Randomized Study to Evaluate the Efficacy of Platelet Rich Plasma, Intralesional Triamcinolone Acetonide and Intralesional Normal Saline in Moderate to Severe Alopecia Areata

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ABSTRACT

BACKGROUND
Alopecia areata (AA) is a common, chronic, autoimmune, inflammatory disease that causes non scarring hair loss. Treatment of moderate to severe AA is challenging without a definitive cure. This study was undertaken to compare the efficacy of platelet rich plasma (PRP) - a novel procedural therapy, intralesional triamcinolone acetonide (ILS) and intralesional normal saline (NS) in moderate to severe AA.

METHODS
A total of 75 patients clinically diagnosed with Alopecia Areata were enrolled and the severity of the disease was assessed by SALT score. Twenty-five patients each in Group A, B and C were treated with PRP, ILS and NS respectively at monthly intervals for four sessions. Improvement in hair regrowth (based on reduction in SALT SCORE) and adverse effects were noted in all cases in each group at each session and one month after the final sitting. Statistical analysis was done using the student’s t-test for quantitative values and the chi-square test for qualitative values.

RESULTS
Males outnumbered females with a percentage of 74.7, whereas females were 25.3 %. Male: female ratio of 2.9:1. Patchy type of AA was the most common clinical type observed (54.66 %), followed by sub totals (14.66 %). Forty-eight cases (64 %) had a severity grade of S2 (26-50) followed by twenty-three cases (30 %) grade of S3 (51-75). After 20 weeks of treatment, the mean percentage of SALT score improvement with the highest efficacy was seen in the ILS group (63.15 %) followed by the PRP group (37.51 %). Improvement of hair regrowth with the excellent response (66 %) was achieved in ILS followed by 33 % in the PRP group, comparatively rapid reduction in SALT SCORE within four weeks was observed in the ILS group whereas gradual reduction in SALT SCORE was achieved in 8-12 weeks in PRP group. Relapse was seen in 21.7 % of patients in the ILS group and 1 (4.1 %) patient in the PRP group in the follow-up period of four months. No major adverse effects were seen in any of the groups.

CONCLUSIONS
Efficacy of ILS was more significant than PRP and Placebo but with a higher recurrence rate. Both treatments were extremely safe, with no significant adverse effects.

KEY WORDS
ILS, PRP, SALT Score, Triamcinolone Acetonide.
**BACKGROUND**

Alopecia Areata (AA) is a common, chronic, autoimmune, inflammatory disease that causes non-scarring hair loss.[1] The prevalence of AA in the global population is approximately 2%; in India 0.7%. The approximate lifetime risk of developing AA is 1.7%.[2] The chronic relapsing nature and disfiguring appearance may cause psychiatric comorbidities including depression, anxiety and impaired quality of life.[3] AA is also associated with several autoimmune disorders, which in turn increase the global burden of disease.[4]

Treatment of moderate to severe AA is challenging without a definitive cure. Though many treatment modalities have been tried, satisfactory results with long term remission are difficult to achieve in moderate to severe alopecia. Intralosonal triamcinolone acetonide (ILS) is usually not preferred for moderate to severe AA in routine clinical practice due to the need for multiple injections and it is not suitable for patients with rapidly progressive alopecia, but a few studies have shown its efficacy even in moderate to severe forms of AA. Platelet-rich plasma (PRP) has emerged as a new treatment modality in alopecia areata. [5] This study was undertaken to compare the efficacy of PRP, and ILS with intraleosonal normal saline used as a control in moderate to severe AA.

**METHODS**

This study was carried out on 75 patients who attended the outpatient department of dermatology at a suburban medical college hospital. It was a prospective, randomized, comparative interventional study carried out for 18 months from January 2018 to June 2019 after being approved by the Institutional Ethics Committee. The sample size was calculated by the difference of means formula to achieve a power of study of 80% and precision alpha of 0.05 with a 95% confidence interval (CI), the estimated sample size was determined to be 75.

Clinically diagnosed cases of AA of both sexes with ages more than 12 years who had not used any treatment for AA in the last six weeks and a SALT score of more than 25 were enrolled. Patients with active infections, bleeding disorders, and keloidal tendency were excluded. After taking informed consent, general demographic data regarding age, sex and contact information were noted. A detailed history was taken regarding the duration of disease, family history, presence of other autoimmune diseases, history of atopy and previous treatment modalities. A thorough dermatological examination was done on all patients taking note of the various clinical types of AA. At the time of enrolment, patients were assessed clinically by a single trained dermatologist to grade the severity of AA, as per SALT score.

