Dermatology Practical & Conceptual

Transepidermal Delivery of Triamcinolone Acetonide or Platelet Rich Plasma Using Either Fractional Carbon Dioxide Laser or Micro-needling in Treatment of Alopecia Areata

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ABSTRACT

Introduction: Trans-epidermal drug delivery, using "laser-assisted drug delivery", or micro-needling, are new treatment modalities, that can improve drug penetration into skin in treatment of alopecia areata patients.

Objectives: To evaluate the use of fractional carbon dioxide laser versus micro-needling in trans-epidermal delivery of triamcinolone acetonide and platelet rich plasma in alopecia areata treatment.

Methods: Interventional comparative study carried out on 60 patients, randomly divided into four equal groups. Group I: Fractional Carbon dioxide laser and triamcinolone acetonide. Group II: micro-needling with Dermapen and triamcinolone acetonide. Group III: fractional carbon dioxide laser and platelet-rich plasma. Group IV: micro-needling with Dermapen and platelet-rich plasma. Patients were evaluated clinically, using Severity of Alopecia Tool score and hair regrowth scale, and dermoscopically.

Results: In all treatment groups, there was improvement in the Regrowth scale, with statistical significance between the different groups at fourth (P = 0.001) and last (P = 0.008) visits, with highest, most significant changes in Pen-Steroid group. Comparing Regrowth scale at last visit, results were in

favor of Dermapen, compared to Carbon dioxide laser for trans-epidermal drug delivery (P = 0.023); and in favor of triamcinolone acetonide, compared to platelet-rich plasma as topical medication (P = 0.015). Dermoscopic signs of improvement included decrease in black dots, and appearance of Upright regrowing hairs (P < 0.001).

Conclusions: Micro-needling and fractional carbon dioxide laser are effective tools for trans-epidermal drug delivery for Alopecia areata treatment. Micro-needling for delivery of Triamcinolone acetonide showed best treatment outcomes. Dermoscopy is highly beneficial in evaluating treatment response in alopecia areata.

Introduction

Trans-epidermal drug delivery (TED) depends on using ablative method (CO_2 laser, erbium lasers or ablative radiofrequency), to create vertical channels through the epidermis. This is followed by applying a medication (eg triamcinolone actenoide, platelet rich plasma) that is delivered through these channels into the skin. "Laser-assisted drug delivery" is the specific use of lasers for TED. Micro-needling technique can be used for the same purpose [1].

Objectives

The aim of this study is to evaluate the use of fractional carbon dioxide laser versus micro-needling in trans-epidermal delivery of triamcinolone acetonide and platelet rich plasma in alopecia areata (AA) treatment, clinically and dermoscopically.

Methods

Patients Group

This interventional comparative study was carried out on 60 patients, of either sex, presenting with AA to the Dermatology, Venereology and Andrology outpatient and Hair clinics in the Main University Hospital. The local Ethics Committee approved the study, and all procedures were in accordance with the Helsinki Declaration of 1975, as revised in 2000. All patients signed an informed written consent. Assent was obtained from minors, and their parents signed written consents.

The inclusion criteria were patients with AA of both sexes, aged 6-60 years, not responding to treatment (topical and/or systemic) for at least 3 months, and off treatment for at least 1 month, prior to the study. The exclusion criteria [2,3] included AA with spontaneous hair regrowth, active scalp inflammation, other scalp or hair diseases, history of hypertrophic scar or keloid, bleeding disorders, and long-term use of anti-coagulant therapy. Pregnant and lactating females and immunocompromised patients were excluded.

Closed envelope method was used to randomly distribute the patients over the study groups. The study included four groups, 15 patients each:

Group I: Fractional Carbon dioxide laser (CO_2 Laser) and triamcinolone acetonide (TrA; 10mg/ ml) [1,4]:

Fractional CO_2 laser was used in ablative mode, using (ATL-250 laser): 10,600nm CO_2 medical laser system built by Advanced Technology Laser Company, Ltd., Shanghai, China. Scanning mode was used with the following parameters: power of 20 Watts, density of PPI 4 (pulses per inch, i.e. array density), and pulse duration/time on of three milliseconds. One pass was applied to the treated area without gaps between pulses, overlap of about 20% was allowed. Scanning area was set to square shape, ratio 9/9, and size 100%. In smaller patches, dimensions were modifiable. Immediately after the laser pass, TrA solution was dripped on the treated area and spread evenly using the blunt end of syringe.

Group II: Micro-needling with Dermapen and triamcinolone acetonide (TrA; 10 mg/ml) [5]:

Dermapen with a 36-needle disposable tip was used, with 2-2.5 mm long needle depth. The speed of the needles' movement and of the Dermapen movement was adjusted to the patient's tolerance to pain. The desired end point was minute pinpoint bleeding points or mild erythema. TrA was applied before, during and after performing micro-needling.

Group III: Fractional carbon dioxide laser (CO_2 Laser) and platelet-rich plasma (PRP) [6]:

The same laser parameters as group I were used, followed by application of freshly prepared PRP. PRP was prepared using double-centrifugation protocol, which results in higher platelet concentrations, compared to single centrifugation protocol [7]. For PRP, 10 cc of venous blood were collected from antecubital vein under aseptic conditions, into tubes containing sodium citrate (10:1) as anticoagulant. The initial centrifugation ("soft"/ light spin) was done at 2000 rpm for 5 min. The second centrifugation step (heavy / "hard" spin) was carried out at 4000 rpm for 15 min.

Group IV: Micro-needling with Dermapen and Platelet-rich plasma (PRP) [2,5]:

Micro-needling was performed as Group II; however, diluted TrA was substituted by PRP.

