COMPARATIVE STUDY OF EFFICACY OF INTRALESIONAL TRIAMCINOLONE ACETONIDE WITH TOPICAL CALCIPOTRIOL VERSUS INTRALESIONAL TRIAMCINOLONE ACETONIDE ALONE IN THE TREATMENT OF ALOPECIA AREATA: RANDOMISED SINGLE BLINDED CLINICAL TRIAL

Thesis

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In partial fulfilment of the requirement for the degree of

DOCTOR OF MEDICINE (MD)

(DERMATOLOGY, VENEREOLOGY AND LEPROLOGY)

JULY, 2020 **DR. THOYYIB PARAMMAL KARAT**

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CERTIFICATE

This is to certify that the thesis titled "**Comparative study of efficacy of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata: Randomised single blinded clinical trial"** is the bonafide work of **Dr. Thoyyib Parammal Karat**, in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

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DECLARATION

I hereby declare that the work reported in the thesis entitled **"Comparative study of efficacy of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata: Randomised single blinded clinical trial"** embodies the result of original work carried out by the undersigned in the Department of Dermatology, Venereology and Leprology, All India Institute of Medical Sciences, Jodhpur.

I further state that no part of this thesis has been submitted either in part or in full for any other degree of All India Institute of Medical Sciences, Jodhpur or any other institution.

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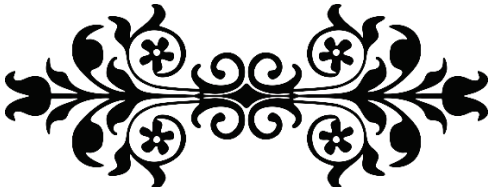

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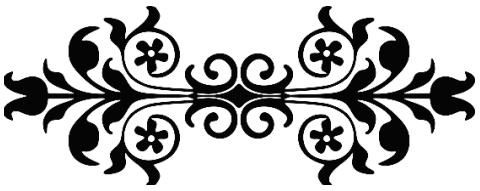
TO MY

PARENTS,

TEACHERS & PATIENTS



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"A thankful heart is not only the greatest virtue, but the parent of all the other virtues."

-Marcus Tullius Cicero

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Dr. Thoyyib Parammal Karat

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LIST OF ABBREVIATIONS

AA	Alopecia Areata
HLA	Human Leukocyte Antigen
MHC	Major Histocompatibility Complex
HF-IP	Hair Follicle Immune Privilege
NK cells	Natural Killer Cells
HF	Hair Follicle
IP	Immune Privilege
MICA	Major Histocompatibility Complex Class I Polypeptide-Related Sequence A
TGF	Transforming Growth Factor
α-MSH	Alpha Melanocyte Stimulating Hormone
IDO	Indoleamine-2,3-Dioxygenase
IL-10	Interleukin-10
CGRP	Calcitonin Gene-Related Peptide
IGF- 1	Insulin-Like Growth Factor 1
VDR	Vitamin D Receptor
ORS	Outer Root Sheath
AU	Alopecia Universalis
AT	Alopecia Totalis
AAT	Alopecia Areata Totalis
AAU	Alopecia Areata Universalis
SALT	Severity of Alopecia Tool
RGS	Regrowth score
AAPI	Alopecia Areata Progression Index
OPD	Outpatient Department
HBV	Hepatitis-B Virus
HCV	Hepatitis-C Virus
HIV	Human Immuno-Deficiency Virus
TSH	Thyroid Stimulating Hormone
RBS	Random Blood Sugar

MP	Megapixel
NS	Normal Saline
W/W	Weight For Weight
DIMT	Diffuse, Irregular, Marginal, Targetoid
BD	Black Dots
BH	Broken Hair
EMH	Exclamation Mark Hairs
TH	Tapered Hairs
PPC	Pohl Pinkus Constrictions
SPSS	Statistical Package for Social Sciences
TNF	Tumor Necrosis Factor
1,25(OH)2D3	1,25-dihydroxy Cholecalciferol
WNT	Wingless- Related Integration Site
CD	Clusters Of Differentiation
JAK/ STAT	Janus Kinase/ Signal Transducers and Activators of Transcription
IFN-γ	Interferon- Gamma
BAA	Alopecia Areata of Beard
DPCP	Diphenylcyclopropenone
SADBE	Squaric Acid Dibutylester
PUVA	Psoralen and Ultraviolet-A
PRP	Platelet Rich Plasma
PDE	Phosphodiesterase
CO2	Carbon Dioxide
ILC	Intralesional Corticosteroids
CF	Clobetasol Foam
PF	Placebo Foam
ITA	Injection Triamcinolone Acetonide
TCA	Trichloroacetic Acid
LA	Local Application
MTX	Methotrexate
NBUV B	Narrow-Band Ultraviolet B

FCO2	Fractional Carbon Dioxide
JAK inhibitors	Janus Kinase Inhibitors
FDA	Food And Drug Administration
RCT	Randomized Control Trial
DNCB	1-Chloro,2,4, Dinitrobenzene
Nd:YAG	Neodymium-Doped Yttrium Aluminum Garnet
8- MOP	8-Methoxypsoralen
UV	Ultraviolet
BMI	Body Mass Index
SD	Standard Deviation
ITT	Intention To Treat
PP	Per Protocol
mg	Milligram
gm	Gram
p- value	Probability Value
µg	Microgram
ml	Milliliter
J/cm2	Joules Per Square Centimeter
IU	International Unit

SUMMARY

Background:

Alopecia areata (AA) is a non-scarring autoimmune condition characterised by round, finely delineated alopecia patches that may occur anywhere on the hair bearing site. There are several therapeutic options for alopecia areata, with intralesional steroid injection being one of the first-line treatment options in localised AA. There is, however, no studies evaluating the combined efficacy and safety profile of topical vitamin D analogues with intralesional steroids in AA.

Aims and objective:

To compare the efficacy and safety profile of topical calcipotriol with intralesional triamcinolone acetonide versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata.

Methods:

This was an interventional, randomized single blinded clinical trial done on 93(225 patches) patients. They were randomly allocated to one of the two treatment groups, Group A received 4 weekly intralesional triamcinolone acetonide injections and topical calcipotriol twice daily, whereas Group B received intralesional triamcinolone acetonide injections alone with 4 weekly follow-ups for 12 weeks. These patches were analysed using clinical and dermoscopic parameters on each follow-up using regrowth score (RGS), dermoscopic activity markers and DIMT-classification of regrowth pattern.

Results:

Total 73 patients (179 patches) completed the study with male to female ratio 3.43:1 and mean age of 26.6 years. At baseline, all study parameters were comparable between the two groups ($p>0.05$). At baseline dermoscopic examination, black dots (52.4%), broken hair (36%), yellow dots (31%), and exclamation mark hair (27.1%) in patches were the most common findings. Regardless of treatment group, study found that the 'diffuse pattern' (Group A- 39.6% and Group B- 38.6%) was the most common, followed by the 'marginal pattern' (Group A- 24.3% and Group B- 25.0%). Regrowth score (RGS) and patches with more than 50% terminal hair regrowth significantly improved in both the groups beginning in

the fourth week and going forward (p value- 0.001, 0.001). The two treatment groups exhibited no statistically significant difference in terminal hair density (p values: 0.930, 0.616, 0.178), however subgroup analysis showed a statistically significant difference in scalp patches of group A compared to group B at the 12th week of study (p = 0.018), but not in beard patches (p value- 0.527). Topical calcipotriol patches exhibited more irritation and dryness. Burning, atrophy, and persistent erythema were comparable in both the groups.

Conclusion:

Topical calcipotriol displayed synergistic action with intralesional steroid injection in scalp patches of alopecia areata in terms of hair regrowth and reduced the incidence of atrophy in patches when compared to intralesional steroid alone, though beard patches did not demonstrate any added benefits with topical calcipotriol.

Limitation:

Potential limitations of this study include the small sample size and patients lost to follow-up.

INTRODUCTION

INTRODUCTION

AA is a prevalent chronic inflammatory condition that causes non-scarring hair loss. There is a wide range in the clinical presentation of AA, from small, well-circumscribed patches of alopecia to total alopecia of the body, beard, eyebrows, and scalp.[1] Lifetime risk is 1.7%, and the prevalence is believed to be between 0.1% and 0.2% in the general population.[2]

Alopecia areata has gone by several names in the past. The Ebers papyrus originally recorded alopecia areata as "bitten" or "bite alopecia," describing its patchy distribution. Celsus was the first to use the phrase "areas" to describe alopecia, which was initially described by Hippocrates.[3] The term alopecia areata (AA) was coined by the French physician Sauvages de Lacroix (1706-1767) in his book "Nosologia Methodica" published in 1763.[4]

Several theories about the disease's genesis exist. Initially, an infectious or poisonous substance was believed to be the cause; however, neuropathic and endocrine problems were later postulated as explanations. The autoimmune theory gained prominence in the 1960s.[5] It has also been shown that environmental factors significantly influence the development, progression, and pattern of AA.[2] The HLA region, which encodes vital human key regulators and major histocompatibility complexes (MHC), has been discovered as a significant genetic factor to disease phenotype.[6]

Normally, an immunosuppressive environment protects anagen hair follicles from autoimmune reactions, a phenomenon known as hair follicle immune privilege (HF-IP).[7] The presence of lymphocytes, dendritic cells, and natural killer (NK) cells in the anagen HF peribulbar region, which is ordinarily free of immune cells, provides compelling evidence of immune privilege collapse in AA.[8] According to this hypothesis, environmental stress can cause a buildup of reactive oxygen species in HF keratinocytes. Genetically susceptible individuals are unable to overcome this effect, so stress accumulates in the cells, promoting MICA (MHC Class I Polypeptide-Related Sequence A) expression.[5] When HF cells begin to present the MHC-I molecules, the immune system is exposed to a large number of secure antigens, and subsequent immunological events become unstoppable.[9]

Evidence suggests that vitamin D deficiency may increase the likelihood of developing AA.

Vitamin D inhibits the function and differentiation of T-helper 17(Th 17) cells, which are the immune regulatory cells. VDR (Vitamin D Receptor) is found in the outer root sheath (ORS), hair follicle bulb, and sebaceous gland of the hair follicle. It helps to distinguish hair follicles in utero.[10] Reduced VDR expression is linked to slower hair follicle development and reduced epidermal differentiation.[7]

AA can be classified depending on the extent and pattern of hair loss.[11] According to its extent alopecia areata is classified as Patchy alopecia, Alopecia totalis, Alopecia universalis, and according to its pattern as Reticular, Ophiasis, and Sisaipho. Acute diffuse and total alopecia has also been described as a new variant.[2]

Alopecia areata (AA) is often characterised by alterations in the nails, which may significantly impair the functionality and cause cosmetic disfigurement of nails.[12] Nail changes are more common in patients with severe alopecia, such as alopecia universalis (AU) and alopecia totalis (AT).[12] Nail changes associated with AA include geometric pitting (multiple, small, superficial pits regularly distributed along transverse and longitudinal lines), geometric punctate leukonychia (multiple white spots in a grill pattern), and trachyonychia (sandpaper nails). Other changes include Beau's lines, onychomadesis, and red lunulae, which indicate acute and severe disease.[2] The histopathologic features of AA-associated nail changes suggest a matrix keratinization disorder. That is, AA-associated nail disease primarily affects the proximal nail matrix, with a minor impact on the distal matrix and negligible effect on the nail bed.[12]

Trichoscopy is a non-invasive technique used to diagnose and monitor scalp and hair diseases.[13] Yellow dots, black dots, broken hairs, tapering hair (exclamation mark hair), and small vellus hairs are trichoscopic characteristics of alopecia areata.[2] Trichoscopic activity indicators may be used to predict the course of the disease as well as the treatment's effectiveness.[14]

Several scoring systems are used in alopecia areata like the Severity of alopecia tool (SALT), and Regrowth scores (RGS). The Alopecia Areata Progression Index (AAPI), which was established in 2016, takes into account the results of the hair pull test and the trichoscopy.[15] These grading methods have a drawback in that they do not account for extra-scalp alopecia. In practice, determination of the percentage of the affected area is not very useful in limited

patchy AA because it usually resolves spontaneously or after local treatment.[16]

Since there is no permanent cure, treatment focuses on controlling disease activity. It is crucial to advise patients and inform them of the potential benefits and risks of different treatment options. Alopecia areata treatment options vary depending on the severity of the condition. Topical corticosteroids, intralesional (IL) corticosteroids, topical minoxidil, topical anthralin, and topical vitamin D analogues are among therapies that have been proposed for limited involvement. Systemic corticosteroids, immunosuppressive medications such azathioprine, cyclosporine, and topical immunotherapy are all proposed treatments for severe alopecia areata.[17]

Since 1958, intralesional corticosteroids have been the therapy of choice for individuals with patchy AA, with 60-75% success rates.[2] They work by preventing T-cell-mediated immune attacks on hair follicles.[18] For intralesional treatment, triamcinolone acetonide is the preferred corticosteroid.[2] If the disease has expanded over more than 50% of the scalp, is very progressive or has been present for more than two years, intralesional therapy shows a poorer outcome.[16] Regrowth is usually visible in 4 weeks. Some patients are resistant to intralesional steroids because they have low levels of thioredoxin reductase 1, an enzyme that activates the glucocorticoid receptor in the outer root sheath.[2]

Calcipotriol, a vitamin D analogue, has shown promising results when used topically to treat AA.[19] However, systemic vitamin D supplementation in the treatment of autoimmune diseases is still being studied.[19] The proposed mechanism of topical calcipotriol is regulation of differentiation of B cells, T cells, dendritic cells, and expression of Toll-like receptors causing the T lymphocyte proliferation inhibition particularly the Th1 arm and tilting the T cell response toward Th2 dominance along with regulation of epidermal cell proliferation and differentiation and modulation of cytokine production.[20]

Intralesional corticosteroids need regular dermatologist visits and have adverse effects. Thus, in this trial, we want to determine if topical calcipotriol combined with intralesional steroids may minimise patient visits to the hospital and lessen local steroid side effects. Thus, this study investigated the clinical and trichoscopic efficacy and safety of topical calcipotriol and intralesional triamcinolone acetonide in alopecia areata.

AIMS AND OBJECTIVES

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AIM OF THE STUDY

To compare the efficacy and safety profile of topical calcipotriol with intralesional triamcinolone acetonide versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata.

OBJECTIVES

Primary Objective:

- To assess and compare the hair regrowth in alopecia areata patch treated with topical calcipotriol-intralesional triamcinolone acetonide versus patch treated with intralesional triamcinolone acetonide alone using hair regrowth score (RGS).

Secondary objectives:

- To compare the pattern of hair regrowth in each selected patch.
- To compare the local side effects in each selected patch.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Alopecia areata (AA) is a complex autoimmune disease that results in non-scarring alopecia. It is characterised by sharply demarcated round patches of alopecia. The disease's prognosis is uncertain. According to literature data, 34%–50% of patients spontaneously recover within a year whereas 14%–25% of patients will advance to AT or AU.[21,22] Patients may experience multiple episodes of hair loss and subsequent regrowth throughout the course of their lives. Factors such as atopy, young age at onset, nail dystrophy, severe disease, ophiasis pattern, progressive hair loss, and presence of other autoimmune disorders are associated with bad prognosis.[11]

Although it can affect any area of the body that has hair, it is most obvious when it impacts areas that are crucial for appearances, like the scalp, moustache, eyebrows, eyelashes, and beard. Involvement of these sites frequently results in psychological problems like stress, low self-esteem, and humiliation.[23] AA affecting the beard in postpubertal men frequently necessitates prompt evaluation and management.[24]

EPIDEMIOLOGY

The prevalence in the general population was estimated to be 0.1-0.2%, with a lifetime risk of 1.7%.[2] While AA affects both sexes equally, men were diagnosed at a younger age than women.[25] Prevalence of AA is highest in the paediatric population and decreases with each subsequent decade of life and few studies suggest that the peak incidence is in the second and third decade of life.[1,2] AA in the Childhood or paediatric age range is a quite commonly diagnosed disorder, with more than half of the total patients diagnosed with AA reported to have acquired the first episode at <20 years of age. Recent meta-analysis has put the prevalence rate of paediatric AA at 1.92%. It is estimated that paediatric AA accounts for 18.1% of all AA patients.[26–28]

PATHOGENESIS OF ALOPECIA AREATA

Several hypotheses have been suggested regarding the pathogenesis of AA throughout the years. Similar to other autoimmune disorders, observational studies reporting family clustering, twin studies, and genome-wide association studies have revealed an AA genetic susceptibility. Breakdown of HF immune privilege is the central pathophysiology of AA.[5,29] Pathobiology of AA is still not completely understood, and not a single notion about the pathogenesis of AA can claim to be widely accepted.

Genetic associations

Very little is known about the genetics underlying the alopecia areata because of paucity of data on genome-wide studies. The initial investigations of individual genes in alopecia areata started with the introduction of HLA analysis and several studies have now established a substantial association between HLA antigens and alopecia areata.

Majority of studies have shown an increase in the incidence of HLA DR4, DR5 (DR11), and DQ3. Serological tests have showed that HLA DR4 and DR5 are associated with severe forms of alopecia areata. This was later confirmed by molecular typing, which also found an increase in the broad antigen DQ3 in all of the patients in the studies, which suggests that this may be a risk factor for alopecia areata.[29,30] The DQB1*0301 allele (a subtype of DQ3 that is in linkage disequilibrium with DR5) was linked to severe alopecia but not newly diagnosed patchy alopecia.[31]

Hair growth regulation may be influenced by immunomodulatory cytokines, which not only mediate immunity and inflammation but also control cell proliferation and differentiation. Interleukin-1 (IL-1) is the principal cytokine that mediates inflammatory reactions in AA. In hair follicle organ cultures, IL-1 suppresses hair follicle development and promotes morphological alterations similar to those found in alopecia areata.[32,33]

Similar to IL-1, tumour necrosis factor alpha (TNF-alpha) has a potent inhibitory effect on in vitro hair growth. TNF-alpha is encoded by a gene in the HLA class III region, and a polymorphism of this gene has been found to be closely linked to specific autoimmune inflammatory disorders. In a short study of 50 cases, TNF-alpha polymorphisms in alopecia areata were explored by Galbraith et al, who found a substantial difference in TNF-alpha genotypes between individuals with patchy disease and those with alopecia totalis and universalis. However, there was no overall difference between the illness and control groups. TNF-alpha's significance in the pathophysiology of alopecia areata has yet to be

determined.[29,34]

Immune Privilege collapse and Local disturbances in HFs

Normally, an immunosuppressive environment protects anagen hair follicles from autoimmune reactions, a phenomenon known as hair follicle immune privilege (HF-IP).[10] The presence of an extracellular matrix and the absence of lymphatic drainage may act as physical barriers to invading immune cells. To protect their sequestered antigens, hair follicle (HF) cells also produce factors that suppress MHC expression. Transforming growth factor beta (TGF-β), alpha melanocyte stimulating hormone (α-MSH), indoleamine-2,3-dioxygenase (IDO), protein red encoded by the IK gene (red/IK), interleukin-10 (IL-10), calcitonin gene-related peptide (CGRP), insulin-like growth factor 1 (IGF-1) and somatostatin are among the immune privilege (IP) guardians.[5] The presence of lymphocytes, dendritic cells and NK cells in the anagen HF peribulbar area, which is normally devoid of immune cells, is strong evidence of immune privilege collapse in AA.[8] According to this hypothesis, environmental stress can cause a buildup of reactive oxygen species in HF keratinocytes. Genetically susceptible individuals are unable to overcome this effect, so stress accumulates in the cells, promoting MICA (MHC Class I Polypeptide-Related Sequence A) expression.[5] When HF cells begin to present MHC-I molecules, the immune system is exposed to a large number of secure antigens, and subsequent immunological events become unstoppable.[9]

Role Of Vitamin D in alopecia areata

Lack of micronutrients, like vitamins and minerals, might be a risk factor that can be changed for the development of AA. Vitamin D binds to the vitamin D receptor (VDR), a nuclear hormone receptor that is widely expressed in the kidney, immune cells, osteocytes, and other cell types. The vitamin D-activated VDR forms a heterodimer with the retinoid X receptor. This complex is recruited to the vitamin D response elements in the target genes, where it interacts with additional co-regulators to regulate the expression of numerous genes. Consequently, vitamin D has many activities and target organs.[35,36]

There is no evidence in the literature that vitamin D has a role in HF cycling. However, recent research has shown that VDR plays a crucial role in the hair cycle process. However, vitamin D doesn't appear to be necessary for this VDR function.[37] VDR may selectively suppress/de-repress gene expression without 1,25(OH)2D3. Wnt/β-catenin signalling induces anagen and maintains cycle transition during HF initiation and regeneration. Wnt/β-catenin

signals may diminish hair cycle-related VDR expression in AA. AA disrupts HF cycling due to reduced VDR expression.[38,39]

An autoimmune response disrupts HF cycling in AA, and there is emerging evidence that vitamin D influences the pathophysiological processes of autoimmunity. As a result, vitamin D may influence HF cycling via its effect on autoimmunity in AA pathogenesis.[1]

The collapse of HF IP in response to autoantigen exposure results in a concentration of autoreactive effector T cells in and around the lesional hair bulb, which is histologically characterised as "swarm of bees." Significant advancement in immunology research supports the notion that AA is an autoimmune reaction characterised by a CD8+ T cell attack on the anagen stage HF, with CD4+ T cell help as the underlying mechanism.[40,41] *1,25 dihydroxyvitamin D* has been shown in several studies to be effective at preventing the proliferation of human CD8 and CD4 T cells.[42]

Other possible roles of vitamin D in AA pathogenesis are found out in different studies as follows[19,43,44]

1. Inhibiting the JAK/ STAT pathway and in turn block the effects of key cytokines in the pathogenesis of AA.
2. Control the overactive self-reactive T cells by enhancing the inhibitory function of Treg cells.
3. Contribute to prevent the skin NKG2D+CD8+ T cell activation and trafficking in AA by down-regulating both NKG2D-activating and CXCR3-activating ligands.
4. Contribute to maintain the IP of HF by decreasing the production of IFN-γ.

However, further research has to be done before a systemic supplement of vitamin D may be utilised effectively in the treatment of human autoimmune disorders like AA.

Vitamin D status in alopecia areata patients

A number of studies have shown that patients with AA had considerably lower levels of vitamin D than the control group.

Table-1: Vitamin D status in alopecia areata

Study	Study design	Sample size	Results
Fawzi et al.,[45] 2016	Case-control	40	Serum and tissue VDR levels significantly lower in AA cases
Bakry et al.,[46] 2016	Case control	60	Serum vitamin D levels were significantly lower in AA cases and severe AA showed significantly the lowest vitamin D levels compared with mild AA cases.
Thompson et al.,[47] 2016	Prospective study	133	No association between serum vitamin D and risk of AA
Daroach et al.,[10] 2017	Prospective observational study	60	96.7% patients were Vitamin D deficient, compared with 73.3% controls and VDR expression was reduced in all patients and was normal in controls
Erpolat et al.,[48] 2017	Case control	41	Serum vitamin D: no significant difference from control. 93.8% patients had vitamin D deficiency in AA patients vs 85.3% in control
d'Ovidio et al.,[49] 2018	Case control	156	Higher vitamin D deficiency prevalence in patients as compared to control
Tsai et al.,[50] 2018	Systematic review & meta-analysis	-	AA patients have higher vitamin D deficiency prevalence and lower serum vitamin D than controls
Lee et al.,[37] 2018	Systematic review & meta-analysis	-	AA patients have higher vitamin D deficiency prevalence than controls

CLINICAL PRESENTATION

The clinical presentation of AA might vary from patient to patient. Patients often describe a sudden onset of hair loss over localized area of scalp.

Scalp is the most common site (90%), but any part of the body may be affected.[2] The characteristic lesion of alopecia areata is typically a round or oval, completely hairless, smooth patch that involves the scalp or any other part of the body that bears hair. At first, only the pigmented hairs are affected, leaving the white hairs alone. In long-term cases, the grey hair also begins to fall out, but in chronic situations white hair loss also occurs.[51] The presence of exclamatory hairs at the border and a positive hair pull test from the periphery suggests that the patch may be active and progressive.[52]

Some people report paresthesia with mild to moderate pruritus, tenderness, burning sensation, or pain prior to the emergence of the patches, despite the fact that hair loss is often asymptomatic.[11]

AA affecting the beard area is well known and is referred to as AA of the beard (BAA) or AA barbae when involvement is limited exclusively to the beard. It is characterized by well-demarcated, smooth-surfaced patches of hair loss on the beard of male patient. Beard is the 2nd most affected region in alopecia areata accounting for 20-30% all cases. Although AA can solely involve the beard (as in AA barbae), it often presents concomitantly with alopecic patches in other hair-bearing regions.[53] Facial involvement has reportedly been linked to depressive, anxious, and neurotic symptoms. It can lead to poor quality of life and lack of coping skills.[23]

AA can be classified depending on extent and pattern of hair loss.

Table-2: Clinical classification of alopecia areata[2,54,55]

Classification	Types	Remarks
Based on extent	Patchy alopecia	Localized, well-demarcated patches of hair loss
	Alopecia totalis	Involve the entire scalp
	Alopecia universalis	Involving total body hair
Based on pattern	Ophiasis	Alopecia along the posterior occipital and temporal margins
	Sisaipho	Alopecia involving the frontal, temporal, and parietal scalp but spares hair along the scalp periphery
	Reticular	Multiple active and regressing patches in a net-like pattern in the scalp
New variants	Acute and diffuse total alopecia	It is distinguished by a predominance of females, generalized thinning, rapid progression, tissue eosinophilia, extensive involvement, a brief clinical course, and a favourable prognosis
Unusual patterns	Perinevoid alopecia	Patches of alopecia around the nevi
	Linear	Appearing as a linear band traversing the center of the scalp

ASSOCIATED CONDITIONS

Alopecia areata had been reported to be associated with atopy and several autoimmune diseases. Atopies, which include allergic rhinitis, asthma, and atopic dermatitis, have been found in more than 40% of AA patients, but they are only found in about 20% of the general population, which is twice as prevalent as in the general population.[56] Serarslan et al., found that there is no significant difference in the prevalence of autoimmune and atopic diseases between the adult patients, the paediatric patients, and the healthy controls.[57]

In India, in a study done by Sharma et al., just 1% of AA patients had autoimmune thyroiditis, whereas in an another study 18.3% of AA patients had thyroid disease.[58,59]

Vitiligo, psoriasis, diabetes mellitus, down's syndrome, addison's disease, autosomal recessive autoimmune polyglandular syndrome, systemic lupus erythematosus, celiac disease, ulcerative colitis, and multiple sclerosis are other associated disorders. These are less prevalent and more likely to be associated with AT/AU.[60]

NAIL CHANGES IN ALOPECIA AREATA

Nail changes may precede or follow the hair loss, and they may be limited to one or most nails.[2] The pathogenesis of AA and the associated nail changes is unspecified. Its onset and severity are linked to a complex interaction of genetic and immunological factors. The nail unit is an immune-protected site as well. Nail changes in AA are most likely caused by lymphocytic infiltrate.[12] In one study, patients with AA-related nail changes had lower levels of 25-hydroxyvitamin D than healthy people though which wasn't significant.[61]

Nail changes caused by AA are frequently asymptomatic. They can be subtle on physical examination and are frequently overlooked. The prevalence of nail changes has been reported to range from 7% to 66%, with an average prevalence of around 30%.[12] Nail changes are associated with more severe forms of AA. Both pitting (11.4–0.6%) and trachyonychia (8–14%) are considered to be the most prevalent nail alterations related with AA in adults. Alterations such as longitudinal ridging, Beau's lines, onycholysis, punctate leukonychia, and red spotted lunulae were also mentioned in the studies.[12]

HAIR REGROWTH PATTERNS IN ALOPECIA AREATA

Despite the fact that there have been several case reports about interesting morphological features of hair regrowth in patients with AA, systematic investigations and classifications of hair regrowth patterns in AA have been studied less.

Lee et al studied the recovery patterns of 106 AA patches in the past. They discovered four different types of hair regrowth patterns: diffuse, irregular, marginal, and targetoid. Based on their findings, they proposed a classification system for hair regrowth patterns known as the 'DIMT classification'.[62]

Lim et al investigated whether the DIMT-classified hair regrowth patterns of AA patches are associated with treatment modality and patch size in 152 AA patches. They found that the associations between the diffuse pattern and patch size >2 cm, between the irregular pattern and intralesional injection of triamcinolone acetonide, between the marginal pattern and systemic and topical corticosteroids, and between the targetoid pattern and patch size >2 cm were statistically significant.[63]

It has to be determined if these hair regrowth patterns are associated with AA clinical outcomes such as hair regrowth rate.

DERMOSCOPY: A TOOL FOR DIAGNOSIS AND PROGNOSIS

Dermoscopic results can be helpful in determining the intensity and varying stages of AA activity. Dermoscopy may also be a useful method for dermatologists to diagnose, monitor, and assess the effectiveness of treatment for AA.

Dermoscopic features of AA include:

- Yellow dots: Round or polycyclic yellow to yellow-pink dots that represent distended follicular infundibula filled with sebum and keratin remnants
- Black dots: Remnant of broken hair shafts inside follicular ostia
- Exclamation mark hairs: Broken hairs that tapered toward follicles
- Short vellus hairs: Thin, nonpigmented hairs with length ≤10 mm may demonstrate early disease remission
- Broken hairs: Due to fracture of dystrophic hair shafts or rapid regrowth of hairs that

formerly manifested as black dots.[64]

In a study by Ganjoo et al[14] it was found that the frequency of black dots, broken hairs, exclamation mark hairs, and tapering hairs in patients given intralesional triamcinolone acetonide during follow-up trichoscopy was lower than it was at baseline.

In a study conducted by Burnat et al[65] comprised a total of 65 participants with patchy alopecia areata. Trichoscopy was done at the beginning of the treatment and two months afterwards. Patients were classified into two groups: responders (27/65) and non-responders (38/65) after six months. In terms of baseline trichoscopy, there were no variations between the groups. After two months, non-responders significantly outnumbered responders in terms of the frequency of black dots, broken hairs, exclamation mark hairs, and tapering hairs.

