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Effects of Combined Platelet-Rich Plasma and Tressfix Serum Therapy on MicroRNA Expression and Clinical Outcomes in Patients with Alopecia Areata: A Randomized Clinical Trial.

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Abstract

Background:
Alopecia Areata (AA) is a chronic autoimmune disorder causing non-scarring hair loss. MicroRNAs (miRNAs) are increasingly recognized as important regulators of immune activity and disease progression in AA. Platelet-rich plasma (PRP) and Tressfix serum may offer therapeutic benefits, but their combined clinical and molecular effects remain unclear. This study compared combined PRP and Tressfix serum therapy with PRP alone and Tressfix serum alone in patients with AA.

Methodology:
This single-blind, three-arm randomized clinical trial included 45 patients with patchy AA. Participants were randomized equally to receive combined PRP and Tressfix serum, PRP alone, or Tressfix serum alone for 14 weeks. Expression of six miRNAs was measured using qRT-PCR before and after treatment. Clinical response was assessed using the Severity of Alopecia Tool (SALT) score at baseline, week 7, and week 14.

Results:
All participants completed the study. Significant reductions in multiple miRNAs were observed across treatment groups, with the greatest changes occurring in the combination therapy group. Group A demonstrated statistically significant reductions in all six evaluated miRNAs (all $p < 0.001$), whereas Group B showed significant reductions in five miRNAs and Group C in three miRNAs. SALT scores improved progressively in all groups throughout the study period. After adjustment for baseline differences, the combination therapy group demonstrated the greatest reduction in SALT score at week 14, followed by the PRP monotherapy group and the Tressfix monotherapy group. Treatment-related adverse events were mild and self-limiting, consisting primarily of transient injection-site pain, burning sensation, and erythema.

Conclusion:
Combined PRP and Tressfix serum therapy produced greater clinical improvement and stronger modulation of selected miRNAs than either treatment alone. These findings suggest potential clinical and molecular benefits of combination therapy in AA.

Keywords:
Alopecia Areata, Platelet-Rich Plasma, Tressfix Serum, MicroRNA, SALT Score, Hair Regrowth

Introduction

Alopecia Areata (AA) is a common autoimmune disorder characterized by non-scarring hair loss resulting from immune-mediated destruction of anagen hair follicles. The disease is associated with disruption of the hair follicle immune privilege and activation of autoreactive cytotoxic T lymphocytes, leading to premature follicular regression and hair shedding [1]. Although AA is not life-threatening, it can substantially impair quality of life, self-esteem, emotional well-being, and social functioning.

The global lifetime risk of Alopecia Areata is estimated to be approximately 2%, making it one of the most prevalent autoimmune dermatological disorders worldwide [1]. Reported prevalence rates vary geographically, ranging from approximately 0.7% in India to over 2% in the United States and certain Asian populations [2,3]. The condition affects both sexes and all age groups, although disease onset most commonly occurs during early adulthood [4,5]. Despite extensive research, the precise etiology of AA remains incompletely understood. Current evidence suggests that genetic susceptibility, environmental triggers, immune dysregulation, psychological stress, infections, and hormonal factors collectively contribute to disease development [6,7].

Clinically, AA exhibits considerable heterogeneity. Patients may present with localized patchy scalp involvement, diffuse thinning, ophiasis pattern hair loss, alopecia totalis, or alopecia universalis [8]. Disease severity ranges from limited patchy involvement to complete loss of scalp and body hair [9–12]. Such variability in presentation often influences treatment response and prognosis, highlighting the need for individualized therapeutic strategies. Conventional treatment options include topical and intralesional corticosteroids, systemic immunomodulatory agents, topical sensitizers, and, more recently, Janus kinase (JAK) inhibitors [12]. Although many patients experience clinical improvement, therapeutic responses remain variable, relapse rates are high, and treatment-associated adverse effects may limit long-term use. Consequently, considerable interest has emerged in identifying safer and more effective therapeutic approaches capable of targeting both the clinical manifestations and underlying biological mechanisms of AA.