For all groups:

Each patient received four treatment sessions, spaced three weeks apart [1,2,5] , followed by a follow up visit, four weeks after the last treatment session. Prior to the procedure topical anesthetic cream, (pridocaine2.5% + lidocaine 2.5%) was applied under occlusion for 15-60 minutes. Patients were instructed not to wash their scalp on the treatment day. No treatments for the alopecia were allowed. Topical post-procedure care, including topical antibiotics, emollient or sunscreen could be used.

On the first visit, thorough history was taken, followed by clinical and trichoscopic evaluation [2-4,8]. Trichoscopic evaluation [9-11] was performed using a DermLite® DL4 (3 Gen), at 10× magnification in polarized mode.

Patient Evaluation

The patients were assessed clinically and dermoscopically for signs of hair regrowth at each visit, and at the follow up visit. Using Samsung J5 Pro 13-megapixel camera with F1.7 lens, serial digital photographs (clinical and dermoscopic) of the alopecic patches were taken prior to commencement of the treatment, during the treatment sessions and at the end of the treatment. Two independent investigators evaluated the photographs.

Severity of Alopecia Tool (SALT) score at baseline, at each visit, and at end of study, and hair regrowth scale, were used to calculate treatment response [2,8]. Global assessment score [8] was used to assess the overall improvement, taking into account extent and density of regrowth by SALT score: A0 = no change or further loss, A1 = 1-24% regrowth, A2 = 25-49% regrowth, A3 = 50-74% regrowth, A4 = 75-99% regrowth, and A5 = 100% regrowth. According to the Regrowth scale (RGS), the degree of clinical improvement was evaluated according to a 6-point semi-quantitative score: RGS 0 (re-growth <10%), RGS1 (re-growth 11%-25%), RGS2 (re-growth 26%-50%), RGS3 (re-growth 51%-75%), RGS4 (re-growth ≥75%), and RGS5 (re-growth =100%) [12].

Any side effects like atrophy and telangiectasia were observed, clinically and dermoscopically. Patient satisfaction with results of the procedure was graded as satisfied, fair, and unsatisfied. Pain during procedure was graded as: no pain - mild -moderate -severe- pain as bad as it could be [13].

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (IBM Corp). Significance of obtained results was judged at 5% level.

Results

Demographic Data (Table (1))

Table (1) represents different demographic data and patients details, with no significant difference between all groups. There was no significant difference between the studied groups as regards the Baseline Hair loss, using SALT score [8,9].

Baseline and Follow-up SALT Scores

There was no significant difference in SALT score at baseline between the 4 groups.

However, SALT score at last follow-up visit showed statistically significant difference between the different groups (P = 0.005). Eighty percent of patients in Pen-Steroid group improved to SALT S0 (no hair loss), compared to only 40% of patients in CO_2-Steroid and Pen-Steroid groups, and only 13.3% in the CO_2-PRP group. Pen-Steroid group showed significantly higher improvement compared to CO_2-PRP group (P = 0.001).

Rate of Hair Regrowth (Figures 1-5)

Over subsequent visits, in all treatment groups, there was a shift in the RGS towards higher scores with improved hair regrowth percentages. However, this improvement showed statistical significance between the different treatment groups only at fourth (P = 0.001) and fifth (P = 0.008) visits. At fourth visit, Pen-Steroid group showed maximum improvement with 53.3% of patients scoring RGS4, followed by Pen-PRP group (40% of patients), then CO_2-Steroid group (33.3% RGS4+5), and finally CO_2-PRP group with only 13.3% of patients. The difference between Pen-Steroid group and CO_2-PRP group was significant (P = 0.003). At the final follow up visit, Pen-Steroid group showed maximum improvement with 80% of patients scoring RGS5, followed by Pen-PRP and CO_2-Steroid group (40% of patients), and finally CO_2-PRP group with 13.3% of patients. The difference between Pen-Steroid group and CO_2-PRP group was statistically significant (P < 0.001).

Hair Regrowth Score (RGS) at the End of Study

Improvements in RGS at the end of study, were in favor of using Dermapen for TED (mean RGS 3.93 ± 1.66), compared to CO_2 laser (mean RGS 3.13 ± 1.68) , with P = 0.023. Moreover, higher RGS were obtained with TrA as topical medication (mean RGS 4.0 ± 1.53), compared to PRP (mean RGS 3.07 ± 1.76), with P = 0.015.

Dermoscopic Evaluation (Table (2); Figures 6-8)

At baseline, most common dermoscopic findings were black dots, in 65% of patients, yellow dots and white dots in 45%

Table 1. Comparison between the four studied groups according to demographic data.

	Steroid CO_2 (N = 15)		Steroid Pen (N = 15)		PRP CO_2 (N = 15)		PRP Pen (N = 15)		Test of Significance	P
	No.	%	No.	%	No.	%	No.	%		
Sex										
Male	6	40.0	9	60.0	6	40.0	10	66.7	χ^2=3.404	0.333
Female	9	60.0	6	40.0	9	60.0	5	33.3		
Age (years)										
<15	5	33.3	2	13.3	2	13.3	3	20.0	χ^2=10.849	(MC) 0.075
15 – 30	8	53.3	6	40.0	7	46.7	2	13.3		
>30	2	13.3	7	46.7	6	40.0	10	66.7		
Duration (years)										
<2 Y	7	46.7	9	60.0	6	40.0	9	60.0	χ^2=1.802	0.614
≥2 y	8	53.3	6	40.0	9	60.0	6	40.0		
Present episode (months)										
<6 months	8	53.3	6	40.0	7	46.7	12	80.0	χ^2=8.192	(MC) 0.135
6 - 1Y	7	46.7	9	60.0	7	46.7	3	20.0		
>1 Y	0	0.0	0	0.0	1	6.7	0	0.0		
No of relapse										
First attack	7	46.7	6	40.0	5	33.3	6	40.0	χ^2=0.556	0.907
Recurrent	8	53.3	9	60.0	10	66.7	9	60.0		
Type of AA										
Patchy	11	73.3	14	93.3	11	73.3	13	86.7	χ^2=10.065	(MC) 0.214
Ophiasis	3	20.0	0	0.0	2	13.3	0	0.0		
Subtotalis	0	0.0	1	6.7	2	13.3	2	13.3		
Universalis	1	6.7	0	0.0	0	0.0	0	0.0		
Family history										
Negative	9	60.0	9	60.0	8	53.3	11	73.3	χ^2=1.340	0.720
Positive	6	40.0	6	40.0	7	46.7	4	26.7		
Hair loss (%)										
Min. – Max.	1.0 – 100.0		1.0 – 80.0		2.0 – 80.0		2.0 – 80.0		H=0.996	0.802
Mean ± SD.	12.80 ± 24.65		11.47 ± 19.50		16.60 ± 25.99		13.53 ± 20.53			
Median (IQR)	4.0 (2.50 – 12.50)		4.0 (3.0 – 12.0)		7.0 (5.0 – 8.0)		5.0 (3.0 – 12.0)			