Table-3: Trichoscopic features in alopecia areata

Activity markers	**Longstanding inactive disease markers**	**Regrowth markers**
Black dots Exclamation mark hairs Broken hairs Tapered hairs Pohl-pinkus constrictions Coudability hairs	Yellow dots	Upright regrowing hairs Short vellus hairs Pigtail hairs

HISTOPATHOLOGY

Histopathological changes in AA are time-dependent. Nonscarring alopecia necessitates the use of TSs since it is associated with abnormalities in the growth cycle of hair follicles. Only on TSs can aspects like change in hair follicle size with miniaturisation and the existence of nanogen follicles in alopecia areata be examined.[66] The single scalp biopsy specimen for TSs may be cut in different ways. The innovative HoVert approach, on the other hand, is an useful strategy for identifying alopecia by integrating the benefits of both VS and TS in a single biopsy sample.[67] This allows us to see the hair follicles at different layers of the dermis and measure the density, diameter, and percentage of hair follicles at different stages.[68]

The peribulbar lymphocytic infiltrate is the first and most important sign of the acute phase of AA histology. The lymphocytic inflammatory infiltrate around anagen follicles, resembling 'swarm of bees,' is characteristic. It can extend into the epithelium and hair matrix.[69]

AA is considered as a non-scarring alopecia. However, the disease's progression is very unpredictable. On the basis of peribulbar lymphocytic infiltrates, prevalence of non-anagen terminal follicles, pigment casts, and perifollicular fibrosis with normal epidermis and interfollicular dermis, pathologists may confidently diagnose AA.[68]

TREATMENT OPTIONS IN ALOPECIA AREATA

The goal of treatment is primarily to reduce the disease activity because there is no known definitive cure or preventive measure. Counseling and explaining potential genuine expectations of available therapies are critical. Predominantly it is a self-limiting disorder that results in spontaneous hair regrowth. However, in some individuals, this regrowth may take many months or years, resulting in psychological distress. Within one year, up to 80% of individuals with patchy alopecia areata (AA) have spontaneous remission.[70]

Depending on severity of involvement, there are a variety of systemic and topical therapy options for AA.[2,17,71–73]

Table-4: Classification of interventions for alopecia areata

CLASSIFICATION	TREATMENT OPTIONS
Topical agents	Corticosteroid Minoxidil Immunotherapy- Diphenylcyclopropenone (DPCP) and Squaric acid dibutylester (SADBE) Topical vitamin D analogues Prostaglandin analogues PUVA Topical retinoids Anthralin
Intralesional	Corticosteroids Platelet rich plasma (PRP) Methotrexate Intralesional carboxy therapy
Systemic agents	Corticosteroids Immunosuppressive drugs- Cyclosporine, Methotrexate, Azathioprine Sulfasalazine Biologicals Janus kinase inhibitors- Tofacitinib, Ruxolitinib, and Baricitinib PDE-4 inhibitor - Apremilast
Procedural treatments	Cryotherapy Lasers- Excimer, CO2 laser, Diode laser Microneedling

Intralesional Corticosteroids

Because of their ability to reduce inflammation, corticosteroids have been the cornerstone of AA therapy. They can be used topically, as intralesional injection, orally and administered parenterally. They act by reducing peri-follicular inflammation mediated by T-cells.[11]

Intralesional corticosteroids (ILCs) have been used since 1958 in the treatment of AA, and for adult patients with less than 50% scalp involvement, ILCSs, preferably triamcinolone acetonide, are considered first-line therapy.[74] ILCs therapy has poor results with more than 50% scalp involvement, very advanced disease and duration more than two years. Local atrophy is the most common side effect.[16,18]

Steroids with low solubility are generally preferred due to their slow absorption from the injection site, which promotes maximum local action with minimal systemic effects. The most commonly used preparations in AA is triamcinolone acetonide,.[11,75] Corticosteroid is injected into the upper subcutis just beneath the dermis. A 0.05 to 0.1 mL injection will result in a tuft of hair growth about 0.5 cm in diameter. Multiple injections may be administered, with the main limitation being patient discomfort.[71] Among the multiple triamcinolone concentrations, 2.5, 5 and 10 mg/dL were all found to be equally effective.[76] Regrowth is usually visible in 4 weeks, and intralesional corticosteroids should be stopped after 6 months if there is no improvement.[2] In adult patients with less than 50% scalp involvement, ILCs, preferably triamcinolone acetonide, is the first-line therapy and the preferred concentration for the scalp is 5 mg/mL, while the concentration for the face and eyebrow is 2.5 mg/ml.[18]

Table-5: Intralesional steroids in treatment of alopecia areata

Study	Type	Sample size	Treatment given	Results	Adverse effects
Intralesional corticosteroids					
Kuldeep et al., [77] 2011	Clinical trial	78 patients Group A- 28, group B &C- 25 each	Group A- ITA 10mg/ml 3weely Group B- Betamethasone valerate 0/1% LA OD Group C- Ointment Tacrolimus 0.1% LA OD x 12 weeks	60% of those treated with ITA reached the end point of > 75% hair regrowth at 12 weeks followed by 53.6% in group B and nil in group C	Group A showed local pain and atrophy in 6 cases, itching and burning in 3 and 2 cases, respectively. Group B&C- itching in 3 cases each local pain, Atrophy
Thappa et al., [14] 2013	Clinical trial	70 patches in 60 patients	Injection Triamcinolone acetonide (ITA) at 4 weeks interval x 24 weeks	28 patients responded early and achieved RGS of 4 within 12 weeks. Dermoscopically, 60 patches demonstrated regrowth of new vellus hair at 4 weeks.	Atrophy in 16% patches and telangiectasia in 3% patches

Kaur et al., [78] 2015	Randomized clinical trial	40 patients with minimum 3 patches of AA.	Group A- ITA 2.5 mg/ml at 3 weeks interval. Group B2 – NB-UVB given twice a week Group 3 - Combination of ITA and NBUVB x 12 weeks.	ITA is more effective than NBUVB and their combination is not synergistic	Pain, minor bleeding, folliculitis, and transient atrophy in group A Transient itching or redness in the treated area in group B
Ustuner et al.,[79] 2017	Randomized Controlled Trial	83 patients with total 231 patches	ITA and intralesional betamethasone dipropionate (BD) in 3 different dilutions; 1/4, 1/8, 1/12 (BD1, BD2, BD3, ITA1, ITA2, ITA3) and Saline (control) randomly applied every 4 weeks X 6 sessions	BD ¼ dilution (1.25 mg/dl) seems best corticosteroid showed better response than othersfor intralesional injection in the treatment of localized alopecia areata in adults.	Adverse effects were more common in ITA groups (24.3%) than in BD groups (10.6%)
Albalat MD et al.,[80] 2019	Randomiszed double-blind study	80 patients	Group A - ITA Group B - Injection PRP - 3 to 5 sessions, once every 2 weeks	No significant difference was found	Erythema and burning sensation in both groups

El-Husseiny et al.,[81] 2020	Randomized Controlled Trial	20 patients with minimum 2 patches of AA	Group A– Fractional CO2 every other week 2 weekly for 3-6 sessions Group B- ITA monthly x 3 sessions	Significant improvement with Fractional CO2 laser rather than ITA 3 months after last session	Side effects with fFractional CO2 laser - mild pain during laser session, transient post-treatment scaling, erythema and edema occurred with all studied patients Mild pain during injection (15 cases) in ITA group
Muhaidat et al., [82] 2020	Retrospective Comparative Study	85 patients of patchy scalp AA.	Group A- ITA 5 mg/mL Group B- ITA 10 mg/mL x 3 treatment sessions	No statistically significant difference found	17-.4% patients in group A and 25.6% patients in group B had atrophy
Kapoor et al., [83] 2020	Randomized controlled study	40	Group A- ITA Group B- Injection PRP The injections in every 3 weeks till 12 weeks	Around 50% patients in triamcinolone group and 5% patients in PRP group showed improvement	Pain during intralesional injection was higher in the PRP group

Hegde et al ., [84] 2020	Randomized, placebo-controlled study	50 patients of patchy scalp AA.	Left side of the scalp received placebo, right side of the scalp received intralesional PRP in one group and ITA in second group at 4-weekly intervals for 3 sessions	The maximum absolute regrowth was shown by the ITA followed by PRP followed by placebo group	27% from group ITA and 20% patients from group PRP reported pain during the injection
Mahgoub et al., [85] 2021	Randomized, active-controlled, parallel-group, interventional therapeutic trial	22 patients with at least 2 AA patches	ITA and topical Trichloroacetic acid (TCA) 35% were performed in two randomly selected patches. Three sessions, 3 weeks apart	Both ITA and TCA 35% treated patches showed measurable improvement with no statistically significant difference.	-
Rajan MB, et al., [86] 2021	Double-blind randomiszed controlled trial.	105 patients with 242 patches AA	4 treatment groups (10, 5, 2.5 mg/ml ITA and NS) every 4-weekly till 12-weeks	Hair regrowth scale of all ITA concentrations was better than NS group ($P < .001$)	Atrophy and telangiectasia were maximum in 10 mg/mL group

Elshahid et al., [87] 2022	Randomized trial	40 patients with multifocal patchy alopecia areata	Group A- ITA Group B- Jessner's solution Group C- Normal saline x 3 sessions for 3 months	Patches coated with Jessner's and those steroids injected showed a significant higher response rate than patches injected with saline but didn't show any significant difference between group A and B	-
Metwally et al., [88] 2022	A randomized trial	30 patients of localized AA with 3 patches	Grp A- ITA Grp B- Injection Carbon dioxide Grp C- Combination of A and B x 2weekly for 12weeks	79.2% hair regrowth following the combined regimen, 69.5% improvement after ILC, 50% improvement after carboxy therapy	Transient pain during injection in both the groups Post injection headache in group B (33.3%)
Hamdino M et al., [89] 2022	Randomized clinical trial	40 patients of localized AA	Group A- ITA Group B- intralesional MTX every 3 weeks, for maximum 4 sessions.	65.0% of patients achieved regrowth≥ 75% in MTX group compared to 50% in the ITA group, but statistically insignificant difference between both groups at 3months after follow-up	Atrophy in 1 patient of group A Erythema in 1 and 2 patients of group A and B respectively

Vitamin D and its analogues in treatment of alopecia areata

A number of studies have shown that patients with AA had considerably lower levels of vitamin D than the control group.[10,37,48,50] The significance of vitamin D and its analogue as a treatment option for alopecia areata has been speculated upon in previous studies.

Regulation of B cell, T cell, and dendritic cell differentiation, as well as expression of Toll-like receptors, is hypothesised to be the mechanism of action of topical vitamin D analogues in hair regrowth. Vitamin D exerts direct effects on T and B cells, inhibiting T lymphocyte growth, especially in the Th1 arm, and skewing the T cell response toward Th2 dominance. Vitamin D derivatives biological activities include epidermal cell proliferation and differentiation control, as well as cytokine production regulation. All of these actions may account for the effectiveness of vitamin D derivatives in AA.[20,90]

Currently available synthetic vitamin D3 analogues used in dermatology include calcipotriol, maxacalcitol, tacalcitol and calcitriol. Due to their minimal systemic absorption, these drugs have little systemic adverse effects.[91] Currently calcipotriol is the only vitamin D analogue that has been investigated in alopecia areata.

Table-6: Vitamin D analogues in treatment of alopecia areata

Study	Type	Sample size	Treatment given	Results	Adverse effects
Topical vitamin D analogues					
Orecchia et al., [92] 2009	Clinical trial	28 patients with severe AA	2% Squaric Acid Dibutylester(SADBE) solution applied to the vertex. After 2 weeks and then weekly, a 0.001% SADBE solution was applied to the whole scalp. Every day, except the day of SADBE application, an ointment containing 50 µg/g calcipotriol was applied to the left side of the scalp	Addition of topical Failure of calcipotriol didn't to potentiate the effectiveness of SADBEsquaric acid dibutylester effectiveness	15 patients had redness/scaling at the application site of topical calcipotriol
Berth-Jones et al., [93] 2009	Clinical trial	20 patients with AT/AU	Calcipotriol ointment (containing 50 µg/g) applied to one side of the scalp and matching vehicle to the other side.	No response to calcipotriol in patients with alopecia totalis and alopecia universalis	Pruritus with or without erythema in 8 patients
Kim et al.,[94] 2012	Case report	7-year-old male boy	Ccalcipotriol solution (Daivonex, 50µg/ml) to be applied once daily for 3 months	After 3 months of calcipotriol therapy, complete hair rregrowth was observed	-

Cerman et al.,[95] 2015	Retrospective study	48 patients with mild to moderate AA	Calcipotriol cream was applied to the affected areas twice a day x 12 weeks	69.2% of patients showed hair regrowth	-
Narang et al.,[20] 2017	Prospective study	22 patients	Calcipotriol lotion 0.005% LA BD x 12 weeks	59.1% patients had hair regrowth 36.4% patients had no response to treatment	Irritation, scaling, erythema, and folliculitis in 7(31.8%)
Jaiswal et al.,[96] 2018	Clinical trial	60 patients	Group A-topical calcipotriol (0.005%) ointment daily Group B- topical calcipotriol (0.005%) ointment daily and NBUVB twice weekly Group C-Placebo x 12 weeks	52.8, 69.6, and 3.9% improvement in SALT scores in groups A, B, and C patients, respectively	Local erythema, burning, itching, and exfoliation
Krueger et al.,[97] 2019	Case report	1 patient	Anthralin 1% cream with calcipotriene 0.005% cream 5 days per week x 32 weeks	visible hair regrowth with satisfactory cosmetic results	scalp irritation pruritus, erythema, and scaling

El Taieb et al.,[7] 2019	Randomized-controlled trial	60 patients with AA of scalp	4 groups; topical calcipotriol, NB-UVB, both and placebo X 12 weeks	SALT score and vitamin D3 levels were signifcantly improved in all groups except placebo. Combination of calcipotriol and NB-UVB is not superior to each line of treatment alone	-
Calcipotriol vs Steroids					
Alam et al.,[98] 2019	Interventional, comparative analytical study	100 patients	Group A -topical mometasone 0.1% cream along with topical calcipotriol 0.005% ointment LA OD Group B -only topical mometasone 0.1% cream once a day x 24 weeks	Significant decrease in mean SALT score in both groups when compared with baseline values	Group A- Erythema (1%), Dermatitis (1%), Folliculitis (1%) and Atrophy (1%) Erythema, dermatitis, folliculitis, atrophy. Group B- Folliculitis (2%) and Atrophy (2%)

Molinelli et al.,[99] 2020	Prospective intrasubject design study	35 patients with scalp AA.	Calcipotriol 0.005% ointment and clobetasol propionate 0.05% ointment were applied twice daily (right vs. left side) x 12 weeks	RGS score of 4, in 62.9% of the scalp sites treated with the calcipotriol and 45.7% of scalp sites treated with the topical clobetasol	Three (8.5%) patients with telangiectasia and pruritus in clobetasol propionate. 2(5.5%) patients had mild erythema pruritus in calcipotriol group
Intralesional Vitamin D analogues					
Rashad et al.,[100] 2022	Randomized control trial	60 Patients with patchy AA	Group A -1ml of intralesional injection of vitamin D3 every 4 weeks for a maximum of 3 sessions. Group B -intralesional injection of normal saline 0.9% every 4 weeks for 3 sessions	Statistically significant difference between two study groups regarding degree of improvement (group A>B).	Negligible and transient
Vitamin D supplementation					
Harvey et al.,[101] 2020	Case report	1 patient 8-year-old male child with AA.	Vitamin D- 900 IU/day + Zinc – 10mg/day + Vitamin A 400 IU/day X 20 weeks	Complete remission of AA was achieved within five months	Transient erythema over cheeks

Other topical and intralesional treatment used in AA

1. Topical corticosteroids

Although the outcomes may be inferior to intralesional therapy, topically applied corticosteroids are probably beneficial in AA, particularly in patients with limited disease. Split scalp studies have demonstrated that the medication's local action rather than its systemic are the ones which causes hair regrowth.[102]

Potent glucocorticosteroids (class 3 or 4) for at least three months are used in treatment.[16] Topical treatment has a relapse rate ranging from 37 to 63%. Folliculitis, atrophy, striae, telangiectasia, and acneiform eruptions are examples of potential adverse effects.[16]

Despite variable efficacy, topical steroids are the preferred first choice agent in the treatment of AA because of its ease of application, especially in children.[2]

2. Minoxidil

When used alone, minoxidil for alopecia areata may not be able to stimulate complete hair growth. Despite this, several studies have shown that it does promote hair growth in patients with AA, however, it is less effective in severe cases.

5% minoxidil outperformed placebo in children and adults with patchy AA in a meta-analysis with moderate quality of evidence. 1%, 3%, and 5% minoxidil vs. placebo in a meta-analysis of patchy AA treatment in children and adults was efficacious for less than 6 months. Due of limited sample sizes and methodologically deficient studies, 1% and 3% minoxidil's efficacy is substantiated.[103]

Collectively, these findings indicate that topical minoxidil may provide some benefit to AA patients, although it is unlikely to modify the disease's progression or cause remission. This drug is simple to administer, and its adverse effects, including scalp irritation and dermatitis, are minimal. Roughly one in five female patients had hypertrichosis, and about ten percent experienced scalp irritation.[104,105]

3. Contact immunotherapy

The contact allergens that have been used in the treatment of alopecia areata include: 1-chloro,2,4, dinitrobenzene (DNCB); squaric acid dibutylester (SADBE); and 2,3-diphenylcyclopropenone (DPCP). DNCB went out of favour after the Ames test revealed that

it caused mutations in Salmonella typhimurium. Both SADBE and DPCP are not mutagenic.[106,107]

They cause allergic contact dermatitis and, via a poorly understood mechanism, may trigger antigenic competition, altering the immune cell environment around hair follicles.[108]
Happle et al., described the protocol for DPCP contact immunotherapy. A small region of the scalp is treated with a 2% solution of DPCP in order to sensitize the patient. Two weeks later, the scalp is painted with a diluted DPCP solution beginning at 0.001%, and this is repeated weekly.[109]

At each visit the dose is increased until a mild dermatitis is observed. Some clinicians initially treat one side of the scalp to differentiate between a therapeutic response and spontaneous recovery if hair regrowth occurs. Once hair regrowth is seen, treatment is administered to both sides of the scalp. This precaution is unnecessary for individuals with severe, long-lasting alopecia, when spontaneous recovery is uncommon. Varying opinions exist on whether patients should be permitted to treat themselves. Once a maximal response has been obtained, the majority of practitioners lessen the treatment frequency. In patients with complete hair regrowth, therapy can be discontinued. However, the disease may relapse after stopping the treatment.[109,110]

When receiving contact immunotherapy, the patients may develop which include persistent dermatitis, severe cervical lymphadenopathy, widespread eczema, blistering, contact leukoderma, and urticarial reactions.[111]

4. Prostaglandin analogues

Topical prostaglandins, such as bimatoprost and latanoprost, may promote hair regrowth on the scalp and eyebrows.
Bhat et al. conducted a study to investigate the efficacy of topical betamethasone diproprionate lotion versus topical latanoprost ophthalmic solution in the treatment of localised alopecia areata. Considerably fewer individuals in the latanoprost group had a full response to therapy (24% vs 56%), and the median hair regrowth score was significantly lower in the latanoprost group as compared to the betamethasone group.[112] In a study of 40 people with AU, 45% had complete or moderate regrowth of eyelashes after using topical latanoprost for 2 years compared with control group.[113] But a 16-week controlled study of 11

people with eyelash alopecia showed that neither latanoprost nor bimatoprost made a significant difference.[114]

While prostaglandins, especially latanoprost, can cause permanent iris and eyelid hyperpigmentation, uveitis, eyelash curling and conjunctival hyperemia, these adverse effects were not recorded in individuals with alopecia areata.[115]

5. Anthralin

Anthralin has an unclear mechanism of action in AA. It is thought to produce hair regrowth by causing irritant contact dermatitis. It generates free radicals, has immunosuppressive and anti-inflammatory properties.[52]

It is used as 0.5-1% cream with brief contact treatment. It is applied once a day for 20-30 minutes for 2-3 weeks, with the contact duration gradually increased by 5 minutes per day up to 1 hour, or until erythema and/or pruritus emerge, and then kept at the same time for 3-6 months.[2]

Due to the limited number of case report series that have been conducted on the use of dithranol (anthralin) or other irritants in the treatment of alopecia areata, as well as the absence of controls, it is difficult to evaluate response rates.

It has the potential to cause severe inflammation, folliculitis, regional lymphadenopathy, as well as discoloration of the skin, clothing, and hair.[2]

6. Phototherapy

The use of Psoralen plus UVA (PUVA) in the treatment of patients with severe forms of AA has been described in the literature. PUVA works by eliminating the inflammatory cell infiltrates around affected hair follicles, which may play a crucial part in the aetiology of AA.[52] However, the use of oral PUVA is often limited because of systemic adverse effects. However, systemic absorption is less with bath/topical/turban PUVA, this provides an alternate method to treat AA.[116] According to a cochrane review there is a paucity of high-quality randomised controlled trials to show the efficacy of phototherapy for AA.[17]

7. Laser therapies for Alopecia Areata

Various laser and light-based devices have been studied in alopecia areata like excimer laser/light, infrared diode, Nd:YAG , fractional CO2, fractional erbium glass infrared irradiation, yellow light emitting diode with varying responses.[117]

Excimer Laser

Excimer lasers may be used to treat AA in addition to inflammatory skin diseases like psoriasis and vitiligo, as they induce T-cell apoptosis.[118] The excimer laser is the most widely investigated laser to date for the treatment of AA. This device generates significant dosages of long-wave monochromatic ultraviolet B radiation.

In prospective trial, Zakaria et al. used the excimer laser (308 nm) in 9 patients. In each patient, one patch on the other side of the scalp was left untreated to serve as the control group. All patients with patchy AA showed hair regrowth, but not those with AU or AT patients.[119]

Diode Laser

Waiz et al. used a pulsed infrared diode laser (904 mm), which they described as "low energy light," on alopecia patches that were resistant to numerous treatment modalities (in the scalp, brows, beard, and moustache), they observed hair regrowth in 94% of treated patches. Although the diode laser may provide a promising therapeutic strategy for patients with recalcitrant AA while simultaneously offering low risk to the patient, more research into the clinical consequences of this treatment modality is required.[120]

Table-7: Other topical and intralesional treatment used in alopecia areata

Study	Type	Sample size	Treatment given	Results	Adverse effects
Topical Corticosteroids					
Tosti et al., [102] 2006	Randomised double blinded placebo-controlled trial	34 patients of moderate to severe AA.	Group A- Clobetasol foam (CF) 0.05% Group B- Placebo foam (PF) x 24 weeks	Hair regrowth was observed in 89% of patients treated with CF vs. 11% of patients treated with PF	Folliculitis occurred in two patients of CF foam
Sardesai et al., [121] 2012	Open, randomiszed, comparative study.	30 patients with less than 5 patches and involvement of scalp less than 25%.	Regime 1- ITA 10 mg/ml Regime 2- Topical betamethasone dipropionate 0.05% Regime 3- Minoxidil 5% Regime 4- Anthralin 1.15%+Salicylic acid 1.15%+Coal tar 5.3% ointment Regime 5- Placebo solution x 12 weeks	Significant improvement in as far as hair growth was considered was seen with both intralesional and topical steroids.	No other modality except anthralin showed side effects which were such as erythema and pruritus.

Lenane et al., [122] 2014	single-centre, randomised, 2-arm, parallel-group, superiority trial	41 children of AA cases with involvement of at least 10% of area	Group A- clobetasol propionate, 0.05% cream, Group -hydrocortisone, 1%, cream x 24 weeks	85% of Group 1 vs 33.3% of Group 2 had a at least a 50% reduction in surface area at 24 weeks	Reversible atrophy in one patient of group A
Topical Minoxidil					
Fenton et al.,[123] 1983	Modified double blind crossover study	30 patients with AA and AT.	Group A- Topical 1% minoxidil Group B- Placebo x12weeks	Cosmetically acceptable response in 55% of patients in group A	No topical or systemic side effects noted
Fiedler-Weiss et al.,[124] 1987	Clinical trial	66 patients	Group A- 1% topical minoxidil daily Group B- 5% topical minoxidil daily X 30 weeks	Response rate of 38% in group A and 81% in Group B	Mild local irritation, allergic contact dermatitis
Price et al.,[104] 1987	Double blinded clinical trial	30 AT/ AU/ extensive patchy AA	Applied minoxidil or placebo to half of the affected scalp area LA BD x 12 months	Response rate was 63.6% in the minoxidil group and 35.7%in the placebo group	3 patients had scalp itching and 1 had dermatitis

Contact immunotherapy					
Dallo'glioa et al.,[125] 2005	Open-label, paired-comparison, clinical trial	108 patients 54 severe AA cases and 54 controls.	SADBE according to standard protocol x 6months vs Placebo	79.6% obtained complete regrowth after a mean period of 34.5 weeks	No adverse events noted
Ajith et al.,[126] 2006	Clinical trial	70 AA cases, not responding to conventional treatments	SADBE according to standard protocol x 4 months and thereafter depending on the response with initial therapy	43% overall successes	-
Lamb et al.,[127] 2016	Retrospective review	133 patients	DPCP according to a standard protocol. Initially, half the scalp was treated. If hair growth was observed, the entire scalp was treated x 6 months	16.5% developed patchy terminal regrowth, 10.5% patients developed complete regrowth	Mild extra-scalp eczema, severe eczema (blistering, weeping, and/or widespread secondary sensitization, Cervical lymphadenopathy, Headache and otalgia
Tiwary et al.,[128] 2016	Randomized, single blinded, uncontrolled, prospective	24 patients	Group A-SADBE Group B-DPCP according to standard protocol x 24 weeks	58.33% of group A patients and in group B 33.33% of patients showed hair regrowth of >50-75%	Regional lymphadenopathy observed in 16.66% of group A and 33.33% of group B patients

Anthralin					
Sasmaz et al., [129] 2005	Randomized clinical trial	31 patients of patchy AA	Group A-20% azelaic acid Group B-0.5% anthralin x12 weeks	Complete response was observed in 53.3% of cases in the azelaic acid group compared with 56.2% in the anthralin group	2 patients in the anthralin and one patient in azelaic acid reported pruritus, burning, and redness.
Nasimi et al., [130] 2019	Retrospective case series	3232 patients who are nonresponsive to DPCP monotherapy.	DPCP+ dithranol x (3-17months)	40.62% of patients had terminal hair regrowth	Bullae (25%), generalized pruritus (3.1%)
Ghandi et al., [131] 2021	Randomised controlled trial	50 patients	Group A - DPCP alone Group B -Combination with anthralin x 6months	25% and 31% of patients in group A and 21% and 47% of patients in group B had > 75% and > 50% hair regrowth respectively	Pruritus (40.0%), hyperpigmentation (40.0%) and erythema (34.3%) with immunotherapy

Phototherapy					
Mohamed et al., [132] 2005	Clinical trial	149 patients 25 patients with AT/AU. 124 cases with AA	Combining topical 8-methoxypsoralen (8-MOP) with UV irradiation of the scalp at a phototoxic dose. The mean energy required was 15 J/cm2 for AA and 42 J/cm2 for AT.	56% had good hair regrowth in AT and 85% had a good or excellent results in AA	Slight erythema, Painful Bburning pain in 4 patients, 1 with bullous reaction and mild erythema in all
Tan et al., [133] 2020	Retrospective review	10 patients	Paint PUVA therapy involved the application of 0.1% 8-methoxypsoralen (8-MOP) solution. Following patients received UVA irradiation Each patient received paint PUVA therapy twice a week x average of 50 therapy sessions	Significant hair regrowth was seen in 60% patients	One patient with alopecia totalis was unable to tolerate higher doses of paint PUVA due to tenderness over treatment area

Table-8: Procedural treatments in alopecia areata

Study	Type	Sample size	Treatment	Results	Adverse effects
Intralesional PRP					
Singh et al.,[134] 2015	Clinical control trial	20 patients	Inj. PRP, 4 weeks interval x 24 weeks	Hair regrowth in all and 1 patient had a relapse, and his hair regrowth was also minimum	No reported side effects in any patients
Ekelem et al., [135] 2020	Case Series	3 patients 2 patients with patchy AA and 1 with AU	Treated with PRP 3 times at 6-week intervals x 24 weeks	Increased in hair density associated with improvement in inflammation	One Patient reported pruritus and tenderness

Micro needling					
Ragab et al., [136] 2020	Clinical trial	60 patients	Monthly sessions of either PRP intradermal injection, Fractional CO2 laser followed by topical PRP, or micro needling followed by topical PRP x 3months	Patients in all groups showed satisfactory results which was statistically insignificant	Intralesional PRP associated with significantly higher pain scores
Aboeldahab et al., [137] 2021	Randomized Controlled Trial	80 patients with mild scalp AA	Group A- superficial cryotherapy using dimethyl ether and propane in three freeze-thaw cycles of 5sec each. Group B- micro needling. Both groups were treated every 2 weeks for 6 sessions	Excellent response in 37.5% of group 1 compared with 35% of group 2 patients	Group A patients reported Crust (10%), Bullae and hypopigmentation (2.5%) Group B- No adverse events

Laser					
Al-Mutairi et al., [138] 2007	Clinical trial	18 patients with 42 recalcitrant patches	308-nm excimer laser twice a week for a period of 12 weeks; one patch on each patient was left as a control for comparison	13 of the 18 patches in scalp showed a complete regrowth of hair	Mild erythema, hyperpigmentation, itching, and mild peeling of skin
Ohtsuki et al., [139] 2013	Clinical Trial	16 patients of single and multiple AA.	Irradiated with a 308-nm excimer lamp at 2-week intervals	62.5 % patients showed more than 50% hair re-growth	Erythema
Kianfar et al., [140] 2021	Randomized Controlled Trial	16 patients with total of 99 patches. Each case having at least 2 patches	Group 1-Weekly excimer laser Group 2-Monthly injections of ITA.	Hair regrowth was achieved in 47% of laser-treated patches and 66% in ITA-treated	-

Cryotherapy					
Jun M et al., [141] 2017	Retrospective, comprehensive review	353 Patients	Cryotherapy 2 weeks interval x 3months	60.9% were responders at the end of study	8 patients with mild pain 3 patients with pruritus and 6 with mild inflammation
El Sayed et al., [142] 2022	Comparative Study	21 patients with patchy AA	Group 1- Superficial cryotherapy by spraying liquid nitrogen 3–4 times for 2–3s per application Group 2- ITA injection once monthly (4 sessions) (triamcinolone acetonide, 5 mg/ml, 1 ml injected)	Terminal hair count improved in lesions treated with cryotherapy more than lesions treated with ILCS but without statistical significance.	No side effects reported in group 1 5 cases with severe pain, burning sensation and headache. One with atrophy and telangiectasia in group 2
Zawar et al., [143] 2016	Case series	11 patients with recalcitrant AA	Cryotherapy every 2 weeks till significant hair regrowth or maximum five sittings 2 weekly	50% showed an excellent response	No serious adverse effects

Systemic agents used in alopecia areata

1. Systemic corticosteroids

Daily, weekly, and monthly pulses of systemic corticosteroids have been administered with effective results in patchy AA and less successful results in ophiasis, AT, and AU.[144]

For certain patients, daily long-term treatment with oral corticosteroids will result in hair growth. In a clinical trial, 30–47% of patients who had received a 6-week tapering course of oral prednisolone (beginning at 40 mg daily) demonstrated more than 25% hair growth.[145] Unfortunately, most patients require ongoing care to maintain hair regrowth, and the benefits are typically insufficient to outweigh the risks.[71] The long-term usage of oral steroids is what causes the majority of its side effects. Weight gain, suppression of the hypothalamo-pituitary-adrenal axis, osteoporosis, ocular abnormalities including cataract and glaucoma, and worsening of hypertension or diabetes are some of them.[146]

Pulse therapy of corticosteroids have been used to avoid the side effects. Pasricha et al used oral mini pulse, betamethasone 5 mg administered after breakfast on two consecutive days each week for six months and observed significant hair regrowth in alopecia areata refractory to other treatments.[147] Several documented case series of high-dose pulsed corticosteroid therapy using various oral and intravenous protocols exist like oral prednisolone 300 mg once a month, intravenous prednisolone 2 g, and intravenous methylprednisolone 250 mg twice daily for three days with varying success rates.[71]

2. Immunosuppressive agents

The dual properties of cyclosporin as an immunosuppressive drug and as a hypertrichotic agent make it a logical choice in treating alopecia areata. However, its effectiveness in AA is debated. To put it simply, it's use restricted due to adverse effects and frequent recurrence.[74]

There was evidence of hair growth in 57% of AA patients after treatment with methotrexate, and the effectiveness of the treatment increased to 63-64% when combined with prednisone.[148]

Farshi et al. studied azathioprine in an open label study, using the SALT score, a dosage of 2 mg/kg/day for 6 months resulted in 52.3% mean regrowth.[149]

3. Sulfasalazine

Sulfasalazine works as immunomodulator. It inhibits inflammatory cell chemotaxis, cytokine and antibody production. Sulfasalazine has been helpful in many uncontrolled case series.[71]
In an uncontrolled open label trial, by Aghaei et al. 27.3% of study participants had full hair regrowth and 40.9% reported partial hair regrowth in patients with AA, and 32% of them had adverse effect like gastrointestinal distress, headache, fever and rash.[150]

4. Biologicals

So far, the response of alopecia areata to biologic drugs has been disappointing. So far, the evidence suggests that biologic drugs intended to reduce levels of tumour necrosis factor (TNF) have failed to show response. Patients using anti-TNF biologic treatments have been reported to develop alopecia areata. In an open-label study performed among people with moderate to severe alopecia areata, following etanercept therapy, no improvement was seen.[151]

5. Janus kinase inhibitors

There have been a number of case reports and small clinical trials indicating promising outcomes with Janus Kinase (JAK) inhibitors tofacitinib, ruxolitinib, and baricitinib for the treatment of alopecia areata. Oral JAK inhibitor treatment was also associated with seven times higher odds of achieving a good response (50–100% regrowth) than a partial response (5–50% regrowth) compared to topical treatment, with no difference between tofacitinib, ruxolitinib or baricitinib. As such, the major determining factor in determining response appears to be the method of drug delivery, with oral agents associated with the best outcomes.[152] Baricitinib (Olumiant) oral tablets were recently approved by the FDA for the treatment of severe alopecia areata.[153]

6. PDE-4 inhibitors

Apremilast, an oral PDE4 inhibitor approved for the treatment of psoriasis and psoriatic arthritis, may have a role as an off-label therapy in alopecia areata. Although recent clinical studies failed to demonstrate the usefulness of apremilast in AA, study conducted by Estebenaz et al., reported four cases of refractory AA successfully treated with apremilast.[154]

Table-9: Systemic agents in treatment of alopecia areata

Study	Type	Sample size	Treatment	Results	Adverse effects
Systemic Corticosteroids					
Kar et al., [144] 2005	Randomised placebo-controlled trial	43 patients with extensive AA	Group A- oral prednisolone 200 mg once weekly Group B -placebo x 12 weeks	Significant hair regrowth was obtained in group A (35%) and none of the patients had significant hair regrowth in the placebo group.	Side effects was noted in 55% patients in group A Generalised weakness was the most common side effect. Others were acneiform eruption, weight gain
Kurosawa et al.,[155] 2006	Open, label randomised, comparative study.	89 patients 51 patients with AA/AA multiplex, 38 patients with AU/ AT.	Group A- Oral dexamethasone (Dex) 0.5 mg/day Group B- intramuscular triamcinolone acetonide (imTA) 40 mg once a month Group C- pulse therapy (PT) using oral prednisolone 80 mg for 3 consecutive days once every 3 months x 6months	Group A- 37%, Group B- 74% and Group C- 66% hair regrowth was present	Dysmenorrhea in imTA and PT groups Abdominal discomfort in all 3 groups

Díaz et al.,[156] 2022	Prospective cohort	40 patients with AA.	Mini-pulses of dexamethasone Mean dexamethasone dose was 2.72 mg/day, two days a week x 12 months	SALT-50 response was achieved in 51.8% of the patients.	Weight gain and altered blood glucose levels
Other systemic agents					
Rashidi et al.,[157] 2008	Clinical trial	39 cases of persistent AA	Oral sulfasalazine 3gm/day x 6 months	25.6% showed good response and 30.7% showed moderate response while 43.5% showed poor or no response	Dizziness and headache in 5.1% patients, which resolved on lowering the dose of sulfasalazine, and dyspepsia in 20 % patients Dizziness, headache Dyspepsia
Farshi et al.,[149] 2010	Clinical trial	20 patients with history of AA for minimum 6 months.	Azathioprine 2mg/kg/day monotherapy for 6 months.	Mean regrowth percentage was 52.3%. Showed significant difference from baseline score($p<0.001$).	Transaminitis in one patient Mild intermittent leukopenia in 3 patients

Crispin M et al., [158] 2016	Multicenter clinical trial	66 patients with involvement of scalp surface area >50%, AT, AU	Tofacitinib citrate 5mg BD x 3 months.	32% showed 50% or more improvement in SALT score. AA and ophiasis subtype responded better than AT and AU.	Mild clinical infections like paronychia and upper respiratory tract infection were reported in 25% of patients
Mackay-Wiggan et al., [159] 2016	Open label clinical trial	12 patients with moderate to severe AA	Oral ruxolitinib 20 mg twice daily for 3-6 months treatment	75% percentage of the patients showed 92% of average hair regrowth by the end of treatment.	No serious adverse effects reported
Saoji et al., [160] 2019	Case study	4 patients	Azathioprine 1mg/kg/day monotherapy x 6–12 months	All cases showed response to short regimens of oral azathioprineAll cases had remission and nearly complete hair regrowth in 6months	No side effects
Phan et al.,[152] 2019	Systematic review and meta-analysis	30 studies which include 289 patients	Oral tofacitinib 5mg BD, oral ruxolitinib & topical tofacitinib	Among 72.4% responded to treatment, 45.7% were good responders and 21.4% were partial responders. Oral route showed significant response than topical therapy	18.2% upper respiratory tract infections, 2.2% urinary tract infections and 24.6% total infections. 1% developed leucopenia and 1.6% transaminitis

Lai et al.,[161] 2019	Randomized control study	32 patients with moderate to severe alopecia areata	Group A- Oral cyclosporine (4mg/kg/day) x 3months Group B- Placebo	Cyclosporine achieved greater proportion of reduction in SALT score by at least 50% than placebo group (31.3% vs 6.3%)	H_eadaches (34.3%) and hirsutism (28.1%) in group A
Mikhaylov et al., [162] 2019	Randomized control trial	30 patients of moderate to severe AA	Group A – Apremilast 30mg BD x 12 to 24 weeks Group B- Placebo	No statistically significant findings between the two groups	Nausea in 3 patients and 1 patient had diffuse arthralgia and diarrhea_
Estebanez et al.,[154] 2019	Case series	4 patients	Apremilast 30 mg twice daily after 5-day initial titration x 6-12 weeks	All the patients showed good clinical response2 patients with significant improvement in SALT score and 1 had complete hair regrowth	DDiarrohea in 1 patient
Nowaczyk et al., [163] 2020	Systematic review	340 patients of AA. 213 with focal, multifocal/ ophiasis AA, 60 AT, 67 AU	Efficacy of cyclosporine for the treatment of AA with and without systemic corticosteroids	Showed response rate of 69.4% in combined vs 57% in cyclosporine monotherapy	Gastrointestinal problems (*8.2%*) hypertrichosis (5.9%) and hypertension (*2.6%*) were the most common side effects of therapyNephrotoxicity Immunosuppression

Dincer et al., [164] 2021	Retrospective pilot study	13 patients with recalcitrant AU and AA.	Oral tofacitinib for a duration of 3-15 months	All 3 cases of alopecia areata responded well to treatment while only 5 out of 10 alopecia universalis showed response to treatment.	Acneiform lesions in 69.2% and transient transaminitis in 15.3% of patients
King et al., [165] 2021	Randomized control study; double blinded	110 patients subdivided in 1:1:1:1 ratio	Group A-Baricitinib 1mg once daily given to 28 patients, 2mg to 27 patients, 4mg to 27 patients. Group B- Placebo	Proportion of patients achieving a SALT score of more than or equal to 20 was significantly greater in baricitinib 2-mg (33.3%) and 4-mg (51.9%) groups versus placebo (3.6%) at 36 weeks.	Baricitinib was well tolerated and no new safety concerns were raised.
Kinoshita-Ise et al., [166] 2021	Retrospective study	15 pediatric patients	Oral methotrexate 15mg/week up to 12 months	86.7% patients showed regrowth but none showed complete recovery	2 patients experienced nausea

MATERIALS & METHODS

MATERIALS & METHODS

STUDY SETTING

This study was conducted on alopecia areata patients, who attended the Dermatology, Venereology and Leprology OPD at AIIMS Jodhpur.