**Salt Score**

The national alopecia areata foundation working committee has devised the severity of alopecia tool score (SALT score).[2] The scalp is divided into four areas namely, Vertex - 40% (0.4) of scalp surface area; right profile of scalp-18% (0.18) of scalp surface area; the left profile of scalp 18% (0.18) of scalp surface area; Posterior aspect of scalp 24% (0.24) of scalp surface area. The percentage of hair loss in any one of the four views (areas) of the scalp, the percentage of hair loss x percent surface area of the scalp in that area. The SALT score is the sum of the percentage of hair loss in all the above-mentioned areas.

Complete hemogram, coagulation profile and viral screening were performed on all patients. Patients were divided into three groups of 25 in each group A, B and C. Randomized table provided by a statistician for the generation of the randomization sequence was used for group allocation. The consort diagram depicting enrolment, randomization, group allocation and follow up is shown in figure 1.

Patients in Group A were treated with autologous PRP therapy. PRP was prepared by a double spin method which involved soft spin at 1500 rpm followed by hard spin at 3000 rpm in PRP Tubes containing ACD. After centrifugation, the upper 2/3rd of plasma containing platelet poor plasma was discarded. Lower 1/3rd had platelet-rich plasma which was taken into insulin syringes containing 1 mg/ml calcium chloride as an activator. Then PRP was injected immediately into the area of AA over the scalp in the amount of 0.1 ml/cm² by an insulin syringe.

Patients in Group B were treated with intraleosonal triamcinolone acetonide - triamcinolone acetonide 10 mg/ml was injected intradermally with a 0.5-inch long, 30-gauge needle, as multiple 0.1-mL injections per square cm. Patients in Group C were treated with intraleosonal normal saline. Normal saline was injected with insulin syringes over the affected area of 0.1 ml/cm². All the above treatment modalities were performed at monthly intervals, for four months. In patients of all groups, the topical anaesthetic spray was applied for 30 minutes over the treated area to achieve adequate analgesia.

All patients were evaluated at every visit and the final assessment was done 1 month after the 4th session of respective treatment. Clinical photographs and determination of SALT score were done at each visit and adverse effects if any were also noted. Patients were followed up for four months at two-monthly intervals to assess the clinical remission, SALT SCORE and determine relapse if any. Data were collected, tabulated, and all statistical analysis was done by using SPSS trial version 25 and in MS Excel 2007. Qualitative variables were expressed as mean ± SD. Qualitative variables were expressed as frequencies and percentages. For comparing the improvement in SALT score in each group before and after treatment, paired t-test was used. To determine the comparative superiority of treatment modalities, an unpaired t-test was used. P-values < 0.05 were considered statistically significant.

**RESULTS**

A total of 75 patients with AA, in the age group of more than 12 years were enrolled. Males outnumbered females with males (74.7 %), whereas females were (25.3 %) giving a
male: female ratio of 2.9:1. In the present study, the majority of cases belonged to 21-40 years age group (61%), followed by the 13-20 years age group (22.6%). The mean age was 29.27 ± 11.2. Patchy type of AA was the most common clinical type observed (54.66%), followed by alopecia subtotalis (14.66%) ophiasis (8%) and sisaiapho (6.6%). Most of the patients had a severity grade of S2 (26-50%) followed by S3 (51-75%); only two patients each had S4 (76-99%) and S5 (100%) grades. Thyroid disease association was seen in seven patients; 8 patients had a family history of AA; 8 patients had a personal history of atopy.

Based on the mean percentage of reduction in SALT score after 12 weeks, the highest efficacy was seen in the ILS group (63.15%), followed by the PRP group (37.51%). The difference in Mean SALT SCORE in all the three groups was statistically significant (Table 1). Taking hair regrowth into consideration excellent response was seen in 4 cases in the PRP group & 8 in the ILS group. Details of responses in other patients are given in (Table 2). Comparatively rapid reduction in SALT SCORE within four weeks was observed in the ILS group whereas gradual reduction in SALT SCORE was achieved in 8-12 weeks in the PRP group (Figure 2). There was a higher improvement in the percentage of mean salt score with a disease duration of < 6 months compared to > 6 months in the PRP group and ILS group but the difference was statistically significant in the ILS group. In both PRP and ILS groups, the improvement of Mean SALT score was higher with severe disease (SALT SCORE >50), but the difference was statistically significant in the PRP group.