H = H for Kruskal Wallis test; IQR = Inter quartile range; MC = Monte Carlo.

of patients, exclamation mark hairs in 38.3%, and the least common finding was vellus hair in 11.7% of patients.

Black dots were present at baseline in all groups, and their incidence decreased with treatment. This decrease in black dot, indicating improvement, was statistically significant in all groups (CO_2-Steroid group P = 0.008, Pen-Steroid group P = 0.002, Pen-PRP group P = 0.031), except in the CO_2-PRP group.

Exclamation mark hairs were present at baseline in all groups, as third most common dermoscopic finding, and decreased with treatment, especially in Pen-Steroid group (p = 0.002), where the exclamation mark hairs completely disappeared in all patients, after the third treatment session.

Yellow dots were present at baseline in all groups, and decreased with treatment, but without statistical significance.

The decrease in white dots at end of treatment was significantly better in Pen-steroid group compared to the other three groups (P = 0.002). White dots disappeared in 53.3% of affected patients in Pen-steroid group (P = 0.008), compared to only 13.3% in CO_2-PRP and Pen-PRP groups, and none of the affected patients in the CO_2-Steroid group.

Vellus hairs showed no statistically significant difference in occurrence, along sessions.

Upright regrowing hairs were the most consistent feature to indicate hair regrowth. It started to appear after the first treatment session, in most patients in all 4 groups,

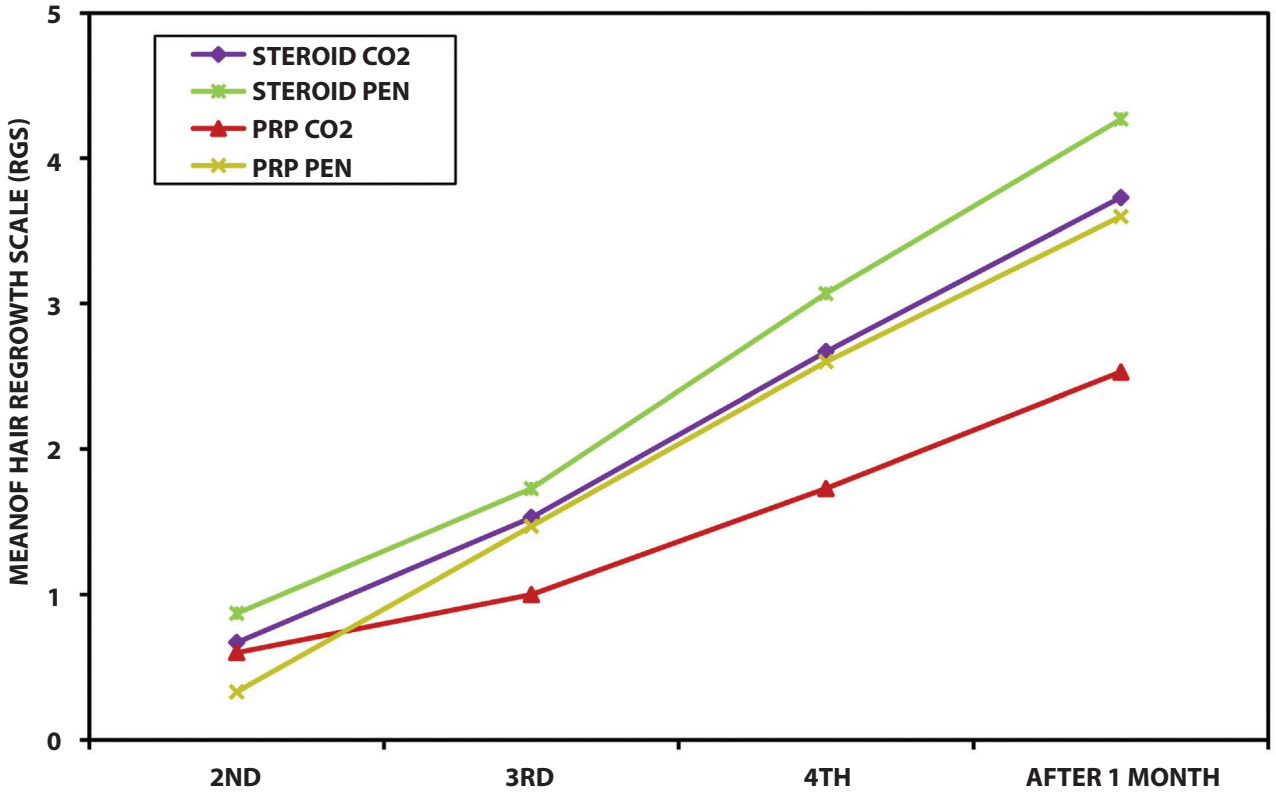

Figure 1. Comparison between the four studied groups according to hair regrowth scale (RGS) over the treatment course. PRP = platelet-rich plasma.