STUDY DESIGN

A single-blinded randomised interventional study

RECRUITMENT

Consenting patients with alopecia areata attending the Department of Dermatology, Venereology and Leprology OPD at AIIMS Jodhpur were recruited.

The inclusion and exclusion criteria were taken as follows:

Inclusion criteria:

Patients fulfilling all the following criteria were included in the study, after informed written consent.

1. Age > 12 years
2. Size of patch >1.0cm x 1.0cm
3. Duration of patch < 2 years
4. Treatment wash-off period of at least 1 month

Exclusion criteria

The following patient were excluded from the study.

1. Known case of uncontrolled systemic illnesses such as diabetes mellitus, hypertension, and coronary artery disease.
2. Pregnancy and Lactation
3. Infection at the lesion site
4. Patches showing obvious evidence of hair regrowth

5. Alopecia totalis, Alopecia universalis, Ophiasis, Sisaipho or area of scalp involvement >50%
6. Known HBV/HCV/HIV patients.
7. Bleeding diathesis

SAMPLING:

The sample size was calculated based on 80% power and an alpha error of 5%. A complete hair regrowth score of 4 was found among 62.86% of patches in the topical calcipotriol group.[99] A complete hair regrowth score of 4 was found among 40% patches in the intralesional triamcinolone acetonide (5mg/ml) group.[80]

The sample size was calculated to be 75 lesions in each group, totaling 166 lesions, taking 10% attrition post-follow-up.

The sample size was calculated using the formula:

$$n = \frac{Z2\ (1-\alpha/2)\ [p1q1+p2q2]}{d2}$$

Where,

P1: Proportion in the first group

P2: Proportion in the second group

d2: Population risk difference

1-α: Desired confidence level.

Randomization and allocation of patients:

The "RESEARCH RANDOMIZER" software (Urbaniak, 19 G.C., & Plous, S. (2013). Research Randomizer (Version 4.0) [Computer software], retrieved on June 22, 2013, from http://www.randomizer.org) was used for random assignment of patients. Patients were

divided into groups by stratified random sampling based on the number of patches they had.

If a patient had more than three patches, they were randomised independently, and if there were three or less patches they were also randomised separately. If a patient had multiple patches, they were numbered anteroposteriorly, and if there were two patches on the same line, the right patch was numbered first as opposed to the left. Numbering for patches over scalp was done first followed by that of patches over beard region.
Patches were assigned to following arms:

Arm 1: Intralesional Triamcinolone acetonide with topical calcipotriol
Arm 2: Intralesional Triamcinolone acetonide with topical placebo (bland emollient)

ETHICAL CONSIDERATIONS

The thesis proposal was approved by the Institutional Ethics Committee, All India Institute of Medical Sciences, Jodhpur [Vide: Certificate reference no. AIIMS/IEC/2021/3389 dated 12th March 2021 (Annexure I)] At the time of recruitment, a detailed explanation of the study protocol was provided to the participants, following which written informed consent was obtained before enrolment.

STUDY DURATION: March 2021 to August 2022 (18 months)

STUDY PROCEDURE:

Evaluation

The diagnosis of alopecia areata was based on the typical clinical presentation (round-oval patches of non-scarring hair loss). After recruitment, the patient's demographic data was collected. Any comorbidities, addictions, family history regarding AA, atopy or other autoimmune diseases were noted. The patient's previous treatment history was obtained. General physical examination, systemic examination and detailed mucocutaneous examination including hair and nails was done.

Examination of the scalp and beard

All of the chosen patients underwent a thorough scalp and beard examination. The overall number of AA patches, each patch's placement and size, and whether or not recovery

indications like obvious regrowth were present were also noted. Baseline SALT Score and Hair Regrowth Score (RGS) were done. TSH and RBS were done in every patient. A dermoscopy (Heine Achromatic HQ Delta 30 Dermatoscope) at 10x magnification in polarized mode was done and the images were captured using a 12 megapixel (MP) mobile camera to further visualize the scalp along with photographic records.

Figure-1: Dermoscope (Heine Achromatic HQ Delta 30) and mobile for clinical and dermoscopic documentation

Examination of nails

The patient's nails were examined for nail changes and patterns such as pitting, longitudinal ridging, and leukonychia along with the types, trachyonychia, splitting of nail plates, onycholysis, onychomadesis and red lunulae.

Documentation of baseline patch characteristics

Using a 12 MP mobile camera at an average distance of one foot, baseline clinical

photographs of chosen regions were collected. Using a Heine Delta 30 dermatoscope coupled with a mobile camera via an adaptor, trichoscopy of the recruited patches was performed at baseline.

Blinding:

The nature of the intervention arms was concealed from the response assessor and statistician.

Intervention

To maintain the homogeneity of the intervention, we used the same brand of triamcinolone acetonide vials (Tricort ^{TM}) in all patients at the standard accessible concentration of 10 mg/ml. Triamcinolone acetonide strengths of 5 mg/ml for scalp patches and 2.5 mg/ml for beard were prepared using normal saline (NS) as a diluent.

Similarly, all patients in Arm 1 received the same brand of topical calcipotriol (0.005% w/w) in ointment preparation (Pasitrex ^{TM}) for self-application twice daily and in Arm 2, petrolatum jelly was used as a placebo for twice daily application over patches.

For patients having both scalp and beard patches, different insulin syringes (Nuovo- fine short needle 30G, POLYMEDTM Insulin syringe (40U), Poly Medicure Ltd., Faridabad) were used to administer the varied strengths.

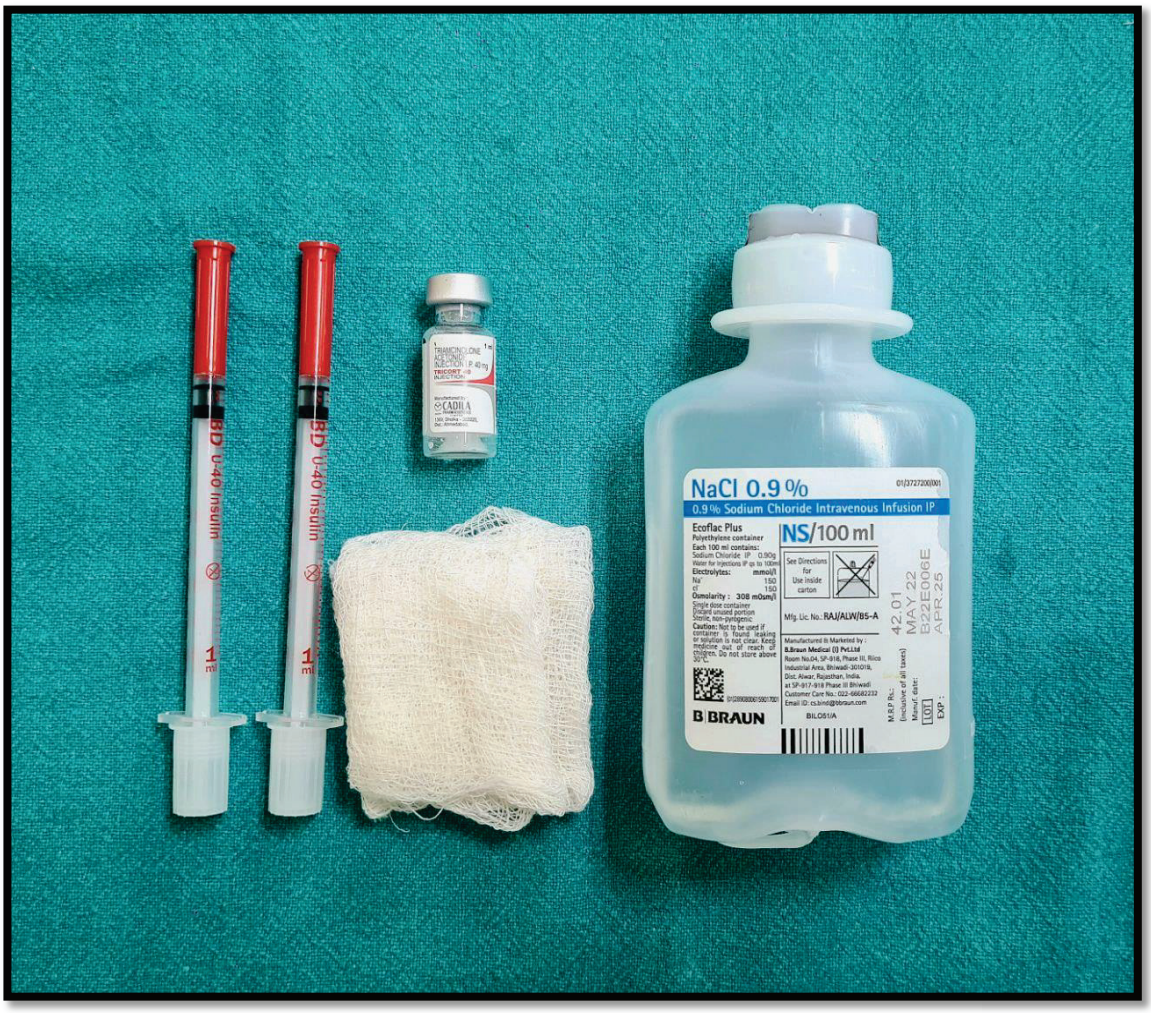


Figure-2: Insulin syringes with triamcinolone acetonide and normal saline for dilution

Follow-up visits and Endpoint

4 weekly analyses by clinical, photographic and trichoscopy documentation, Hair Regrowth score (RGS) was done. Treatment was continued for 12 weeks or until a cosmetically acceptable hair regrowth had occurred, whichever came first. Cosmetically acceptable hair regrowth was sufficient to cover or conceal the patches.

Analysis of the parameters

To lessen bias, one of the investigators evaluated the outcome parameters while being blinded to the intervention arms. During each visit, the following parameters were evaluated.

- Type of hair– Vellus / Terminal/ Intermediate
- The pattern of hair regrowth

- The density of terminal hairs
- Trichoscopic activity
- Local adverse effects

1. Type of hair

On subsequent visits, we looked at each patch to determine the prevalent hair type. Vellus, Terminal, Mixed, and No Growth were the variables that were employed in the assessment.

2. Pattern of hair regrowth

Serial analyses of the regrowth patterns in all patches were done at 4 weeks intervals. For assessment purposes, the "DIMT" classification system was taken into account.[62]
(D- Diffuse, I-Irregular, M- Marginal, T- Targetoid)

3. Density of terminal hairs

Hair regrowth score (RGS), a semi-quantitative score, was used to measure the terminal hair density.[79]

The regrowth score has the following growth parameters and interpretation

Table-10: Hair regrowth score (RGS)

RGS	Percentage of Hair regrowth
0	<10%
1	11-25%
2	26-50%
3	51-75%
4	>75%

4. Trichoscopic activity

Black dots (BD), broken hairs (BH), exclamation mark hairs (EM), tapered hairs (TH), and Pohl- Pinkus constrictions (PP) were among the dermoscopic features of alopecia areata that had previously been regarded to be signs of disease activity.[14,167]

5. Local adverse effects

On each visit, every patch was checked for any local side effects like atrophy, telangiectasia, erythema, burning sensation and scaling.

STATISTICAL ANALYSIS

The results were analyzed by a statistician who was blinded for the entire randomization process including numerical coding.

Descriptive statistics were calculated & graphical representation of data was done. Non-parametric variables such as sex, occupation, co-morbidities, previous treatment, nail changes, baseline dermoscopic findings, hair regrowth, predominant hair type during regrowth, patterns of hair regrowth, dermoscopic signs of disease activity, hair regrowth score, and local adverse effects such as itching, burning sensation and atrophy were described using proportions and percentage. Parametric variables such as age, duration of illness, anthropometric parameters, random blood sugar level and thyroid parameters were described using mean ± Standard deviation.

For continuous variables such as age and SALT score, the independent student t test was used for comparing between the two groups. For nominal variables such as sex, co-morbidities, family history, dermoscopic signs of activity, predominant hair type, 50% hair regrowth, and regrowth type, chi square and fischer exact tests were used for comparison. The mann-whitney test was used to compare categorical variables such as regrowth score (RGS) and total duration of illness. The Cochrane q test and Friedman's test were used for sequentially comparing follow-up visits within the same group.

p value<0.05 was considered significant. Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) v.26.0.

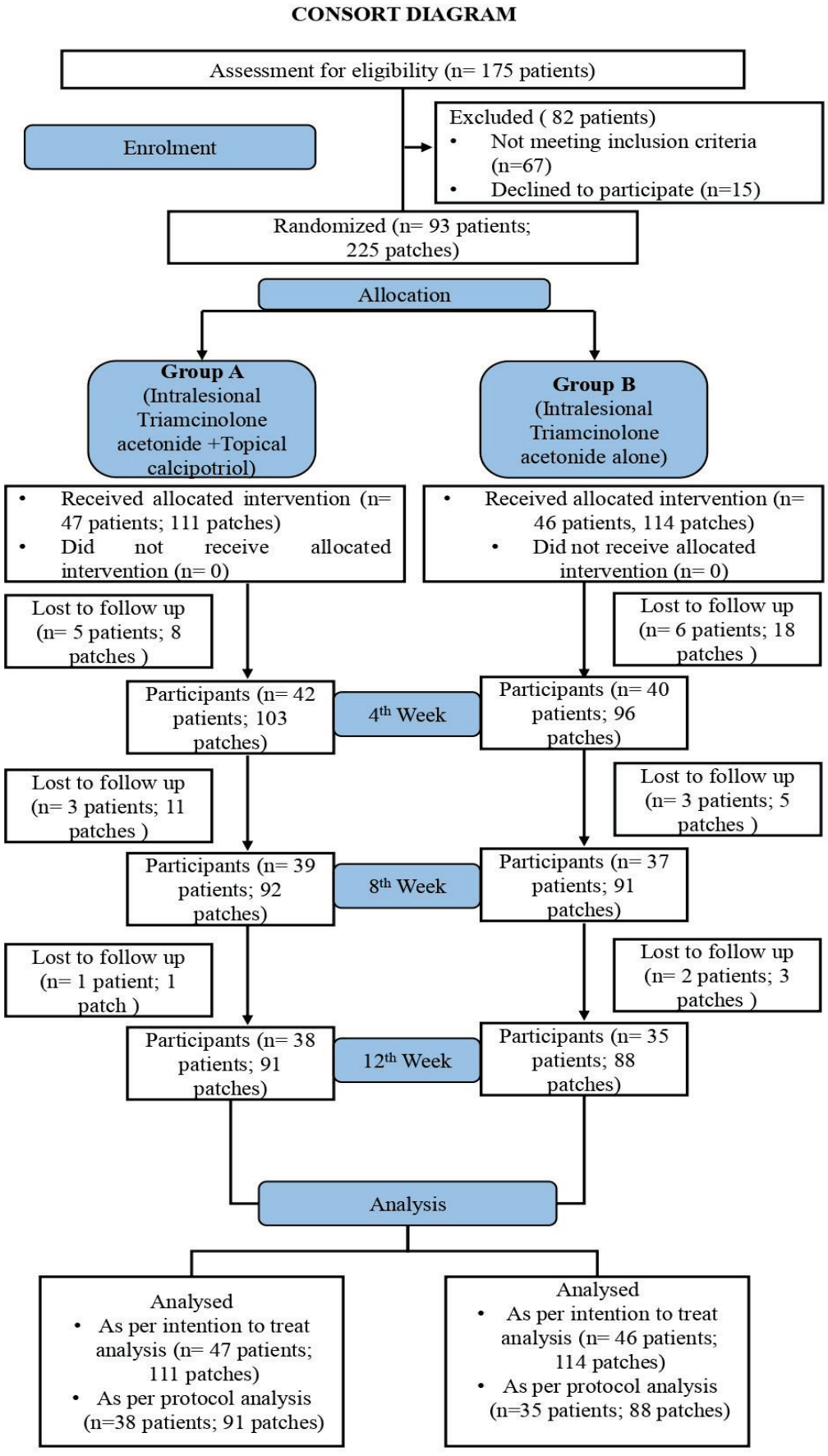


Figure-3: Consort flow diagram

RESULTS

RESULTS

Overall results are presented as intention to treat analysis (ITT), wherever specified, per protocol analysis (PP) has been presented.

DEMOGRAPHIC DATA AND BASIC CLINICAL DETAILS:

We recruited 93 patients and the trial enrolled 225 patches in total. The trial was completed by 73 patients (179 patches), with 38(91 patches) and 35(88 patches) patients in groups A and B, respectively. Participants in Group A received intralesional triamcinolone acetonide (scalp 5.0 mg/ml and beard 2.5 mg/ml) and topical calcipotriol 0.005%, whereas those in group B received intralesional triamcinolone acetonide (scalp 5.0 mg/ml and beard 2.5 mg/ml) alone.

The age of the participants ranged from 14-49 years and the mean age was 26.6+/-7.58 years with a male preponderance (3.43:1). The majority of the patients, 43(46.2%) in total were students. The mean disease duration of the participants was 1.3±0.62 months.

Out of 93 patients, 9 (9.7%) patients had a history of recurrent AA lesions involving the scalp or beard. Co-morbidities such as diabetes mellitus, hypertension, hypothyroidism and tuberculosis were noted in 12 patients. History of atopy was noted in 17 patients (18.3%). Only 5 patients (5.4%) had a family history of AA and 9 (9.7%) had a family history of atopy (Table-11).

Thirty-one patients (33.3%) had previously received treatment for alopecia areata; thirteen (14.0%) had received intralesional steroids, ten (10.8 %) had received topical steroids, six (6.5 %) had received topical minoxidil, and five (5.4 %) had received oral steroids. Five patients (5.8%) went for a multimodal approach.

Anthropometric parameters of the participants (mean height: 167+/- 9.53 cm, mean weight: 64.84+/-10.61 kg and mean BMI: 23.00 +/- 3.32) were normal. General physical examination revealed mild pallor in 4 participants.

Mean serum TSH levels of 71 patients were 1.88 mIU/L (Chemiluminescence immunoassay using DiaSorin/ ADVIA Centaur XP) and abnormally elevated levels were noted in 5(7.1%) participants. Mean Random blood sugar levels of 71 patients were 105+/- 17.40 mg/dl and abnormally elevated levels were noted in 3(4.20 %) participants.

Nail findings were seen in 38(40.9%) of the individuals (Figure-4). Punctate leukonychia was the most prevalent nail feature in 17 individuals (18.3%), followed by fine pitting in 8 patients (8.6%), and longitudinal ridges and trachonychia in 7 patients (7.5%) each. There was no significant difference between the groups in terms of nail changes (Chi square test p-value >0.05). A few patients had multiple nail abnormalities, such as four individuals with nail pitting and leukonychia, two patients with leukonychia and trachonychia, and one patient with both leukonychia and trachonychia.

All groups were comparable (p>0.05) in terms of demographic parameters and basic clinical details as given in Table-11.

Table-11: Demographic data and clinical characteristics as per ITT analysis

Sl No	Demographic and clinical characteristics	Total	Group A	Group B	P-value
1	**Total number of participants - n (%)**	93 (100)	47 (50.5)	46 (49.5)	
2	**Total number of alopecia patches- n (%)**	225 (100)	111 (49.3)	114 (50.7)	
3	**Total number of scalp patches- n (%)**	136 (60.5)	66 (48.5)	70 (51.5)	
4	**Total number of beard patches- n (%)**	89 (39.5)	45 (50.5)	44 (49.5)	
5	**Age (in year), Mean±SD**	26.6±7.58	26.8±7.36	26.4±7.87	0.781¶
6	**Total duration of illness in months, Mean±SD**	1.30±0.62	1.30±0.58	1.30±0.66	0.768 †
	< 6 months	73 (78.5)	36 (76.6)	37 (80.4)	
	6-12 months	12 (12.9)	8 (17.0)	4 (8.7)	
	1-2 years	8 (8.6)	3 (6.4)	5 (10.9)	
7	**Male/Female n(ratio)**	72/21 (3.43:1)	35/12 (2.92:1)	37/9 (4.11:1)	0.473 *
	Co- morbidities				
	a. Diabetes mellitus	3 (3.2)	1 (2.1)	2 (4.3)	0.545 *
	b. Hypertension	3 (3.2)	2 (4.3)	1 (2.2)	0.570 *
	c. Hypothyroidism	5 (5.4)	3 (6.4)	2 (4.3)	0.664 *
	d. Tuberculosis	1 (1.1)	0 (0.0)	1 (2.2)	0.309 *
	Personal history of Atopy	17 (18.3)	9 (19.1)	8 (17.4)	0.826 *
	Family history				
	a. Alopecia areata	5 (5.4)	3 (6.4)	2 (4.3)	0.664*
	b. Atopy	9 (9.7)	5 (10.6)	4 (8.7)	0.751*
P value = * - Chi square test, † -Mann-Whitney test, - ¶ T- Independent samples t-test					

Table-12: Comparison of baseline nail changes

Sl No	Nail changes	Total n (%)	Group A n (%)	Group B n (%)
1	**Nail pitting**	8(8.6)	4 (8.85)	4 (8.85)
2	**Longitudinal ridges**	7 (7.5)	3 (6.4)	4 (8.7)
3	**Trachonychia**	7 (7.5)	4 (8.5)	3 (6.5)
4	**Leukonychia**	17 (18.3)	9 (19.1)	8 (17.4)
5	**Nail splitting**	1 (1.1)	1 (2.1)	0 (0.0)
6	**Onycholysis**	3 (3.2)	2 (4.3)	1 (2.2)
7	**Onychomadesis**	0 (0)	0 (0)	0 (0)
8	**Red lunula**	2 (2.2)	1 (2.1)	1 (2.2)

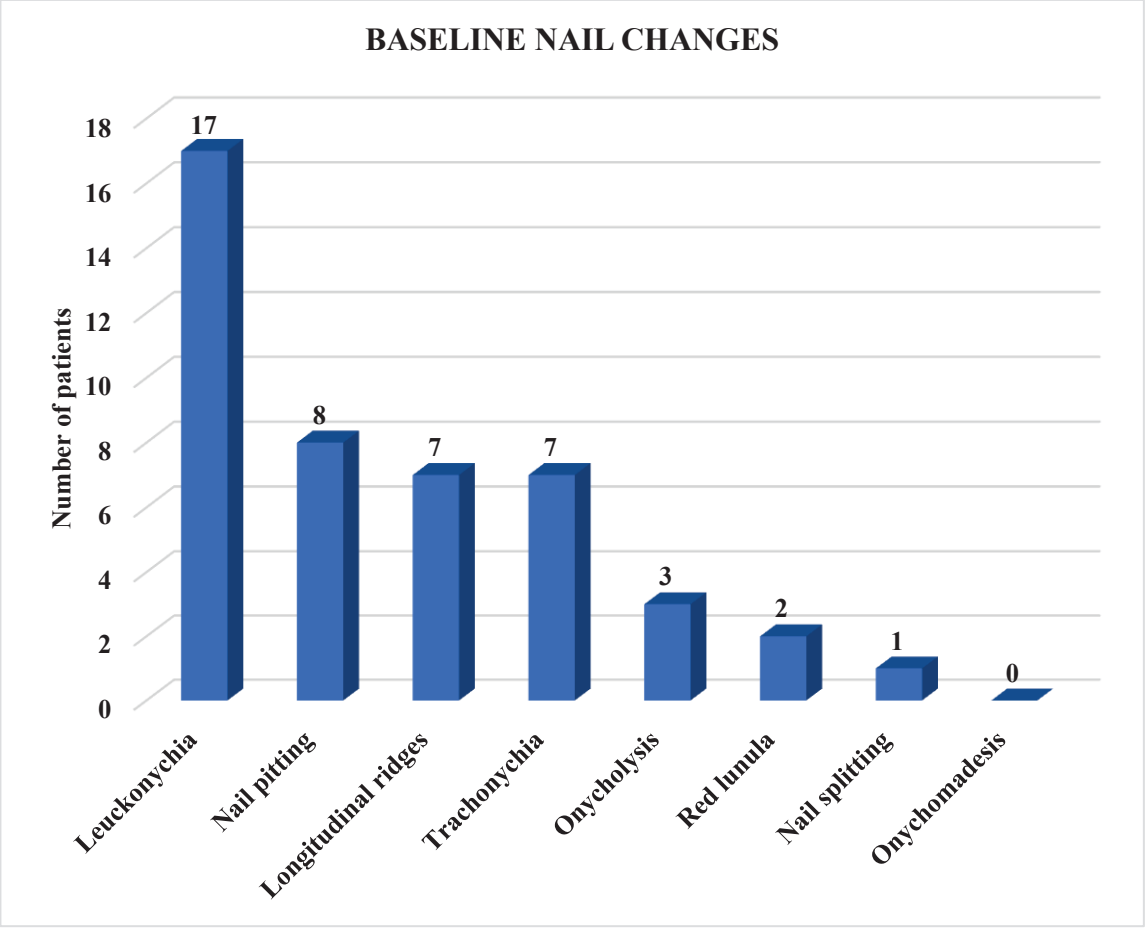


Figure-4: Overall baseline nail changes

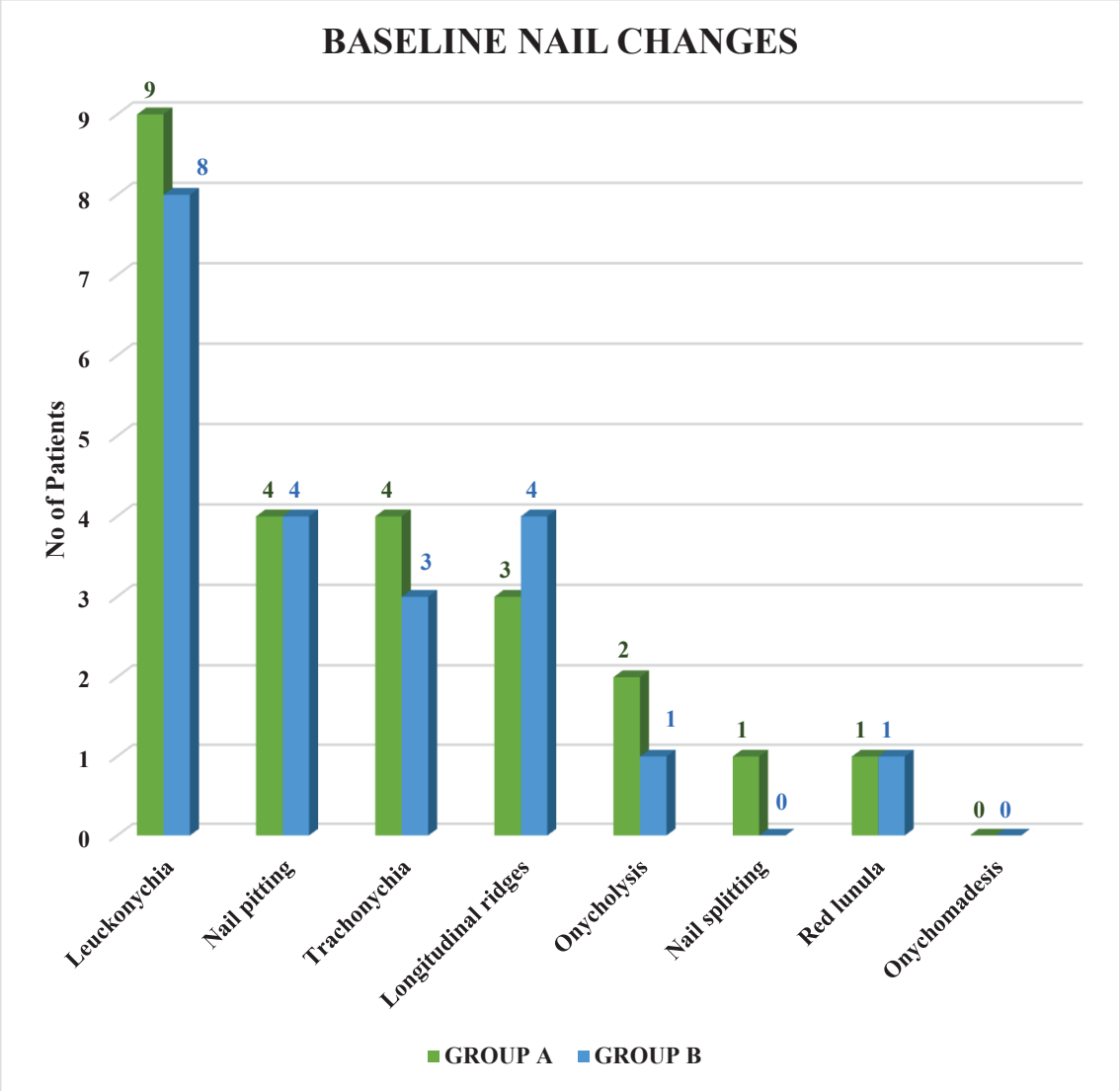


Figure-5: Comparison of baseline nail changes in group A and group B

BASELINE CHARACTERISTICS OF SELECTED PATCHES

Out of 225 alopecia areata patches, 179 alopecia areata patches completed the study. The number of patches selected from each participant ranged from 1 to 13 with a mean of 2.41 +/- 1.21. Patches were seen in a variety of anatomical regions on the scalp, including the parietal region (36.8%), the occipital region (28.2%), the right temporal region (10.3%), the left temporal region (9.5%), the fronto-parietal region (9.3%), and the frontal region (5.9%).

