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**Original Research Article**

# A clinical study on alopecia areata

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## ABSTRACT

**Background:** Alopecia areata (AA) is a common auto-immune condition, causing hair loss. This disease has limited treatment modalities. Through this study, comparison between established modalities of treatment such as oral mini pulse therapy, intralesional corticosteroids and platelet-rich plasma (PRP) as a newer modality has been done. The objective of the study was to evaluate the efficacy and safety of various treatment modalities in alopecia areata.
**Methods:** 45 patients with alopecia areata presenting to the OPD of Dermatology, Dhiraj hospital, Pipariya were included in this study. It was conducted as a randomized prospective study for a period of 16 weeks. After taking informed consent, patients were randomly distributed into three treatment groups. Group 1 patients were treated with Tab. Betamethasone 0.1 mg/kg every Saturday and Sunday, Group 2 was treated with Inj. Triamcinolone acetonide 10 mg/ml for scalp and 2.5 mg/ml for eye brows and face was injected into deep dermis; Group 3 was treated with Dermaroller followed by application of activated platelet rich plasma (PRP). SALT score was calculated at first visit and 16 weeks. Regrowth was calculated using Mac Donald and Null Horis grading Scale at 16 weeks.
**Results:** The SALT score reduced from 13.27 to 7.52 in Group 1, 13.93 to 8.16 in Group 2 and 42.32 to 21.12 in Group 3. Mean grade of regrowth of hairs observed was 3.47, 3.80, 2.93 in Group 1, 2, 3 respectively. The results were analysed using paired t test.
**Conclusions:** Intralesional corticosteroids viz. triamcinolone acetonide still remains the first choice of therapy for AA in adults with limited involvement. Systemic corticosteroids give lower response than intralesional steroids. Platelet Rich Plasma (PRP) showed promising results, especially in children and in extensive involvement. Compared to other treatment modalities Inj. PRP is safe and with less side effects.

**Keywords:** Alopecia areata, PRP, Treatment modalities

## INTRODUCTION

AA is hypothesized to be an organ specific autoimmune disease mediated by T lymphocytes directed to the hair follicles.[1] Although genetic predispositions and environmental factors may trigger the initiation of the disease, the exact cause is still unknown. The peribulbar and lower one third of the follicle show a lymphocytic infiltrate ('swarm of bees') appearance. There is no scarring at any stage which is a characteristic finding. Fifty percent will regrow their hair entirely within a year without treatment; however 7-10% eventually end up with severe chronic form of the condition. Abundance of therapeutic modalities reflects the lack of any one safe and consistently effective approach. Nevertheless, corticosteroids; (topical, intralesional and systemic) topical irritants, topical immunotherapy photochemo-

therapy, systemic immunotherapy, minoxidil, cyclosporine, isoprinosine, azathioprine, sulfasalazine, prostaglandin analogues and combination therapies which takes up the best of each modality are used to treat alopecia areata.[2-20] Recently, the use of biologics and laser therapy in alopecia areata have shown some beneficial results.[21-23] There has been a recent spurt in application of platelet-rich plasma (PRP) in dermatology and aesthetic medicine.[24] Overall, PRP seems to be a promising therapeutic modality but the level of evidence as of now, from the available published data is low.

## METHODS

A randomized prospective study was conducted on 45 patients with features of alopecia areata, presenting to the dermatology outpatient department of Dhiraj General Hospital, Smt. B.K. Shah Medical Institute and Research Centre after the approval from ethics committee, in which the patients were enrolled from December 2012 to May 2014.

Patients of all ages and both sexes with clinical diagnosis of alopecia areata were included. Patients whom had taken any treatment for hair-loss in the past 3 months, pregnant and lactating mothers were excluded from the study.