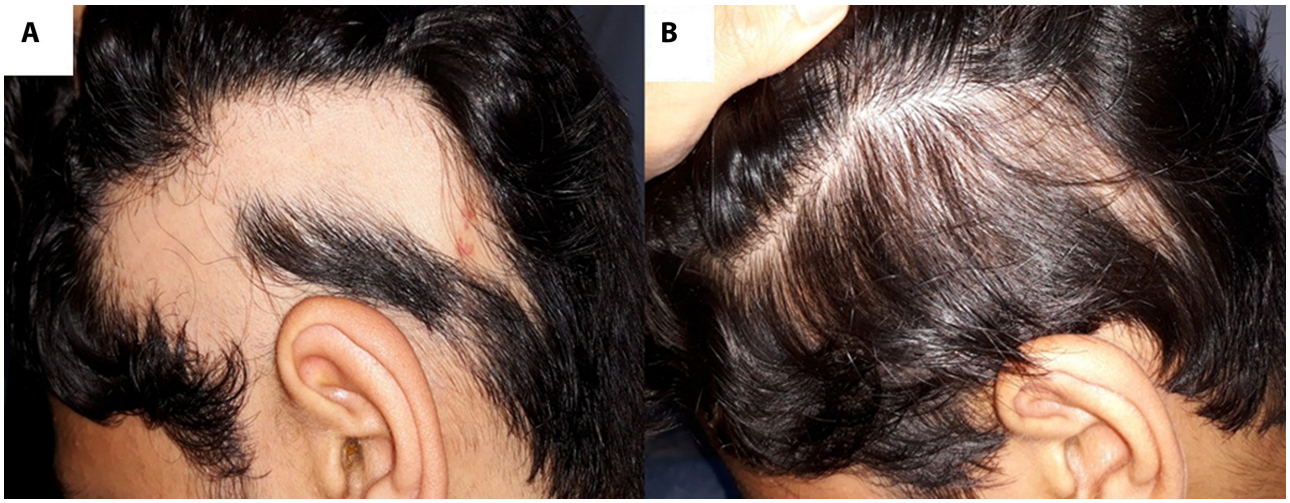

Figure 2. Pen-Steroid group. (A) At baseline. (B) At last follow up visit with complete hair regrowth (100%), hair regrowth scale score 5.

with a statistically significant increase in all study groups ($P < 0.001$).

Terminal hairs, also started to appear after first treatment session, indicating hair regrowth. There was statistically significant rise from baseline to follow up, in all treatment groups ($P < 0.001$ in CO_2-Steroid, Pen-Steroid and Pen-PRP groups; $P = 0.002$ in CO_2-PRP group).

Pig tail hairs appeared transiently in the course of treatment. In Pen-Steroid group 60% of patients showed pig tail hairs, which was significantly higher than only 13.3% of patients in CO_2-PRP group ($P = 0.008$). Also, patients who showed pig tail hairs in Pen-PRP group where significantly more compared to CO_2-PRP group (53.3% versus 13.3%, $P = 0.020$).

The study procedure caused no complications that could be observed by dermoscopy. However, telangiectasia and areas of fibrosis, due to previous intralesional steroid injection, could be visualized dermoscopically.