Table-13: Characteristics of alopecia areata patches

Sl No	**Patch characteristics**	**Total**	**Group A**		**Group B**		**P-value**
			Scalp	**Beard**	**Scalp**	**Beard**	
1	**Total number of patches recruited**	225	66	45	70	44	
2	**Total number of patches completed the study**	179	54	37	58	30	
3	**Baseline SALT score Mean±SD**	4.71± 3.93	4.76±4.04	-	4.65±3.88	-	0.914¶
P value = ¶ Independent samples t-test							

BASELINE DERMOSCOPIC FEATURES:

Dermoscopy of the patches showed various features of alopecia areata. The most common finding noted were black dots in 118(52.4%) patches, broken hair in 81(36%) patches, yellow dots in 70(31%) patches and exclamation mark hair in 61(27.1%) patches. Pohl-Pinkus constriction (0.4%) was the least commonly noted finding in our study. All the baseline dermoscopic parameters were comparable across treatment groups ($p > 0.05$).

Table-14: Comparison of baseline dermoscopic features as per ITT analysis

Sl No	Dermoscopic features	Total n(%)	Group A n(%)	Group B n(%)	P-value
1	**Yellow dots**	70 (31.1)	36 (32.4)	34 (29.8)	0.673*
2	**Black dots**	118 (52.4)	60 (54.1)	58 (50.9)	0.633*
3	**Exclamation mark hair**	61 (27.1)	35 (31.5)	26 (22.8)	0.141*
4	**Tapered hair**	27 (12.0)	14 (12.6)	13(11.4)	0.780*
5	**Broken hair**	81 (36.0)	41 (36.9)	40 (35.1)	0.773*
6	**Short vellus hair**	18 (8.0)	10 (9.0)	8 (7.0)	0.582*
7	**Upright regrowing hair**	4 (1.8)	2 (1.8)	2 (1.8)	1.00 ٠
8	**Pigtail hair**	4 (1.8)	2 (1.8)	2 (1.8)	1.00 ٠
9	**Pohl pinkus constriction**	1 (0.4)	0 (0.0)	1 (0.9)	1.00 ٠
P value = * - Chi square test, ٠ - Fischer exact test					

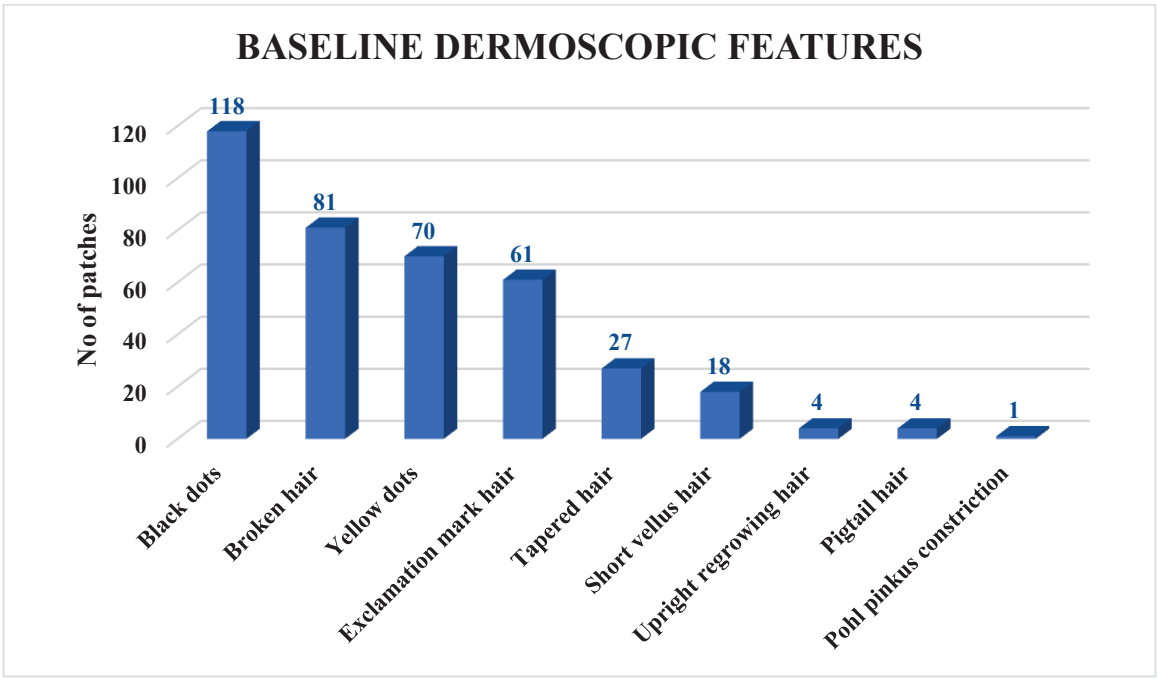


Figure-6: Dermoscopic features of AA patches at baseline

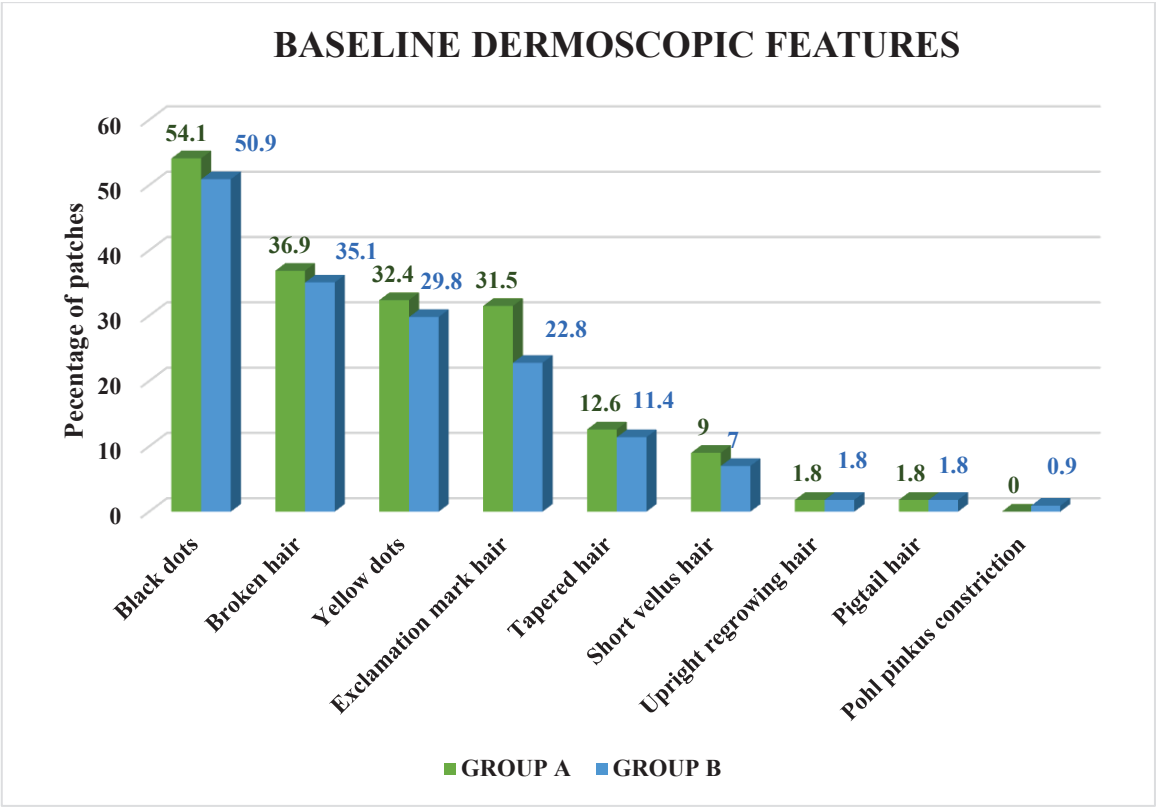


Figure-7: Dermoscopic features of AA patches at baseline in group A and group B

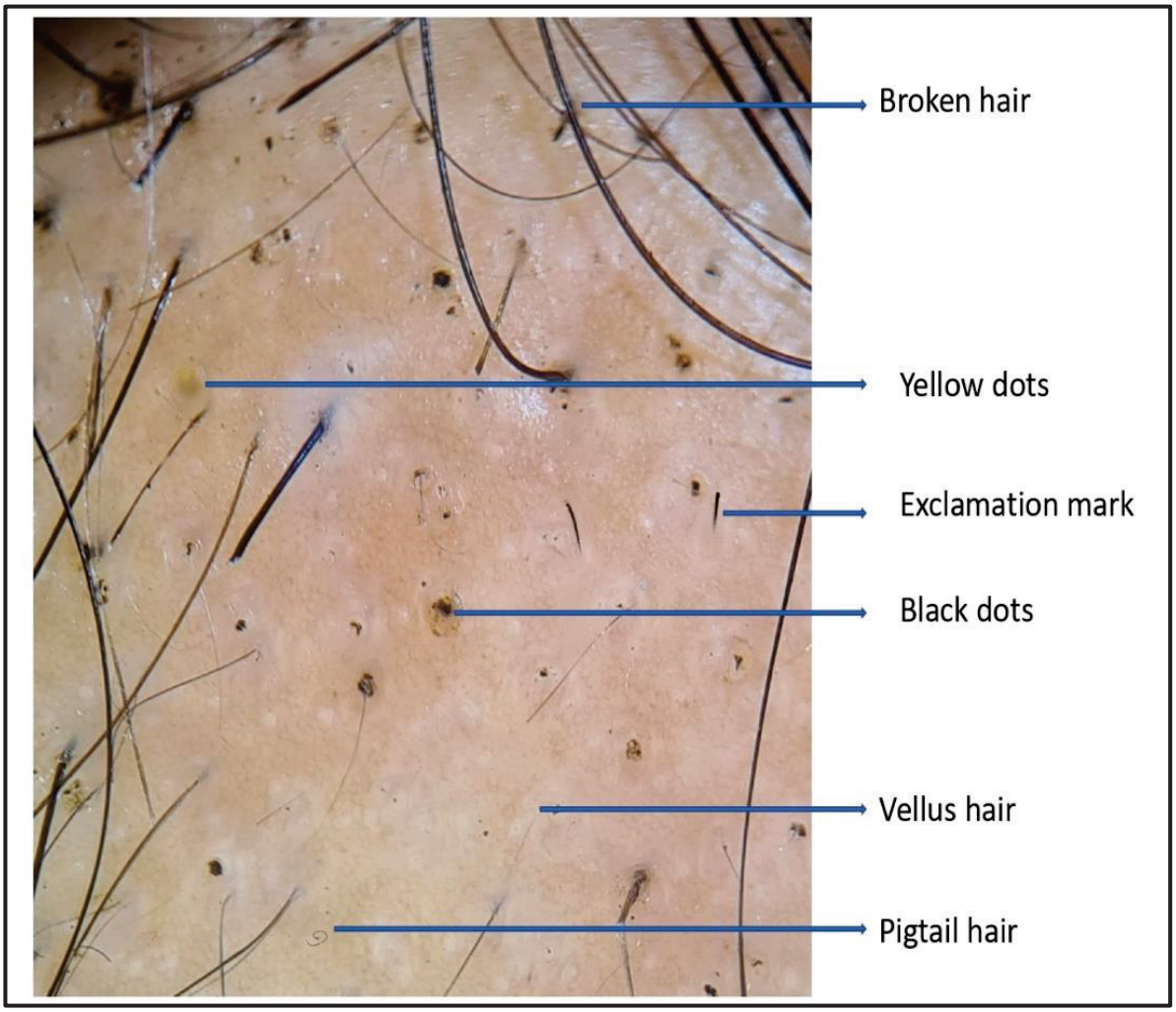


Figure-8: Dermoscopic features of an alopecia areata patch (Heine Delta 30- 10X Dermatoscope, Polarized mode)

TYPE OF PREDOMINANT HAIR:

At baseline, both the groups showed mixed hair type (vellus and terminal) as predominant hair type. On further visits, the terminal hairs showed significantly increase in both the groups (Cochran's Q test; p<0.001, <0.001) with maximum at 12th week (Group A- 82.4% and Group B- 77.3%) (Figure-9). The intergroup analysis didn't show any significant difference in terminal hair in any of the follow-up visits (Chi square test; p=0.514, 0.469, 0.391). (Table-15)

In scalp patches, at baseline, both the groups showed mixed hair type (Vellus and terminal) as predominant hair type. On further visits, the terminal hairs showed significantly increase in both the groups (Cochran's Q test; p<0.001, <0.001) with

maximum at 12th week (Group A- 79.6% and Group B- 77.6%) (Figure-10). The intergroup analysis didn't show any significant difference in any of the follow-up visits (Chi square test; p=0.204, 0.994, 0.792).

In beard patches, at baseline, both the groups showed mixed hair type (Vellus and terminal) as predominant hair type. On further visits, the terminal hairs showed significantly increase in both the groups (Cochran's Q test; p<0.001, <0.001) with maximum at 12th week (Group A- 86.5% and Group B- 76.7%) (Figure-11). The intergroup analysis didn't show any significant difference in any of the follow-up visits (Chi square test; p=0.682, 0.178, 0.297).

Table-15: Type of predominant hair as per ITT analysis

Predominant hair type		Treatment groups				p* - Value
		GROUP A		GROUP B		
		n	%	n	%	
Baseline	Vellus	27	24.4	27	23.7	0.963
	Mixed	50	45	55	48.2	
	Terminal	26	23.5	27	23.7	
	Nil	8	7.1	5	4.4	
	Total	111	100	114	100	
4th week	Vellus	16	15.5	16	16.7	0.514
	Mixed	46	44.7	45	46.8	
	Terminal	40	38.8	33	34.4	
	Nil	1	1.0	2	2.1	
	Total	103	100	96	100	
8th week	Vellus	3	3.3	5	5.5	0.469
	Mixed	18	19.6	19	20.9	
	Terminal	71	77.2	66	72.5	
	Nil	0	0.0	1	1.1	
	Total	92	100	91	100	
12th week	Vellus	1	1.1	2	2.3	0.391
	Mixed	15	16.5	18	20.5	
	Terminal	75	82.4	68	77.3	
	Nil	0	0.0	0	0.0	
	Total	91	100	88	100	
p ♯		<0.001		<0.001		

P value = * - Chi square test, ♯ - Cochran's Q test

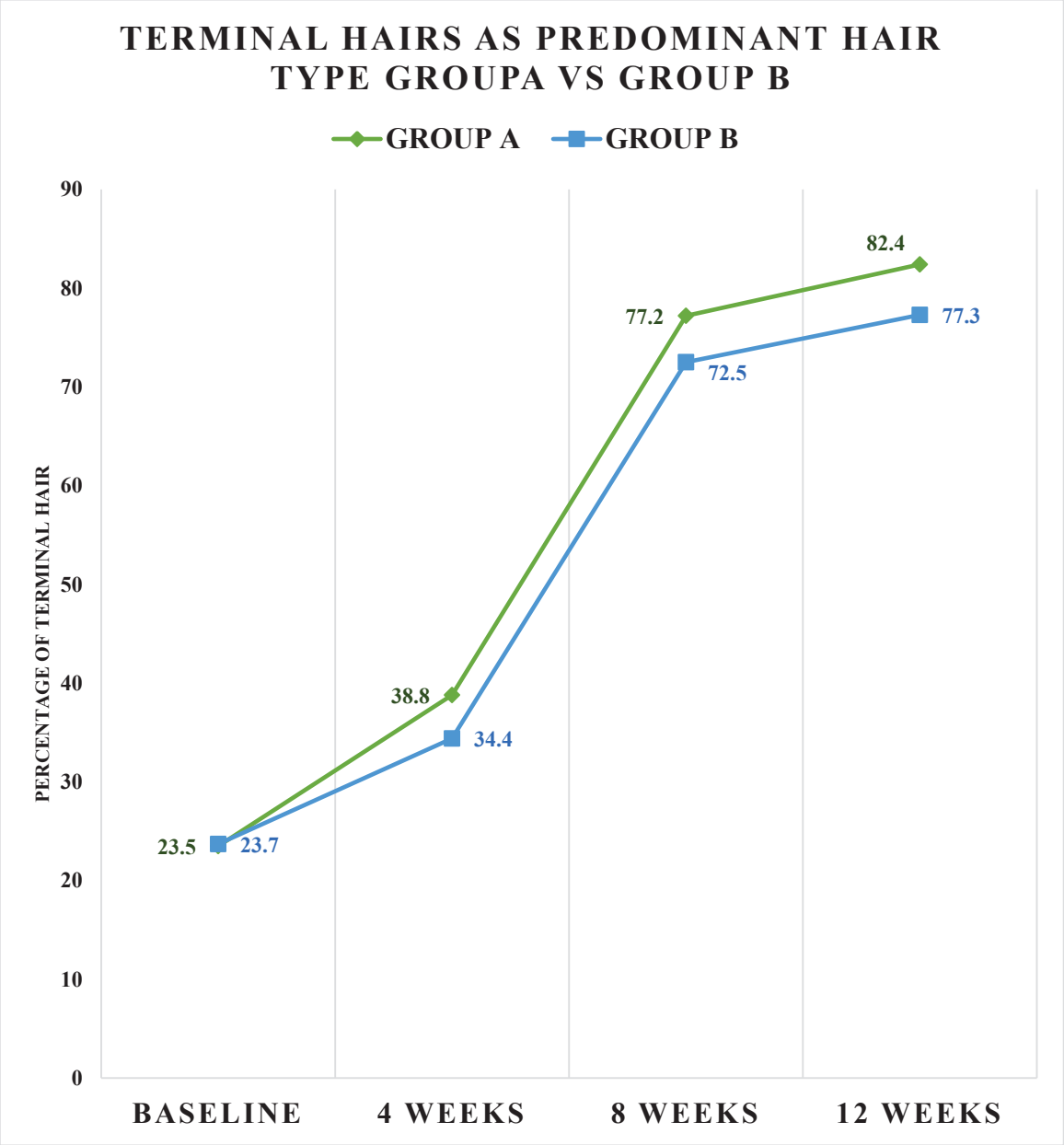


Figure-9: Terminal hairs as predominant hair type at each follow-up visits across different treatment groups

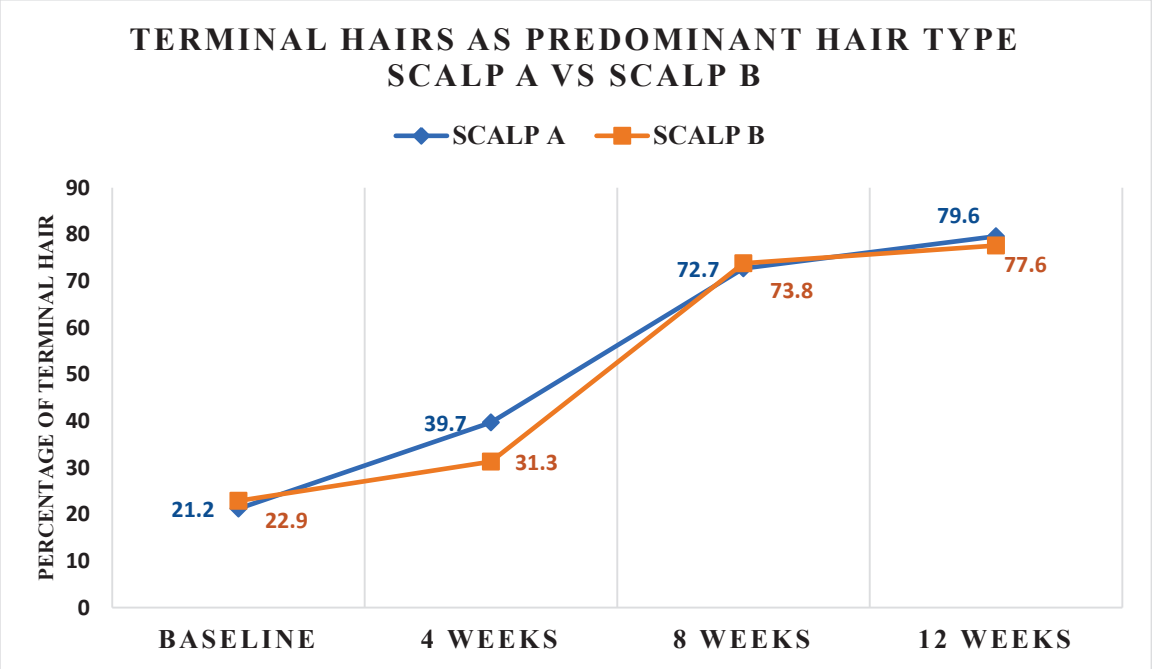


Figure-10: Terminal hairs as predominant hair type at each follow-up visits across different treatment group of scalp patches

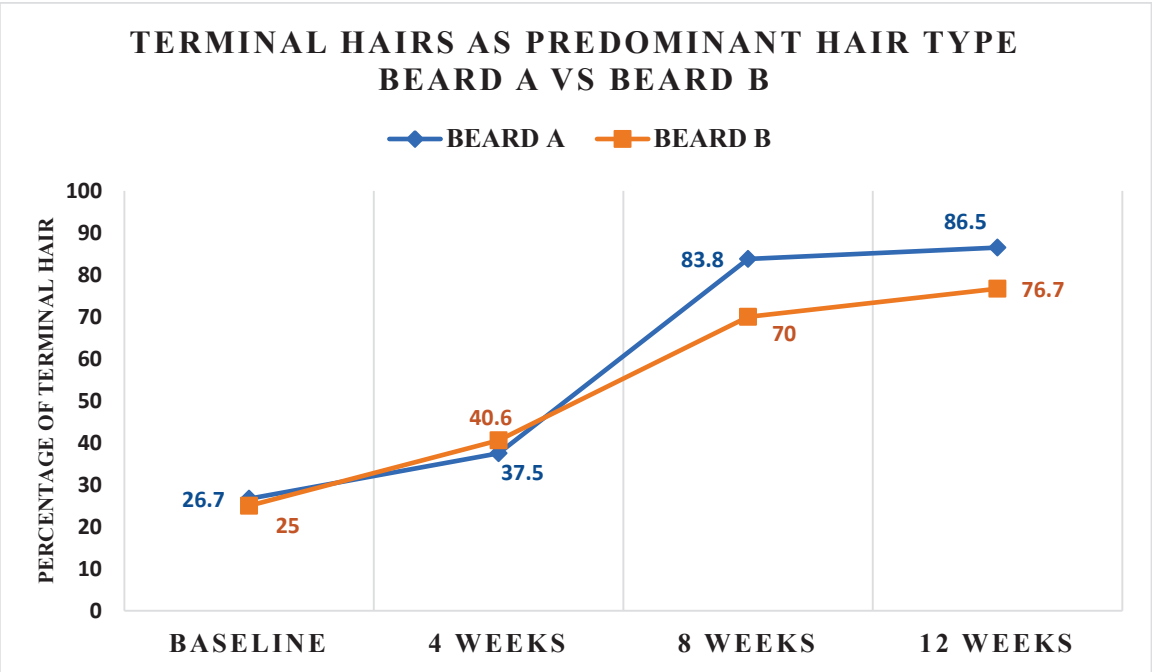


Figure-11: Terminal hairs as predominant hair type at each follow-up visits across different treatment group of beard patches

PATTERNS OF HAIR REGROWTH

Both of these groups exhibited a wide variety of patterns of hair regrowth, as classified by the DIMT classification system, on their scalp and in the beard patches. Regardless of the treatment group, the 'diffuse pattern' was the most prevalent (Group A- 39.6% and Group B- 38.6%), followed by the 'marginal pattern' (Group A- 24.3% and Group B- 25.0%), while the 'irregular pattern' was the pattern that was noticed the least. Regarding the pattern of regrowth, none of the two-treatment groups showed a statistically significant difference from one another (Chi square test p-value 0.899, 0.611, 0.833, 0.660).

In group A, it was found that the irregular and targetoid patterns on the scalp and beard patches were significantly different (Chi square test p-value 0.004, 0.008) (Table-19), whereas in group B, it was found that the only targetoid pattern on the scalp and beard patches was significantly different (Chi square test p-value 0.006) (Table-20).

Comparison of the scalp and beard patches indicated significant difference in irregular and targetoid pattern. (Chi square test p-value 0.019, 0.0002) (Table-21).

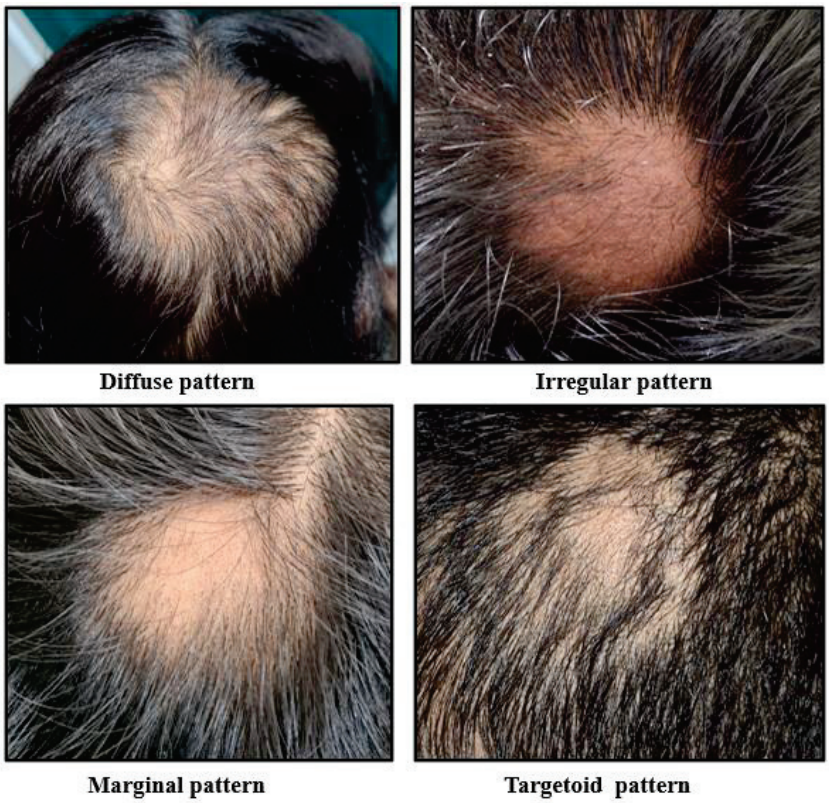


Figure-12: Alopecia areata patches showing different patterns of hair regrowth (DIMT classification)

Table-16: Comparison of hair regrowth patterns in two groups as per PP analysis

TYPE	GROUP A n (%)	GROUP B n (%)	P*- Value
Diffuse	36 (39.6)	34 (38.6)	0.899
Irregular	13 (14.3)	15 (17.0)	0.611
Marginal	22 (24.2)	22 (25.0)	0.833
Targetoid	20 (22.0)	17 (19.3)	0.660
Total	91 (100)	88 (100)	
P value = * - Chi square test			

Table-17: Comparison of hair regrowth patterns in scalp patches as per PP analysis

TYPE	SCALP A n (%)	SCALP B n (%)	P*- Value
Diffuse	18 (33.3)	22 (37.9)	0.612
Irregular	3 (5.6)	9 (15.5)	0.089
Marginal	16 (29.6)	11 (19.0)	0.126
Targetoid	17 (31.5)	16 (27.6)	0.651
Total	54 (100)	58 (100)	
P value = * - Chi square test			

Table-18: Comparison of hair regrowth patterns in beard patches as per PP analysis

TYPE	BEARD A n (%)	BEARD B n (%)	P*- Value
Diffuse	18 (48.6)	12 (40.0)	0.479
Irregular	10 (27.0)	6 (20.0)	0.502
Marginal	6 (16.2)	11 (36.7)	0.103
Targetoid	3 (8.1)	1 (3.3)	0.622
Total	37 (100)	30 (100)	
P value = * - Chi square test			

Table-19: Comparison of hair regrowth patterns in scalp and beard of group A as per PP analysis

TYPE	SCALP A n (%)	BEARD A n (%)	P*- Value
Diffuse	18 (33.3)	18 (48.6)	0.142
Irregular	3 (5.6)	10 (27.0)	0.004
Marginal	16 (29.6)	6 (16.2)	0.182
Targetoid	17 (31.5)	3 (8.1)	0.008
Total	91 (100)	37 (100)	
P value = * - Chi square test			

Table-20: Comparison of hair regrowth patterns in scalp and beard of group B as per PP analysis

TYPE	SCALP B n (%)	BEARD B n (%)	P*- Value
Diffuse	**22 (37.9)**	**12 (40.0)**	**0.850**
Irregular	**9 (15.5)**	**6 (20.0)**	**0.596**
Marginal	**11 (19.0)**	**11 (36.7)**	**0.069**
Targetoid	**16 (27.6)**	**1 (3.3)**	**0.006**
Total	**88 (100)**	**30 (100)**	
P value = * - Chi square test			

Table-21: Comparison of hair regrowth patterns in scalp and beard as per PP analysis

TYPE	SCALP n (%)	BEARD n (%)	P*- Value
Diffuse	**40 (35.7)**	**30 (44.8)**	**0.229**
Irregular	**12 (10.7)**	**16 (23.9)**	**0.019**
Marginal	**28 (24.1)**	**17 (25.4)**	**0.782**
Targetoid	**33 (29.5)**	**4 (5.9)**	**< 0.001**
Total	**112 (100)**	**67 (100)**	
P value = * - Chi square test			

