

Platelet-rich plasma in alopecia areata: intradermal injection versus topical application with transepidermal delivery via either fractional carbon dioxide laser or microneedling

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Abstract

Introduction: Alopecia areata (AA) is a common cause of non-scarring alopecia with variable response to treatment. Platelet-rich plasma (PRP) stimulates proliferation and differentiation of stem cells in the hair-follicle bulge via multiple mechanisms. Although beneficial, pain during injection in addition to unequal delivery and coverage is a major drawback of intralesional PRP in alopecia, particularly for extensive lesions and in patients with a low pain threshold. This study evaluates intradermal injection of PRP versus its topical use with enhanced transepidermal delivery through either fractional CO₂ laser (FCL) or microneedling in treatment of AA.

Methods: Sixty AA patients were randomized into three equal groups to receive monthly sessions of either PRP intradermal injection, FCL followed by topical PRP, or microneedling followed by topical PRP for 3 consecutive months. Assessment was done through the Severity of Alopecia Tool (SALT) score and patient satisfaction at the end of sessions (2 weeks after the last session) and after 3 additional months of follow-up.

Results: Patients in all groups showed satisfactory results of PRP treatment, with statistically insignificant differences in the degree of improvement among patients of the three groups studied according to the two assessment parameters. Intralesional PRP injection was associated with significantly higher pain scores.

Conclusions: PRP is potentially effective and safe for treatment of AA. FCL and microneedling could facilitate topical PRP delivery and considerably decrease pain associated with intradermal injection while preserving PRP efficacy. These methods could be helpful for extensive lesions and in children.

Keywords: alopecia areata, platelet-rich plasma, transepidermal delivery

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Introduction

Alopecia areata (AA) is a common non-scarring alopecia. It affects 1.7% of the population at some point in life. AA is an autoimmune condition characterized by dense peribulbar lymphocytic infiltrate. Its exact cause and triggering factors are still unknown. Many therapeutic options exist, but none are curative or preventive (1, 2).

Platelet-rich plasma (PRP) is an autologous concentration of platelets contained in a small volume of plasma that accelerates circulation to hair follicles and promotes growth and even thickening of the hair shaft due to the presence of more than 20 types of growth factors in addition to cellular adhesion molecules (3, 4). Recently, utilizing PRP has become more prevalent as a method to support hair growth and prevent hair loss (5). Unfortunately, intralesional injection of PRP could be troublesome with pain and poor coverage of the area involved, especially in AA involving multiple, wide scalp areas or in pediatric patients.

Fractional lasers are commonly used devices in dermatologic practice, with the concept of fractional photothermolysis first described in 2004 (6). Similar to conventional lasers, fractional lasers ablate the skin in minute fractions, splitting the laser beam into microbeams to create microscopic vertical channels of ablation in the skin surrounded by thin layers of coagulated tissue referred to as the microthermal zone (MTZ) (7, 8). The creation of these channels theoretically provides access pathways for topically applied drug molecules and cellular materials that would otherwise

be too large to traverse the epidermal layer while minimizing the healing time (9, 10). Laser-assisted drug delivery has been found to enhance the local intake of any remedy or substance applied to the skin without considerable exception thus far (10).

Microneedling with a dermaroller is an advantageous treatment modality for treatment of acne scars, stretch marks, wrinkles, and facial rejuvenation. It is a simple and relatively cheap modality that can also be used for transdermal drug delivery. The medical dermaroller needles are 0.5 to 1.5 mm in length. During treatment, the needles pierce the stratum corneum and create microconduits (holes) without damaging the epidermis (11). Microneedling has been reported to facilitate the penetration of high-molecular-weight drugs through the stratum corneum and hair follicles (12).

This study evaluates the use of PRP in treatment of AA as a sole treatment option via intradermal injection versus its use as a topical application in combination with accelerating its transepidermal delivery through either fractional CO₂ laser (FCL) or microneedling.