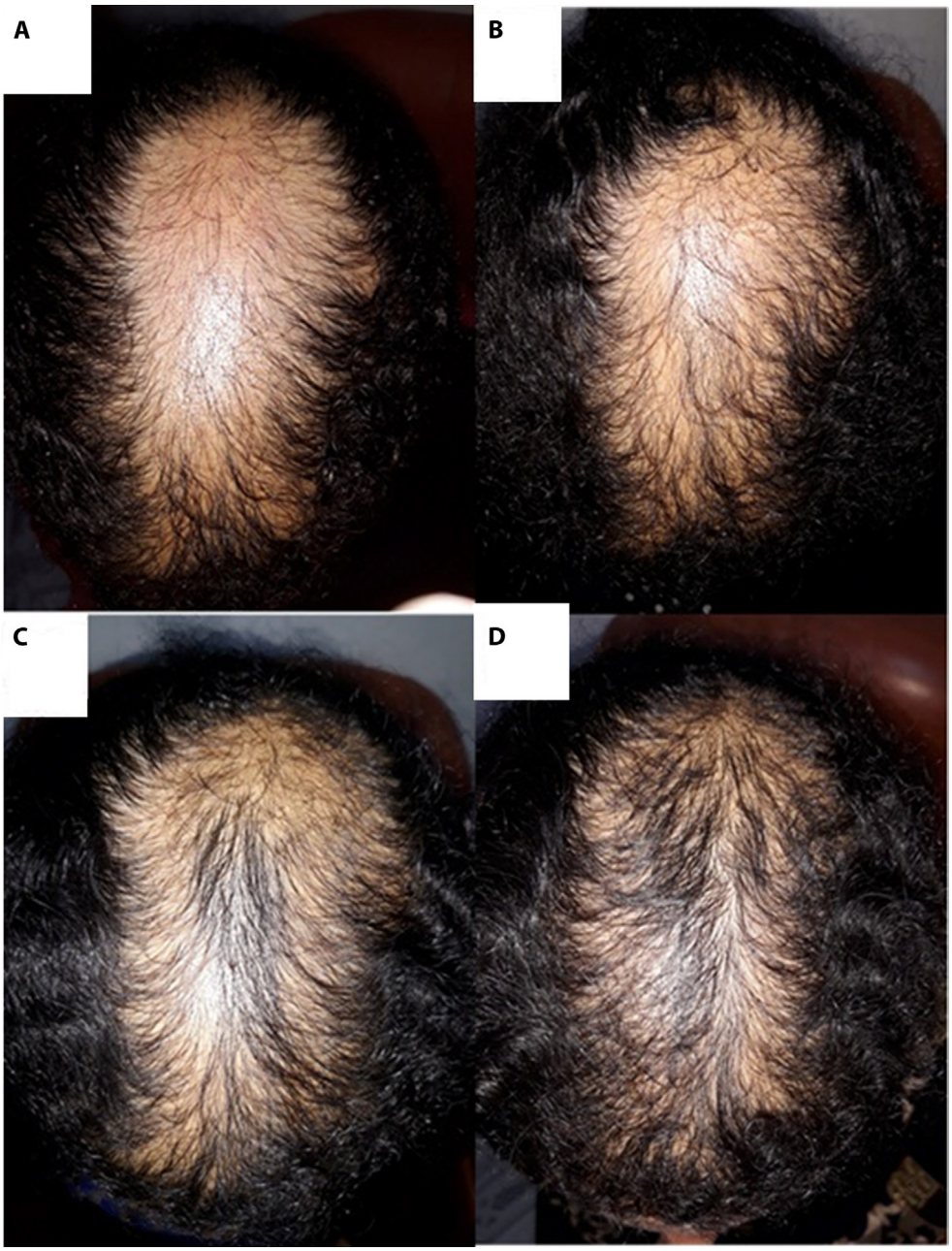

Figure 3. Pen-PRP group. (A) At base line. (B) At 3rd visit. (C) At 4th visit. (D) At last follow-up visit with complete hair regrowth (100%), hair regrowth scale score 5.

Relation Between Clinical Response and Dermoscopic Features at Baseline (Table 6)

Amongst all 60 patients, presence of black dots at baseline, could not indicate response to treatment. However, presence of Exclamation mark hairs at base line was significantly related to response (P = 0.024), where it was present in 48.7% of responder patients, compared to only 19% of non-responder ones. Yellow dots were significantly related to poor response to treatment (P = 0.003), present in 71.4% of non-responder patients at baseline, compared to only 30.8% of responder ones. White dots and vellus hairs were represented insignificantly among responder patients and non-responder ones at baseline.

Responders were those with hair regrowth ≥ 75% (ie ≥ RGS 4/ global assessment score A4) [14].

Onset of Dermoscopic Improvement

Most patients across all treatment groups showed first signs of dermoscopic improvement after first treatment session. Out of the 30 patients receiving TrA, 86.7% showed dermoscopic improvement after first treatment session, compared to 80% of the 30 patients receiving PRP, without statistical significance. The number of patients in Dermapen groups, who showed dermoscopic improvement after first treatment session, was significantly higher than in CO_2 groups (P = 0.010). This indicates that

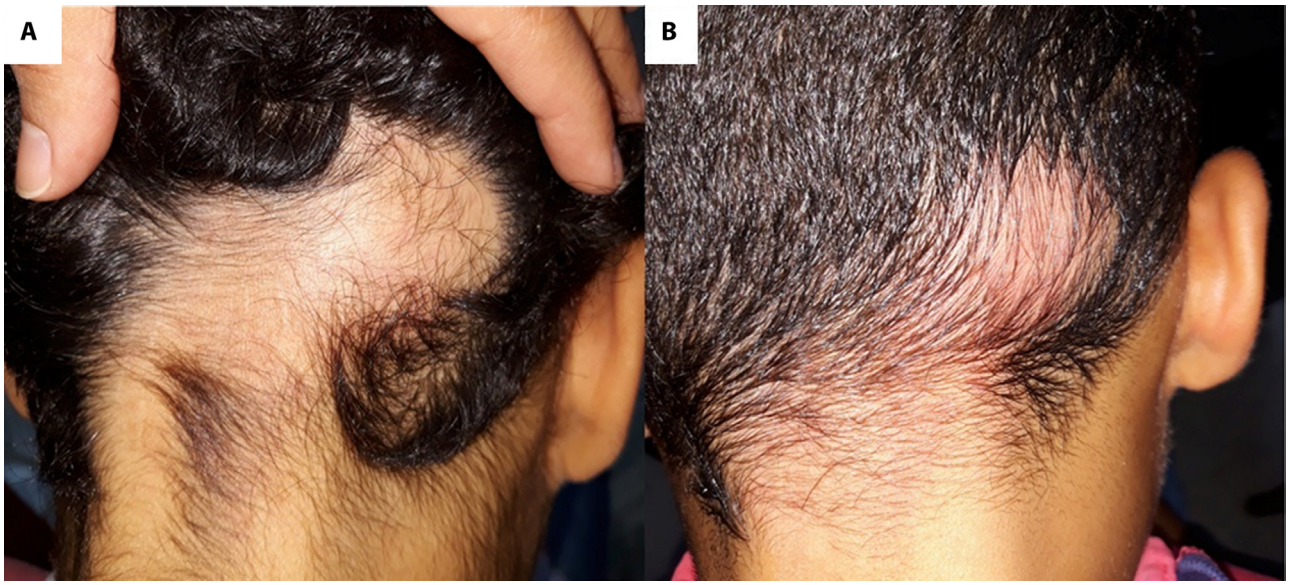

Figure 4. CO_2-Steroid group. (A) At baseline. (B) At last follow up visit with complete hair regrowth (100%), hair regrowth scale score 5.