Each patient was subjected to detailed relevant clinical history regarding site, onset, duration, progression, associated diseases, history of similar lesion in the past, family history & personal history. All patients were counselled about modalities of treatment available, thoroughly explained about possible outcome and complications. Informed consent and baseline photographs were taken. Pre-treatment severity of alopecia tool (SALT) score was recorded as follows:[25]

The entire scalp was divided into 4 parts based on the surface area, top (40%-0.4), posterior (24%-0.24), right side (18%-0.18), and left side of scalp (18%-0.18). Percentage of hair loss in each area was determined independently and is multiplied by the percentage of scalp covered in that area of the scalp, and summing the products of each area will give the SALT score.

The patients were randomly grouped serially into 3 groups (group1, 2, 3) and chosen treatment modality was given serially according to the group.

***Group 1: 15 patients (Corticosteroid oral mini pulse therapy)***

The patients were started with Tab. Betamethasone 0.1 mg/kg every Saturday and Sunday for maximum of 16 weeks.

***Group 2: 15 patients (Intralesional Corticosteroid injection)***

Inj. Triamcinolone acetonide 10 mg/ml for scalp and 2.5 mg/ml for eye brows and face was injected into deep dermis or upper subcutaneous tissue using a 0.5 inch long 30-gauge needle at the sites, 1 cm apart and 0.1 ml into each site, once in 4 weeks for maximum of 16 weeks.

***Group 3: 15 patients (Dermaroller with PRP)***

Patients were counselled for micro-needling with dermaroller containing 192 needles of needle size 1 mm. Then, a topical anaesthetic cream containing a eutectic mixture of topical tetracaine and lignocaine in a cream base was applied for 1 hour on the treatment area to achieve a satisfactory anaesthetic effect. Then the treatment area was cleaned with a mild cleanser followed by 70% ethanol solution. Treatment was performed by rolling the dermaroller in vertical, horizontal and diagonal directions in the affected area until the appearance of fine pinpoint bleeding spots.

Treatment with dermaroller was followed by application of activated platelet rich plasma (PRP) over the treated area. A 0gentle massage was done to allow penetration and the roller was again quickly rolled over the treatment area.

Before using the PRP, it was activated with 0.425 ml of 10% calcium chloride ($CaCl_2$) per 1 ml of PRP.

***Method of preparation of PRP:***

- For preparation of PRP, under strict aseptic conditions, 10 ml of patients own venous blood was withdrawn and collected in a sterile vaccutainer containing anticoagulant sodium citrate (acid citrate dextrose –(ACD), can also be used). Anticoagulants bind calcium and prevent the coagulation proteins from initiating the clotting cascade. This collected whole blood was sent for centrifugation to separate the PRP by double spin centrifugation method.

*Double spin method*[26]

- The whole blood is centrifuged by ‘light spin’ centrifugation (1600 rpm for 10 minutes). This centrifugation is slow to avoid spinning down platelets due to isolate plasma. Platelets are mostly concentrated right on top of buffy coat layer.
- Subsequently, the platelets were concentrated by ‘heavy spin’ centrifugation (4000 rpm for 10 minutes) with removal of supernatant plasma. This centrifugation is faster, so that platelets are spun down and separated as a pellet at the bottom of tube from PRP above.
- The final concentration depends on the volume reduction of PRP.
- Approximately $3/4^{th}$ of the supernatant volume is discarded and the platelet rich pellet is resuspended in remaining amount of plasma.
- The resultant suspension is used as PRP.

For the post procedure care the patients were kept under the cover of systemic antibiotic (tablet azithromycin 500 mg) for three consecutive days post treatment.

This procedure was carried out once in 4 weeks for maximum of 16 weeks.

All the patients from all three groups were adjunctively given Tab. Biotin (5 mg.) – 1 OD every day, Tab. Levamisole 3 mg/kg- 1 OD every 3 days and topically mometasone furoate 0.1% cream– applied once a day over the affected site.

- All patients were followed up at every 4 weeks and reevaluated after 16 weeks by using SALT Score and Mac Donald Hull and Norris Grading system to evaluate the results of treatment as follows:[27,28]
- Grade 1- Regrowth of vellus hair.
- Grade 2- Regrowth of sparse pigmented terminal hair.
- Grade 3- Regrowth of terminal hair with patches of alopecia.
- Grade 4- Regrowth of terminal hair on scalp.