Platelet-rich plasma (PRP) has attracted increasing attention as a regenerative treatment modality in dermatology. PRP is an autologous blood-derived product enriched with platelets and growth factors, including platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor-β, and epidermal growth factor. These bioactive molecules are believed to stimulate follicular stem cells, enhance angiogenesis, promote tissue repair, and prolong the anagen phase of the hair cycle [12]. Several clinical studies have reported encouraging outcomes with PRP in patients with Alopecia Areata, although the mechanisms underlying these effects remain incompletely understood [13–18]. Tressfix serum represents another emerging therapeutic option. The formulation contains a combination of biologically active compounds including Ginkgo biloba, Procapil, panthenol, biotin, castor oil, glycerin, and wheat proteins, which may support hair follicle function through antioxidant, anti-inflammatory, and follicular trophic effects. Although individual components have demonstrated potential benefits for hair health, evidence regarding their molecular effects in Alopecia Areata remains limited. In recent years, microRNAs (miRNAs) have emerged as important regulators of immune responses, inflammation, apoptosis, cellular differentiation, and tissue remodeling. These small non-coding RNA molecules regulate gene expression at the post-transcriptional level and have been implicated in the pathogenesis of numerous autoimmune and inflammatory disorders. Several miRNAs, including miR-223, miR-146b, miR-155, miR-143, miR-421, and miR-29b-1, have been associated with inflammatory signaling pathways and immune dysregulation relevant to Alopecia Areata. Consequently, alterations in their expression may provide valuable insights into treatment-associated molecular responses and disease activity. Despite growing interest in PRP and topical biologically active therapies, evidence regarding their combined effects on molecular biomarkers and clinical outcomes in Alopecia Areata remains scarce. Furthermore, few studies have evaluated treatment-associated changes in miRNA expression as potential indicators of therapeutic response. Therefore, the present randomized clinical trial was conducted to compare the effects of combined PRP and Tressfix serum therapy, PRP monotherapy, and Tressfix serum monotherapy on selected miRNA expression profiles and disease severity measured by SALT score in patients with Alopecia Areata. We hypothesized that combined therapy would result in greater clinical improvement and more pronounced modulation of disease-associated miRNAs than either treatment administered alone.

Methodology

Trial Design

This study was conducted as a single-center, single-blind, parallel-group randomized clinical trial with three intervention arms and a 1:1:1 allocation ratio. The trial was designed to evaluate the comparative effects of combined platelet-rich plasma (PRP) and Tressfix serum therapy, PRP monotherapy, and Tressfix serum monotherapy on clinical and molecular outcomes in patients with Alopecia Areata (AA). The intervention period lasted 14 weeks, during which participants underwent scheduled treatment sessions and outcome assessments. The primary objective was to compare treatment-associated changes in the expression levels of six selected microRNAs (miRNAs), while secondary objectives included assessment of clinical improvement using the Severity of Alopecia Tool (SALT) score.

Participants

Participants were recruited consecutively from the Dermatology Department of Isra University Hospital, Hyderabad, Pakistan. Adult patients aged 20–40 years with clinically diagnosed patchy Alopecia Areata involving localized scalp lesions were screened for eligibility. Baseline evaluation included detailed clinical history, assessment of disease duration and activity, prior treatment history, number and approximate size of alopecic patches, and baseline SALT score. Patients with alopecia totalis, alopecia universalis, ophiasis pattern alopecia, extensive or severe AA, scarring alopecia, active scalp infection, bleeding disorders, platelet dysfunction, anticoagulant therapy, pregnancy, lactation, or any contraindication to PRP therapy were excluded. To minimize the influence of prior therapies, patients who had received topical treatments for alopecia within the preceding four weeks, intralesional corticosteroids within eight weeks, or systemic/procedural therapies including PRP, microneedling, laser treatment, or systemic immunosuppressive medications within the preceding three months were not eligible. Relevant comorbidities, including thyroid disorders, autoimmune diseases, and nutritional deficiencies, were documented whenever available.

Interventions

Group A: PRP plus Tressfix Serum

Participants assigned to Group A received intradermal PRP injections every two weeks for a total of seven treatment sessions over 14 weeks and simultaneously applied Tressfix serum topically once daily throughout the treatment period.

Group B: PRP Monotherapy

Participants assigned to Group B received PRP injections according to the identical protocol and schedule used in Group A but did not receive Tressfix serum.

Group C: Tressfix Serum Monotherapy

Participants assigned to Group C applied Tressfix serum once daily for 14 consecutive weeks without receiving PRP injections. The serum formulation contained Ginkgo biloba (4%), Procapil (10%), Panthenol (1%), Biotin (2 mg), Castor Oil (2%), Propylene Glycol (5%), Glycerin (5%), and Wheat Protein (5%).

PRP Preparation

For each treatment session, approximately 15 mL of venous blood was collected using a 21-gauge butterfly needle into five sterile tubes containing 3.8% sodium citrate anticoagulant. PRP was prepared using a standardized double-spin centrifugation protocol. The first centrifugation was performed at 150 × g for 10 minutes to separate erythrocytes from plasma and buffy coat. The plasma fraction was transferred into sterile tubes and centrifuged again at 1500–2000 × g for 10 minutes. The upper platelet-poor plasma layer was discarded, and the lower platelet-rich fraction was retained. PRP activation was achieved by adding 0.1 mL calcium chloride ($CaCl_2$) to every 0.9 mL PRP, yielding approximately 3 mL activated PRP per session.[14]

PRP Administration

Activated PRP was administered intradermally using sterile 30-gauge needles attached to 1-mL syringes. Following local anesthesia, approximately 0.2 mL PRP was injected at 2-cm intervals throughout the affected scalp regions, including frontal, parietal, and vertex areas. Participants underwent seven treatment sessions at two-week intervals across the 14-week study period.[15]

As part of quality-control procedures, platelet and leukocyte counts were measured in both whole blood and final PRP preparations using an automated hematology analyzer. Platelet enrichment factor was calculated by dividing the platelet concentration in PRP by baseline whole-blood platelet concentration. PRP preparations were categorized as leukocyte-rich or leukocyte-poor based on leukocyte content. Final PRP volume, platelet concentration, leukocyte concentration, and enrichment factor were recorded for each preparation.

