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Original Article

Effect of Topical Liquid Nitrogen on Pain Reduction during Intradermal Platelet-Rich Plasma Therapy Scalp in Androgenic Alopecia

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ABSTRACT

Objective: To compare the effect of topical liquid Nitrogen and normal saline on pain during intradermal Platelet-rich Plasma therapy in androgenic alopecia.

Study Design: Prospective Comparative study.

Place and Duration of Study: Tertiary Care Hospital, Gujranwala Pakistan, from Mar to Nov 2021.

Methodology: Sixty-four patients with the clinical diagnosis of androgenic alopecia were enrolled after informed consent. After liquid Nitrogen contact application, Group- A patients opted to receive intradermal Platelet-rich Plasma on the scalp. In contrast, patients in Group-B opted to receive Platelet-rich Plasma after normal Saline topical application for improved pain tolerance.

Results: Out of Sixty-four patients, there were 45(70.3%) males, 19(29.7%) females. Pain in intradermal Platelet-rich Plasma injection of the scalp in Group-A had a median (IQR) score of 4 (3-5), while Group-B had a median (IQR) of 5(3-7.5). Patient satisfaction score at the end of three sessions of PRP in group-A had a median (IQR) of 1.5(1-2), and in Group-B, a median (IQR) of 2(1-2).

Conclusion: Topical liquid Nitrogen application improved pain tolerance during intradermal Platelet-rich Plasma therapy in androgenic alopecia compared to normal saline. Satisfaction of patients with Platelet-rich Plasma therapy between the two groups had no significant difference.

Keywords: Androgenic alopecia, Platelet-rich plasma therapy, Painless platelet-rich plasma therapy.

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INTRODUCTION

Platelet-rich Plasma (PRP) is autologous plasma rich in platelets. It stimulates fibroblasts, hence increasing the concentration of collagen fibres. It is anti-inflammatory and is practised in various dermatological conditions such as androgenic alopecia (AGA), acne scarring and aesthetic rejuvenation. Non-healing ulcers and vitiligo other conditions treated with PRP.[1]

Androgenic alopecia is androgen-dependent progressive miniaturization of hair follicles. The exact pathophysiology is unknown. The genetic locus for AGA is on X-chromosome AR/EDA2R and chromosome 20p11.[2] Hyperandrogenism in females is most commonly reported in Polycystic Ovarian syndrome (PCOS). It also accounts for irregularities in the menstrual cycle and polycystic ovaries. The management is based on reproductive function, hirsutism, alopecia, and acne.[3] Platelet-rich Plasma has favourable results in AGA.[4] It modifies the hair cycle signalling pathways and increases the number of hair and their thickness.[5] Regenerative parameters of PRP are attributed to growth factors released by platelet granules. A randomized control trial documented pain in 91.4% of subjects with intradermal PRP in androgenic alopecia in the scalp with topical lignocaine.[6] Irrespective of fine needles, topical numbing creams, and chilling devices, patients may experience injection-related pain distress. The present study aims to compare the effect of topical liquid Nitrogen and normal saline on pain during intradermal Platelet-rich Plasma therapy in androgenic alopecia. It would compare patients' satisfaction with Platelet-rich Plasma therapy between topical liquid Nitrogen and normal saline. The study would find practical, inexpensive delivery of PRP to the scalp while minimizing pain.

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METHODOLOGY

This prospective comparative study was conducted from March to November 2021 at the Dermatology Department of the Tertiary Care Hospital, Gujranwala Pakistan. The Ethical Committee approved the study (ERB No 02/2021). All the patients during the study were recruited by consecutive sampling after informed consent. The topical application of liquid Nitrogen on

pain for intradermal PRP injection in the scalp was assessed. Patients were given a choice to opt for topical liquid Nitrogen or normal saline with the cotton applicator on the scalp, depending on their pain threshold. Group-A included patients who opted to receive intradermal PRP scalp after topical application of liquid Nitrogen with the cotton tip for pain relief.

In contrast, Group-B patients chose to receive PRP after the light touch of normal saline through a cotton tip applicator.

Inclusion Criteria: Patients of either gender, with the clinical diagnosis of androgenic alopecia were included in the study.

Exclusion Criteria: Pregnant women, nursing mothers, age less than 23 years, patients with active scalp infections, non-androgenic alopecia, patients on immunosuppressive therapies, autoimmune diseases, on oral anticoagulants, chemotherapy, systemic treatment with finasteride in the past three months, hair transplantation and those with comorbid diseases were excluded from the study.