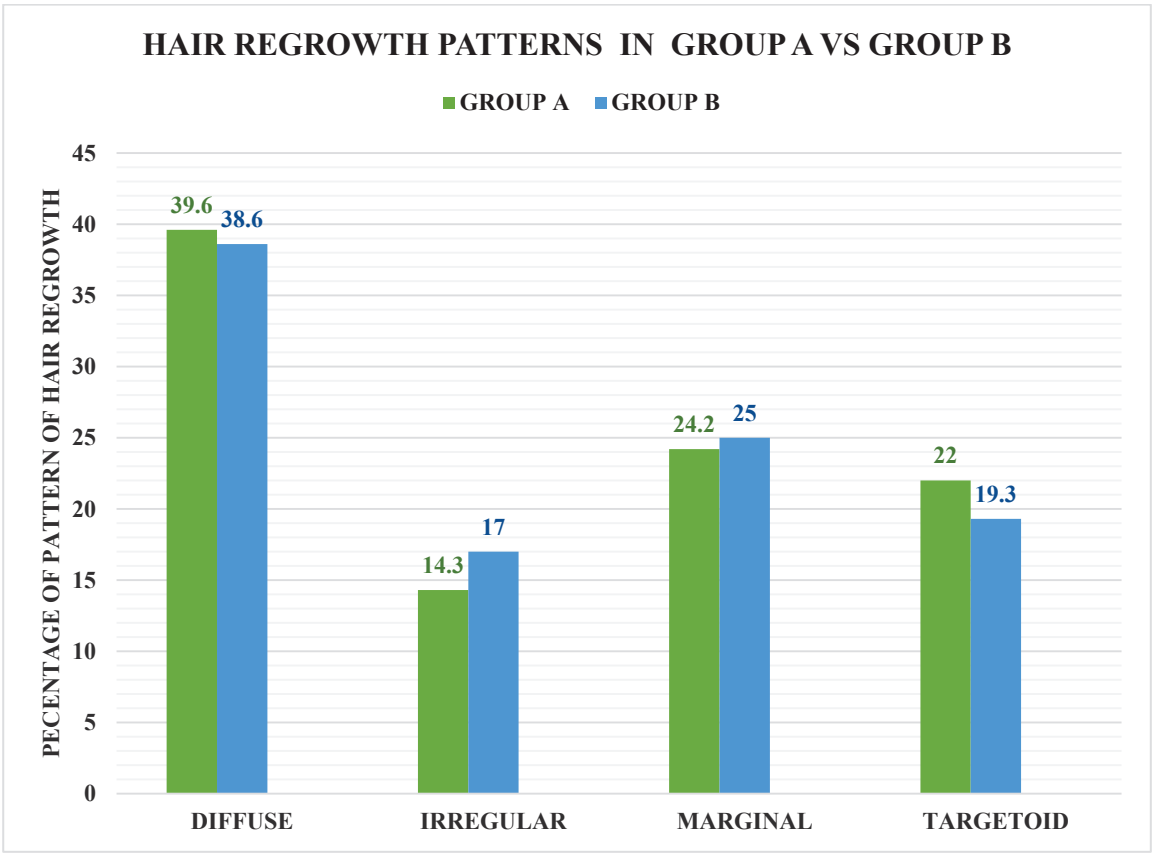


Figure-13: Pattern of hair regrowth in two treatment groups

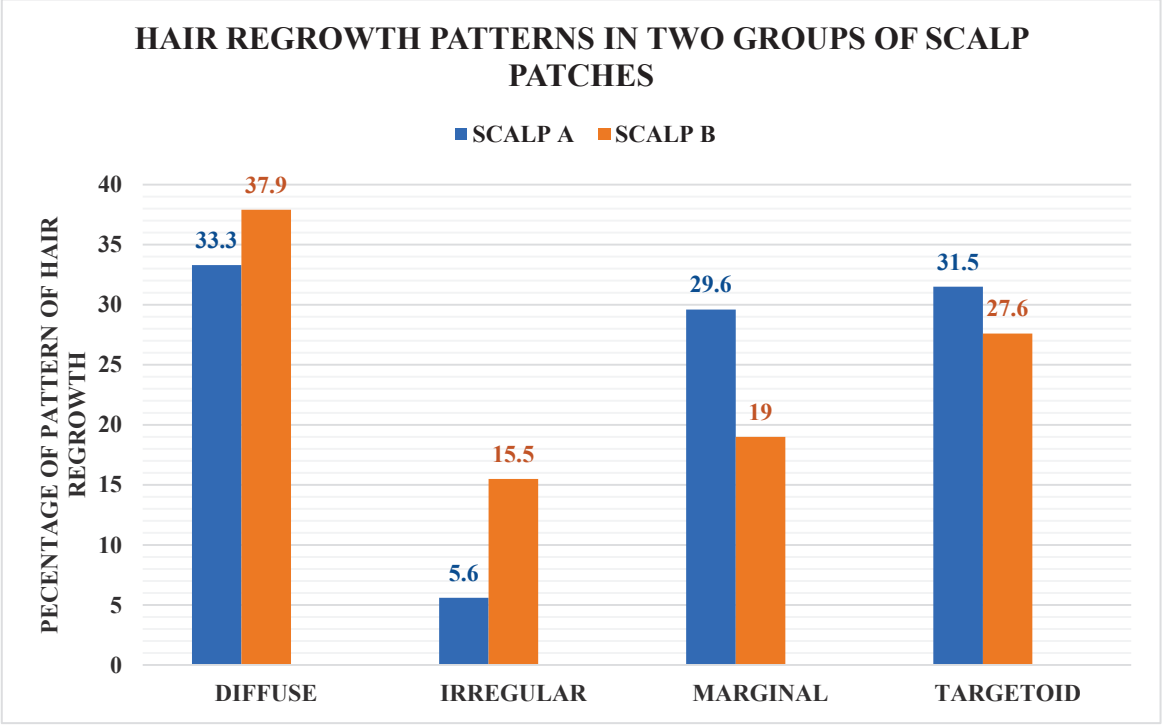


Figure-14: Pattern of hair regrowth in two groups of scalp patches

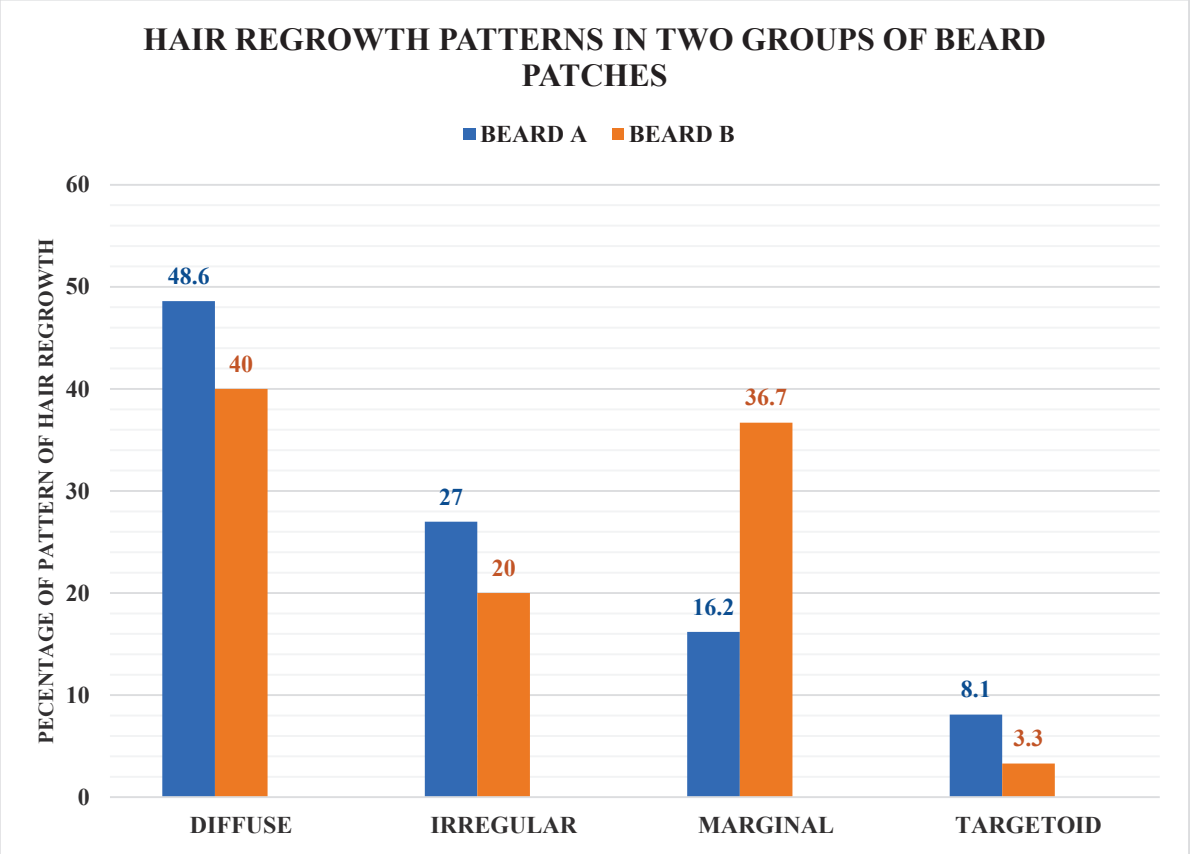


Figure-15: Pattern of hair regrowth in two groups of beard patches

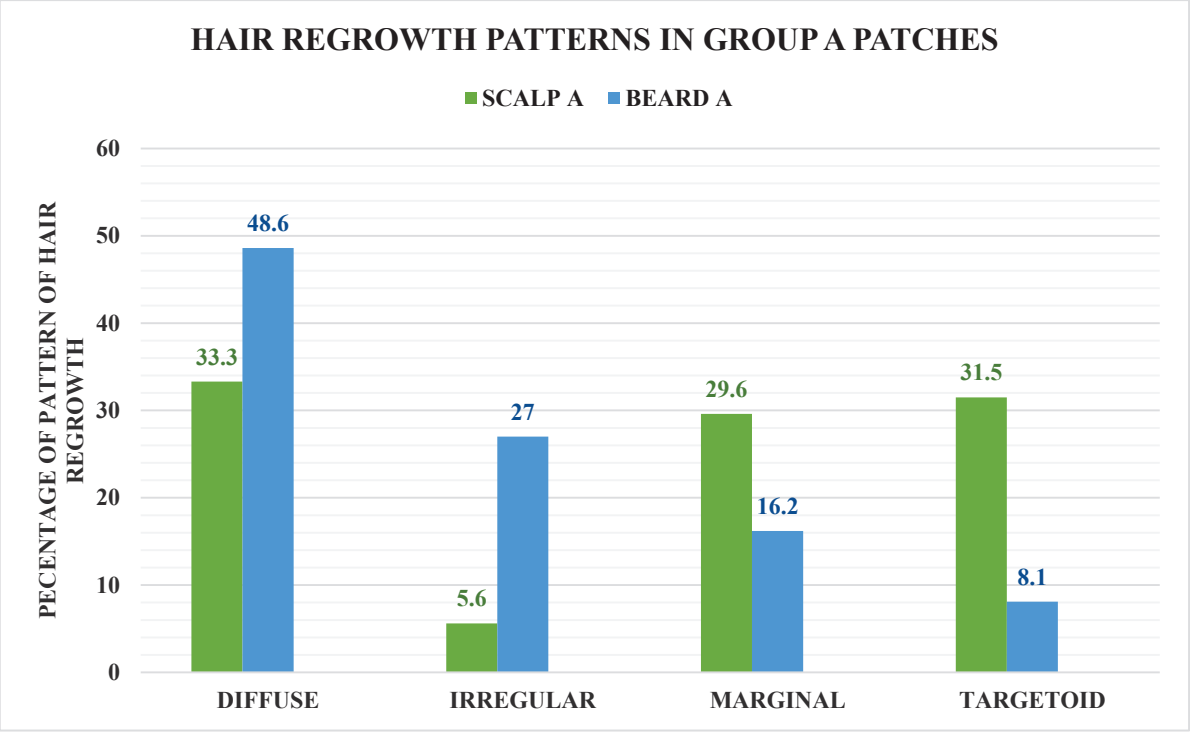


Figure-16: Pattern of hair regrowth in scalp and beard patches of group A

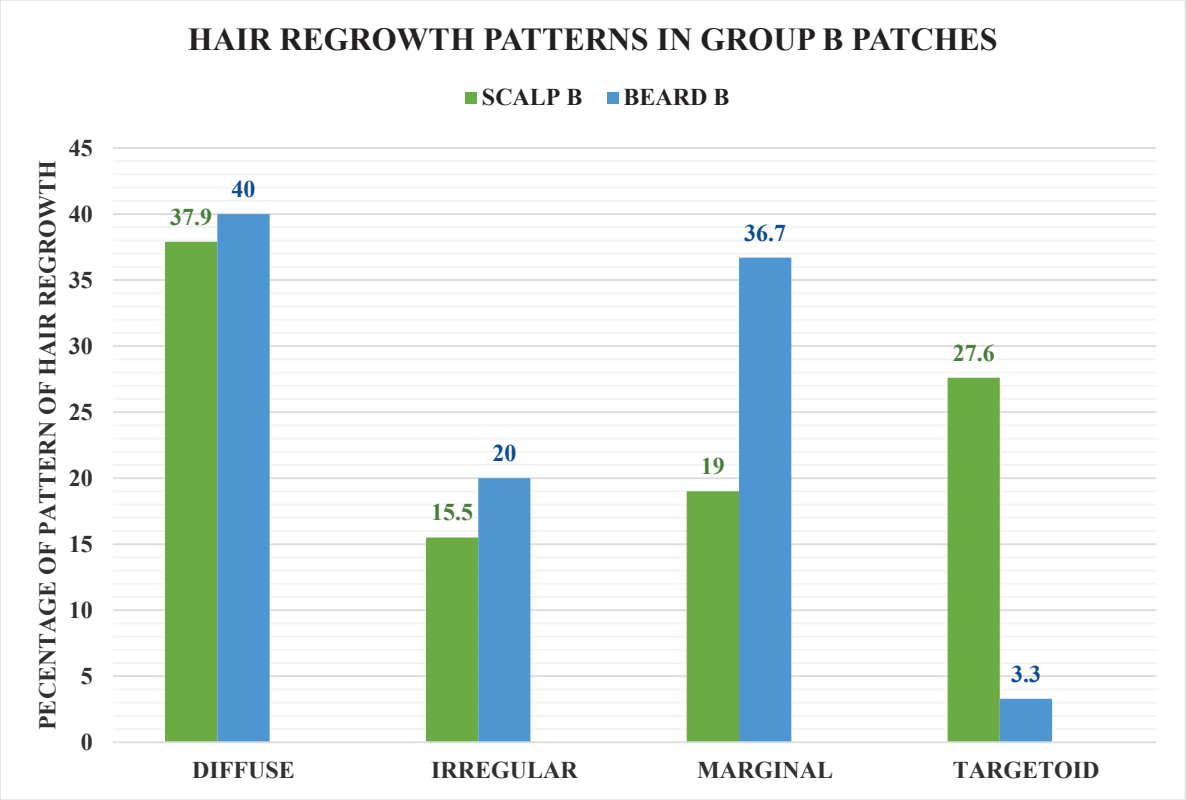


Figure-17: Pattern of hair regrowth in scalp and beard patches of group B

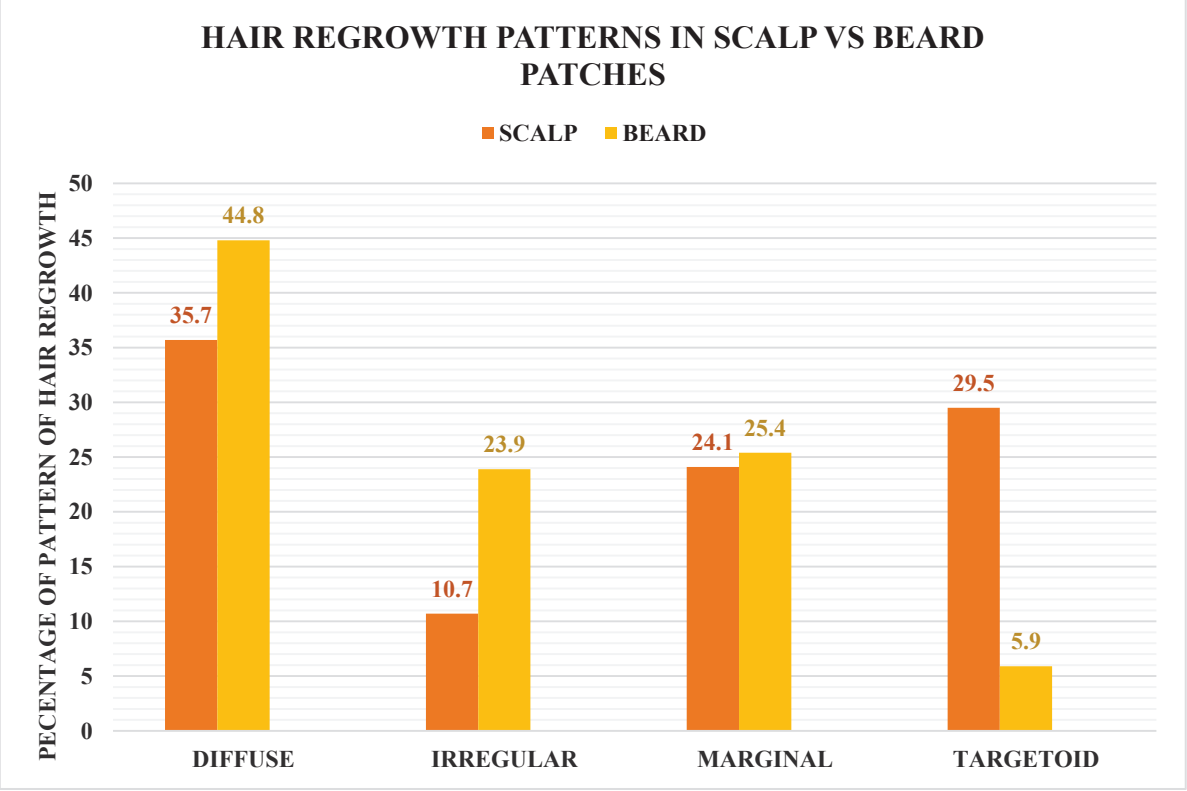


Figure-18: Pattern of hair regrowth in the scalp and beard patches

DENSITY OF TERMINAL HAIRS:

The density of terminal hairs was calculated using the Hair Regrowth score (RGS). Baseline parameters between the groups were comparable (Mann-Whitney test; p=0.728) (Table-22)

The density of terminal hairs showed a significantly increasing trend throughout the follow-up visits in all treatment groups (Friedman test; p<0.001, <0.001) (Table-22). The inter-group analysis did not reveal any statistically significant differences in the density of terminal hairs between the groups at any point during the follow-up period (Mann-Whitney test; 0.930, 0.616, 0.178) (Table-22).

The subgroup analysis did reveal statistically significant differences in the scalp patches of the two groups during the 12^{th} week of the follow-up period (Mann-Whitney test; 0.018) (Table-23). However, the regrowth score of the beard patches did not reveal any significant differences at any time of follow-up visits (Mann-Whitney test; 0.546, 0.705, 0.517, 0.527) (Table-24).

In both groups, no patch exhibited more than 50% hair regrowth at the baseline. It revealed an increasing tendency in both the groups from the first to the final follow-up of the study (Cochran's Q test; p<0.001, <0.001) (Table-25). There was no statistically significant difference between the groups at any point of study follow-up (Fischer exact test; 4^{th} week- 0.052. Chi square test; 8^{th} week-0.575 and 12^{th} week- 0.088) (Table-25).

The subgroup analysis revealed a statistically significant difference in scalp patches (Chi square test p= 0.002) towards the end of the trial (Table-26), while beard patches did not exhibit any significant difference (Fischer exact test at 4^{th} week- p=0.581, Chi square test 8^{th} week and 12^{th} week; p= 0.602 and 0.615 respectively) at any point of study (Table-27).

Table-22: Density of terminal hairs as per ITT analysis

Density of terminal hairs (RGS)		GROUPS				P† - Value
		GROUP A		GROUP B		
		n	%	n	%	
Baseline	0 (0-10%)	108	97.3	110	96.5	**0.728**
	1 (11-25%)	3	2.7	4	3.5	
	2 (26-50%)	0	0	0	0	
	3 (51-75%)	0	0	0	0	
	4 (>75%)	0	0	0	0	
	Total	111	100	114	100	
4th week	0 (0-10%)	40	38.8	42	43.8	**0.930**
	1 (11-25%)	42	40.8	29	30.2	
	2 (26-50%)	19	18.4	17	17.7	
	3 (51-75%)	1	1.0	7	7.3	
	4 (>75%)	1	1.0	1	1.0	
	Total	103	100	96	100	
8th week	0 (0-10%)	12	13.0	18	19.8	**0.616**
	1 (11-25%)	27	29.3	21	23.1	
	2 (26-50%)	18	19.6	21	23.1	
	3 (51-75%)	19	20.7	14	15.4	
	4 (>75%)	16	17.4	17	18.7	
	Total	92	100	91	100	
12th week	0 (0-10%)	2	2.2	4	4.5	**0.178**
	1 (11-25%)	13	14.3	14	15.9	
	2 (26-50%)	16	17.6	23	26.1	
	3 (51-75%)	22	24.2	16	18.2	
	4 (>75%)	38	41.8	31	35.2	
	Total	91	100	88	100	
P≠ - VALUE		< 0.001		< 0.001		
P- VALUE-≠ Friedman test, † -Mann-Whitney test						

Table-23: Density of terminal hairs in scalp patches as per ITT analysis

Density of terminal hairs (RGS)		GROUPS				P† - Value
		SCALP A		SCALP B		
		n	%	n	%	
Baseline	0 (0-10%)	64	97.0	68	97.1	**0.953**
	1 (11-25%)	2	3.0	2	2.9	
	2 (26-50%)	0	0.0	0	0.0	
	3 (51-75%)	0	0.0	0	0.0	
	4 (>75%)	0	0.0	0	0.0	
	Total	66	100	70	100	
4th week	0 (0-10%)	20	31.7	26	40.6	**0.773**
	1 (11-25%)	27	42.9	20	31.3	
	2 (26-50%)	15	23.8	12	18.8	
	3 (51-75%)	1	1.6	5	7.8	
	4 (>75%)	0	0.0	1	1.6	
	Total	63	100	64	100	
8th week	0 (0-10%)	2	3.6	12	19.7	**0.210**
	1 (11-25%)	13	23.6	10	16.4	
	2 (26-50%)	14	25.5	17	27.9	
	3 (51-75%)	15	27.3	8	13.1	
	4 (>75%)	11	20.0	14	23.0	
	Total	55	100	61	100	
12th week	0 (0-10%)	0	0.0	2	3.4	**0.018**
	1 (11-25%)	3	5.6	10	17.2	
	2 (26-50%)	6	11.1	13	22.4	
	3 (51-75%)	18	33.3	11	19.0	
	4 (>75%)	27	50.0	22	37.9	
	Total	54	100	58	100	
P≠ - VALUE		< 0.001		< 0.001		
P- VALUE-≠ Friedman test, † -Mann-Whitney test						

Table-24: Density of terminal hairs in beard patches as per ITT analysis

Density of terminal hairs (RGS)		GROUPS				P† - Value
		Beard A		Beard B		
		n	%	n	%	
Baseline	0 (0-10%)	44	97.8	42	95.5	**0.546**
	1 (11-25%)	1	2.2	2	4.5	
	2 (26-50%)	0	0.0	0	0.0	
	3 (51-75%)	0	0.0	0	0.0	
	4 (>75%)	0	0.0	0	0.0	
	Total	45	100	44	100	
4th week	0 (0-10%)	20	50	16	50.0	**0.705**
	1 (11-25%)	15	37.5	9	28.1	
	2 (26-50%)	4	10	5	15.6	
	3 (51-75%)	1	2.5	2	6.3	
	4 (>75%)	0	0.0	0	0	
	Total	40	100	32	100	
8th week	0 (0-10%)	10	27.0	6	20.0	**0.517**
	1 (11-25%)	14	47.8	11	36.7	
	2 (26-50%)	4	10.8	4	13.3	
	3 (51-75%)	4	10.8	6	20.0	
	4 (>75%)	5	13.5	3	10.0	
	Total	37	100	30	100	
12th week	0 (0-10%)	2	5.4	2	6.7	**0.527**
	1 (11-25%)	10	27.0	4	13.3	
	2 (26-50%)	10	27.0	10	33.3	
	3 (51-75%)	4	10.8	5	16.7	
	4 (>75%)	11	29.7	9	30.0	
	Total	37	100	30	100	
P≠ - VALUE		**< 0.001**		**< 0.001**		
P- VALUE-≠ Friedman test, † -Mann-Whitney test						

Table-25: More than 50% terminal hair density at each follow-up visit across different treatment groups as per ITT analysis

Density of terminal hairs (RGS)		GROUPS				P - Value
		Group A		Group B		
		n	%	n	%	
Baseline	50% or below	111	100	114	100	-
	Above 50%	0	0	0	0	
	Total	111	100	114	100	
4th week	50% or below	101	98.0	88	91.6	0.052ᵠ -
	Above 50%	2	2.0	8	8.4	
	Total	103	100	96	100	
8th week	50% or below	57	62.0	60	66.0	0.575*
	Above 50%	35	38.0	31	34.0	
	Total	92	100	91	100	
12th week	50% or below	31	34.0	41	46.6	0.088*
	Above 50%	60	66.0	47	53.4	
	Total	91	100	88	100	
P♯ - VALUE		0.001		0.001		

P- VALUE- ♯ - Cochran's Q test, * - Chi square test, ᵠ - Fischer exact test

Table-26: More than 50% terminal hair density at each follow-up visit across different treatment group of scalp patches as per ITT analysis

Density of terminal hairs (RGS)		GROUPS				P - Value
		SCALP A		SCALP B		
		n	%	n	%	
Baseline	50% or below	66	100	70	100	-
	Above 50%	0	0	0	0	
	Total	66	100	70	100	
4th week	50% or below	62	98.4	58	90.6	0.115 ٭
	Above 50%	1	1.6	6	9.4	
	Total	63	100	64	100	
8th week	50% or below	29	52.7	39	63.9	0.221 *
	Above 50%	26	47.3	22	36.1	
	Total	55	100	61	100	
12th week	50% or below	9	16.7	25	43.1	0.002 *
	Above 50%	45	83.3	33	56.9	
	Total	54	100	58	100	
P♯ - VALUE		0.001		0.001		
P- VALUE- ♯ - Cochran's Q test, * - Chi square test, ٭ - Fischer exact test						

Table-27: More than 50% terminal hair density at each follow-up visit across different treatment groups of beard patches as per ITT analysis

Density of terminal hairs (RGS)		GROUPS				P- Value
		Beard A		Beard B		
		n	%	n	%	
Baseline	50% or below	45	100	44	100	-
	Above 50%	0	0	0	0	
	Total	45	100	44	100	
4th week	50% or below	39	97.5	30	93.8	0.581 ‧
	Above 50%	1	2.5	2	6.2	
	Total	40	100	32	100	
8th week	50% or below	28	75.6	21	70.0	0.602 *
	Above 50%	9	24.4	9	30.0	
	Total	37	100	30	100	
12th week	50% or below	22	59.4	16	53.3	0.615 *
	Above 50%	15	40.6	14	46.7	
	Total	37	100	30	100	
P♯ - VALUE		0.001		0.001		
P- VALUE-♯ Cochran's Q test, * - Chi square test, ‧ - Fischer exact test						

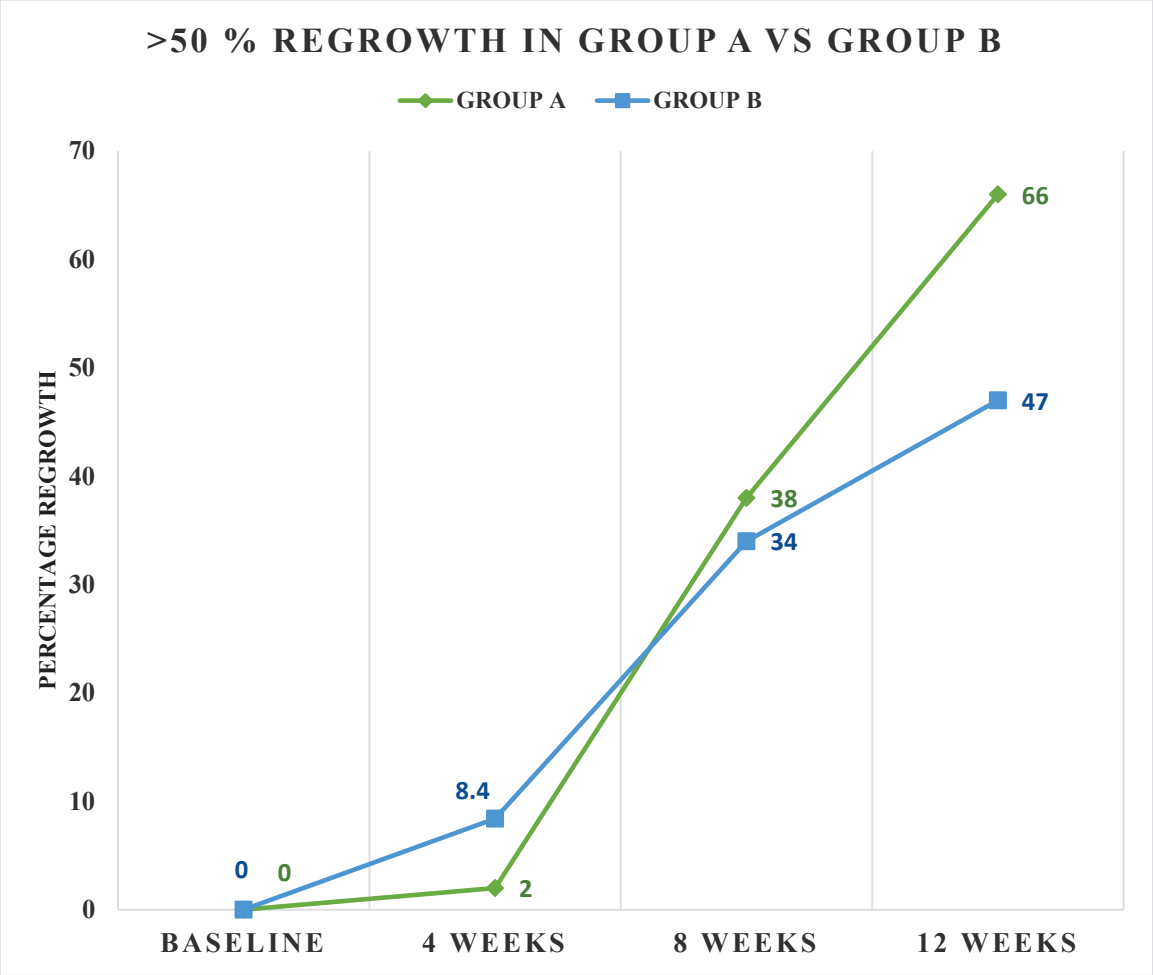


Figure-19: More than 50% terminal hair density at each follow-up visit across different treatment groups

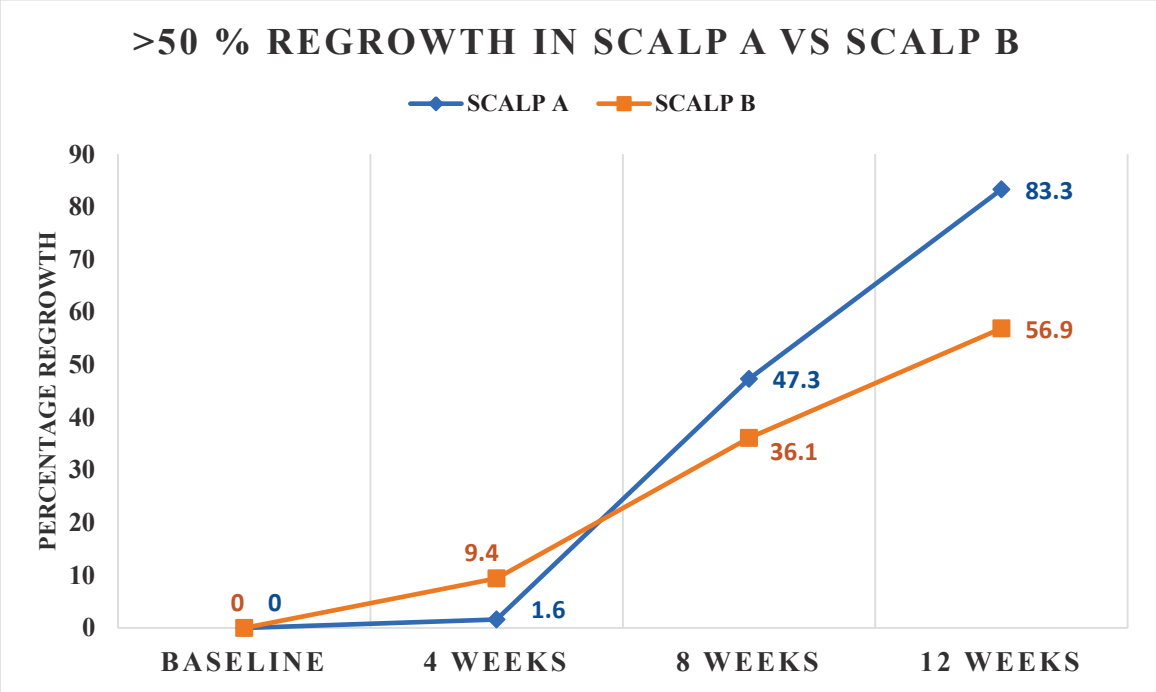


Figure-20: More than 50% terminal hair density at each follow-up visit across different treatment Group of scalp patches

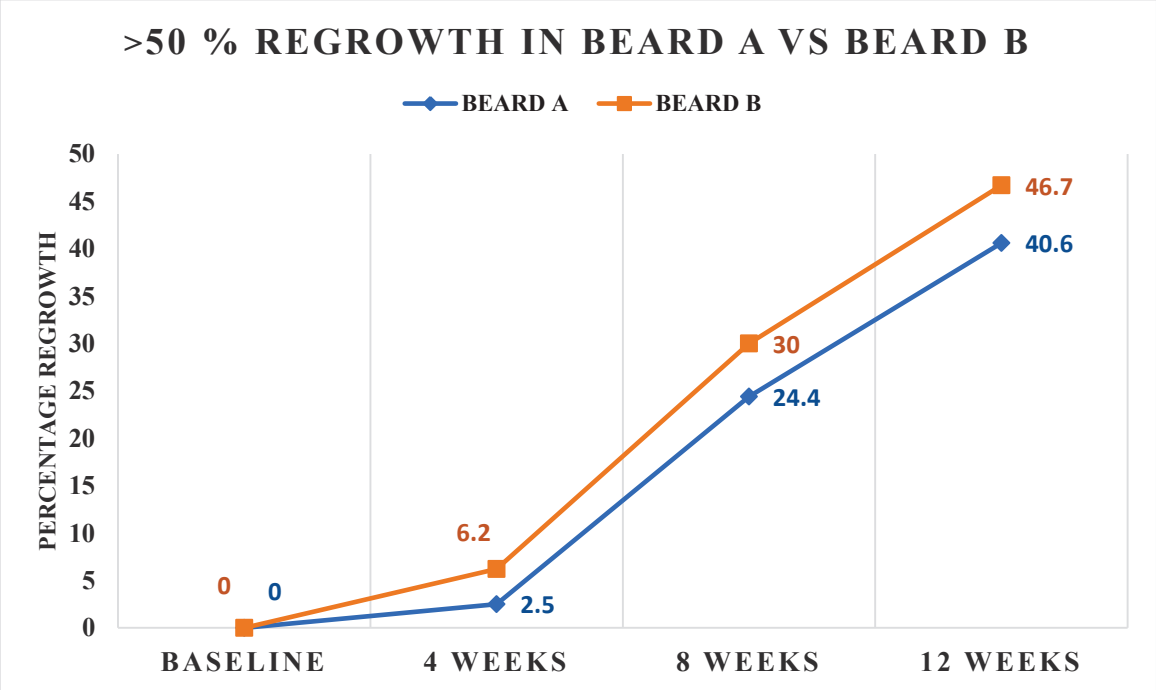


Figure-21: More than 50% terminal hair density at each follow-up visit across different treatment group of beard patches

DERMOSCOPIC SIGNS OF DISEASE ACTIVITY:

Dermoscopic signs of disease activity (Presence of any Black dots (BD), Broken hair (BH), Exclamation mark hair (EM), Pohl-pinkus constriction (PP), and Tapered hair (TH)) were sequentially evaluated in both the groups. In both treatment groups, the reduction was significant throughout the follow-up period in comparison to the baseline (Cochran's Q test- p-value <0.001, 0.001). Both groups were comparable at the beginning (Chi square test p-value - 0.320) and showed no statistically significant differences in subsequent visits (Chi square test p-value 0.581, 0.833, 0.431) (Table-28).