Methods

Sixty patients with AA of different clinical types—clinically diagnosed and trichoscopically confirmed—were randomized into three equal groups (using the “coin tossing” method) after excluding those receiving topical or systemic treatment for alopecia in the preceding 6 weeks, those with alopecia totalis or universalis, patients with uncontrolled systemic infection or chronic illness (e.g.,

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debilitating disease, coagulation disorder, diabetes, hepatitis, or thyroid disease), pregnant or lactating females, and those receiving hormonal treatment. Patients receiving systemic retinoids during the preceding 3 months and those with a history of malignancy or keloid tendency were also excluded. A complete blood picture was performed for each participant at study initiation, and those with thrombocytopenia (less than 100,000 per ml) or anemia (hemoglobin < 12 g/dl in females, < 14 g/dl in males) were also excluded. Patients in Group A received intraslesional PRP intradermal injection using a 30 gauge syringe; patients in Group B received FCL (HiScan DOT tip of SmartXide DOT®, DEKA, Italy) using a power of 9 to 11 W, spacing of 200 µm, dwell time of 800 µs, and smart stack level 1 followed by PRP topically applied and gently rubbed onto the skin; and patients in Group C received micro-needling by dermaroller (1.5 mm needles) performed in horizontal, vertical, and oblique lines in several passes until mild erythema appeared followed by PRP applied topically and gently rubbed onto the skin. Treatment sessions were performed monthly for 3 consecutive months or until complete recovery, whichever came first. The area to be treated was anesthetized with a local anesthetic cream (5% lidocaine) for 30 minutes under occlusion in all groups before the procedure, and the skin was then disinfected using ethyl alcohol 75% spray.

The study protocol was approved by the Institutional Review Board of the ethical committee of Faculty of Medicine at Tanta University, and written informed consent was obtained from all study participants before study initiation.

PRP preparation

For PRP preparation, 10 ml of autologous blood was collected from each patient into a sterile tube containing 1.5 ml of sodium citrate anticoagulant solution. The citrated blood was centrifuged for 15 min at 1,000 rpm. The yellow plasma was taken up using a micropipette, and a second round of centrifugation was performed for 10 min at 4,000 rpm. A thrombocyte pellet in 1.0 ml of plasma (at the bottom) was used as PRP. Three percent calcium chloride was added at a concentration of 0.1 ml for each 1 ml of PRP to activate platelets.

All patients were instructed not to shampoo their hair on the day of session, to use a topical antibiotic cream for at least 3 days, and to report any side effects.

All patients were digitally photographed at baseline, at the end of the treatment sessions (2 weeks after the last session), and 6 months after the start of sessions for follow-up (Cybershot, DSC-HX5V, Sony Corp, Tokyo, Japan). The extent of scalp hair loss was determined according to the Severity of Alopecia Tool (SALT) score (13). The degree of improvement was graded according to the change in SALT score percentage as follows: 0 to 25% change = no improvement, 26 to 50% = mild improvement, 51 to 75% = moderate improvement, and 76 to 100% = excellent improvement.

Patient satisfaction with the treatment outcome in comparison to baseline was recorded at the end of the treatment protocol and 3 months after the last session as follows: 0 to 25% change = not satisfied, 26 to 50% = mildly satisfied, 51 to 75% = moderately satisfied, and 76 to 100% = very satisfied.

Pain during the procedure was evaluated after each session according to a numeric pain rating scale (NRS-10), which ranged from 0 (no pain) to 10 (most severe pain), and the mean value for the three sessions was then calculated for each patient (14).

Statistical analysis

Qualitative data were described using numbers and percentages. Quantitative data were described using ranges (minimum and maximum), mean, and standard deviation (SD). Chi-squared (χ^2) tests were carried out for comparison between groups in categorical variables, and numerical data were compared through an unpaired t-test by Statistical Package for the Social Sciences (SPSS) version 18 (SPSS Inc., Chicago, IL, USA). The significance of the results obtained was judged at the 5% level.

Results

The demographic and baseline clinical characteristics of AA patients included are presented in Table 1.

Most of the AA patients included (80%, 80%, and 70% in groups A, B, and C, respectively) experienced improvement of their condition to varying degrees after the three treatment sessions (Fig. 1). There was no significant difference in the degree of improvement between the groups studied at the end of the sessions by physicians' clinical assessment ($p = 0.268$, Table 2) or by patient satisfaction ($p = 0.147$, Table 3).