The demographic data of the patients' age and gender were recorded. A total of 25ml of blood was drawn from the antecubital vein under aseptic measures in the closed tube containing Sodium Citrate (dilution 1:9). Blood was then centrifuged in True tabletop low-speed centrifuge (serial number: W 1920 0020090814) for 1800rpm for 3 minutes. This would sediment red blood cell and separates them from whole blood. The whole blood has a top layer rich in platelets and WBC, an intermediate buffy coat and the bottom layer has RBCs. The upper layer and buffy coat are switched to a sterile plain tube. The second centrifugation (3000rpm for 7min) would form platelet pellets at the bottom of the tube. The top one-third of plasma was removed; the remaining 8.0mL of supernatant was then agitated to re-suspend the platelets within the plasma. The PRP was then transferred to a 32-gauge insulin syringe.

The scalp skin to be treated was disinfected with a 75% Methylated alcohol swab. Patients were given a choice to choose either topical liquid Nitrogen or normal saline for pain relief. First, liquid Nitrogen was applied with a cotton applicator on the scalp skin for 1sec by skin trained assistant. After 1-2seconds, an intradermal injection of 0.1ml/cm^2 of PRP was injected at a depth of 3 to 4mm with a 32G needle by the dermatologist in Group-A. Liquid Nitrogen was then applied to cool the next area to be treated on the scalp. Next, a cotton applicator dipped in normal saline was applied to scalp skin for 1-2sec, followed by intradermal PRP scalp injection to Group-B patients. Finally, the patients were asked to wash their scalps with regular shampoo three hours post-procedure. A total of three procedures were performed within one month. Pain tolerance was assessed using a 10-point numerical rating scale by patients (0 as no pain, ten as the worst possible pain). Additionally, patients' satisfaction with the PRP procedure using liquid Nitrogen or normal saline was evaluated in accordance with a 4-grade scale (very satisfied, somewhat satisfied, somewhat dissatisfied and very dissatisfied).

The data were analyzed using the statistical package for the social sciences (SPSS) version 23:00. Descriptive statistics were provided for age, gender, occupation, and family history of androgenic alopecia, menstrual cycle regularity and patient satisfaction. Median (IQR) was calculated for pain and satisfaction in both groups. Mann-Whitney U test was applied to compare mean ranks of the numerical score of pain and satisfaction. The p-value of ≤0.05 was taken as significant.

RESULTS

Sixty-four patients with a clinical diagnosis of AGA were enrolled in the study. Males were 45 (70.3%), and females were 19(29.7%). Age ranged from 25 years to 50 years with the mean age of 34.92±3.24 years. Twenty-five (80.65%) male patients had a family history of androgenic alopecia. None of the female patients had menstrual irregularities.

A total of 32 patients had intradermal PRP with topical liquid Nitrogen (Group-A), whereas 32 patients had PRP with normal topical saline (Group-B). The pain reported in Group-A had a median (IQR) score of 4(3-5), while in Group-B median (IQR) of 5(3-7.5). Patient satisfaction score at the end of three sessions of PRP in Group-A had a median (IQR) of 1.5(1-2), and in Group-B, a median (IQR) of 2(1-2). Pain tolerance of intradermal PRP scalp with liquid Nitrogen improved significantly than with normal saline (p=0.03). However, as in the Table, patient satisfaction with the PRP procedure in Groups A and B had a statistically insignificant difference (p=0.197) (Table).

Patients' satisfaction with intradermal PRP scalp with topical liquid Nitrogen and normal saline was shown in the bar graph (Figure).

DISCUSSION

Androgenic alopecia is a common condition consulted in the Dermatology outpatient department.