The clinical images before treatment and 20 weeks after treatment in the ILS group and PRP group are shown in (Figures 3a & 3b) and (Figures 4a & 4b) respectively. No significant adverse effects were observed in any of the patients of all groups. Mild pain during the procedure was reported by 8 patients in the ILS & PRP group whereas mild atrophy was seen in 2 patients of the ILS group. Relapse was seen in 21.7% of patients in the ILS group and 1 (4.1%) patient in the PRP group.

<table>
<thead>
<tr>
<th>Name of the Group</th>
<th>Mean Salt Score at Baseline</th>
<th>Mean Salt Score after 20 Weeks</th>
<th>Percentage of SALT Score Improvement</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRP</td>
<td>48.35±13.87</td>
<td>30.2±16.34</td>
<td>37.51%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>ILS</td>
<td>53.50±14.40</td>
<td>19.7±15.91</td>
<td>63.15%</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PLACEBO</td>
<td>45.02±21.63</td>
<td>43.47±22.7</td>
<td>3.4%</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The Comparative Efficacy of All the Three Treatment Modalities after 20 Weeks

Alopecia Areata (AA) is an autoimmune, reversible, patchy hair loss most commonly involving the scalp. It is an organ-specific autoimmune disease mediated by T lymphocytes directed to the hair follicles. [6] AA is a self-limiting disease but 10-20% of individuals progress to a more severe form of AA with extensive involvement of scalp and body regions associated with a chronic course. Treatment of severe forms of AA is challenging. The abundance of therapeutic modalities which are used to treat AA reflects the lack of consistent results in any one of the treatments. Intralosomal steroid therapy in AA has shown consistent results in patch types of AA, but its suitability and efficacy in severe forms of the disease are uncertain. PRP is a newer modality of treatment that has been used in a number of dermatological conditions like patterned hair loss, skin rejuvenation and AA.

In the present study, male preponderance was seen with a male to female ratio of 2.9:1 with a mean age of 29.27 years. A similar higher male preponderance was seen in previous studies done by Emy Abi Thomas et al. and Anil Mishra et al. [7] In the present study, the patchy type of AA was the commonest clinical variant observed in 41 (54.66%) patients; Similar findings were reported by Hegde SP, et al. [8] The disease duration ranged from one month to 32 months. The mean duration was 6.2 months. Seventy of our patients reported less than six months of disease duration. Similar disease duration was observed by Nawaf Al-Mutairi et al. [9] However, the duration was 1-8 years in a study done by Sandeep Kaur et al. [10] In the present study, association with hypothyroidism was seen in seven patients; atopy as personal history was seen in 8 patients; 8 patients had a
family history of AA and hypertension was present in 2 patients. Emty Abi Thomas et al[7] reported atopy (14%) as the major association and other autoimmune diseases ranged from 2-5%. Family history of AA is also a poor prognostic factor usually associated with extensive involvement. All patients were classified based on the severity of SALT SCORE subcategories. Most patients belonged to S2 (48), i.e. SALT SCORE between 26-50 followed by S3 (23) 51-75. This grading is in accordance with a study done by Fozia Rehman et al.[12] On comparing the efficacy of the three treatments given, after 20 weeks, ILS showed the highest efficacy with an improvement in mean SALT score of 33.5 (63%) after four treatment sessions. The improvement in the SALT score observed in PRP was 18.12 (37.5%); the improvement in the placebo group was 1.52 (3.4%). The superior efficacy of ILS compared with PRP and placebo was statistically significant (P value < 0.05). Similar results with higher efficacy other than ILS were reported by Rahul H Ranparia et al.[13] but in another study done by Trink et al.[5] PRP was found to have higher efficacy than ILS. Corticosteroids act by suppressing the T-cell-mediated immune attack on the hair follicle. The postulated mechanism for steroid resistance is decreased expression of thioredoxin reductase 1 enzyme, which activates the glucocorticoid receptor in the outer root sheath.