It should be noted that all patients in different groups that experienced improvement of their AA maintained their improvement without recurrence or regression of hair growth at follow-up visits after 3 months (Table 2).

Despite the use of adequate topical anesthesia prior to the sessions, discomfort and sometimes marked pain was detected in all patients during the sessions, which was significantly more obvious in the patients in Group A (treated by intradermal injection of PRP, mean NRS = 5.2 ± 0.9) than in those in Group B (treated with FCL followed by topical PRP, mean NRS = 3.1 ± 0.6 , $p < 0.05$) and Group C (treated with microneedling followed by topical PRP, mean NRS = 2.8 ± 0.4 , $p < 0.019$). All other reported side effects

Table 1 | Baseline demographic and clinical characteristics of groups of alopecia areata patients studied.

Characteristic	Group A (n = 20)	Group B (n = 20)	Group C (n = 20)
Age, mean \pm SD	30.20 \pm 11.49	34.20 \pm 14.55	29 \pm 7.64
Sex			
Female, n (%)	2 (10)	6 (30)	4 (20)
Male, n (%)	18 (90)	14 (70)	16 (80)
Disease duration, months, mean \pm SD	8.2 \pm 5.79	12 \pm 2.27	9.95 \pm 6.87
Type of AA, n (%)			
AA monolocularis	12 (60)	12 (60)	14 (70)
AA multilocularis	5 (25)	6 (30)	2 (10)
AA reticularis	2 (10)	1 (5)	2 (10)
Ophiasis	1 (5)	1 (5)	2 (10)
Distribution of AA, n (%)			
Parietal	6 (30)	6 (30)	2 (10)
Occipital	2 (10)	8 (40)	4 (20)
Vertex	4 (20)	0	6 (30)
Diffuse	4 (20)	4 (20)	6 (30)
Beard area	4 (20)	2 (10)	2 (10)
SALT score at baseline, n (%)			
S1 (< 25% hair loss)	12 (60)	14 (70)	12 (60)
S2 (25–49% hair loss)	4 (20)	4 (20)	4 (20)
S3 (50–74% hair loss)	4 (20)	2 (10)	2 (10)
S4 (75–99% hair loss)	0	0	2 (10)
S5 (100% hair loss)	0	0	0

AA = alopecia areata, SALT = Severity of Alopecia Tool score, Group A = intradermal PRP injection, Group B = fractional carbon dioxide laser + topical PRP, Group C = microneedling + topical PRP, SD = standard deviation.

Table 2 | Outcome of treatment modalities by physician assessment after three treatment sessions and at follow-up.

Improvement, <i>n</i> (% change)	Physician clinical assessment by group					
	End of sessions (2 weeks after third monthly session)			Follow-up (6 months after first session)		
	A (<i>n</i> = 20)	B (<i>n</i> = 20)	C (<i>n</i> = 20)	A (<i>n</i> = 20)	B (<i>n</i> = 20)	C (<i>n</i> = 20)
None (< 25%)	4 (20)	4 (20)	6 (30)	4 (20)	4 (20)	6 (30)
Mild (26–50%)	2 (10)	4 (20)	2 (10)	2 (10)	4 (20)	1 (5)
Moderate (51–75%)	4 (20)	6 (30)	2 (10)	2 (10)	4 (20)	3 (15)
Excellent (76–100%)	10 (50)	6 (30)	10 (50)	12 (60)	8 (40)	10 (50)
χ^2		7.609			5.403	
<i>p</i> -value		0.268			0.494	

Group A = intradermal PRP injection, Group B = fractional carbon dioxide laser + topical PRP, Group C = microneedling + topical PRP, χ^2 = chi-squared test.

Table 3 | Outcome of treatment modalities by patient satisfaction after three treatment sessions and at follow-up.