Outcomes

Primary Outcome

The primary outcome was the change in expression levels of six miRNAs implicated in Alopecia Areata pathogenesis: hsa-miR-223, hsa-miR-421, hsa-miR-29b-1, hsa-miR-146b, hsa-miR-155, and hsa-miR-143. Peripheral venous blood samples were collected at baseline and after completion of the 14-week treatment period. Plasma was separated by centrifugation and stored at −80°C until analysis. Total RNA, including small RNA fractions, was extracted using a commercially available serum/plasma miRNA extraction kit according to the manufacturer's instructions. RNA quantity and purity were assessed spectrophotometrically using A260/A280 and A260/A230 ratios. Samples failing predefined quality criteria were excluded from molecular analyses.

Reverse transcription was performed using a miRNA-specific reverse transcription kit according to manufacturer protocols. Quantitative real-time polymerase chain reaction (qRT-PCR) was subsequently conducted using a Bio-Rad CFX96 Real-Time PCR Detection System and miRNA-specific primers/probes targeting the selected miRNAs and endogenous control 5S rRNA. All reactions were performed in technical triplicate. Negative controls included no-template controls and no-reverse-transcription controls.

Amplification efficiency was evaluated using serial dilution standard curves, and only assays demonstrating efficiencies between 90–110% with coefficient of determination (R^2) ≥0.98 were included. Relative miRNA expression was normalized using 5S rRNA and calculated using the comparative $2^{-\Delta\Delta Ct}$ method. Fold-change values represented relative expression changes between baseline and post-treatment samples.

Secondary Outcome

The secondary outcome was change in hair-loss severity measured using the Severity of Alopecia Tool (SALT). SALT assessments were performed at baseline (week 0), midway through treatment (week 7), and upon completion of treatment (week 14). SALT scores range from 0 to 100, with higher scores indicating greater scalp hair loss severity.

Safety Outcomes

Treatment tolerability and adverse events were monitored throughout the study period. Adverse events including injection-site pain, erythema, burning sensation, edema, infection, allergic reactions, vasovagal episodes, and treatment discontinuations were documented at each visit.

Sample Size

Sample size estimation was performed using G*Power software for one-way analysis of variance (ANOVA) comparing three independent treatment groups. Based on previous randomized studies evaluating PRP in Alopecia Areata and assuming a large effect size (f = 0.50), a significance level of 0.05, statistical power of 80%, and three comparison groups, a minimum sample size of 42 participants was required. To account for potential attrition, 45 participants were enrolled and equally allocated into three groups of 15 participants each.

Randomization

Random allocation was performed using a sealed opaque envelope technique. Allocation assignments were generated before participant recruitment by an investigator not involved in enrollment, treatment administration, laboratory procedures, outcome assessment, or statistical analysis. Allocation codes were placed in sequentially numbered opaque sealed envelopes. Following confirmation of eligibility and informed consent, the next available envelope was opened to determine treatment assignment. Participants were allocated in a 1:1:1 ratio to Group A, Group B, or Group C.

Blinding

Because of the obvious differences between injectable and topical treatment modalities, blinding of participants and treating clinicians was not feasible. Therefore, the trial was conducted using a single-blind design. Outcome assessors responsible for SALT scoring remained blinded to treatment allocation throughout the study. Laboratory personnel performing miRNA analyses and statisticians conducting data analyses were also blinded using anonymized sample identifiers and coded group assignments until completion of final analyses.

Ethics

The study protocol was reviewed and approved by the Institutional Ethics Committee of Isra University Hospital, Hyderabad, Pakistan (Reference No. IU/CP.REC(FCS)/2025/712). The trial was conducted in accordance with the principles outlined in the Declaration of Helsinki and applicable ethical guidelines for human

research. Written informed consent was obtained from all participants prior to enrollment. Participants were informed about study procedures, potential risks, expected benefits, confidentiality measures, and their right to withdraw from the study at any time without affecting their medical care.

Statistical Methods

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 25.0. Continuous variables were summarized as mean ± standard deviation (SD) or median with interquartile range (IQR), depending on distribution characteristics. Categorical variables were reported as frequencies and percentages. Within-group changes in miRNA expression between baseline and week 14 were evaluated using paired-samples t-tests. Between-group comparisons of mean changes from baseline were performed using one-way ANOVA followed by post-hoc pairwise comparisons with appropriate correction for multiple testing. To account for simultaneous evaluation of multiple miRNAs, p-values were adjusted using the Benjamini–Hochberg false discovery rate procedure. Effect sizes for within-group changes were reported as Cohen's dz, and 95% confidence intervals (CI) were calculated for mean differences. For SALT score analysis, baseline, week 7, and week 14 measurements were evaluated. Because baseline SALT scores differed significantly between groups, analysis of covariance (ANCOVA) was performed to compare week-14 SALT scores while adjusting for baseline SALT values. Additionally, longitudinal changes in SALT scores were examined using linear mixed-effects models with participant included as a random effect and treatment group, time, and group-by-time interaction included as fixed effects. Adjusted mean differences with corresponding 95% confidence intervals were reported where appropriate. All statistical tests were two-sided, and statistical significance was defined as $p < 0.05$.