The results were presented in mean±SD and difference between pre and post treatment in all groups was found by paired t-test and $p<0.05$ was considered as significance level.

## RESULTS

Forty five consecutively clinically diagnosed cases of AA were enrolled in the study. More than 75% of the total cases were males (Table 1). Majority of the patients belonged to the age group of 20-40 years (55.6%) (Table 2). The most common pattern observed was "patchy" (71.1%) (Table 3).

**Table 1: Sex distribution.**

| Sex | Frequency | Percentage (%) |
|---|---|---|
| Male | 34 | 75.6 |
| Female | 11 | 24.4 |
| Total | 45 | 100.0 |

**Table 2: Age distribution**

| Age group | Frequency | Percentage (%) |
|---|---|---|
| ≤10 | 7 | 15.6 |
| 11-20 | 6 | 13.3 |
| 21-30 | 16 | 35.6 |
| 31-40 | 9 | 20.0 |
| 41-50 | 4 | 8.9 |
| 51-60 | 1 | 2.2 |
| 61-70 | 2 | 4.4 |
| Total | 45 | 100.0 |

**Table 3: Pattern of alopecia areata.**

| Pattern | Frequency | Percentage (%) |
|---|---|---|
| Patchy | 32 | 71.1 |
| ophiasis | 4 | 8.9 |
| Sisaphio | 0 | 0 |
| Reticulate | 3 | 6.7 |
| Diffuse | 2 | 4.4 |
| Totalis | 1 | 2.2 |
| Universalis | 3 | 6.7 |

**Table 4: SALT score.**

| Groups | N | Mean SALT score at start of treatment | Mean SALT score after 16 weeks | P value |
|---|---|---|---|---|
| Group-1 | 15 | 13.27 | 7.52 | 0.069 |
| Group-2 | 15 | 13.93 | 8.16 | 0.032 |
| Group-3 | 15 | 42.32 | 21.12 | 0.019 |

The SALT score of all the patients included in the study with group-wise mean SALT score was 13.27, 13.93, 42.32 in Group 1, 2, 3 respectively which was reduced after 16 weeks of treatment as 7.52, 8.16, 21.12 in group 1, 2, 3 respectively (Table 4). P value was found to be significant in Group 2 (0.032) and Group 3 (0.019). Type of hair regrowth was assessed by using Mac Donald Hull and Norris grading system (Table 5). P value was found to be significant in group 1 (<0.0001), Group 2 (<0.0001) and Group 3 (0.02).

In Group 1, 10/15 patients (66.6%) showed grade 4 (excellent) response after 4 months of treatment while 3/15 patients (20%) showed grade 3 (good) response, with the mean grade of 3.47 after 16 weeks of treatment.

In Group 2, 13/15 patients (86.6%) showed grade 4 (excellent) response after 4 months of treatment while 1/15 patients (6.6%) showed grade 3 (good) response, with the mean grade of 3.80 after 16 weeks of treatment. In Group 3, 4/15 patients (26.6%) showed grade 4 (excellent) response after 4 months of treatment while 7/15 patients (46%) showed grade 3 (good) response, with the mean grade of 2.93 after 16 weeks of treatment.

Adverse effects were observed in 11.11% patients, largely attributed to systemic corticosteroids. 3 patients showed acneform eruptions out of which 2 patients were in group 2 and 2 patients showed atrophy over the treated site, both of which were in group 2. Recurrence was seen in 11.11% patients, despite continuation of treatment.