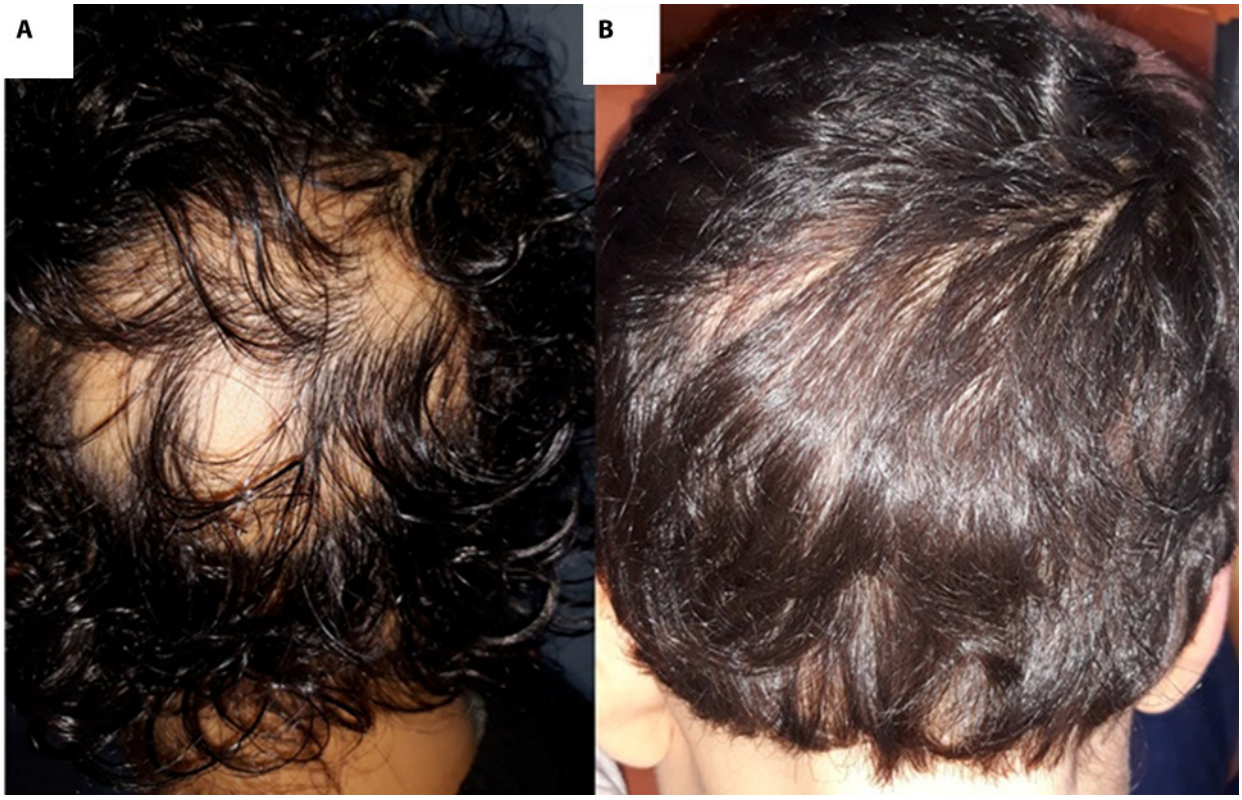

Figure 5. CO_2-PRP group. (A) At baseline. (B) At last follow-up visit with complete hair regrowth (100%), hair regrowth scale score 5.

Dermapen might show faster improvement, compared to fractional CO_2.

Onset of Hair Growth Clinically

Seventy percent of patients in Steroid groups started to show hair regrowth after first treatment session versus 66.7% in PRP groups, without statistical significance. The number of patients in Dermapen group, who started to show hair regrowth after first treatment session, was significantly higher (P = 0.046), than CO_2 groups (80.0% versus 56.7%).

Relation Between Onset of Dermoscopic and Clinical Improvement

Across all 60 patients included in the study, 82% who sowed dermoscopic improvement after first session also showed clinical hair regrowth after first session (statistically significant).

Table 2. Agreement (sensitivity, specificity and accuracy) for Exclamation mark hairs and Yellow Dots.

1st	Non responders (n = 21)		Responders (n = 39)		Sensitivity	Specificity	PPV	NPV	Accuracy
	No.	%	No.	%					
Exclamation mark hairs									
No	17	81.0	20	51.3	48.72	80.95	82.61	45.95	60.0
Yes	4	19.0	19	48.7					
Yellow Dots									
No	6	28.6	27	69.2	30.77	28.57	44.44	18.18	30.0
Yes	15	71.4	12	30.8					

PPV: Positive predictive value
NPV: Negative predictive value

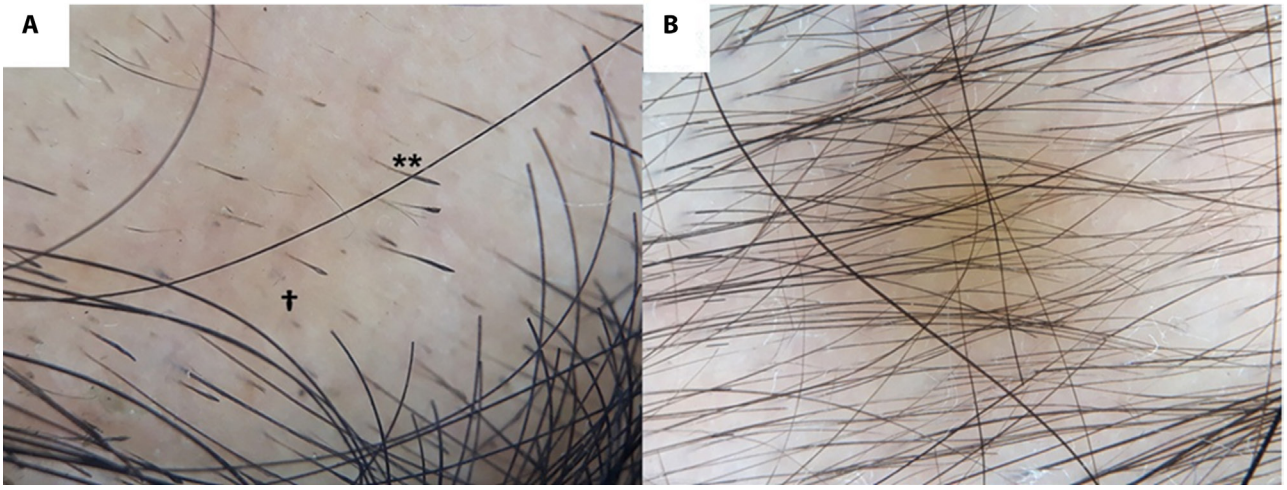

Figure 6. Pen-Steroid group: Black dots and Exclamation mark hairs. (A) At baseline. (), (B) Disappearing at 4th follow up visit (), that features mainly upright regrowing and terminal hairs.
† Black dot
** Exclamation mark hairs