Results

Recruitment

Participant recruitment was conducted at the Dermatology Department of Isra University Hospital, Hyderabad, Pakistan. A total of 45 eligible patients with clinically diagnosed patchy Alopecia Areata were enrolled during the study period and randomized equally into three intervention groups (n = 15 per group). Group A received combined Platelet-Rich Plasma (PRP) and Tressfix serum therapy, Group B received PRP monotherapy, and Group C received Tressfix serum monotherapy. All randomized participants completed the 14-week intervention period and were included in the final analyses. No participant was lost to follow-up, withdrew consent, or discontinued treatment because of adverse events. Consequently, outcome data were available for all enrolled participants, resulting in complete follow-up and analysis of the randomized cohort.

Baseline Data

The baseline demographic characteristics of the study population are summarized in Table 1. The overall mean age of participants was 35.3 ± 2.5 years. Group-specific mean ages were comparable, measuring 34.56 ± 1.3 years in Group A, 35.55 ± 1.1 years in Group B, and 35.78 ± 2.1 years in Group C. The study population consisted predominantly of males, with 30 male participants (66.7%) and 15 female participants (33.3%). The distribution of sex across treatment groups was generally comparable, although Group B included a slightly higher proportion of male participants. Baseline SALT scores demonstrated statistically significant differences among treatment groups (Table 3), indicating that complete equivalence between groups was not achieved despite randomization. Therefore, subsequent analyses of treatment outcomes incorporated baseline-adjusted statistical methods to account for these initial differences.

Numbers Analyzed

All 45 randomized participants were included in the final statistical analyses according to their assigned treatment groups. Fifteen participants were analyzed in each intervention arm for both clinical and molecular outcomes. Complete datasets were available for all participants, including baseline and post-treatment microRNA measurements as well as serial SALT score assessments performed at baseline, week 7, and week 14. Consequently, no imputation procedures for missing data were required.

Outcomes and Estimation

MicroRNA Expression Outcomes

Changes in the expression levels of six selected microRNAs following treatment are presented in Table 2. Within-group analyses demonstrated significant treatment-associated reductions in multiple miRNAs across all intervention groups, although the magnitude of response varied substantially.

Participants receiving combination therapy (Group A) exhibited the largest reductions in expression levels for all six evaluated microRNAs. Statistically significant decreases were observed for hsa-miR-223, hsa-miR-421, hsa-miR-29b-1, hsa-miR-146b, hsa-miR-155, and hsa-miR-143 (all $p < 0.001$). Mean reductions ranged from 15.33 units for hsa-miR-29b-1 to 32.02 units for hsa-miR-223, suggesting a broad treatment-associated modulation of molecular pathways implicated in Alopecia Areata.

In Group B (PRP monotherapy), significant reductions were observed for five of the six studied microRNAs. The greatest reductions were noted for hsa-miR-223 (25.18 ± 4.21), hsa-miR-143 (18.43 ± 4.56), and hsa-miR-421 (17.13 ± 4.22). However, the reduction observed in hsa-miR-146b did not reach statistical significance ($p = 0.06$).

Participants receiving Tressfix serum alone (Group C) demonstrated comparatively smaller molecular changes. Significant reductions were identified for hsa-miR-421, hsa-miR-29b-1, and hsa-miR-143, whereas changes in hsa-miR-223, hsa-miR-146b, and hsa-miR-155 were not statistically significant. These findings indicate that although Tressfix serum monotherapy may influence selected molecular pathways, its overall effect appeared less pronounced than that observed with PRP-containing interventions.

Between-group comparison of mean changes from baseline demonstrated the greatest reductions in miRNA expression among participants receiving combination therapy, followed by PRP monotherapy and Tressfix serum monotherapy. These comparative findings are illustrated in Figure 4. Although statistically significant differences were observed, the relatively small sample size and exploratory molecular analyses warrant cautious interpretation of these findings.

SALT Score Outcomes

Changes in SALT scores over the 14-week treatment period are summarized in Table 3 and graphically illustrated in Figure 5. All treatment groups demonstrated progressive reductions in SALT scores over time, indicating clinical improvement in scalp hair loss severity. At baseline, mean SALT scores were highest in Group A (84.2 ± 2.56), followed by Group C (82.55 ± 2.47) and Group B (80.25 ± 1.89). By week 7, substantial reductions had occurred in all groups, with Group A demonstrating the largest improvement (40.55 ± 1.58), followed by Group B (52.65 ± 3.10) and Group C (69.70 ±

1.20). At the completion of treatment (week 14), Group A exhibited the lowest mean SALT score (28.78 ± 4.32), indicating the greatest degree of clinical improvement. Corresponding week-14 scores were 38.95 ± 2.69 in Group B and 53.12 ± 3.69 in Group C. Because baseline SALT scores differed significantly between treatment groups, post-treatment comparisons were adjusted using analysis of covariance (ANCOVA). Baseline-adjusted analyses demonstrated that Group A achieved significantly greater clinical improvement than Groups B and C. Longitudinal evaluation using mixed-effects repeated-measures modelling demonstrated significant effects of time and significant group-by-time interaction effects, indicating that treatment response trajectories differed among intervention groups. The most favorable pattern of improvement was consistently observed in the combination therapy group.