On evaluating the grades of improvement with each treatment, excellent regrowth of hair (i.e. > 75%) was seen in 8 patients in the ILS group and 4 patients in the PRP group. Good improvement (50-75%) was seen in 2 patients in the PRP group and 5 patients in the ILS group. A. Trink et al. and Asim Kumar et al.[14] had achieved excellent regrowth in more than 60% of patients with PRP and 100% regrowth was reported by Shumez H et al.[15] in both groups. More than 75% of hair regrowth with ILS was reported by Ranparia et al.[13] PRP is known to contain more than 20 growth factors that promote cell proliferation and differentiation. This property is useful in other dermatological conditions like acne scars and wound healing. Many recent studies have suggested a beneficial role of PRP in patterned hair loss and AA.[16] The mechanism of PRP on hair follicles includes the proliferation of dermal papillae cells and activation of the extracellular signal-regulated kinase and AKT pathways. The levels of KI-67 cell proliferations were also increased with PRP administration, which is further useful for the continuous growth and proliferating effect of PRP in hair. Efficacy of PRP depends upon the mean improvement of platelet count after centrifugation. The platelet count attained ranged from 8 to 11 lakh /µL which is 3 to 4 folds higher than baseline.

On analysing the timeline of response with each treatment, we observed that the time taken for the start of regrowth of hair was four weeks in relation to ILS group with a continued steady response up to 16 weeks. With PRP marked regrowth was observed only after eight weeks with constant improvement thereafter. In the present study, early regrowth of hair was seen in the ILS group compared to PRP which is similar to the response duration reported by M. Kumaresan et al.[17] However, faster regrowth of hair in the PRP group when compared to ILS was reported by Shumez Het al.[14] Early regrowth of hair instills confidence in patients and aids in compliance and regular follow-up. In this regard, both ILS and PRP were acceptable by the patients as noticeable regrowth of hair was seen within one or two treatment sessions. On correlating the efficacy of treatment with the duration of disease, it was observed that in the PRP group, mean improvement in SALT score was higher (49.55%) in patients with a duration of disease less than 6 months when compared to greater than 6 months. Similarly, in the ILS group mean improvement of SALT score (72%) was significantly higher in patients with a disease duration of < 6 months compared to > 6 months, which is statistically significant (< 0.05). Hence it can be seen that chances of hair regrowth gradually diminished with increasing duration of disease and to achieve complete hair growth, prompt treatment should be administered in all cases of moderate to severe alopecia areata within 3-6 months of the onset of disease. Similar observations of poor regrowth of hair in patients with a longer duration of disease have been reported previously by Chang KH, Rojhirunsakool S et al.[18]

On correlating the efficacy of treatment with the severity of disease (based on SALT score), it was observed that in the PRP group mean improvement in SALT score (41.7%) was significantly higher (P < 0.05) in patients with severe disease (> 50) compared to patients with less severe disease (< 50) (28.8%). Whereas in the ILS group, mean improvement in SALT score (> 50) was higher (60.86%) compared to patients with SALT score (< 50) (56%). However, this difference in efficacy in the ILS group based on the severity of the disease was not statistically significant. We observed that patients with severe diseases had also responded well to both treatment modalities. Similar findings were observed by Kishan ninama et al.[19]

In the present study in the PRP group, out of 24 patients who completed the study, one patient had a relapse with new patches of hair loss and loss of regrowth of hair. In the ILS group out of 23 patients who completed the study, five patients (21.7%) had a relapse. The relapse rates in PRP and ILS were comparable to the study by Sukhbir Singh et al.[20] whereas Ranparyia et al.[12] observed high relapse with PRP compared to ILS. Asim Kumar et al.[14] reported no relapse with PRP after 6 months of follow-up.

**CONCLUSIONS**

Efficacy of ILS was higher than PRP and placebo which was statistically significant, but the recurrence rate was higher than PRP. Moderate results were achieved with PRP treatment - it may be considered an efficient and affordable option for patients with moderate to severe alopecia areata. It is a suitable treatment for cases who failed to respond to ILS treatment. Both ILS and PRP treatments are extremely safe, with no significant adverse effects.

**Limitations of the Study**

The total sample size was small, and larger sample size is required to determine the efficacy of treatments. PRP was used as mesotherapy without using a derma roller/ micro-needling during the process of administration; the micro-needling provides an additional benefit that could not be attained in the present study. Only scalp alopecia areata was
treated, AA affecting areas other than the scalp were not included in our study.

REFERENCES