Patient satisfaction, <i>n</i> (% change)	Patient satisfaction by group					
	End of sessions (2 weeks after third monthly session)			Follow-up (6 months after first session)		
	A (<i>n</i> = 20)	B (<i>n</i> = 20)	C (<i>n</i> = 20)	A (<i>n</i> = 20)	B (<i>n</i> = 20)	C (<i>n</i> = 20)
None (< 25%)	6 (30)	5 (25)	7 (35)	6 (30)	6 (30)	5 (25)
Mild (26–50%)	0	4 (20)	1 (5)	0	4 (20)	2 (10)
Moderate (51–75%)	4 (20)	6 (30)	4 (20)	2 (10)	5 (25)	3 (15)
Very (76–100%)	10 (50)	5 (25)	8 (40)	12 (60)	5 (25)	10 (50)
χ^2		9.5			8.5	
<i>p</i> -value		0.147			0.204	

Group A = intradermal PRP injection, Group B = fractional carbon dioxide laser + topical PRP, Group C = microneedling + topical PRP, χ^2 = chi-squared test.

after treatment sessions in the form of erythema, edema, itching, ecchymosis, headache, or crusting were minimal, transient, and tolerable.

There was no significant correlation between the degree of improvement at the end of sessions and patient age or disease duration ($p = 0.862$, 0.367 , respectively). There was no significant relation between degree of improvement at the end of the sessions and patient sex ($p = 0.717$) or type of AA ($p = 0.4$). There was a significant relation between the degree of improvement at the end of the sessions and different AA distributions (Z -test = 17.382 , $p = 0.004$), whereby lesions on the beard followed by those on the vertex of scalp showed the best degree of improvement with therapy.

Discussion

PRP is a controversial therapy for treating AA. In this study, most of the AA patients included showed an adequate response and variable degrees of improvement with PRP therapy in various treatment protocols. Previous investigators claimed that PRP diminishes hair loss and stimulates hair regrowth with subjective improvement in the mean number of hairs and mean hair density in patients with severe chronic AA (15, 16). The beneficial effect of PRP in AA could be attributed to its activating effect on antiapoptotic regulators, such as Bcl-2 protein and Akt signaling, leading to prolonged survival of dermal papilla cells during the hair cycle and a prolonged anagen phase. PRP was also found to have a potent anti-inflammatory effect. It suppresses cytokine release and limits local tissue inflammation, which makes PRP potentially beneficial in treating inflammatory hair conditions such as AA (17, 18).

In this study, the combination of FCL and topical PRP in group B yielded a convenient therapeutic effect in AA, which was comparable to intradermal PRP. Some studies have shown fractional lasers to have an effective role in hair growth in cases of AA (19, 20). Yoo et al. (20) concluded that fractional photothermolysis laser was effective alone in treatment of AA. The mechanism of fractional laser in inducing hair regrowth in AA was thought to be

through induction of T-cell apoptosis, decrease in perifollicular lymphocytic infiltration through scattering of perifollicular lymphocytes, and enhancement of hair growth. Moreover, fractional laser therapy may halt AA disease progression by arresting the hair follicles in the telogen stage of the hair cycle and increasing the anagen stage (20). Furthermore, minor trauma and wound healing itself can drive hair growth by increasing the blood flow in the dermal papilla (21). In this study, the combination of FCL and topical PRP provided channels for transepidermal delivery of topical PRP and was associated with significantly lower pain scores than intradermal PRP injection while preserving the therapeutic effect of PRP in AA.

It should be noted that laser-assisted drug delivery of PRP—with or without the use of bimatoprost and/or minoxidil—has had very encouraging results in patients with androgenetic alopecia due to potential maximization of stimulation to the bulge area of the hair follicles with consequent hair growth (22). The author recommended considering this combination to ensure the uniform deposition of PRP in the dermis with avoidance of multiple painful injections (22).