Ancillary Analyses

Additional exploratory analyses were conducted to compare treatment-associated changes among the three intervention groups. Between-group comparisons demonstrated consistently larger reductions in miRNA expression among participants receiving combined PRP and Tressfix therapy relative to either monotherapy. Similarly, the magnitude of SALT score improvement was greatest in Group A throughout the treatment period. These findings suggest a potential additive or synergistic effect of combining PRP with Tressfix serum. However, because the study was not specifically powered to evaluate mechanistic interactions between treatments, these observations should be regarded as exploratory and hypothesis-generating.

Harms

All three treatment strategies were generally well tolerated throughout the study period. The most frequently reported adverse event was mild injection-site pain among participants receiving PRP therapy. Additional minor adverse events included transient burning sensation and temporary scalp erythema. These events were self-limiting, resolved spontaneously without intervention, and did not necessitate treatment discontinuation. Importantly, no serious adverse events were observed during the study period. There were no reported cases of scalp infection, folliculitis, persistent edema, allergic reactions, scarring, vasovagal episodes, hospitalization, or other clinically significant complications. No participant withdrew from the study because of treatment-related adverse effects, supporting the overall tolerability of the investigated interventions.

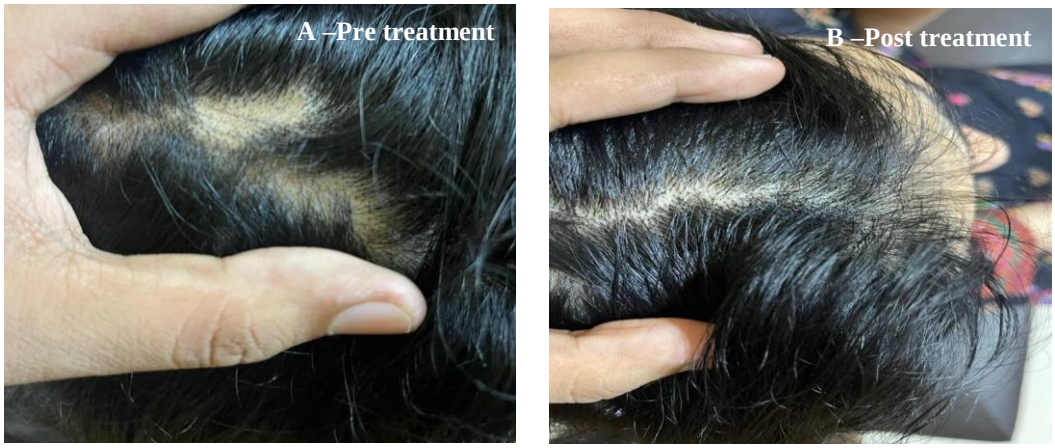

Figure 1: Group A (PRP and Tressifx Serum Combination Therapy).

Figure 2: Group B (PRP alone Therapy).

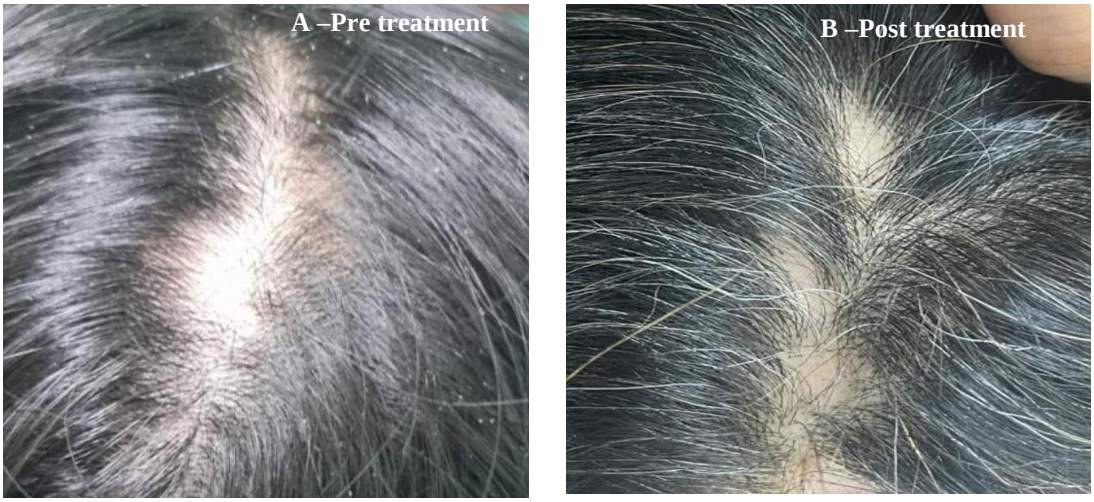

Figure 3: Group C (Tressifix Serum alone).