PRP is considered safe and novel management for AGA.[8] Gentile *et al*. administered PRP for AGA. However, they did not anaesthetize the scalp.[7-9] PRP scalp injections are painful, and clinicians require anaesthesia to ease patients. Local anaesthetics should be carefully selected, as they may compromise the therapeutic efficacy of PRP by interfering with platelet functions.[10]

Table: Pain and satisfaction score with Intradermal Platelet-Rich Plasma Therapy of scalp (n=64)

Parameters	Intra Dermal Platelet-Rich Plasma Therapy Scalp Treatment Groups		*p*-value
	Group-A Liquid Nitrogen (n=32)Median(IQR)	Group- B Normal saline (n=32)Median(IQR)	
Pain Score	4(3-5)	5(3-7.5)	0.030
Satisfaction score	1.5(1-2)	2(1-2)	0.197

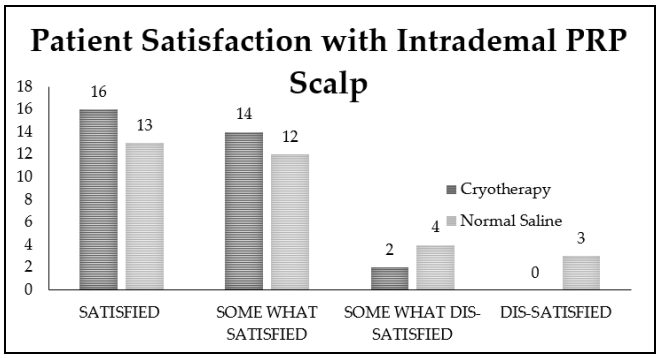

Figure: Patients Satisfaction with Intradermal PRP Scalp with topical liquid Nitrogen Application and normal Saline (n=64)

The US Food and Drug Administration (FDA) has approved Minoxidil and Finasteride in treating AGA. Minoxidil increases anagen duration in the hair cycle and hair follicle diameter through prostaglandin E2. It also improves the survival of dermal papillae cells. Finasteride reduces dihydrotestosterone levels by 65% in serum, prostrate and scalp. It also prolongs the anagen phase.[11] More recently, PRP has gained attention as therapy for AGA.[12] PRP therapy has the advantage of being minimally invasive, safe, and cost-effective in comparison with hair transplant. However, there are various methods of PRP preparation and administration that lack standardization.[13] Hesseler *et al*. in a systematic review, stressed the need for standardized treatment protocol for better reproducibility.[14]

Platelet-rich Plasma is prepared by centrifuging peripheral blood, which separates red blood cells and generates concentrated platelets. The quality of PRP is influenced by the speed of centrifugation and the anticoagulants. The use of local anaesthetics should be limited as they affect platelet aggregation. Thirty gauge needles are recommended in order to minimize pain experienced in scalp PRP. Cold air devices have been practiced but are unsafe due to the risk of scattering PRP in air.[15]

A randomized control trial in California evaluated the efficacy of PRP in AGA; they used Lidocaine 23%/Tetracaine 7% topically on the scalp. One-third of PRP patients reported it as a painful procedure, whereas 74.4% reported it tolerable, with a pain score of 2.1.[16] These findings are consistent with our results in patients receiving PRP injections following liquid Nitrogen. Alves *et al*. in Spain, in a randomized control trial, did not use any anaesthesia for PRP injections in the scalp and pain at the injection site was the most commonly reported adverse reaction.[17] Our study population in group B reported a median(IQR) pain score of 5(3-7.5). Chen *et al.* injected PRP in AGA with or without an anaesthetic agent. Pain, erythema and headache were reported as the common post-procedure adverse reactions.[18] Khatu *et al.* in Maharashtra injected PRP on the scalp. They used topical anaesthetic cream one hour before the PRP injection. Reported undesirable effects of post-PRP injections were pain, erythema and minute hemorr-hages.[19] Reducing pain and stress in nonsurgical procedures is important for patient satisfaction. The anticoagulant used in our study was sodium citrate. Görgü *et al.* in a clinical trial, documented sodium cit-rate as a better anticoagulant compared to acid citrate dextrose-A (ACD-A) for causing less pain with PRP.[20]

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LIMITATIONS OF STUDY

Pain is a subjective phenomenon. Additional factors such as needle size, pressure on the needle, and anticoagulants used in PRP preparation influence pain in PRP therapy. Another limitation of the study was that the patients were questioned to score pain for the whole application area. It would be better to chart pain scores in a specific scalp region.

CONCLUSION

Topical liquid Nitrogen application improved pain tolerance during intradermal Platelet-rich Plasma therapy in androgenic alopecia compared to normal saline. Satisfaction of patients with Platelet-rich Plasma therapy procedure between the two groups had, however, insignificant difference.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

MT & SM: Conception, Study design, Data acquisition, data analysis, drafting the manuscript, approval of the final version to be published.

KK & NH: Drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

AR & SJ: Critical review, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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