**Table 5: Mac Donald Hull and Norris grading system.**

| | Group | N | Mean grade | Std. deviation | Std. Error | 95% confidence interval for Mean | | Min | Max |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Lower Bound | Upper Bound | | |
| **Grading at 1st follow up** | Group-1 | 11 | 1.18 | .405 | .122 | .91 | 1.45 | 1 | 2 |
| | Group-2 | 10 | 1.30 | .483 | .153 | .95 | 1.65 | 1 | 2 |
| | Group-3 | 6 | 1.17 | .408 | .167 | .74 | 1.60 | 1 | 2 |
| | Total | 27 | 1.22 | .424 | .082 | 1.05 | 1.39 | 1 | 2 |
| **Grading at 2nd follow up** | Group-1 | 14 | 2.29 | .914 | .244 | 1.76 | 2.81 | 1 | 4 |
| | Group-2 | 14 | 2.29 | .726 | .194 | 1.87 | 2.71 | 1 | 3 |
| | Group-3 | 12 | 1.75 | .754 | .218 | 1.27 | 2.23 | 1 | 3 |
| | Total | 40 | 2.12 | .822 | .130 | 1.86 | 2.39 | 1 | 4 |
| **Grading at 3rd follow up** | Group-1 | 15 | 3.07 | 1.033 | .267 | 2.49 | 3.64 | 1 | 4 |
| | Group-2 | 15 | 3.07 | .884 | .228 | 2.58 | 3.56 | 1 | 4 |
| | Group-3 | 14 | 2.36 | .929 | .248 | 1.82 | 2.89 | 1 | 4 |
| | Total | 44 | 2.84 | .987 | .149 | 2.54 | 3.14 | 1 | 4 |
| **Grading at 4th follow up** | Group-1 | 15 | 3.47 | .915 | .236 | 2.96 | 3.97 | 1 | 4 |
| | Group-2 | 15 | 3.80 | .561 | .145 | 3.49 | 4.11 | 2 | 4 |
| | Group-3 | 15 | 2.93 | .884 | .228 | 2.44 | 3.42 | 1 | 4 |
| | Total | 45 | 3.40 | .863 | .129 | 3.14 | 3.66 | 1 | 4 |