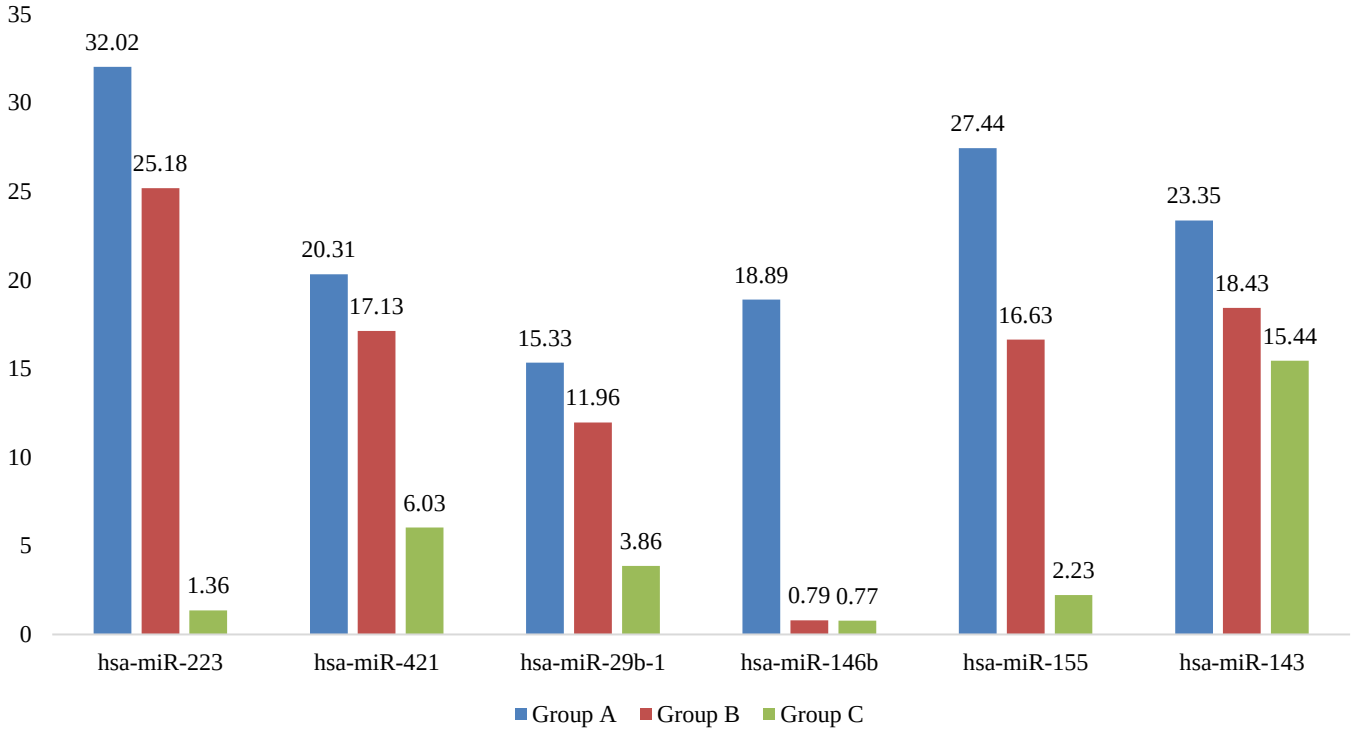

Figure 4: Comparative analysis of mean between the group for 06 miRNAs.

Table 1: Demographic Description of participants included in the study.

Variables		Combine	Group A	Group B	Group C
Age (years); Mean ± SD		35.3±2.5	34.56±1.3	35.55±1.1	35.78±2.1
Gender; n(%)	Male	30 (66.66)	9(60)	12(80)	11(73.33)
	Female	15 (33.33)	6(40)	3(20)	4(26.66)

Group A (PRP + Tressifix Serum), Group B (PRP only), Group C (Tressifix Serum only)

Table 2: Levels of MicroRNA before and after intervention.