In our study, most patients in Group C with AA (60%) experienced moderate to excellent improvement. Some studies used a dermaroller as a tool for transepidermal drug delivery of different drugs in AA with various results. Deepak et al. (23) used a dermaroller for delivery of a mixture of 1 ml of triamcinolone acetonide, 0.5 ml of meso-solution, and 0.5 ml of injectable minoxidil 2 to 5% in AA. They showed that a dermaroller was useful and safe in treating multiple patches or alopecia totalis, but it could not be ascertained strongly with the role of each modality (23). Moreover, a scalp roller has been suggested as a sole treatment option that helps stimulate hair growth by breaking down the dermal capillaries so that platelets start to form a plug at the site of damage with release of chemotactic factors or growth factors (23). We think that this process could be improved by adding topical PRP as a nutritional elixir to enhance hair regrowth in AA after the almost painless skin perforation with dermarolling. In this study, pain scores were significantly lower in patients in Group C than in



Figure 1 | Photos before treatment and 2 weeks after third session at monthly intervals with excellent response: a, b) 33-year-old male with alopecia areata (AA) monocularis affecting beard region treated with intraslesional platelet-rich plasma (PRP); c, d) 16-year-old male with AA affecting vertex region treated with intraslesional PRP; e, f) 34-year-old female with AA of vertex region treated with fractional CO₂ laser + topical PRP; g, h) 23-year-old female with AA monocularis of vertex region treated with dermaroller + topical PRP.

those in Group A with intradermal PRP injection.

In this study, there was no significant relation between improvement at the end of the sessions and patients' sex. On the other hand, females were previously reported to have a greater likelihood of extensive AA and poorer prognosis than males (24). This was attributed to the significantly increased prevalence of anti-thyroid antibodies in female patients, reported to be present in 30% overall and in 44% of the youngest age group (11–17 years), in addition to smooth muscle antibodies (25).

In this study, there was a significant relation between improvement at the end of the sessions and different AA distributions, with better results for lesions on the beard and vertex. In a recent case report, the authors treated a healthy 30-year-old male suffering from AA of the beard area with only three injections of PRP at 6-week intervals. They reported stabilization of AA 6 weeks after the first injection and initial minimal hair regrowth before the third injection (26). Moreover, AA of the beard area was the only type that showed complete resolution after four sessions of photodynamic therapy, whereas none of the patients with AA of the scalp achieved complete hair regrowth (27). The authors thought that it may be that AA of beard hair responds better to photodynamic therapy, but we believe that further studies on larger patient series are required to prove whether there is location vari-

ability in response to different treatment modalities.

This study has some limitations. The placebo-like effect of different treatment modalities in different AA patients cannot be overlooked, and the variation in patient response and even in every lesion response to treatment should be considered. The small sample size, short period of follow-up, and absence of a control group are also limitations of this study.

Conclusions

Autologous PRP either delivered through injection or applied topically after FCL or microneedling is a potentially effective, inexpensive, and safe treatment modality for AA. Apart from the pain associating intradermal injection, side effects are minimal. FCL and microneedling could help with AA through a dual mechanism: first, through their suggested roles in enhancing hair regrowth in AA and, second, by opening conduits for topical PRP delivery, thus bypassing painful injection, and preserving the efficacy of intralesional PRP injection. Microneedling has an additional advantage of being less costly than FCL. Topical PRP after FCL or microneedling could be helpful for more extensive AA lesions affecting wider areas, particularly in anxious patients or in children to decrease the discomfort and pain associated with PRP injection.