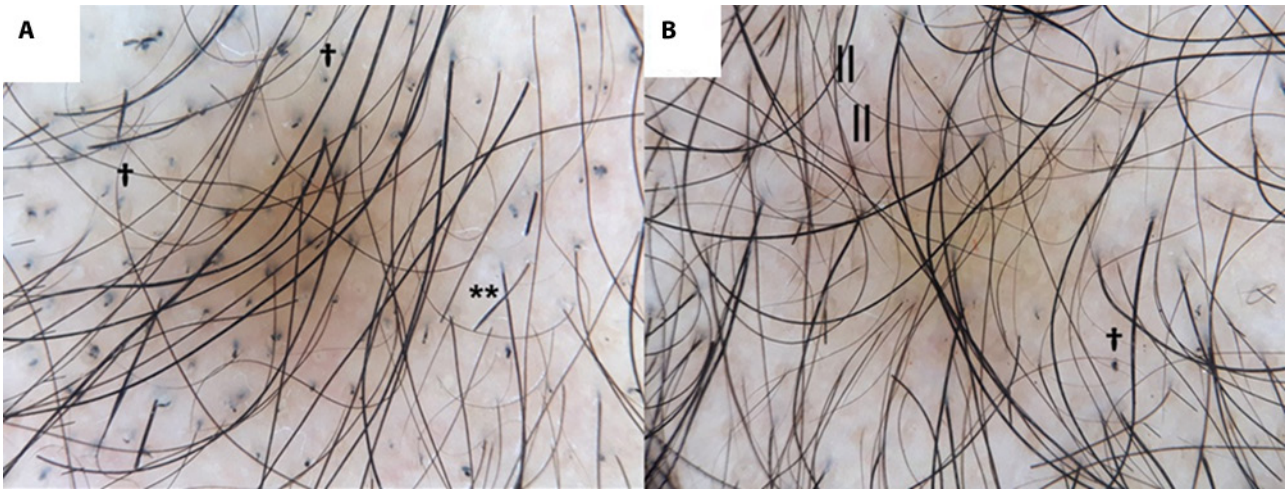

Figure 7. Pen-PRP group: Black dots and Exclamation mark hairs. (A) At baseline. ((B) Significant decrease at 4th follow up visit (), with appearance of upright regrowing and terminal hairs.
† Black dot
** Exclamation mark hairs
|| Upright regrowing

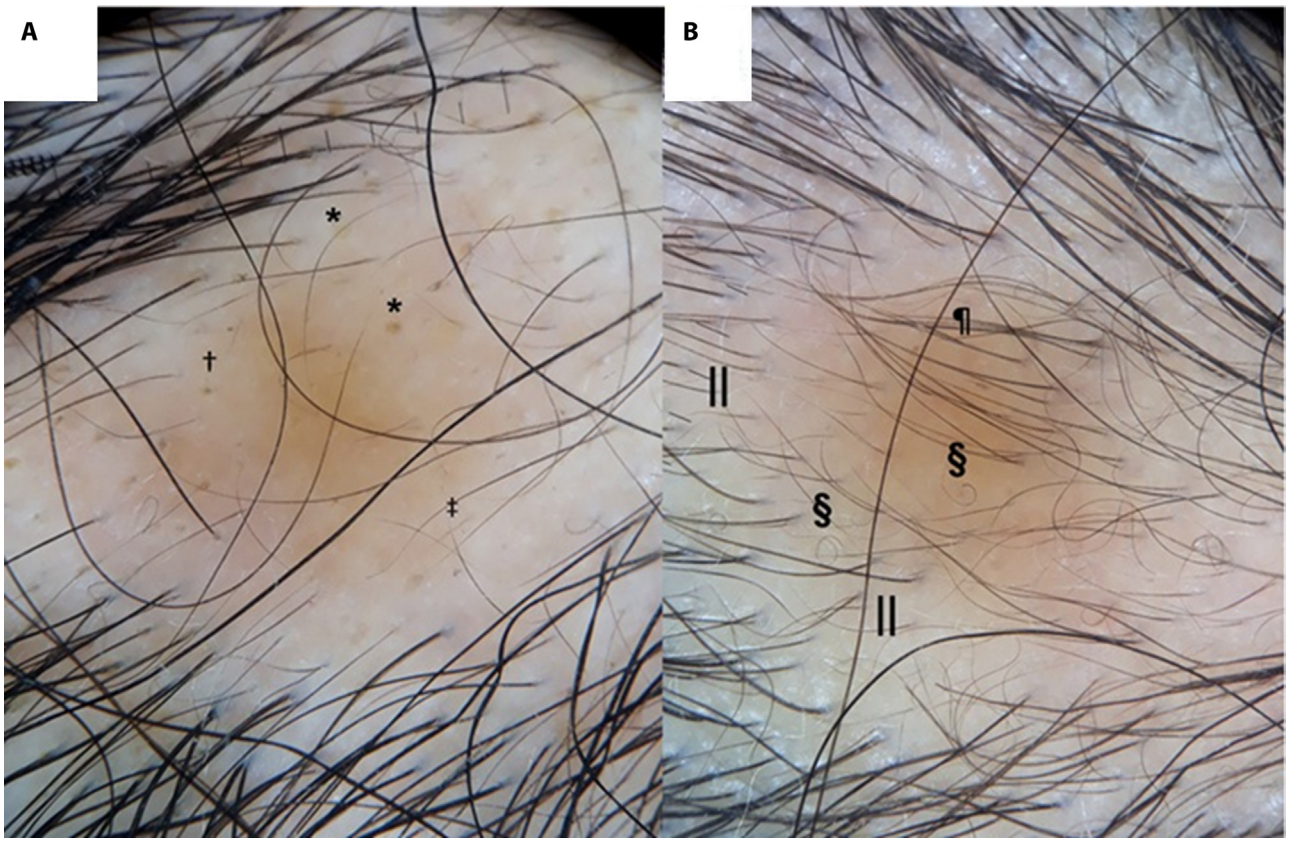

Figure 8. CO_2-Steroid group: Yellow dots, few vellus hairs and black dots. (A) At baseline. (B)Disappearing at last follow up visit, with appearance of pigtail, upright regrowing and terminal hairs.
* Yellow dot
† Black dot
‡ Vellus hair
§ Pig tail hair
‖ Upright regrowing
¶ Terminal hair

