signaling pathway.[10] To our knowledge, this is the first study to examine the effect of platelet-rich plasma on immune dysregulation in alopecia areata. Our results suggest an immunomodulatory effect of platelet-rich plasma on alopecia areata patches, and may provide some basis for its potential role as an adjuvant treatment.

Our study was conducted in accordance with the National Alopecia Areata Foundation guidelines, using validated outcome measures.[18,19] The split-head study design has the advantage of nullifying any interindividual variations to treatment response. However, our study was limited by a small sample size, a relatively short follow-up, and a large dropout rate. It remains to be seen whether more frequent or additional treatment sessions and a longer follow-up period can produce better hair regrowth. Furthermore, because the study was conducted in a tertiary care center, we may have included more patients with severe disease, with 82% of patients (n = 22/27) having extensive alopecia areata, including alopecia subtotalis, totalis, or universalis; nonetheless, these patients are the ones in need of newer treatment options. The validity of our results needs to be tested in other less severe types of alopecia areata.

CONCLUSION

Although better than placebo, platelet-rich plasma was found to have limited efficacy in causing hair regrowth in alopecia areata as a stand-alone treatment in our study. However, the immunomodulatory effect of platelet-rich plasma on the lesional T-cell cytokine profile suggests its potential as an adjuvant therapy, which may be further evaluated.

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Conflicts of interest

None disclosed.

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