In addition, subgroup analysis also failed to identify a statistically significant difference between two groups of scalps (Table-29) and beard (Table-30) patches at any visits.

Table-28: Dermoscopic signs of disease activity in each group as per ITT analysis

Dermoscopic signs of activity		GROUPS				p* - Value
		Group A		Group B		
		n	%	n	%	
Baseline	Present	78	70.3	73	64.0	**0.320**
	Absent	33	29.7	41	36.0	
	Total	111	100	114	100	
4th week	Present	55	53.4	55	57.3	**0.581**
	Absent	48	46.6	41	42.7	
	Total	103	100	96	100	
8th week	Present	36	39.1	37	40.7	**0.833**
	Absent	56	60.9	54	59.3	
	Total	92	100	91	100	
12th week	Present	22	24.2	17	19.3	**0.431**
	Absent	69	75.8	71	80.7	
	Total	91	100	88	100	
p ♯ - VALUE		< 0.001		< 0.001		
P value = * - Chi square test, ♯ - Cochran's Q test						

Table-29: Dermoscopic signs of disease activity in scalp patches as per ITT analysis

Dermoscopic signs of activity		GROUPS				p* - Value
		SCALP A		SCALP B		
		n	%	n	%	
Baseline	Present	49	74.2	48	68.6	**0.614**
	Absent	17	25.8	22	31.4	
	Total	66	100	70	100	
4th week	Present	40	63.5	37	57.8	**0.512**
	Absent	23	36.5	27	42.2	
	Total	63	100	64	100	
8th week	Present	24	43.6	21	34.4	**0.309**
	Absent	31	56.4	40	65.6	
	Total	55	100	61	100	
12th week	Present	11	20.4	10	17.2	**0.672**
	Absent	43	79.6	48	82.8	
	Total	54	100	58	100	
p ♯ - VALUE		< 0.001		< 0.001		
P value = * - Chi square test, ♯ - Cochran's Q test						

Table-30: Dermoscopic signs of disease activity in beard patches as per ITT analysis

Dermoscopic signs of activity		GROUPS				p* -Value
		Beard A		Beard B		
		n	%	n	%	
Baseline	Present	21	46.7	25	56.8	**0.338**
	Absent	24	53.3	19	43.2	
	Total	45	100	44	100	
4th week	Present	15	37.5	18	56.3	**0.113**
	Absent	25	62.5	14	43.8	
	Total	40	100	32	100	
8th week	Present	12	32.4	14	46.7	**0.085**
	Absent	25	67.6	16	53.4	
	Total	37	100	30	100	
12th week	Present	11	29.7	7	23.3	**0.557**
	Absent	26	70.3	23	76.7	
	Total	37	100	30	100	
p ♯ - VALUE		0.010		< 0.001		
P value = * - Chi square test, ♯ - Cochran's Q test						

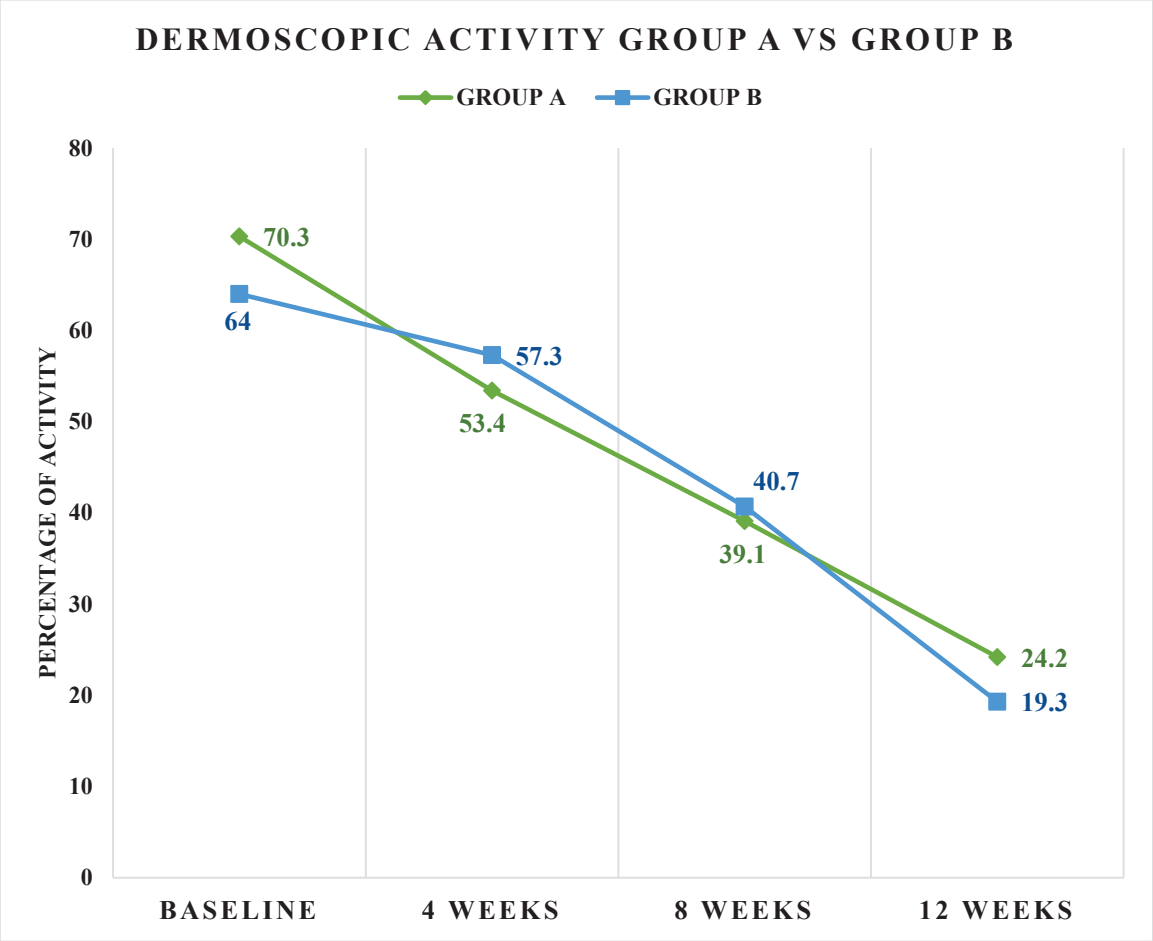


Figure-22: Dermoscopic signs of disease activity in each group

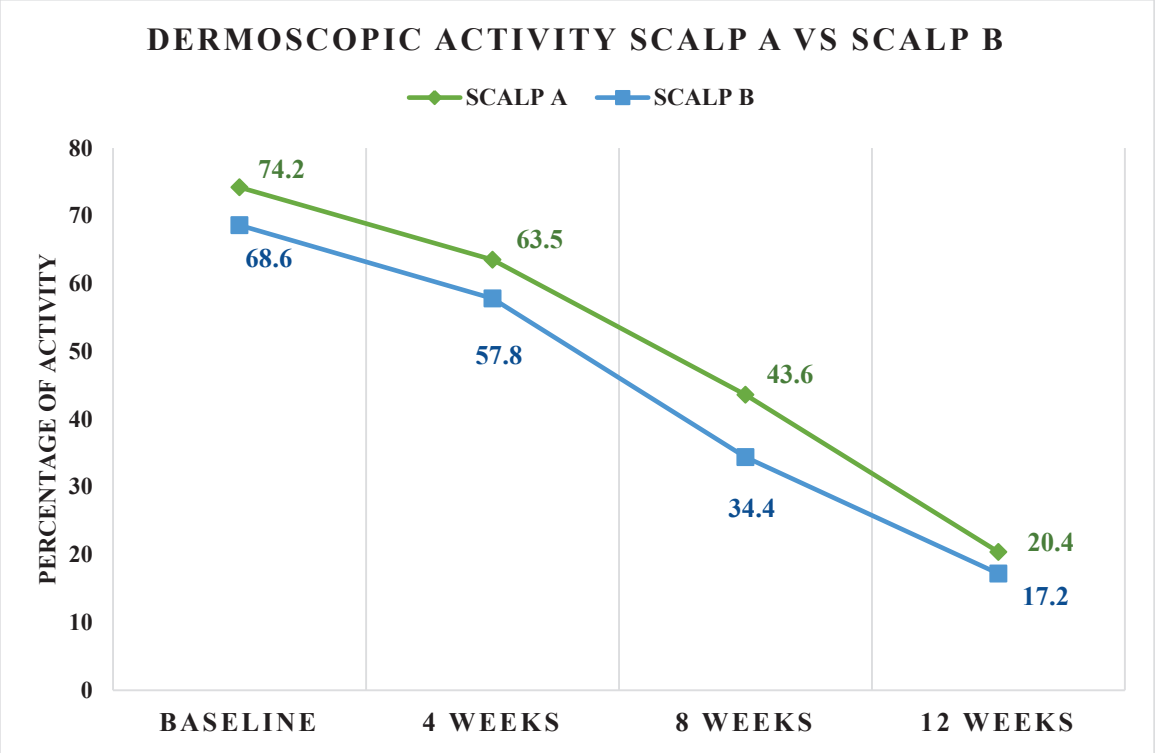


Figure-23: Dermoscopic signs of disease activity in scalp patches

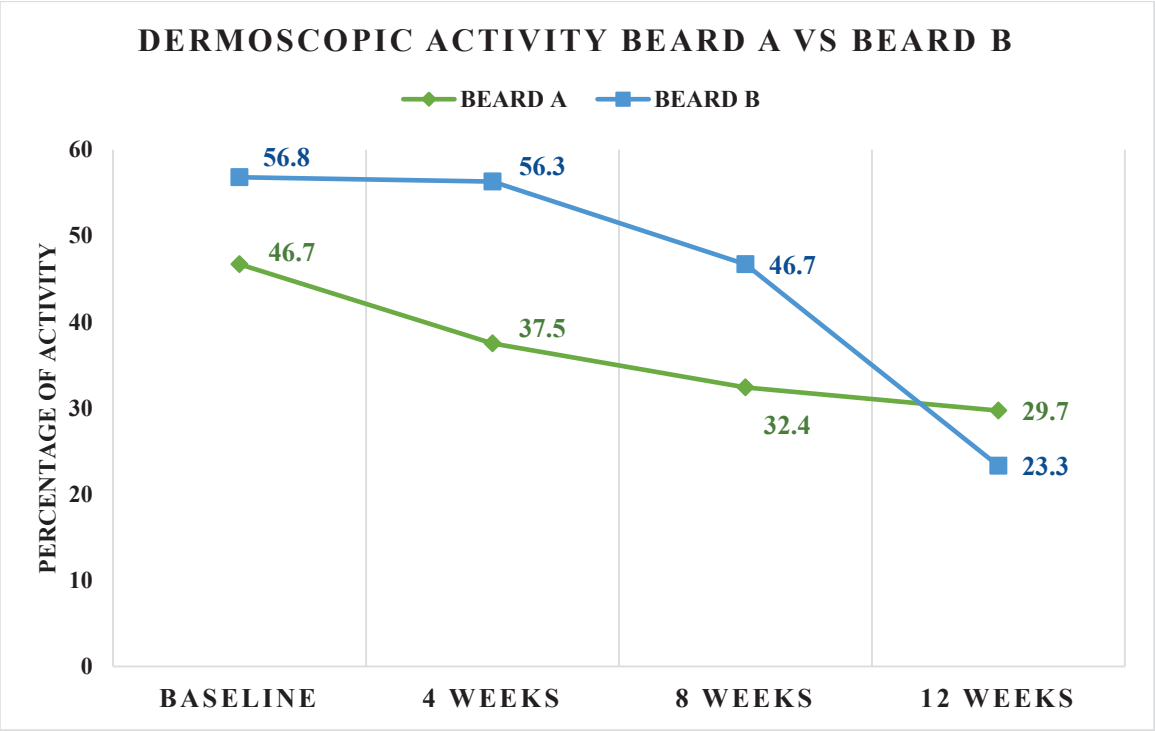


Figure-24: Dermoscopic signs of disease activity in beard patches

ADVERSE EFFECTS AT EACH VISIT:

The most often reported adverse events in the trial were itching, dryness, followed by burning sensation and atrophy of patches. Two scalp patches in Group A exhibited persisting erythema at 4 weeks, and one of those patches continued to be erythematous until the end of the trial.

A statistically significant difference in itching was found between patches of group A and B in all subsequent visits (Chi square test- $p< 0.016, 0.001, 0.001$), and dryness was also shown to be statistically significant between patches of group A and patches of group B at the 8th and 12th weeks of the trial (Chi square test- $p< 0.006, 0.006$)) with both the side effects were more pronounced in group A patches. At any point during the trial, the burning sensation, atrophy, and persistent erythema were comparable in both treatment groups.

Subsequent visits revealed statistically significant improvements in both burning sensation and itching for Group A patches, and reductions in both itching and dryness for Group B patches.

Both groups of scalp patches had atrophy as a side effect, with a statistically significant increase in atrophy in group B scalp patches seen on subsequent visits (Cochrane Q test p value - 0.006). During any of the visits, no beard patches exhibited signs of atrophy.

Table-31: Adverse effects at each visit: as per ITT analysis

A/E	Visit	Group A n (%)	Group B n (%)	P-value (Group A vs B)	Scalp A n (%)	Scalp B n (%)	Beard A n (%)	Beard B n (%)
Itching	**Base line**	17 (18.7)	11 (12.5)	0.225*	12 (22.2)	10 (17.2)	5 (13.5)	1 (3.3)
	4th weeks	23 (25.3)	10 (11.4)	0.016*	14 (25.9)	9 (15.5)	9 (24.3)	1 (3.3)
	8th weeks	26 (28.6)	6 (6.8)	0.001*	15 (27.8)	5 (8.6)	11 (29.7)	1 (3.3)
	12th weeks	21 (23.1)	5 (5.7)	0.001*	12 (22.2)	4 (6.9)	9 (24.3)	1 (3.3)
P - value#		**0.008**	**0.015**		**0.277**	**0.015**	**0.016**	**1.000**
Dryness	**Base line**	11 (12.1)	10 (11.4)	0.880*	3 (5.6)	10 (17.2)	8 (21.6)	5 (16.5)
	4th weeks	14 (15.4)	9 (10.2)	0.303*	6 (11.1)	9 (15.5)	8 (21.6)	0(0)
	8th weeks	16 (17.6)	4 (4.5)	0.006*	7 (13.0)	4 (6.9)	9 (24.3)	0(0)
	12th weeks	16 (17.6)	4 (4.5)	0.006*	7 (13.0)	4 (6.9)	9 (24.3)	0(0)
P - value#		**0.233**	**0.019**		**0.160**	**0.019**	**0.909**	-
Burning sensation	**Base line**	2 (2.2)	2 (2.3)	0.973*	1 (1.9)	2 (3.4)	1 (2.7)	0(0)
	4th weeks	8 (8.8)	3 (3.4)	0.134*	5 (9.3)	2 (3.4)	1 (2.7)	0(0)
	8th weeks	6 (6.6)	2 (2.3)	0.278•	5 (9.3)	2 (3.4)	1 (2.7)	0(0)
	12th weeks	5 (5.5)	2 (2.3)	0.444•	4 (7.4)	2 (3.4)	1 (2.7)	0(0)
P - value#		**0.029**	**0.392**		**0.035**	**0.392**	**0.308**	-
Atrophy	**Base line**	0 (0)	0 (0)	-	0 (0)	0 (0)	0 (0)	0 (0)
	4th weeks	0 (0)	1 (1.1)	0.492•	0 (0)	1 (1.7)	0 (0)	0 (0)
	8th weeks	1 (1.1)	3 (3.4)	0.362•	1 (1.9)	3 (5.2)	0 (0)	0 (0)
	12th weeks	2 (2.2)	6 (6.8)	0.164•	2 (3.7)	6 (10.3)	0 (0)	0 (0)
P - value#		**0.194**	**0.006**		**0.194**	**0.006**	-	-
Redness	**Base line**	0 (0)	0 (0)	-	0	0 (0)	0 (0)	0 (0)
	4th weeks	2 (2.2)	0 (0)	0.497•	2 (3.7)	0 (0)	0 (0)	0 (0)
	8th weeks	1(1.1)	0 (0)	1.000•	1 (1.9)	0 (0)	0 (0)	0 (0)
	12th weeks	1 (1.1)	0 (0)	1.000•	1 (1.9)	0 (0)	0 (0)	0 (0)
p- value#		**0.261**	**-**		**0.261**	**-**	**-**	**-**

P- value - # - Cochran's Q test, * - Chi square test, • - Fischer exact test

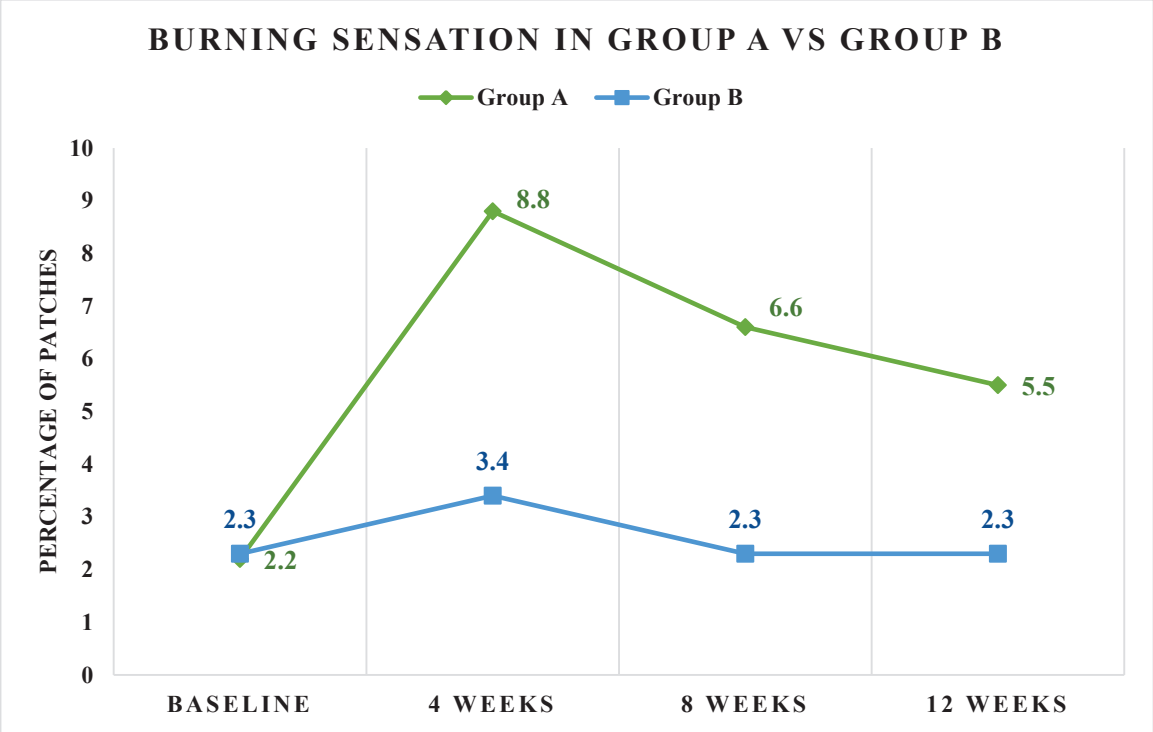


Figure-25: Burning sensation at each follow-up visit across different treatment groups

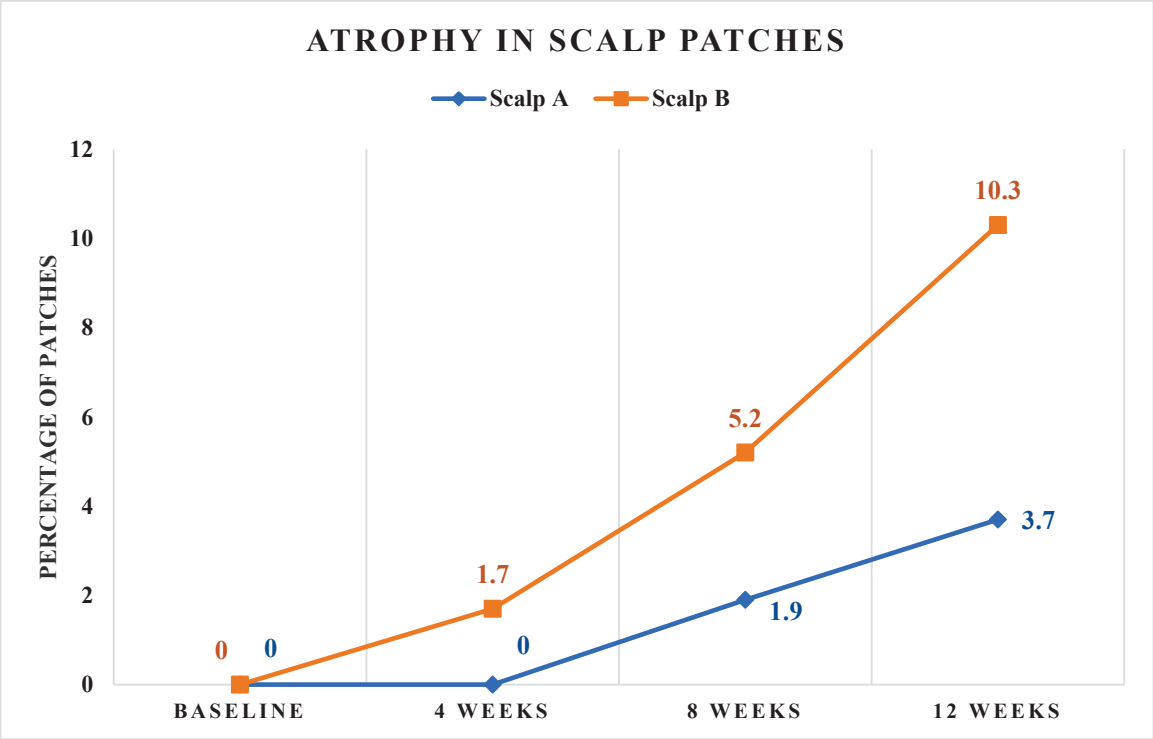
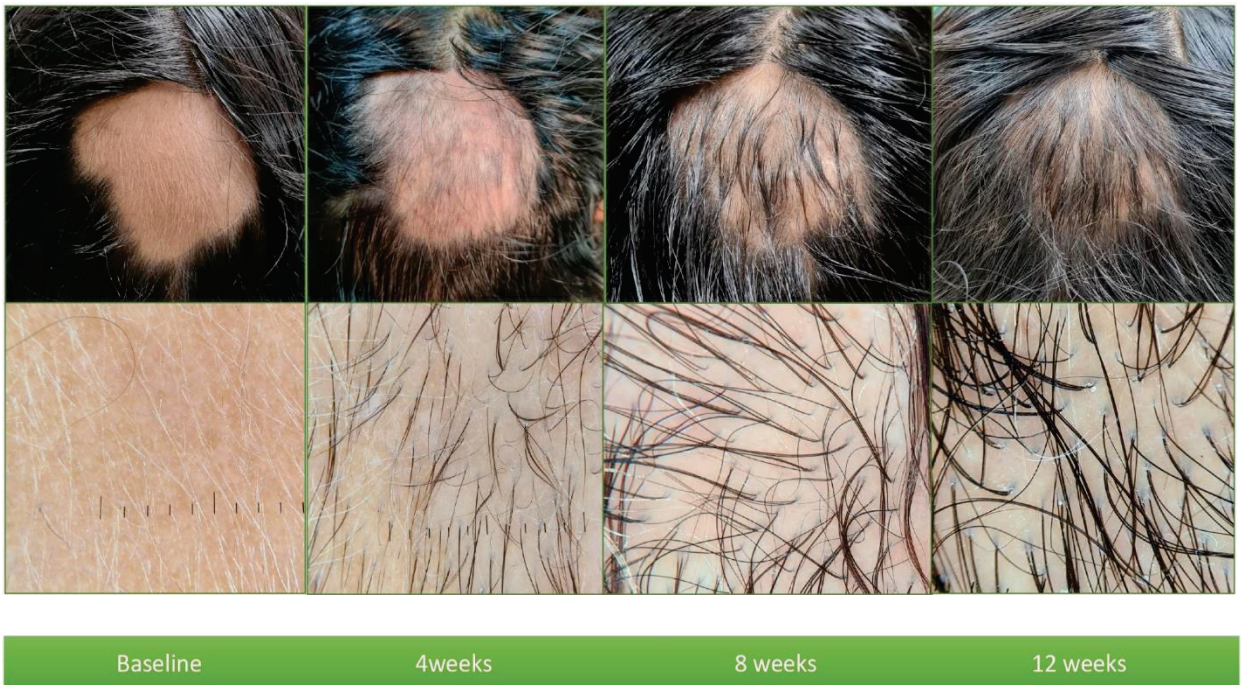
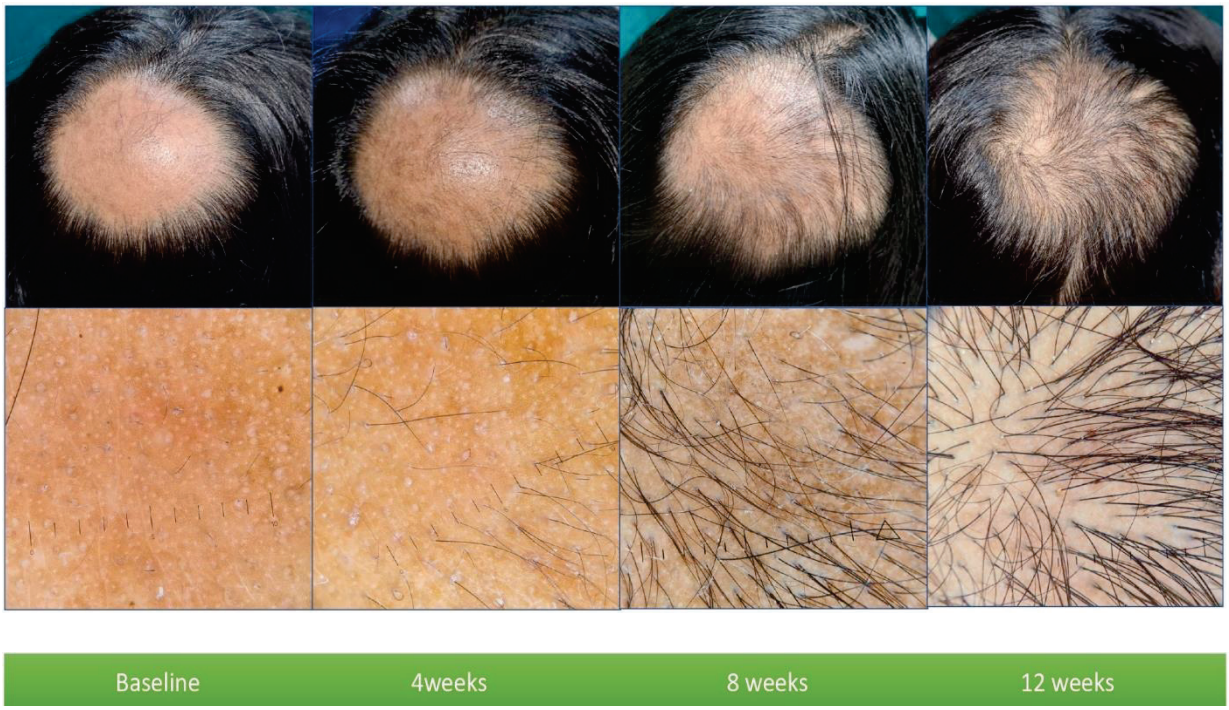


Figure-26: Atrophy at each follow-up visit across different treatment groups in scalp patches



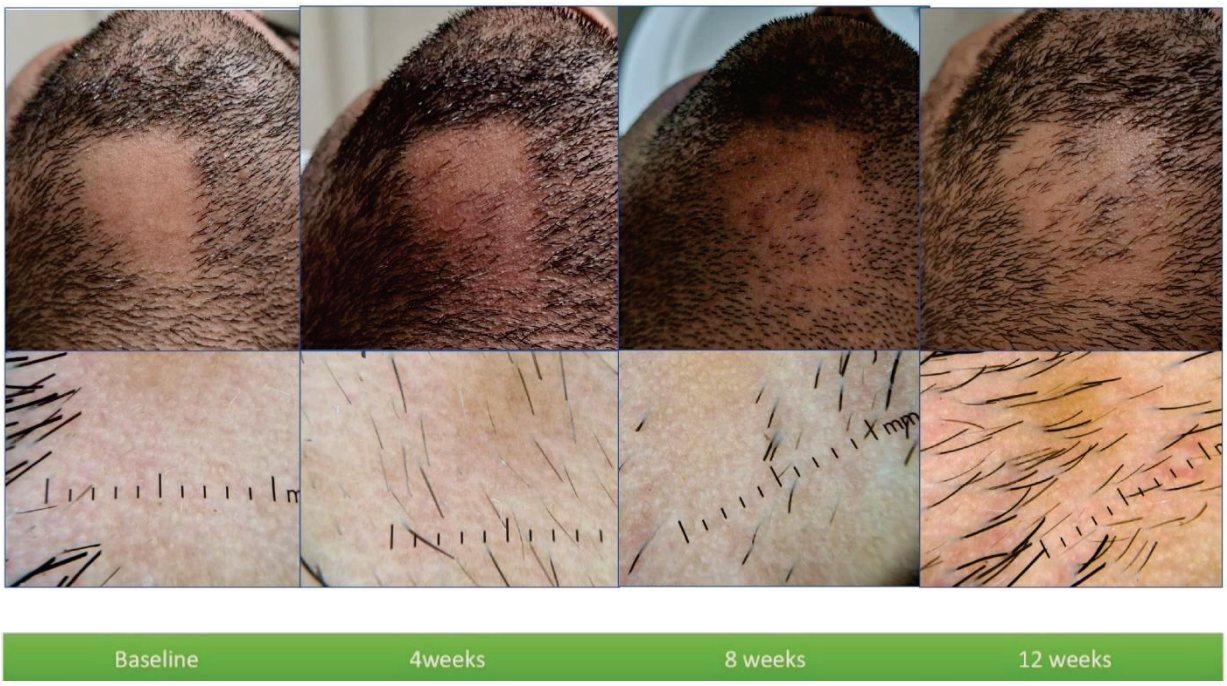
RGS - 0 **RGS- 1** **RGS- 2** **RGS- 4**

Figure-27: Clinical and dermoscopic images of a scalp patch at serial visits in group A with RGS score (Patch No-30)



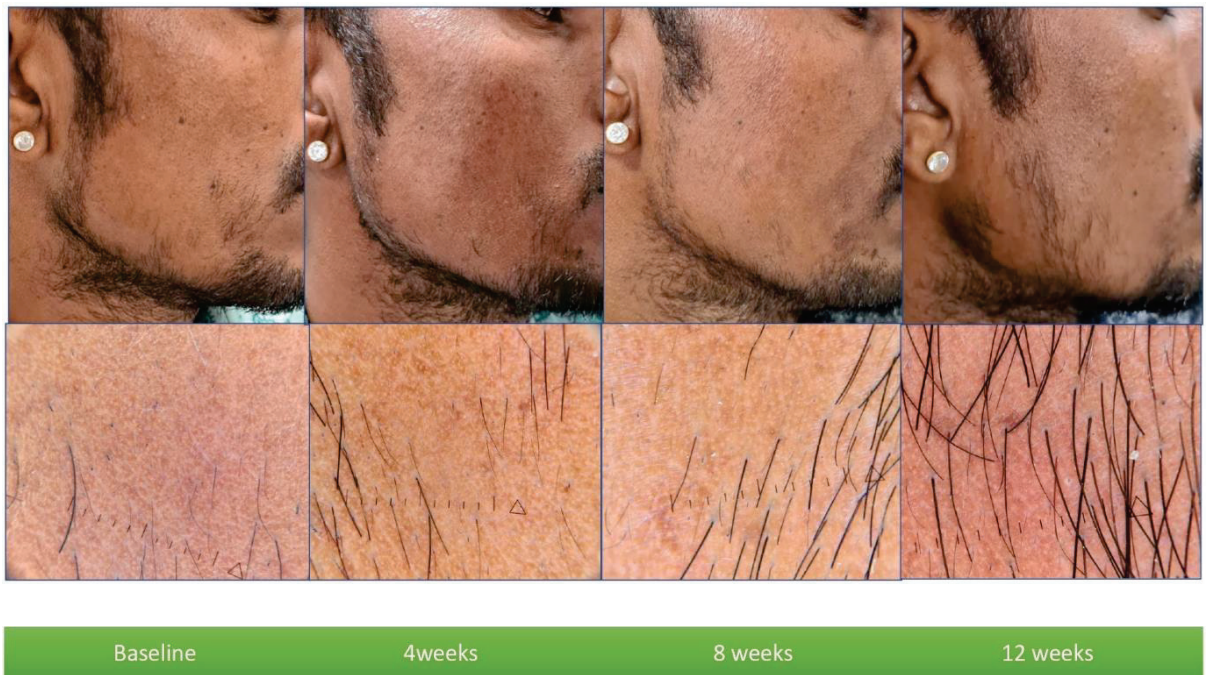
RGS - 0 **RGS- 0** **RGS- 2** **RGS- 3**

Figure-28: Clinical and dermoscopic images of a scalp patch at serial visits in group B with RGS score (Patch No-134)



RGS - 0 **RGS- 0** **RGS- 1** **RGS- 2**

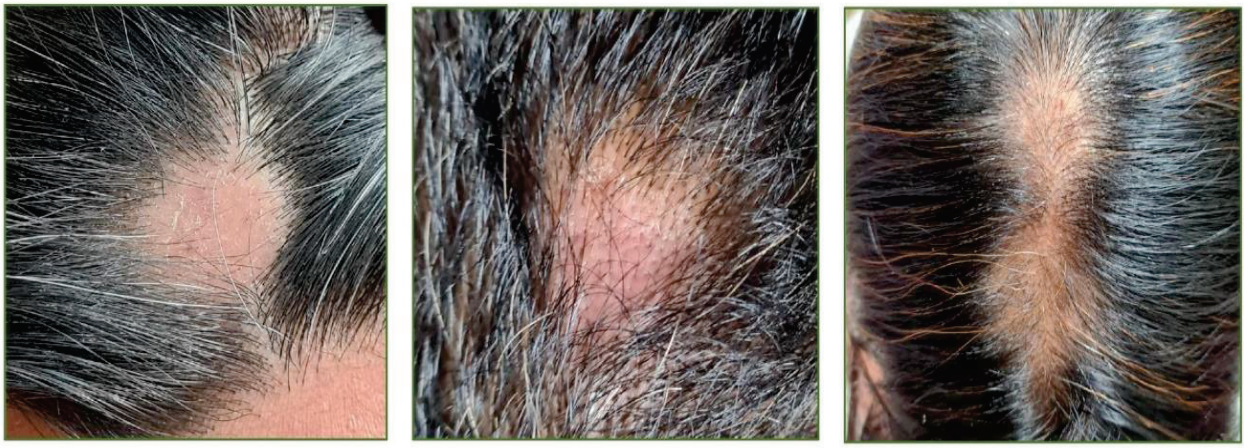
Figure-29: Clinical and dermoscopic images of a beard patch at serial visits in group A with RGS score (Patch No-69)



RGS - 0 **RGS- 0** **RGS- 0** **RGS- 1**

Figure-30: Clinical and dermoscopic images of a beard patch at serial visits in group B with RGS score (Patch No-143)

ADVERSE EVENTS IN SELECTED PATCHES



A B C

Figure-31: Clinical images showing adverse effects in patches

A- Dryness (Patch No-74)

B- Redness (Patch No-34)

C- Atrophy in anterior patch (Patch No-106)

DISCUSSION

DISCUSSION

Based on our inclusion criteria, 93 consenting patients with alopecia areata were recruited from Dermatology, Venereology and Leprology Out patient department (OPD) at All India Institute of Medical Sciences, Jodhpur between May 2021 and August 2022 out of which 73 patients completed the follow up.