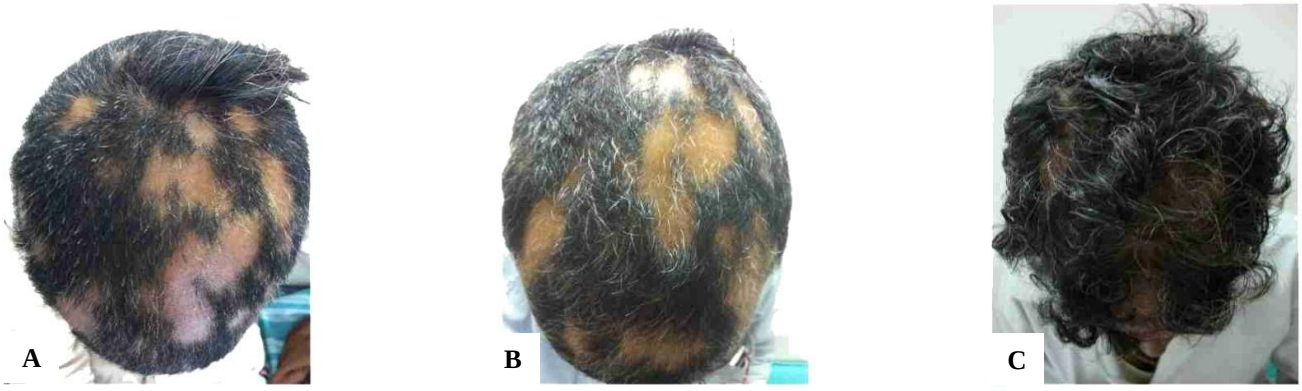


**Figure 1: Corticosteroids oral mini pulse therapy; A=Baseline, B=After 3 months, C=After 5 months.**

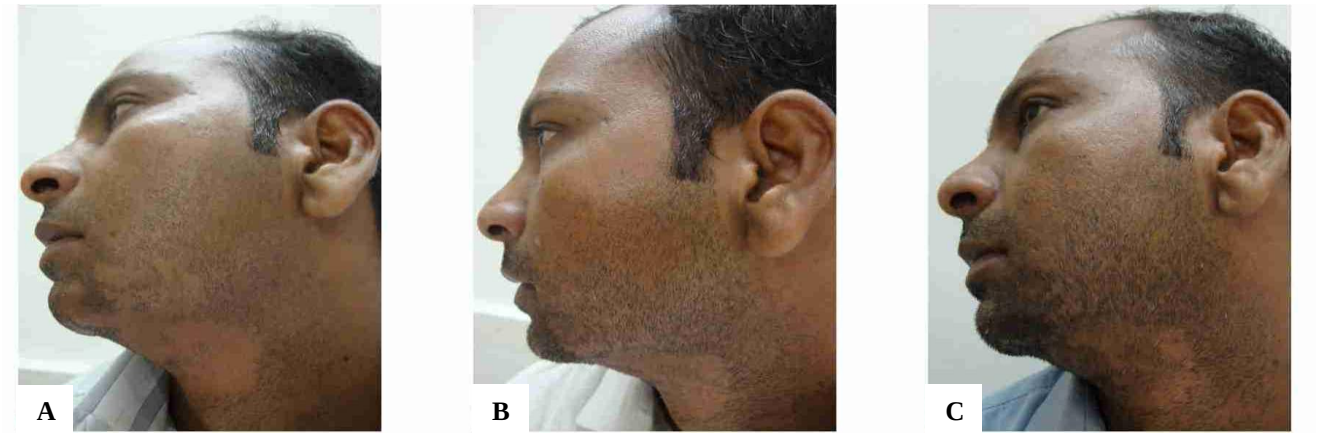


**Figure 2: Intralesional corticosteroid injection; A=Baseline, B=After 2 months, C=After 4 months.**


**Figure 3: Dermaroller with PRP; A=Baseline, B=After 3 months, C=After 5 months.**

## DISCUSSION

Out of 15 patients enrolled in group 1 (oral mini pulse therapy) 66.6% showed grade 4 (excellent) response and 20% showed grade 3 (good) responses after 16 weeks of treatment. Pasricha has reported remarkable hair growth in one patient, refractory to other therapies with oral mini pulse, betamethasone 5 mg given as a single oral dose after breakfast on two consecutive days every week for 6 months.[8] In another study by Khaitan et al, 75% of extensive AA patients showed acceptable hair growth with betamethasone oral mini pulse.[9] Among the patients enrolled in group 2 (intralesional corticosteroid therapy), 86.6% showed grade 4 (excellent) response and 6.6% showed grade 3 (good) response after 16 weeks of treatment. Intralesional corticosteroids have been used since 1958 in the treatment of AA with approximate success rates of 60-75%.[3] Porter and Burton showed that hair regrowth was possible in 64% of AA sites treated by intralesional injections of triamcinolone acetonide.[29] Abell and Munro reported that 52 of 84 patients (62%) showed regrowth of hair at 12 weeks after three injections of triamcinolone acetonide, using the Porto Jet needleless device, compared to one of 15 (7%) control subjects injected with isotonic saline.[30] In Group 3 (dermaroller with PRP therapy), 26.6% showed grade 4 (excellent) response and 46% showed grade 3 (good) response after 16 weeks of treatment. In this study Patients with age <20 years showed good to excellent response with sustainable hair growth, while the patients with age >40 years showed poor with non-sustainable growth of hair. As it has autologous the inherent risk of intralesional and systemic steroids are not encountered. Study by Trink et al, a randomized, double-blind, placebo- and active-controlled, half-head study to evaluate the effects of platelet-rich plasma on alopecia areata.[31] In a study by Singh, role of platelet-rich plasma in chronic alopecia areata: Our centre experience.[32]

## CONCLUSION

Systemic corticosteroids are one of the commonly prescribed therapies in patients of alopecia areata. However, the adverse systemic effects limit long term use of the same. Our study shows that intralesional corticosteroids and intralesional platelet rich plasma to be superior to oral mini pulse therapy in treatment of alopecia areata. Intralesional corticosteroids still remains the first choice of therapy for AA in adults with limited involvement, giving a prompt response. The adverse effects in conjunction with the associated pain, restricts its use in children and in extensive involvement. PRP opens gateways to newer treatments in management of this chronic disease.

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