Variables		Pre	Post	Mean Difference	T-stats	p-value
		Mean ± SD				
Group A	hsa-mir- 223	161.34±4.23	129.32±3.38	32.02±3.6	28.12	<0.001*
	hsa-mir- 421	80.12±2.38	59.81±8.21	20.31±6.33	14.12	<0.001*
	hsa-mir-29b-1	125.65±5.56	110.32±6.2	15.33±3.56	8.51	<0.001*
	hsa-mir-146b	132.45±8.85	113.56±5.56	18.89±1.25	12.56	<0.001*
	hsa-mir-155	79.65±1.22	52.21±3.56	27.44±1.89	16.32	<0.001*
	hsa-mir-143	75.56±4.8	52.21±3.11	23.35±3.05	15.55	<0.001*
Group B	hsa-mir- 223	160.59±7.58	135.41±3.24	25.18±4.21	16.58	<0.001*
	hsa-mir- 421	81.25±9.33	64.12±6.36	17.13±4.22	11.65	<0.001*
	hsa-mir-29b-1	130.52±2.25	118.56±5.98	11.96±5.21	7.58	<0.001*
	hsa-mir-146b	130.78±4.55	129.99±6.58	0.79±0.012	0.55	0.06
	hsa-mir-155	75.58±5.44	58.95±10.52	16.63±4.51	12.32	<0.001*
	hsa-mir-143	77.56±2.8	59.13±4.1	18.43±4.56	11.55	<0.001*
Group C	hsa-mir- 223	159.25±6.59	157.89±4.56	1.36±1.02	2.1	0.05*
	hsa-mir- 421	83.21±7.13	77.18±4.61	6.03±3.11	6.73	<0.001*
	hsa-mir-29b-1	131.42±3.14	127.56±4.58	3.86±1.14	3.1	<0.001*
	hsa-mir-146b	130.63±4.18	129.86±6.11	0.77±0.05	0.025	0.083
	hsa-mir-155	77.25±3.54	75.02±5.47	2.23±1.65	1.54	0.05*
	hsa-mir-143	79.22±2.18	63.78±4.23	15.44±3.45	8.43	<0.001*

*p<0.05 is considered statistically significant

Table 3: Analyses of the effects of treatment strategies on SALT score

Variables	Group A	Group B	Group C	p-value
Baseline; Mean ± SD	84.2±2.56	80.25±1.89	82.55±2.47	
At Week 7; Mean ± SD	40.55±1.58	52.65±3.1	69.7±1.2	<0.001*
At Week 14; Mean ± SD	28.78±4.32	38.95±2.69	53.12±3.69	

*p<0.05 is considered statistically significant

Discussion

This randomized clinical trial evaluated the effects of combined platelet-rich plasma (PRP) and Tressfix serum therapy, PRP monotherapy, and Tressfix serum monotherapy on both molecular and clinical outcomes in patients with Alopecia Areata (AA). The principal findings demonstrated that all three treatment strategies were associated with varying degrees of improvement in disease severity and modulation of selected microRNA (miRNA) expression profiles. However, the combination therapy group consistently demonstrated the greatest reductions in SALT scores and the most pronounced changes in miRNA expression, suggesting that simultaneous administration of PRP and Tressfix serum may provide greater therapeutic benefit than either intervention alone.

Alopecia Areata is increasingly recognized as an immune-mediated disorder characterized by collapse of hair follicle immune privilege, activation of autoreactive T lymphocytes, and dysregulation of inflammatory cytokine pathways, particularly those involving IFN-γ, IL-15, and JAK-STAT signaling [19-21]. Growing evidence indicates that miRNAs play critical regulatory roles in these pathways by modulating immune responses, inflammatory signaling, cellular differentiation, and tissue repair processes [22]. Therefore, evaluation of treatment-associated changes in miRNA expression may provide important insights into the biological mechanisms underlying clinical improvement.

In the present study, the combination therapy group exhibited significant downregulation of all six investigated miRNAs, including hsa-miR-223, hsa-miR-421, hsa-miR-29b-1, hsa-miR-146b, hsa-miR-155, and hsa-miR-143. Several of these miRNAs have been implicated in inflammatory and immune regulatory pathways. For example, miR-155 has been associated with activation of inflammatory immune responses, whereas miR-146 family members participate in feedback regulation of inflammatory signaling cascades. Similarly, miR-223 has been linked to immune-cell regulation and inflammatory processes, while miR-29b-1 and miR-143 may contribute to tissue remodeling and repair mechanisms [22]. The observed reductions in these miRNAs may therefore reflect treatment-associated modulation of inflammatory pathways relevant to AA pathogenesis.

The favorable outcomes observed in PRP-treated groups are consistent with previous literature demonstrating the regenerative and immunomodulatory properties of PRP in Alopecia Areata [13–19]. PRP contains numerous growth factors and cytokines capable of stimulating dermal papilla cells, promoting angiogenesis, enhancing follicular vascularization, prolonging anagen phase activity, and supporting tissue regeneration [16]. Previous randomized and controlled studies have reported superior hair regrowth and disease improvement among AA patients receiving PRP compared with conventional therapies [12,13].

Furthermore, a recent systematic review and meta-analysis concluded that PRP represents an effective steroid-sparing treatment option for Alopecia Areata with a favorable safety profile [18]. The present findings provide additional support for these observations and suggest that PRP may also influence molecular pathways reflected by alterations in miRNA expression.