Nevertheless, dermoscopic improvement can herald clinical hair growth. Out of the 22/30 patients in CO_2 groups who showed dermoscopic improvement after first session, 3 started to show clinical hair regrowth after second session and 2 after third session. In Dermapen groups, among the 28 patients who showed dermoscopic improvement after first session, clinical hair regrowth was delayed after second session in 4 patients. Clinical hair regrowth was also delayed to after the second treatment session in four patients in the PRP group. Out of the 26 patients in the Steroid groups who showed dermoscopic improvement after first session, 3 patients showed clinical improvement after the second session and 2 after the third session.

Relation Between Hair Regrowth and Different Parameters

There was no significant correlation between final hair regrowth and patients age, Family history and site of AA patches , in all study groups. Only in CO_2-Steroid group, Hair regrowth at end of treatment, was significantly higher in patients with first attack of alopecia (100 %), compared to patients who had recurrent disease (65%) (P = 0.031). In CO_2-Steroid, Pen-Steroid, and Pen-PRP groups there was a negative correlation between the total duration of disease and overall hair regrowth. However, this correlation was significant only in CO_2-Steroid (rs = –0.678, P = 0.005) and Pen-PRP (rs = –0.593, p = 0.020) groups. The shorter the duration of the current episode of alopecia, the higher the hair regrowth. This negative correlation was significant only in Pen-PRP group (rs = –0.702, P = 0.004). Patients without body hair affection scored higher hair regrowth rates, in all groups. This negative correlation was statistically significant only in the Pen-PRP group (rs = –0.577, P = 0.024). Although not statistically significant, the less the hair loss at baseline, the better the improvement at end of treatment. This was the case in all the groups, with the exception of the Pen-Steroid one. Hair regrowth, at one month follow up, was higher in patients with patchy hair loss. This was significant in Pen-Steroid (P = 0.021) and Pen-PRP (P = 0.022) groups.

Side Effects and Patient Satisfaction

Most patients were satisfied by the results, with no statistical significance between the four study groups (CO_2-steroid 86.7%, CO_2-PRP 73.3%, CO_2-Steroid and Pen-Steroid 80%).

Unlike intralesional steroid injection, no atrophy or telangiectasia were observed. Pain during the procedures, was appreciated as more tolerable compared to injection, in patients who experienced intralesional steroid or PRP injection before. In CO_2 groups, the patients expressed their discomfort as related to heat generated from laser procedure.

No major complications, including secondary infection, ulceration or scaring, occurred in any patient.

Conclusions

A main challenge in AA treatment is directing therapies to the hair follicle. Stratum corneum forms a barrier to topical drug penetration, especially hydrophilic and large molecule drugs [15] . Fractional lasers [4] and micro-needling devices may be used deliver drugs to deeper skin layers [16], by creating small channels through the stratum corneum to the dermis– microscopic treatment zones (MTZ) for ablative fractional lasers, and physical puncturing in micro-needling [17,18].

PRP is thought to release growth factors, cytokines, and proteins, from alpha granules, hence stimulating folliculo-genesis and anagen phase [19]. One limitation in evaluating PRP efficacy for AA is lack of standardized protocols [20]. Therefore, vertical uniform channels from skin surface into the dermis, may promote uniform placement of PRP in the dermis and eliminate injection-associated pain [21]. TED can also be used for TrA for the same purposes, with additional advantage of reducing incidence of skin atrophy [4]. This was in accordance with our study, where atrophy and telangiectasia were not observed.

RGS at end of the study were in favor of using Dermapen, compared to CO2 for TED (P = 0.023). A possible explanation may be occurrence of border of carbonization surrounded by coagulated tissue around the MTZ of fractional CO2 laser, which may partially hinder drug penetration [22]. On the other hand, dermapen creates transient aqueous microchannels in the stratum corneum, allowing drug permeation by passive diffusion [23]. Moreover, size of dermapen microchannels are in the range of microns, whereas the macromolecules delivered are usually nanometers in size [24]. Also, drugs are applied prior, during and after micro-needling, that is more efficacious in drug delivery, compared to spreading the drug only after laser procedure [25].

Trichoscopy can be used in the diagnosis and monitoring of treatment in AA[26,27]. Appearance of new Short vellus hair as sign of improvement in some studies [26,28] may actually correspond to the upright regrowing hairs, that were the most consistent features of hair regrowth in our study, as the differentiation between both may be difficult [29]. Exclamation mark hairs and black dots decreased significantly with treatment. Yellow dots decreased mildly, but without statistical significance. This was in accordance with a study by Ganjoo and Thappa [26], indicating that exclamation mark hairs, and black dots are markers of disease activity, and are the first parameters to change in response to therapy, whereas yellow dots were the least responsive [26]. Trichoscopy is useful in identification of early atrophy and telangiectasia in patients treated with TrA injections. This allowed avoiding reinjection in these areas [26].

As with our study, changes in the dermoscopic findings as well as hair RGS were observed from the first follow up [30].

Hence, from the present study it can be concluded that, micro-needling and fractional CO_2 laser can be effectively used for TED for AA treatment, and that trichoscopy can be used in AA for evaluation of treatment response.

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