References

- Simakou T, Butcher JP, Reid S, Henriquez FL. Alopecia areata: a multifactorial autoimmune condition. *J Autoimmun.* 2019;98:74–85.
- Cranwell WC, Lai VW, Photiou L, Meah N, Wall D, Rathnayake D, et al. Treatment of alopecia areata: an Australian expert consensus statement. *Australas J Dermatol.* 2019;60:163–70.
- Marchitto MC, Qureshi A, Marks D, Awosika O, Rengifo-Pardo M, Ehrlich A. Emerging nonsteroid-based procedural therapies for alopecia areata: a systematic review. *Dermatol Surg.* 2019;45:1484–1506.
- Garg S, Manchanda S. Platelet-rich plasma—an “elixir” for treatment of alopecia: personal experience on 117 patients with review of literature. *Stem Cell Investig.* 2017;4:64.
- Harrison J. Advances in aesthetic therapies: plasma-rich protein procedure for the treatment of alopecia. *Plast Surg Nurs.* 2017;37:52–5.
- Manstein D, Herron GS, Sink RK, Tanner H, Anderson RR. Fractional photothermolysis: a new concept for cutaneous remodeling using microscopic patterns of thermal injury. *Lasers Surg Med.* 2004;34:426–38.
- Perper M, Aldahan AS, Fayne RA, Emerson CP, Nouri K. Efficacy of fractional lasers in treating alopecia: a literature review. *Lasers Med Sci.* 2017;32:1919–25.
- Laubach HJ, Tannous Z, Anderson RR, Manstein D. Skin responses to fractional photothermolysis. *Lasers Surg Med.* 2006;38:142–9.
- Sklar LR, Burnett CT, Waibel JS, Moy RL, Ozog DM. Laser assisted drug delivery: a review of an evolving technology. *Lasers Surg Med.* 2014;46:249–62.
- Waibel JS, Rudnick A. Laser-assisted delivery to treat facial scars. *Facial Plast Surg Clin North Am.* 2017;25:105–17.
- Doddaballapur S. Microneedling with dermaroller. *J Cut Aesthet Surg.* 2009;2:110.
- Prausnitz MR. Microneedles for transdermal drug delivery. *Adv Drug Deliv Rev.* 2004;56:581–7.
- Olsen EA, Hordinsky MK, Price VH, Roberts JL, Shapiro J, Canfield D, et al. Alopecia areata investigational assessment guidelines—Part II. *J Am Acad Dermatol.* 2004;51:440–7.
- Jensen MP. Measurement of pain. In: Fishman SM, Ballantyne JC, Rathmell JP, editors. *Bonica's management of pain.* Media, PA: Williams & Wilkins; 2010. p. 251–70.
- Vekris A, Zafeiratou D, Andriopoulou A, Tsiatoura A. Total regrowth in chronic severe alopecia areata treated with platelet rich plasma: a case report and literature review. *Dermatol Surg.* 2015;25:190–1.
- Singh S. Role of platelet-rich plasma in chronic alopecia areata: our centre experience. *Indian J Plast Surg.* 2015;48:57–9.
- Mubki T. Platelet-rich plasma combined with intralesional triamcinolone acetate for the treatment of alopecia areata: a case report. *J Saudi Soc Dermatol Dermatologic Surg.* 2016;20:87–90.
- Li J, Choi HI, Choi DK, Sohn KC, Im M, Seo YJ, et al. Autologous platelet-rich plasma: a potential therapeutic tool for promoting hair growth. *Dermatol Surg.* 2012;38:1040–6.
- Eckert MM, Gundin NL, Crespo RL. Alopecia areata: good response to treatment with fractional laser in 5 cases. *J Cosmo Trichol.* 2016;2:108.
- Yoo K, Kim M, Kim B, Kim C. Treatment of alopecia areata with fractional photothermolysis laser. *Int J Dermatol.* 2010;49:845.
- Chuong CM. Regenerative biology: new hair from healing wound. *Nature.* 2007;447:265–6.
- Cohen PR. Laser-assisted drug delivery for the treatment of androgenetic alopecia: ablative laser fractional photothermolysis to enhance cutaneous topical delivery of platelet-rich plasma—with or without concurrent bimatoprost and/or minoxidil. *Dermatol Online J.* 2019;25:13030/qt7243d5h4.
- Deepak SH, Shwetha S. Scalp roller therapy in resistant alopecia areata. *J Cutan Aesthet Surg.* 2014;7:61–2.
- Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part I. Clinical picture, histopathology, and pathogenesis. *J Am Acad Dermatol.* 2010;62:177–88.
- Ucak H, Cicek D, Demir B, Erden I, Ozturk S. Prognostic factors that affect the response to topical treatment in patchy alopecia areata. *J Eur Acad Dermatol Venereol.* 2014;28:34–40.
- Pototschnig H, Madl MT. Successful treatment of alopecia areata barbae with platelet-rich plasma. *Cureus.* 2020;12:e7495.
- Fernández-Guarino M, Harto A, García-Morales I, Pérez-García B, Arrazola JM, Jaén P. Failure to treat alopecia areata with photodynamic therapy. *Clin Exp Dermatol.* 2008;33:585–7.