The addition of Tressfix serum appeared to enhance both molecular and clinical responses. Although the precise mechanisms remain uncertain, the serum contains several biologically active compounds with potential antioxidant, anti-inflammatory, and follicular-supportive properties. Ingredients such as Ginkgo biloba, Procapil, biotin, panthenol, and wheat proteins may contribute to improved scalp microenvironment, enhanced follicular metabolism, and reduced oxidative stress. When combined with the regenerative effects of PRP, these mechanisms may theoretically produce complementary or synergistic effects that facilitate hair follicle recovery and immune regulation.

Clinically, all treatment groups demonstrated progressive reductions in SALT scores throughout the 14-week study period. However, participants receiving combination therapy achieved the largest improvement after adjustment for baseline differences. These findings suggest that molecular changes observed in miRNA expression may be accompanied by clinically meaningful reductions in disease severity. Nevertheless, because mechanistic analyses were not performed, a direct causal relationship between miRNA modulation and clinical improvement cannot be established from the present data. An important strength of this study is the simultaneous assessment of both molecular and clinical outcomes. Most previous studies evaluating PRP in Alopecia Areata have focused primarily on hair regrowth and clinical response, whereas the present investigation incorporated quantitative analysis of selected miRNAs associated with disease pathogenesis. The randomized design, standardized treatment protocols, blinded outcome assessment, and complete follow-up of all enrolled participants further strengthen the validity of the findings.

Despite these strengths, the results should be interpreted cautiously. Although significant treatment-associated changes were observed, the study was not designed to establish mechanistic pathways, and the observed miRNA alterations cannot be assumed to represent direct therapeutic targets. Furthermore, spontaneous remission is a well-recognized feature of Alopecia Areata and may have contributed to some of the observed improvements. Consequently, the findings should be regarded as preliminary evidence supporting further investigation rather than definitive proof of superiority of any intervention.

Limitations

Several limitations should be considered when interpreting the findings of this study. First, although the sample size was determined through an a priori power calculation and was sufficient to meet the planned statistical requirements, the relatively small number of participants in each treatment group may have reduced the precision of effect estimates and increased the likelihood that treatment effects were overestimated. Second, this was a single-center study conducted in a tertiary-care dermatology setting, which may limit the generalizability of the findings to broader populations with different demographic, ethnic, geographic, and clinical characteristics. Third, the absence of a placebo or untreated control group limits the ability to definitively distinguish treatment-related improvements from spontaneous remission, natural fluctuations in disease activity, regression to the mean, or placebo effects. Because spontaneous hair regrowth is a recognized phenomenon in patchy Alopecia Areata, the true magnitude of the treatment effect cannot be determined with certainty. Fourth, despite random allocation, significant differences in baseline SALT scores were observed between treatment groups. Although statistical adjustments using analysis of covariance and mixed-effects modeling were applied to minimize the impact of these differences, residual confounding related to baseline imbalance cannot be entirely excluded. Furthermore, the follow-up period was limited to 14 weeks, which precluded assessment of the long-term durability of clinical improvement and treatment-associated changes in microRNA expression. Given the chronic and relapsing nature of Alopecia Areata, longer follow-up studies are required to determine whether the observed benefits are sustained over time. In addition, only six selected microRNAs were evaluated. Although these biomarkers were chosen because of their reported involvement in immune and inflammatory pathways relevant to Alopecia Areata, numerous other microRNAs and molecular mediators contribute to disease pathogenesis. Therefore, the present findings provide only a partial understanding of the molecular mechanisms underlying treatment response. Another limitation is that, although adverse events were monitored throughout the study period, a formal standardized dermatology-specific safety assessment instrument was not employed. Consequently, subtle, delayed, or less frequently occurring treatment-related adverse effects may have been underreported. Finally, the study was not specifically powered to perform subgroup analyses based on sex, disease duration, baseline disease severity, or associated comorbid conditions. Future multicenter randomized controlled trials with larger sample sizes, placebo-controlled comparison groups, longer follow-up periods, and more comprehensive molecular assessments are needed to validate these findings and identify patient characteristics associated with optimal treatment response.

Conclusion

This randomized clinical trial demonstrated that combined platelet-rich plasma and Tressfix serum therapy was associated with greater improvement in clinical disease severity and more pronounced modulation of selected microRNA expression profiles compared with PRP monotherapy or Tressfix serum monotherapy in patients with Alopecia Areata. Participants receiving combination therapy exhibited the largest reductions in SALT scores and the most substantial changes in disease-associated miRNAs, suggesting potential therapeutic benefits at both clinical and molecular levels.

These findings support the hypothesis that combined regenerative and topical biologically active therapies may influence inflammatory and immune pathways involved in Alopecia Areata. However, given the small sample size, single-center design, baseline imbalance among treatment groups, absence of a placebo control group, and short follow-up duration, the results should be considered preliminary and hypothesis-generating rather than definitive evidence of treatment superiority. Future multicenter randomized controlled trials with larger sample sizes, placebo-controlled comparison groups, longer follow-up periods, comprehensive molecular profiling, and standardized safety assessment are required to confirm these findings and further clarify the biological mechanisms underlying treatment response. Such studies may contribute to the development of more effective and targeted therapeutic strategies for patients with Alopecia Areata.

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