The patients were randomly distributed in to two groups namely group A and group B. Participants in group A received intralesional triamcinolone acetonide (scalp 5.0 mg/ml and beard 2.5 mg/ml) and topical calcipotriol 0.005%, whereas those in group B received intralesional triamcinolone acetonide (scalp 5.0 mg/ml and beard 2.5 mg/ml) alone.

The age of the participants ranged from 14 to 49 years, with a mean age of 26.6±7.58 years. These findings were in line with a prior study by Tan E et al, in which the mean age at which AA initially presented was 25.2 years.[168] Despite the fact that patients might present at any age, the age range with the highest prevalence was noted to be 30-59 years in other studies.[169]

In the span of our study, there were considerably higher male patients than female patients (3.43:1). This male preponderance was consistent with multiple prior studies (Rajan M et al, Sharma et al, and Ustuner et al) in which the ratio varied from 1.68:1 to 2.61:1 respectively.[58,79,86] The lower incidence among women may be attributable to their unwillingness to travel and contact physicians, their habit of keeping long hair, their domestic and parental obligations according to the predominant culture of western Rajasthan, and their financial dependency on the family head. These factors may have contributed to the study's uneven gender ratio.

Nearly half (46.2%) of the sample population were graduate or postgraduate students. These results may infer, in an indirect manner, a greater level of awareness and potentially an increased propensity to seek treatment among literate patients.

Only twelve patients (12.9%) in our study had concurrent co-morbidities like diabetes mellitus, hypertension etc. Atopy was diagnosed in 17 individuals (18.3%). Only 5 patients (5.4%) had an AA family history, whereas 9 (9.7%) had family history of atopy. Few prior studies showed a higher incidence of atopy in family members of alopecia patients which ranged from 29.8 to 39.0%.[57,170] where as our study was in concordance with other study in terms of family history of alopecia areata which was noted 4.6 % of patients.[168]

In our study, 31 patients had previously received several treatment modalities (33.3%), the most prevalent of which were intralesional steroids in 13 patients (14.0%), followed by topical steroids in 10 patients (10.8%). Since the one-month wash off interval was addressed prior to enrollment, the impact of the previous treatment on our sample group has been negligible.

In our trial, 5(5.4%) subjects were previously diagnosed cases of clinical hypothyroidism, while 5 of subjects (7.1%) had abnormal TSH levels. The prevalence of thyroid disease varies across studies, with 2.3% to 16% of patients with patchy AA having thyroid abnormalities .[60,168]

Our study showed that three (3.2%) patients also had Type 2 diabetes mellitus while three (4.2%) patients had abnormally elevated blood glucose levels. This was contradictory when compared with the findings of Serarslan et al., in which no patients were found to have diabetes despite a high proportion of diabetic family members.[57]

38(40.9%) individuals in our study had nail changes. The reported prevalence of nail alterations in the clinical studies varies from 7% to 66%, with an average prevalence of around 30%.[12] In a study conducted by Goh et al., a similar proportion (38%) of nail changes were observed.[60]

In our trial punctate leukonychia was the predominant nail finding and it was observed in 17 patients (18.3%). This was followed by fine geometric pitting in 8 patients (8.6%), and longitudinal ridges and trachonychia in 7 patients (7.5%) each. Rajan M et al.,[86] similarly reported punctate leukonychia as the most prevalent nail change which was noted in around 40% patients, however the majority of the studies demonstrated a lower prevalence of leukonychia as compared to our study.[171–173] When compared to our results, we found a greater frequency of nail pitting in prior studies which ranged from 11-34%. However this higher range was observed more commonly in paediatric cases of alopecia areata.[12] Similar prevalence of trachonychia and longitudinal ridging (4–9%) have also been identified in other studies.[168,171–173]

We selected 225 patches for our study on the basis of inclusion and exclusion criteria. Mean number of patches selected from each patient was 2.41. We enrolled only limited AA patients due to the poor response of extensive AA to intralesional steroids and need for systemic therapy in these patients.[174]

The baseline dermoscopic examination of our study patches exhibited a variety of dermoscopic features. Black dots (52.4%) were the most frequently observed finding, followed by broken hair (36%), yellow dots (31%), and exclamation mark hair (27.1%) in patches. Pohl-Pinkus constriction (0.4%) was the least common finding. A review article by Waskiel et al., revealed a comparable prevalence of black dots, although other dermoscopic features were present in higher number than in our study. Pohl-pinkus constriction was the least common finding in most of the studies(0-4%), which was similar to our study.[13,65]

At baseline, all study parameters were comparable between the two groups ($p>0.05$), which ensured the comparability of study outcomes.

At baseline, the predominant type of hair was mixed (vellus and terminal) (45-48.2%) in both groups ($p=0.963$). In both treatment groups, the proportion of patches with terminal hair as the major hair type increased significantly throughout the period of the follow-up visits in both scalp and beard patches (p 0.001, 0.001). Intergroup comparison failed to reveal any statistically significant differences in the increment in terminal hairs in any of the follow-up visits. These findings suggest that topical calcipotriol may have no significant qualitative influence on the predominant hair type at 12th week in patients receiving monthly triamcinolone acetonide injections. A similarly significant increase in terminal hairs was also observed in the study conducted by Rajan M et al. in the scalp patches treated with 5 mg/ml intralesionsal triamcinolone acetonide.[86]

The pattern of hair regrowth was another parameter which we investigated. We looked for the pattern of hair regrowth in both groups using the DIMT (Diffuse, Irregular, Marginal and Targetoid) pattern of classification. Both of these groups had a broad range of hair regrowth patterns. Regardless of treatment group, our study found that the 'diffuse pattern' (Group A- 39.6% and Group B- 38.6%) was the most common, followed by the 'marginal pattern' (Group A- 24.3% and Group B- 25.0%). This was consistent with the study findings of Lee et al, who discovered diffuse (34%) and marginal (33%) patterns as the most prevalent hair regrowth patterns in alopecia patches.[62]

However, neither the scalp nor the beard patches showed any evidence of a statistically significant difference in regrowth pattern between the two treatment groups, regardless of which treatment modality was used. But a study carried out by Lim et al., found a significant correlation between the DIMT-classified hair regrowth patterns and the treatment modalities that were used in patches. The researchers noted that there was a difference in the pattern of

hair regrowth in the intralesional and topical steroid groups in their study. Likewise, this was revealed that an irregular hair regrowth pattern was more frequently observed in AA patches treated with intralesional steroid than in those treated by any other modality.[63]

After thorough literature review, we couldn't find any study that investigates the difference in pattern of regrowth between scalp and beard patches, but our data suggests that this difference exists regardless of treatment group. Compared to beard patches, scalp patches had a significantly larger percentage of the "targetoid" pattern (p value- <0.001). Similarly, compared to scalp patches, beard patches exhibited a significantly greater percentage of "irregular" regrowth (p value- 0.019).

The semi-quantitative study of hair regrowth using RGS (regrowth score) as well as patches with more than 50% terminal hair regrowth revealed a substantial improvement in both the groups beginning in the fourth week and continuing forward (p value- 0.001, 0.001) indicating that the therapy instituted in both the groups was efficacious. This was the case for both the scalp (p value- 0.001, 0.001) and the beard (p value- 0.001, 0.001) patches in their own right. These findings are consistent with the findings of Ustuner et al, who demonstrated a substantial improvement in RGS score (69.0%) from baseline in the ITA (5mg/ml) group compared to NS group.[79] The use of topical calcipotriol ointment alone in scalp patches exhibited an RGS score of 4 in 62.9% of the patches, however our study found an RGS score of 4 in 50.0% of the scalp patches treated with the combination of intralesional steroid and topical calcipotriol.[99]

Our study observed no statistically significant difference in the density of terminal hairs at any time during the study between the two treatment groups (p value- 0.930, 0.616, 0.178), but subgroup analysis eventually showed a statistically significant synergistic action of topical calcipotriol with intralesional steroids in scalp patches (p value- 0.018) but not in beard patches (p value- 0.527) at the 12th week of study. This lesser advantage of calcipotriol in beard patches could not be explained, however in a study measuring the vitamin D concentration in human hair found that there was low quantity of 25(OH)D3 in beard hair samples (231 pg/mg), in comparison to the samples of scalp hair (421 pg/ml).[175] However, the exact molecular mechanism and the clinical significance needs to be further studied.

After thorough literature review, we were unable to find any study that investigates the synergistic action of topical calcipotriol with intralesional steroids in alopecia areata. But in a trial comparing the synergistic effects of topical calcipotriol with topical steroids, a

statistically significant improvement was observed when topical calcipotriol was added to the topical steroid regimen. It was a comparative analytical study on 100 scalp patch AA patients, with 50 patients instructed to use topical mometasone 0.1% cream combined with topical calcipotriol 0.005% (group A) and the remaining 50 patients advised to apply just topical mometasone 0.1% cream (group B). Over a 24-week period, the mean SALT score reduced in Group A and Group B patients with a statistically significant difference in Group A patients. [98]

In both treatment groups, dermoscopic markers of disease activity (BD, BH, EM, PP, and TH) were comparable at baseline (p=0.320). During subsequent visits, a decreasing trend was observed with the frequency of patches exhibiting disease activity in both groups (p value- <0.001, <0.001). Similar results were noted in a comparable study by Wasiki et al. who discovered that patients who responded to treatment showed a reduction in dermoscopic signs of activity in follow-up visits.[65] During the follow-up visits in our study, there was no substantial difference between the treatment groups in terms of the dermoscopic markers of disease activity. Despite the fact that scalp patches exhibited a considerably greater improvement in terminal hair regrowth at the 12th week as compared to beard patches, but the subgroup analysis did not indicate any significant differences in activity at any point in time. It's possible that the notion that we analysed the data qualitatively rather than quantitatively might be the reason why there was no discernible difference between the two groups.

In our study, we investigated the adverse events of intralesional steroid treatment, as well as the synergistic side effects of combining topical calcipotriol with intralesional steroid injections in alopecia patches of the scalp and beard. Itching and dryness were the adverse events that were reported by participants in the trial the most often, followed by a burning sensation and atrophy of patches.

Itching was shown to be statistically significant between patches of group A and patches of group B at the 4th, 8th and 12th weeks of the trial (p 0.016, 0.001,0.001), and dryness was also shown to be statistically significant between patches of group A and patches of group B at the 8th and 12th weeks of the trial (p 0.006, 0.006). Both of these side effects were more pronounced in the group that was treated with topical calcipotriol. Similarly, patients treated with calcipotriol exhibited more burning sensation as compared with placebo group, although it wasn’t statistically significant. This spectrum of adverse effects, including scaling,

dryness, irritation and burning sensation associated with topical calcipotriol have been noted in various of studies in the past as well (Alam et al, Narang et al, and Jaiswal et al).[20,96,98]

Patients who were treated with topical calcipotriol showed statistically significant improvement in both the burning sensation and the itching during subsequent visits, which suggests that patients may tolerate these side effects when the treatment is continued over long term. In our trial, no patients discontinued medication owing to any adverse effects.

Both groups of scalp patches experienced atrophy as a side effect, but group B's scalp patches encountered a statistically significant increase in atrophy (p value 0.006) on subsequent visits while group A's topical calcipotriol combination treatment did not (p value 0.194). This may suggest that combining topical calcipotriol with an intralesional steroid regimen for the treatment of alopecia areata may lower the likelihood of side effects like atrophy, even if there isn't any study in the literature exploring the same idea.

Small sample size and patient's lost to follow-up are potential limitations of this study. Also, quantitative assessment for hair regrowth was not done by using automated software due to financial constraints. Follow up of the patients was not done to look for any relapse.

CONCLUSION

CONCLUSION

The study was a prospective, interventional, randomized single blinded clinical trial which recruited patients from the out-patient department of Dermatology, Venereology and Leprology at AIIMS, Jodhpur. The study included 93 patients (225 patches) with alopecia areata. They were randomly allocated to one of the two groups, participants in group A received intralesional triamcinolone acetonide (scalp 5.0 mg/ml and beard 2.5 mg/ml) and topical calcipotriol 0.005%, whereas those in group B received intralesional triamcinolone acetonide (scalp 5.0 mg/ml and beard 2.5 mg/ml) alone.

These patches were analyzed using clinical and dermoscopic parameters on each follow-up. The response assessor and the statistician were blinded to the intervention groups. The results led us towards the following key interpretations.

At baseline all the clinical and dermoscopic parameters were comparable in both the groups. All the parameters of hair regrowth showed a progressively increasing trend in both the treatment groups. Qualitative regrowth parameters such as the type of predominant hair (terminal hair) and disappearance of dermoscopic disease activity signs were significantly achieved in both the groups in comparison at 12th week.

Both of the groups didn't exhibit any significant difference in hair regrowth pattern as it was evaluated using the 'DIMT' pattern of hair regrowth, however regardless of the treatment groups, the differences in hair regrowth pattern in the scalp and the beard patches were significant.

Initially, the study didn’t observe any significant difference in the density of terminal hairs at any time during the study between the two treatment groups. However, the subgroup analysis eventually showed a statistically significant synergistic action of topical calcipotriol with intralesional steroids in scalp patches but not in beard patches at the 12th week of the study. This synergistic action was only observed in the scalp patches, not in the beard patches.

In both of the therapy groups, there have been reports of a diverse range of side effects. A greater percentage of irritation and dryness was seen in patches that had been treated with combination of topical calcipotriol and intralesional steroids. Both groups had a burning

sensation, atrophy, and persistent erythema in a similar manner. When compared to patches treated with intralesional triamcinolone acetonide alone, the atrophy exhibited by the topical calcipotriol and intralesional triamcinolone acetonide group did not demonstrate a significant increase when tracked until the end of the study.

Further, randomized control trials with longer follow-up, systematic reviews and meta-analyses are required in more population subsets to establish the study findings.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Strazzulla LC, Wang EHC, Avila L, Lo Sicco K, Brinster N, Christiano AM, et al. Alopecia areata: Disease characteristics, clinical evaluation, and new perspectives on pathogenesis. *J Am Acad Dermatol*. 2018;78(1):1-12.

2. Seetharam KA. Alopecia areata: an update. *Indian J Dermatol Venereol Leprol*. 2013;79(5):563-75.

3. Broadley D, McElwee KJ. A "hair-raising" history of alopecia areata. *Exp Dermatol*. 2020;29(3):208-22.

4. Callander J, Yesudian PD. Nosological Nightmare and Etiological Enigma: A History of Alopecia Areata. *Int J Trichology*. 2018;10(3):140-1.

5. Rajabi F, Drake LA, Senna MM, Rezaei N. Alopecia areata: a review of disease pathogenesis. *Br J Dermatol*. 2018;179(5):1033-48.

6. Simakou T, Butcher JP, Reid S, Henriquez FL. Alopecia areata: A multifactorial autoimmune condition. *J Autoimmun*. 2019;98:74-85.

7. El Taieb MA, Hegazy EM, Ibrahim HM, Osman AB, Abualhamd M. Topical calcipotriol vs narrowband ultraviolet B in treatment of alopecia areata: a randomized-controlled trial. *Arch Dermatol Res*. 2019;311(8):629-36.

8. Todes-Taylor N, Turner R, Wood GS, Stratte PT, Morhenn VB. T cell subpopulations in alopecia areata. *J Am Acad Dermatol*. 1984;11(2 Pt 1):216-23.

9. Yenin JZ, Serarslan G, Yonden Z, Ulutaş KT. Investigation of oxidative stress in patients with alopecia areata and its relationship with disease severity, duration, recurrence and pattern. *Clin Exp Dermatol*. 2015;40(6):617-21.

10. Daroach M, Narang T, Saikia UN, Sachdeva N, Sendhil Kumaran M. Correlation of vitamin D and vitamin D receptor expression in patients with alopecia areata: a clinical paradigm. *Int J Dermatol*. 2018;57(2):217-22.

11. Madani S, Shapiro J. Alopecia areata update. *J Am Acad Dermatol*. 2000;42(4):549-66

12. Chelidze K, Lipner SR. Nail changes in alopecia areata: an update and review. *Int J Dermatol*. 2018;57(7):776-83.

13. Waskiel A, Rakowska A, Sikora M, Olszewska M, Rudnicka L. Trichoscopy of alopecia areata: An update. *J Dermatol*. 2018;45(6):692-700.

14. Ganjoo S, Thappa DM. Dermoscopic evaluation of therapeutic response to an intralesional corticosteroid in the treatment of alopecia areata. *Indian J Dermatol Venereol Leprol*. 2013;79(3):408-17.

15. Jang YH, Moon SY, Lee WJ, Lee SJ, Lee WK, Park BC, et al. Alopecia areata progression index, a scoring system for evaluating overall hair loss activity in alopecia areata patients with pigmented hair: A development and reliability assessment. *Dermatology.* 2016;232(2):143-9.

16. Sterkens A, Lambert J, Bervoets A. Alopecia areata: a review on diagnosis, immunological etiopathogenesis and treatment options. *Clin Exp Med*. 2021;21(2):215-30.

17. Fukumoto T, Fukumoto R, Magno E, Oka M, Nishigori C, Horita N. Treatments for alopecia areata: A systematic review and network meta-analysis. *Dermatol Ther.* 2021;34(3):e14916.

18. Kumaresan M. Intralesional steroids for alopecia areata. *Int J Trichology*. 2010;2(1):63-5.

19. Lin X, Meng X, Song Z. Vitamin D and alopecia areata: possible roles in pathogenesis and potential implications for therapy. *Am J Transl Res*. 2019;11(9):5285-300

20. Narang T, Daroach M, Kumaran MS. Efficacy and safety of topical calcipotriol in management of alopecia areata: A pilot study. *Dermatol Ther*. 2017;30(3):e12464.

21. Gip L, Lodin A, Molin L. Alopecia areata. A follow-up investigation of outpatient material. *Acta Derm Venereol*. 1969;49(2):180-8.

22. MacDonald Hull SP, Wood ML, Hutchinson PE, Sladden M, Messenger AG; British Association of Dermatologists. Guidelines for the management of alopecia areata. *Br J Dermatol*. 2003;149(4):692-9.

23. Bhandary DJ, Girisha BS, Mahadevappa BN. Clinico-Dermoscopic Pattern of Beard Alopecia Areata: A Cross-Sectional Study. *Indian Dermatol Online J*. 2019;10(6):644-9.

24. Saceda-Corralo D, Grimalt R, Fernandez-Crehuet P, Clemente A, Bernardez C, Garcia-Hernandez MJ, et al. Beard alopecia areata: a multicentre review of 55 patients. *J Eur Acad Dermatol Venereol*. 2017;31(1):187-92.

25. Kyriakis KP, Paltatzidou K, Kosma E, Sofouri E, Tadros A, Rachioti E. Alopecia areata prevalence by gender and age. *J Eur Acad Dermatol Venereol*. 2009;23(5):572-3.

26. Hon KL, Luk DCK, Leung AKC, Ng C, Loo SKF. Childhood Alopecia Areata: An Overview of Treatment and Recent Patents. *Recent Pat Inflamm Allergy Drug Discov.* 2020;14(2):117-32.

27. Lee HH, Gwillim E, Patel KR, Hua T, Rastogi S, Ibler E, et al. Epidemiology of alopecia areata, ophiasis, totalis, and universalis: A systematic review and meta-analysis. *J Am Acad Dermatol*.2020;82(3):675-82.

28. Bertolini M, McElwee K, Gilhar A, Bulfone-Paus S, Paus R. Hair follicle immune privilege and its collapse in alopecia areata. *Exp Dermatol*. 2020;29(8):703-25.

29. McDonagh AJ, Tazi-Ahnini R. Epidemiology and genetics of alopecia areata. *Clin Exp Dermatol*. 2002;27(5):405-9.

30. Colombe BW, Price VH, Khoury EL, Garovoy MR, Lou CD. HLA class II antigen associations help to define two types of alopecia areata. *J Am Acad Dermatol*. 1995;33(5 Pt 1):757-64.

31. de Andrade M, Jackow CM, Dahm N, Hordinsky M, Reveille JD, Duvic M. Alopecia areata in families: association with the HLA locus. *J Investig Dermatol Symp Proc*. 1999;4(3):220-3.

32. Harmon CS, Nevins TD. IL-1 alpha inhibits human hair follicle growth and hair fiber production in whole-organ cultures. *Lymphokine Cytokine Res*. 1993;12(4):197-203.

33. Philpott MP, Sanders DA, Bowen J, Kealey T. Effects of interleukins, colony-stimulating factor and tumour necrosis factor on human hair follicle growth in vitro: a possible role for interleukin-1 and tumour necrosis factor-alpha in alopecia areata. *Br J Dermatol*. 1996;135(6):942-8.

34. Galbraith GM, Pandey JP. Tumor necrosis factor alpha (TNF-alpha) gene polymorphism in alopecia areata. *Hum Genet*. 1995;96(4):433-6.

35. Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, et al. Vitamin D and Chronic Diseases. *Aging Dis*. 2017;8(3):346-53.

36. Umar M, Sastry KS, Chouchane AI. Role of Vitamin D Beyond the Skeletal Function: A Review of the Molecular and Clinical Studies. *Int J Mol Sci*. 2018;19(6):1618.

37. Lee SM, Pike JW. The vitamin D receptor functions as a transcription regulator in the absence of 1,25-dihydroxyvitamin D_3. *J Steroid Biochem Mol Biol*. 2016;164:265-70.

38. Zhang H, Nan W, Wang S, Zhang T, Si H, Yang F, et al. Epidermal Growth Factor Promotes Proliferation and Migration of Follicular Outer Root Sheath Cells via Wnt/β-Catenin Signaling. *Cell Physiol Biochem*. 2016;39(1):360-70.

39. Afvari S, Kazlouskaya M, Cline A. Reply to "Vitamin D status in scarring and nonscarring alopecia". *J Am Acad Dermatol*. 2022;87(2):e89-90.

40. Guo H, Cheng Y, Shapiro J, McElwee K. The role of lymphocytes in the development and treatment of alopecia areata. *Expert Rev Clin Immunol*. 2015;11(12):1335-51.

41. Tanemura A, Oiso N, Nakano M, Itoi S, Kawada A, Katayama I. Alopecia areata: infiltration of Th17 cells in the dermis, particularly around hair follicles. *Dermatology.* 2013;226(4):333-6.

42. Cantorna MT, Snyder L, Lin YD, Yang L. Vitamin D and 1,25(OH)2D regulation of T cells. *Nutrients*. 2015;7(4):3011-21.

43. Lee S, Kim BJ, Lee CH, Lee WS. Increased prevalence of vitamin D deficiency in patients with alopecia areata: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol*. 2018;32(7):1214-21.

44. Liu Y, Li J, Liang G, Cheng C, Li Y, Wu X. Association of Alopecia Areata with Vitamin D and Calcium Levels: A Systematic Review and Meta-analysis. *Dermatol Ther (Heidelb).* 2020;10(5):967-83.

45. Fawzi MM, Mahmoud SB, Ahmed SF, Shaker OG. Assessment of vitamin D receptors in alopecia areata and androgenetic alopecia. *J Cosmet Dermatol*. 2016;15(4):318-23.

46. Bakry OA, El Farargy SM, El Shafiee MK, Soliman A. Serum Vitamin D in patients with alopecia areata. *Indian Dermatol Online J*. 2016;7(5):371-7.

47. Thompson JM, Li T, Park MK, Qureshi AA, Cho E. Estimated serum vitamin D status, vitamin D intake, and risk of incident alopecia areata among US women. *Arch Dermatol Res*. 2016;308(9):671-6.

48. Erpolat S, Sarifakioglu E, Ayyildiz A. 25-hydroxyvitamin D status in patients with alopecia areata. *Postepy Dermatol Alergol*. 2017;34(3):248-52.

49. d'Ovidio R, Vessio M, d'Ovidio FD. Reduced level of 25-hydroxyvitamin D in chronic/relapsing Alopecia Areata. *Dermatoendocrinol*. 2013;5(2):271-3.

50. Tsai TY, Huang YC. Vitamin D deficiency in patients with alopecia areata: A systematic review and meta-analysis. *J Am Acad Dermatol*. 2018;78(1):207-9.

51. Nahm M, Navarini AA, Kelly EW. Canities subita: a reappraisal of evidence based on 196 case reports published in the medical literature. *Int J Trichology*. 2013;5(2):63-8.

52. Wasserman D, Guzman-Sanchez DA, Scott K, McMichael A. Alopecia areata. *Int J Dermatol*. 2007;46(2):121-31.

53. Wolff H, Fischer TW, Blume-Peytavi U. The Diagnosis and Treatment of Hair and Scalp Diseases. *Dtsch Arztebl Int*. 2016;113(21):377-86.

54. Lew BL, Shin MK, Sim WY. Acute diffuse and total alopecia: A new subtype of alopecia areata with a favorable prognosis. *J Am Acad Dermatol*. 2009;60(1):85-93.

55. Shetty S, Rao R, Kudva RR, Subramanian K. Linear Alopecia Areata. *Int J Trichology*. 2016;8(3):144-5.

56. Hordinsky M, Ericson M. Autoimmunity: alopecia areata. J *Investig Dermatol Symp Proc*. 2004;9(1):73-8.

57. Serarslan G, Savas N, Yenin JZ. Is atopy and autoimmunity more prevalent in patients with alopecia areata? A comparative study. *J Eur Acad Dermatol Venereol*. 2012;26(6):720-3.

58. Sharma VK, Dawn G, Kumar B. Profile of alopecia areata in Northern India. *Int J Dermatol*. 1996;35(1):22-7.

59. Thomas EA, Kadyan RS. Alopecia areata and autoimmunity: a clinical study. *Indian J Dermatol*. 2008;53(2):70-4.

60. Goh C, Finkel M, Christos PJ, Sinha AA. Profile of 513 patients with alopecia areata: associations of disease subtypes with atopy, autoimmune disease and positive family history. *J Eur Acad Dermatol Venereol*. 2006;20(9):1055-60.

61. Soheila, N., Zahra, S., Shima, Y. Association of vitamin D level with alopecia areata. *Iran J Dermatol*. 2013; 16(1): 1-5.

62. Lee H, Lee S, Lee WS. Morphological classification system of hair regrowth patterns in alopecia areata patches: DIMT classification. *J Eur Acad Dermatol Venereol*. 2019;33(3):e96-7.

63. Lim SH, Lee H, Lee S, Lee JW, Lee SH, Lee WS. Relationship of Hair Regrowth Pattern in Alopecia Areata Patches According to DIMT Classification with Treatment Modalities and Patch Size: A Retrospective Cross-Sectional Analysis. *Ann Dermatol*. 2022;34(1):1-6.

64. Miteva M, Tosti A. Hair and scalp dermatoscopy. *J Am Acad Dermatol*. 2012;67(5):1040-8.

65. Waskiel-Burnat A, Rakowska A, Sikora M, Olszewska M, Rudnicka L. Alopecia areata predictive score: A new trichoscopy-based tool to predict treatment outcome in patients with patchy alopecia areata. *J Cosmet Dermatol*. 2020;19(3):746-51.

66. Gurusamy U, Venkataswamy C, Sivaraman A. Evaluation of Alopecia: A New Processing Technique Combining Vertical and Transverse Sections from a Single Scalp Biopsy Specimen. *Int J Trichology*. 2018;10(1):11-6.

67. Nguyen JV, Hudacek K, Whitten JA, Rubin AI, Seykora JT. The HoVert technique: a novel method for the sectioning of alopecia biopsies. *J Cutan Pathol*. 2011;38(5):401-6.

68. Chaitra V, Rajalakshmi T, Kavdia R. Histopathologic profile of alopecia areata in Indian patients. *Int J Trichology*. 2010;2(1):14-7.

69. Stefanato CM. Histopathology of alopecia: a clinicopathological approach to diagnosis. *Histopathology*. 2010;56(1):24-38.

70. Ito T. Advances in the management of alopecia areata. *J Dermatol*. 2012;39(1):11-7.

71. Messenger AG, McKillop J, Farrant P, McDonagh AJ, Sladden M. British Association of Dermatologists' guidelines for the management of alopecia areata 2012. *Br J Dermatol*. 2012;166(5):916-26.

72. Lee S, Lee WS. Management of alopecia areata: Updates and algorithmic approach. *J Dermatol*. 2017;44(11):1199-211.

73. Delamere FM, Sladden MM, Dobbins HM, Leonardi-Bee J. Interventions for alopecia areata. *Cochrane Database Syst Rev*. 2008;(2):CD004413.

74. Alkhalifah A, Alsantali A, Wang E, McElwee KJ, Shapiro J. Alopecia areata update: part II. Treatment. *J Am Acad Dermatol*. 2010;62(2):191-202

75. Orentreich N, Sturm HM, Weidman AI, Pelzig A. Local injection of steroids and hair regrowth in alopecias. *Arch Dermatol*. 1960;82:894-902.

76. Chu TW, AlJasser M, Alharbi A, Abahussein O, McElwee K, Shapiro J. Benefit of different concentrations of intralesional triamcinolone acetonide in alopecia areata: An intrasubject pilot study. *J Am Acad Dermatol*. 2015;73(2):338-40.

77. Kuldeep C, Singhal H, Khare AK, Mittal A, Gupta LK, Garg A. Randomized comparison of topical betamethasone valerate foam, intralesional triamcinolone acetonide and tacrolimus ointment in management of localized alopecia areata. *Int J Trichology*. 2011;3(1):20-4.

78. Kaur S, Mahajan BB, Mahajan R. Comparative Evaluation of Intralesional Triamcinolone Acetonide Injection, Narrow Band Ultraviolet B, and their Combination in Alopecia Areata. *Int J Trichology*. 2015;7(4):148-55.

79. Ustuner P, Balevi A, Ozdemir M. Best dilution of the best corticosteroid for intralesional injection in the treatment of localized alopecia areata in adults. *J Dermatolog Treat*. 2017;28(8):753-61.

80. Albalat W, Ebrahim HM. Evaluation of platelet-rich plasma vs intralesional steroid in treatment of alopecia areata. *J Cosmet Dermatol*. 2019;18(5):1456-62

81. El-Husseiny R, Elframawy S, Abdallah M. Comparative study between fractional carbon dioxide laser vs intralesional steroid injection in treatment of alopecia areata. *Dermatol Ther*. 2020;33(4):e13742.

82. Muhaidat JM, Al-Qarqaz F, Khader Y, Alshiyab DM, Alkofahi H, Almalekh M. A Retrospective Comparative Study of Two Concentrations of Intralesional Triamcinolone Acetonide in the Treatment of Patchy Alopecia Areata on the Scalp. *Clin Cosmet Investig Dermatol*. 2020;13:795-803.

83. Kapoor P, Kumar S, Brar BK, Kukar N, Arora H, Brar SK. Comparative Evaluation of Therapeutic Efficacy of Intralesional Injection of Triamcinolone Acetonide versus Intralesional Autologous Platelet-rich Plasma Injection in Alopecia Areata. *J Cutan Aesthet Surg*. 2020;13(2):103-11.

84. Hegde P, Relhan V, Sahoo B, Garg VK. A randomized, placebo and active controlled, split scalp study to evaluate the efficacy of platelet-rich plasma in patchy alopecia areata of the scalp. *Dermatol Ther*. 2020;33(6):e14388.

85. Mahgoub DA, Dhannoon TI, El-Mesidy MS. Trichloroacetic acid 35% as a therapeutic line for localized patchy alopecia areata in comparison with intralesional steroids: Clinical and trichoscopic evaluation. *J Cosmet Dermatol*. 2021;20(6):1743-9.

86. Rajan M B, Bhardwaj A, Singh S, Budania A, Bains A, Thirunavukkarasu P, et al. Identification of novel step-up regimen of intralesional triamcinolone acetonide in scalp alopecia areata based on a double-blind randomized controlled trial. *Dermatol Ther*. 2021;34(1):e14555.

87. Elshahid AR, Kadah AS, Hassan EA, Elsaie ML. (2022). Efficacy of Jessener solution versus intralesional steroid in treatment of alopecia areata. *Journal of cosmetic dermatology*. Advance online publication. https://doi.org/10.1111/jocd.15546

88. Metwally D, Abdel-Fattah R, Hilal RF. Comparative study for treatment of alopecia areata using carboxy therapy, intralesional corticosteroids, and a combination of both. *Arch Dermatol Res*. 2022;314(2):167-82.

89. Hamdino M, El-Barbary RA, Darwish HM. Intralesional methotrexate versus triamcinolone acetonide for localized alopecia areata treatment: A randomized clinical trial. *J Cosmet Dermatol*. 2022;21(2):707-15.

90. Nancy AL, Yehuda S. Prediction and prevention of autoimmune skin disorders. *Arch Dermatol Res*. 2009;301(1):57-64.

91. O'Neill JL, Feldman SR. Vitamine D analogue-based therapies for psoriasis. *Drugs Today (Barc)*. 2010;46(5):351-60.

92. Orecchia G, Rocchetti GA. Topical use of calcipotriol does not potentiate squaric acid dibutylester effectiveness in the treatment of alopecia areata. *J Dermatolog Treat. 2019;6(1):21-3.*

93. Berth-Jones J, Hutchinson PE. Alopecia totalis does not respond to the vitamin-D analogue calcipotriol. *J Dermatol Treat*. 2009;1(6):293-4.

94. Kim DH, Lee JW, Kim IS, Choi SY, Lim YY, Kim HM, et al. Successful treatment of alopecia areata with topical calcipotriol. *Ann Dermatol*. 2012;24(3):341-4.

95. Çerman AA, Solak SS, Altunay I, Kucukunal NA. Topical Calcipotriol Therapy for Mild-to-Moderate Alopecia Areata: A Retrospective Study. *J Drugs Dermatol*. 2015;14(6):616-20.

96. Jaiswal CSRP, Mehta HH, Barvaliya M. Effectiveness of topical calcipotriol (0.005%) ointment with or without narrowband ultraviolet B phototherapy in patients with alopecia areata: a randomized evaluator-blinded placebo-controlled trial. *J Egypt Women's Dermatol Soc*. 2018;15(3):127-32.

97. Krueger L, Peterson E, Shapiro J, Lo Sicco K. Case report of novel combination of anthralin and calcipotriene leading to trichologic response in alopecia areata. *JAAD Case Rep*. 2019;5(3):258-60.

98. Alam M, Amin SS, Adil M, Arif T, Zahra FT, Varshney I. Comparative Study of Efficacy of Topical Mometasone with Calcipotriol versus Mometasone Alone in the Treatment of Alopecia Areata. *Int J Trichology*. 2019;11(3):123-7.

99. Molinelli E, Campanati A, Brisigotti V, Sapigni C, Paolinelli M, Offidani A. Efficacy and Safety of Topical Calcipotriol 0.005% Versus Topical Clobetasol 0.05% in the Management of Alopecia Areata: An Intrasubject Pilot Study. *Dermatol Ther (Heidelb)*. 2020;10(3):515-21.

100. Rashad AF, Elgamal E, Fouda I. Intralesional vitamin D3 in treatment of alopecia areata: A randomized controlled clinical trial. *J Cosmet Dermatol*. 2022;21(10):4617-22.

101. Harvey CJ. Combined Diet and Supplementation Therapy Resolves Alopecia Areata in a Paediatric Patient: A Case Study. *Cureus*. 2020;12(11):e11371.

102. Tosti A, Piraccini BM, Pazzaglia M, Vincenzi C. Clobetasol propionate 0.05% under occlusion in the treatment of alopecia totalis/universalis. *J Am Acad Dermatol*. 2003;49(1):96-8.

103. Freire PCB, Riera R, Martimbianco ALC, Petri V, Atallah AN. Minoxidil for patchy alopecia areata: systematic review and meta-analysis. *J Eur Acad Dermatol Venereol*. 2019;33(9):1792-9.

104. Price VH. Double-blind, placebo-controlled evaluation of topical minoxidil in extensive alopecia areata. *J Am Acad Dermatol.* 1987;16(3 Pt 2):730-6.

105. Price VH. Topical minoxidil (3%) in extensive alopecia areata, including long-term efficacy. *J Am Acad Dermatol.* 1987;16(3 Pt 2):737-44.

106. Summer KH, Goggelmann W. 1-chloro-2,4-dinitrobenzene depletes glutathione in rat skin and is mutagenic in Salmonella typhimurium. *Mutat Res*. 1980;77(1):91-3.

107. Wilkerson MG, Connor TH, Henkin J, Wilkin JK, Matney TS. Assessment of diphenylcyclopropenone for photochemically induced mutagenicity in the Ames assay. *J Am Acad Dermatol*. 1987;17(4):606-11.

108. Happle R. Topical immunotherapy in alopecia areata. *J Invest Dermatol.* 1991;96(5):S71-2.

109. Happle R, Hausen BM, Wiesner-Menzel L. Diphencyprone in the treatment of alopecia areata. *Acta Derm Venereol.* 1983;63(1):49-52.

110. Ohlmeier MC, Traupe H, Luger TA, Bohm M. Topical immunotherapy with diphenylcyclopropenone of patients with alopecia areata--a large retrospective study on 142 patients with a self-controlled design. *J Eur Acad Dermatol Venereol*. 2012;26(4):503-7.

111. Singh G, Lavanya M. Topical immunotherapy in alopecia areata. *Int J Trichology.* 2010;2(1):36-9.

112. Bhat S, Handa S, De D. A randomized comparative study of the efficacy of topical latanoprost versus topical betamethasone diproprionate lotion in the treatment of localized alopecia areata. *Indian J Dermatol Venereol Leprol*. 2021;87(1):42-8.

113. Coronel-Perez IM, Rodriguez-Rey EM, Camacho-Martinez FM. Latanoprost in the treatment of eyelash alopecia in alopecia areata universalis. *J Eur Acad Dermatol Venereol*. 2010;24(4):481-5.

114. Roseborough I, Lee H, Chwalek J, Stamper RL, Price VH. Lack of efficacy of topical latanoprost and bimatoprost ophthalmic solutions in promoting eyelash growth in patients with alopecia areata. *J Am Acad Dermatol*. 2009;60(4):705-6.

115. Barton VR, Toussi A, Awasthi S, Kiuru M. Treatment of pediatric alopecia areata: A systematic review. *J Am Acad Dermatol*. 2022;86(6):1318-34.

116. Behrens-Williams SC, Leiter U, Schiener R, Weidmann M, Peter RU, Kerscher M. The PUVA-turban as a new option of applying a dilute psoralen solution selectively to the scalp of patients with alopecia areata. *J Am Acad Dermatol.* 2001;44(2):248-52.

117. Mlacker S, Aldahan AS, Simmons BJ, Shah V, McNamara CA, Samarkandy S, et al. A review on laser and light-based therapies for alopecia areata. *J Cosmet Laser Ther.* 2017;19(2):93-9.

118. Byun JW, Moon JH, Bang CY, Shin J, Choi GS. Effectiveness of 308-nm Excimer Laser Therapy in Treating Alopecia Areata, Determined by Examining the Treated Sides of Selected Alopecic Patches. *Dermatology*. 2015;231(1):70-6.

119. Zakaria W, Passeron T, Ostovari N, Lacour JP, Ortonne JP. 308-nm excimer laser therapy in alopecia areata. *J Am Acad Dermatol*. 2004;51(5):837-8.

120. Waiz M, Saleh AZ, Hayani R, Jubory SO. Use of the pulsed infrared diode laser (904 nm) in the treatment of alopecia areata. *J Cosmet Laser Ther.* 2006;8(1):27-30.

121. Sardesai VR, Prasad S, Agarwal TD. A study to evaluate the efficacy of various topical treatment modalities for alopecia areata. *Int J Trichology*. 2012;4(4):265-70.

122. Lenane P, Macarthur C, Parkin PC, Krafchik B, DeGroot J, Khambalia A, et al. Clobetasol propionate, 0.05%, vs hydrocortisone, 1%, for alopecia areata in children: a randomized clinical trial. *J Am Acad Dermatol*. 2014;150(1):47-50.

123. Fenton DA, Wilkinson JD. Topical minoxidil in the treatment of alopecia areata. *Br Med J (Clin Res Ed).* 1983;287(6398):1015-7.

124. Fiedler-Weiss VC. Topical minoxidil solution (1% and 5%) in the treatment of alopecia areata. *J Am Acad Dermatol*. 1987;16(3 Pt 2):745-8.

125. Dalloglio F, Nasca MR, Musumeci ML, La Torre G, Ricciardi G, Potenza C, et al. Topical immunomodulator therapy with squaric acid dibutylester (SADBE) is effective treatment for severe alopecia areata (AA): results of an open-label, paired-comparison, clinical trial. *J Dermatolog Treat*. 2005;16(1):10-4.

126. Ajith C, Gupta S, Kanwar AJ. Efficacy and safety of the topical sensitizer squaric acid dibutyl ester in Alopecia areata and factors influencing the outcome. *J Drugs Dermatol.* 2006;5(3):262-6.

127. Lamb RC, Young D, Holmes S. Retrospective review of diphencyprone in the treatment of alopecia areata. *Clin Exp Dermatol*. 2016;41(4):352-8.

128. Tiwary AK, Mishra DK, Chaudhary SS. Comparative Study of Efficacy and Safety of Topical Squaric Acid Dibutylester and Diphenylcyclopropenone for the Treatment of Alopecia Areata. *N Am J Med Sci*. 2016;8(6):237-42.

129. Sasmaz S, Arican O. Comparison of azelaic acid and anthralin for the therapy of patchy alopecia areata: a pilot study. *Am J Clin Dermatol*. 2005;6(6):403-6.

130. Nasimi M, Ghandi N, Abedini R, Mirshamsi A, Shakoei S, Seirafi H. Efficacy and safety of anthralin in combination with diphenylcyclopropenone in the treatment of alopecia areata: a retrospective case series. *Arch Dermatol Res*. 2019;311(8):607-13.

131. Ghandi N, Daneshmand R, Hatami P, Abedini R, Nasimi M, Aryanian Z, et al. A randomized trial of diphenylcyclopropenone (DPCP) combined with anthralin versus DPCP alone for treating moderate to severe alopecia areata. *Int Immunopharmacol*. 2021;99:107971.

132. Mohamed Z, Bhouri A, Jallouli A, Fazaa B, Kamoun MR, Mokhtar I. Alopecia areata treatment with a phototoxic dose of UVA and topical 8-methoxypsoralen. *J Eur Acad Dermatol Venereol*. 2005;19(5):552-5.

133. Tan LS, Chan MMH, Tan DJA, Lee JSS, Chong WS. Effectiveness of Paint Psoralen and Ultraviolet-A in Alopecia Areata - Our Experience in the National Skin Center. *Indian J Dermatol.* 2020;65(3):199-203.

134. Singh S. Role of platelet-rich plasma in chronic alopecia areata: Our centre experience. *Indian J Plast Surg*. 2015;48(1):57-9.

135. Ekelem C, Juhasz M, Yu J, Hosking AM, Csuka E, Choi F, et al. Monitoring Response to Platelet-Rich Plasma in Patients with Alopecia Areata with Optical Coherence Tomography: A Case Series. *J Investig Dermatol Symp Proc*. 2020;20(1):S50-4.

136. Ragab SEM, Nassar SO, Morad HA, Hegab DS. Platelet-rich plasma in alopecia areata: intradermal injection versus topical application with transepidermal delivery via either fractional carbon dioxide laser or microneedling. *Acta Dermatovenerol Alp Pannonica Adriat.* 2020;29(4):169-73.

137. Aboeldahab S, Nada EEA, Assaf HA, Gouda ZA, Abu El-Hamd M. Superficial cryotherapy using dimethyl ether and propane mixture versus microneedling in the treatment of alopecia areata: A prospective single-blinded randomized clinical trial. *Dermatol Ther.* 2021;34(5):e15044.

138. Al-Mutairi N. 308-nm excimer laser for the treatment of alopecia areata. *Dermatol Surg.* 2007;33(12):1483-7.

139. Ohtsuki A, Hasegawa T, Komiyama E, Takagi A, Kawasaki J, Ikeda S. 308-nm Excimer Lamp for the Treatment of Alopecia Areata: Clinical Trial on 16 Cases. *Indian J Dermatol.* 2013;58(4):326.

140. Kianfar N, Dasdar S, Mahmoudi H, Abedini R, Fahim S, Hosseini SA, et al. Comparison of the efficacy and safety of 308-nm excimer laser with intralesional corticosteroids for the treatment of alopecia areata: A randomized controlled study. *Lasers Surg Med*. 2022;54(4):502-10.

141. Jun M, Lee NR, Lee WS. Efficacy and safety of superficial cryotherapy for alopecia areata: A retrospective, comprehensive review of 353 cases over 22 years. *J Dermatol*. 2017;44(4):386-93.

142. El Sayed MH, Ibrahim NE, Afify AA. Superficial Cryotherapy versus Intralesional Corticosteroids Injection in Alopecia Areata: A Trichoscopic Comparative Study. *Int J Trichology.* 2022;14(1):8-13.

143. Zawar VP, Karad GM. Liquid Nitrogen Cryotherapy in Recalcitrant Alopecia Areata: A Study of 11 Patients. *Int J Trichology*. 2016;8(1):15-20.

144. Kar BR, Handa S, Dogra S, Kumar B. Placebo-controlled oral pulse prednisolone therapy in alopecia areata. *J Am Acad Dermatol.* 2005;52(2):287-90.

145. Olsen EA, Carson SC, Turney EA. Systemic steroids with or without 2% topical minoxidil in the treatment of alopecia areata. *Arch Dermatol*. 1992;128(11):1467-73.

146. Alsantali A. Alopecia areata: a new treatment plan. *Clin Cosmet Investig Dermatol.* 2011;4:107-15.

147. Pasricha JS, Kumrah L. Alopecia totalis treated with oral mini-pulse (OMP) therapy with betamethasone. *Indian J Dermatol Venereol Leprol.* 1996;62(2):106-9.

148. Joly P. The use of methotrexate alone or in combination with low doses of oral corticosteroids in the treatment of alopecia totalis or universalis. *J Am Acad Dermatol*. 2006;55(4):632-6.

149. Farshi S, Mansouri P, Safar F, Khiabanloo SR. Could azathioprine be considered as a therapeutic alternative in the treatment of alopecia areata? A pilot study. *Int J Dermatol*. 2010;49(10):1188-93.

150. Aghaei S. An uncontrolled, open label study of sulfasalazine in severe alopecia areata. *Indian J Dermatol Venereol Leprol*. 2008;74(6):611-3.

151. Strober BE, Siu K, Alexis AF, Kim G, Washenik K, Sinha A, et al. Etanercept does not effectively treat moderate to severe alopecia areata: an open-label study. *J Am Acad Dermatol.* 2005;52(6):1082-4

152. Phan K, Sebaratnam DF. JAK inhibitors for alopecia areata: a systematic review and meta-analysis. *J Eur Acad Dermatol Venereol*. 2019;33(5):850-6.

153. Rubin R. First Systemic Treatment for Severe Alopecia Is Approved. *J Am Acad Dermatol*. 2022;328(3):235.

154. Estebanez A, Estebanez N, Martin JM, Montesinos E. Apremilast in Refractory Alopecia Areata. *Int J Trichology*. 2019;11(5):213-5.

155. Kurosawa M, Nakagawa S, Mizuashi M, Sasaki Y, Kawamura M, Saito M, et al. Comparison of the efficacy, relapse rate and side effects among three modalities of systemic corticosteroid therapy for alopecia areata. *Dermatology*. 2006;212(4):361-5.

156. Sanchez-Diaz M, Montero-Vilchez T, Bueno-Rodriguez A, Molina-Leyva A, Arias-Santiago S. Alopecia Areata and Dexamethasone Mini-Pulse Therapy, A Prospective Cohort: Real World Evidence and Factors Related to Successful Response. *J Clin Med.* 2022;11(6):1694.

157. Rashidi T, Mahd AA. Treatment of persistent alopecia areata with sulfasalazine. *Int J Dermatol*. 2008;47(8):850-2.

158. Kennedy Crispin M, Ko JM, Craiglow BG, Li S, Shankar G, Urban JR, et al. Safety and efficacy of the JAK inhibitor tofacitinib citrate in patients with alopecia areata. *JCI Insight.* 2016;1(15):e89776.

159. Mackay-Wiggan J, Jabbari A, Nguyen N, Cerise JE, Clark C, Ulerio G, et al. Oral ruxolitinib induces hair regrowth in patients with moderate-to-severe alopecia areata. *JCI Insight*. 2016;1(15):e89790.

160. Saoji V, Kulkarni S, Madke B. Alopecia Areata Treated with Oral Azathioprine: A Case Series. *Int J Trichology*. 2019;11(5):219-22.

161. Lai VWY, Chen G, Gin D, Sinclair R. Cyclosporine for moderate-to-severe alopecia areata: A double-blind, randomized, placebo-controlled clinical trial of efficacy and safety. *J Am Acad Dermatol.* 2019;81(3):694-701.

162. Mikhaylov D, Pavel A, Yao C, Kimmel G, Nia J, Hashim P, et al. A randomized placebo-controlled single-center pilot study of the safety and efficacy of apremilast in subjects with moderate-to-severe alopecia areata. *Arch Dermatol Res*. 2019;311(1):29-36.

163. Nowaczyk J, Makowska K, Rakowska A, Sikora M, Rudnicka L. Cyclosporine with and Without Systemic Corticosteroids in Treatment of Alopecia Areata: A Systematic Review. *Dermatol Ther (Heidelb)*. 2020;10(3):387-99.

164. Dincer Rota D, Emeksiz MAC, Erdogan FG, Yildirim D. Experience with oral tofacitinib in severe alopecia areata with different clinical responses. *J Cosmet Dermatol.* 2021;20(9):3026-33.

165. King B, Ko J, Forman S, Ohyama M, Mesinkovska N, Yu G, et al. Efficacy and safety of the oral Janus kinase inhibitor baricitinib in the treatment of adults with alopecia areata: Phase 2 results from a randomized controlled study. *J Am Acad Dermatol*. 2021;85(4):847-53.

166. Kinoshita-Ise M, Sachdeva M, Martinez-Cabriales SA, Shear NH, Lansang P. Oral Methotrexate Monotherapy for Severe Alopecia Areata: A Single Center Retrospective Case Series. *J Cutan Med Surg*. 2021;25(5):490-7.

167. Fawzy MM, Abdel Hay R, Mohammed FN, Sayed KS, Ghanem MED, Ezzat M. Trichoscopy as an evaluation method for alopecia areata treatment: A comparative study. *J Cosmet Dermatol.* 2021;20(6):1827-36.

168. Tan E, Tay YK, Goh CL, Chin Giam Y. The pattern and profile of alopecia areata in Singapore--a study of 219 Asians. *Int J Dermatol*. 2002;41(11):748-53.

169. McMichael AJ, Pearce DJ, Wasserman D, Camacho FT, Fleischer AB Jr, Feldman SR, et al. Alopecia in the United States: outpatient utilization and common prescribing patterns. *J Am Acad Dermatol*. 2007;57(2 Suppl):S49-51.

170. Gilhar A, Etzioni A, Paus R. Alopecia areata. *N Engl J Med*. 2012;366(16):1515-25.

171. Tosti A, Morelli R, Bardazzi F, Peluso AM. Prevalence of nail abnormalities in children with alopecia areata. *Pediatr Dermatol*. 1994;11(2):112-5.

172. Sharma VK, Dawn G, Muralidhar S, Kumar B. Nail changes in 1000 Indian patients with alopecia areata. *J Eur Acad Dermatol Venereol*. 1998;10(2):189-91.

173. Sharma VK, Kumar B, Dawn G. A clinical study of childhood alopecia areata in Chandigarh, *India. Pediatr Dermatol.* 1996;13(5):372-7.

174. Abell E, Munro DD. Intralesional treatment of alopecia areata with triamcinolone acetonide by jet injector. *Br J Dermatol*. 1973;88(1):55-9.

175. Zgaga L, Laird E, Healy M. 25-Hydroxyvitamin D Measurement in Human Hair: Results from a Proof-of-Concept study. *Nutrients vol.* 2019;11(2):423.

ANNEXURES

ANNEXURE-I

INSTITUTIONAL ETHICS CERTIFICATE

अखिल भारतीय आयुर्विज्ञान संस्थान, जोधपुर
All India Institute of Medical Sciences, Jodhpur
संस्थागत नैतिकता समिति
Institutional Ethics Committee

No. AIIMS/IEC/2021/3554 **Date: 12/03/2021**

ETHICAL CLEARANCE CERTIFICATE

Certificate Reference Number: **AIIMS/IEC/2021/3389**

Project title: **"Comparative study of efficacy of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata: Randomised single blinded clinical trial"**

Nature of Project:	**Research Project Submitted for Expedited Review**
Submitted as:	**M.D. Dissertation**
Student Name:	**Dr. Thoyyib Parammal Karat**
Guide:	**Dr. Anupama Bains**
Co-Guide:	**Dr. Abhishek Bhardwaj, Dr. Saurabh Singh, Dr. Anil Budania & Dr. Suman Patra**

Institutional Ethics Committee after thorough consideration accorded its approval on above project.

The investigator may therefore commence the research from the date of this certificate, using the reference number indicated above.

Please note that the AIIMS IEC must be informed immediately of:

- Any material change in the conditions or undertakings mentioned in the document.
- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research.
- In case of any issue related to compensation, the responsibility lies with the Investigator and Co-Investigators.

The Principal Investigator must report to the AIIMS IEC in the prescribed format, where applicable, bi-annually, and at the end of the project, in respect of ethical compliance.

AIIMS IEC retains the right to withdraw or amend this if:

- Any unethical principle or practices are revealed or suspected
- Relevant information has been withheld or misrepresented

AIIMS IEC shall have an access to any information or data at any time during the course or after completion of the project.

Please Note that this approval will be rectified whenever it is possible to hold a meeting in person of the Institutional Ethics Committee. It is possible that the PI may be asked to give more clarifications or the Institutional Ethics Committee may withhold the project. The Institutional Ethics Committee is adopting this procedure due to COVID-19 (Corona Virus) situation.

If the Institutional Ethics Committee does not get back to you, this means your project has been cleared by the IEC. On behalf of Ethics Committee, I wish you success in your research.

Dr. Praveen Sharma
Member Secretary

Member secretary
Institutional Ethics Committee
AIIMS,Jodhpur

Basni Phase-2, Jodhpur, Rajasthan-342005; **Website:** www.aiimsjodhpur.edu.in; **Phone:** 0291-2740741 Extn. 3109
E-mail : ethicscommittee@aiimsjodhpur.edu.in; ethicscommitteeaiimsjdh@gmail.com

ANNEXURE-II

PROFORMA

Patient Name: ……. AIIMS ID: ……...

Age/ Sex: …… Address: …….

Father's name: ……. Mob NO: ……

Occupation: …….

History:

Presenting complaints: ……

……..

1. Age of onset:

2. Total duration of illness:

□< 6 months □ 6-12 months □ 1-2 years □>2 years

3. H/O diabetes/ hypertension/ tuberculosis/ thyroid illness: □ Yes □ No

If, yes→ Specify: Duration: Treatment taken:

4. H/O associated autoimmune diseases:□ Yes □ No If, yes→ Specify:

5. H/O atopy:

6. H/O scalp/beard infections:

7. H/O fever/ stress/ any other illness in the immediate past:

8. LMP, UPT or Lactating mother:

9. Family history:

`□ Alopecia areata □ Atopy □other autoimmune disease □ Nil significant

Details:

10. H/O any other cutaneous illness: □ Yes □ No

If, yes Details: Duration:

INVESTIGATIONS:

RBS level: ……...

TSH level: ……

Other investigations: …….

Treatment History:

S.NO	TREATMENT DETAILS	DURATION	RESPONSE

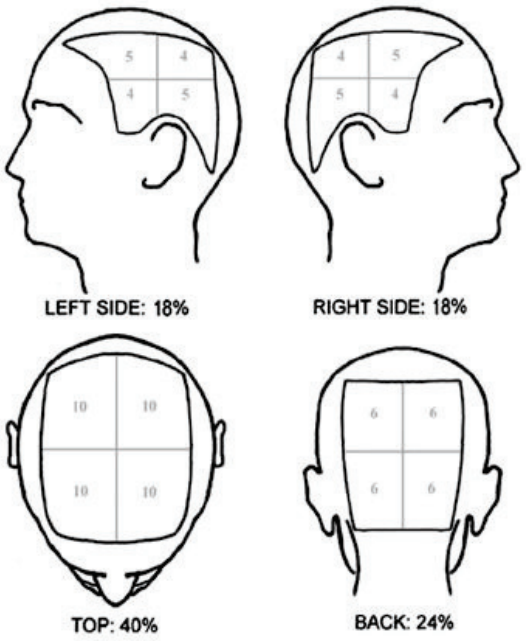
Examination:

General physical examination:

Height: cm Weight: kg BMI:

Pallor/ Icterus/Clubbing/ Cyanosis/ Pedal edema/ Generalized lymphadenopathy

Examination of scalp:



Total Number of alopecia patches:
SALT score:
Number of patches in each quadrant:

1. Top side:
2. Back side:
3. Right side:
4. Lest side:

Number of patches >1.0 x 1.0cm (with site of patches):

Examination of Nail:

- **Nail pitting:** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

- **Longitudinal ridges:** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

- **Trachyonychia (sand paper nails):** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

- **Leukonychia:** □ Yes □ No

  If, yes→ Distribution: Type:

- **Splitting of nail plate:** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

- **Onycholysis:** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

- **Onychomadesis (Periodic shedding):** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

- **Red/mottled Lunulae:** □ Yes □ No

  If, yes→ Distribution: Pattern (if any):

PATCH NO		**AT BASELINE**	**4 WEEKS**	**8 WEEKS**	**12 WEEKS**
	CLINICAL				
	SIDE EFFECTS				
	CLINICAL				
	SIDE EFFECTS				
	CLINICAL				
	SIDE EFFECTS				

TRICHOSCOPIC FINDINGS

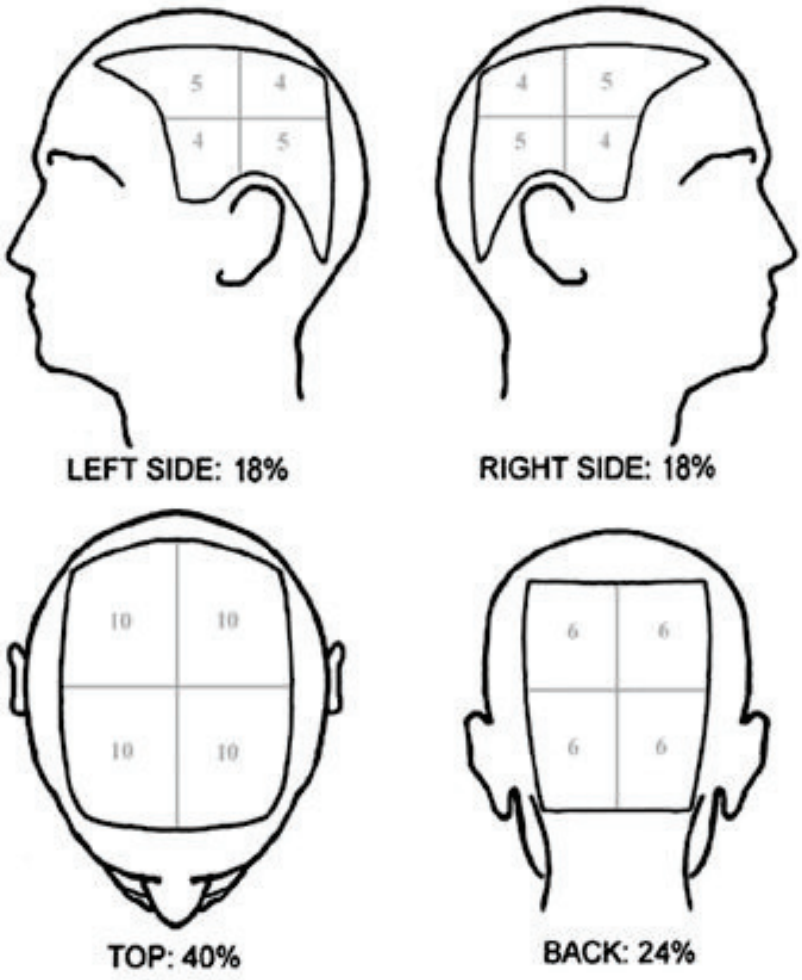
Patch No		Yellow dots	Black dots	Exclamation mark hairs	Tapered hairs	Broken hairs	Short vellus hairs	Upright regrowing hairs	Pigtail hairs	Pohl-Pinkus constrictions
	At Baseline									
	4 weeks									
	8 weeks									
	12 weeks									
	At Baseline									
	4 weeks									
	8 weeks									
	12 weeks									

HAIR REGROWTH SCORE (RGS) AND SALT SCORE

Patch number	At Baseline		4 weeks		8 weeks		12 weeks	
	RGS	SALT	RGS	SALT	RGS	SALT	RGS	SALT

ANNEXURE-III

SALT SCORE FOR SEVERITY OF ALOPECIA



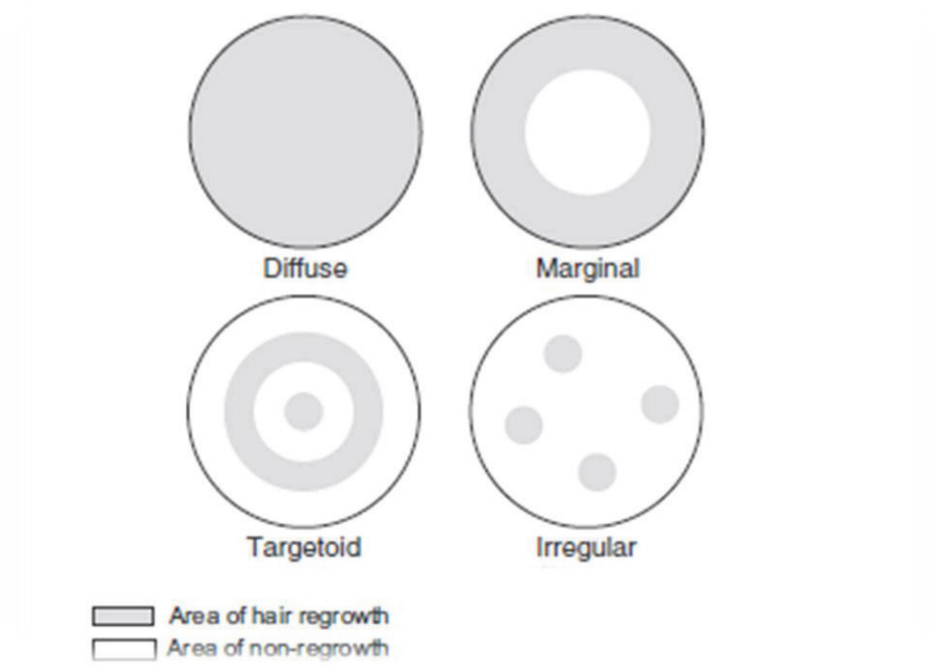
ANNEXURE-IV

HAIR REGROWTH SCORE (RGS)

Regrowth	Score
<10%	1
11-25%	2
26-50%	3
51-75%	4
>75%	5

ANNEXURE-V

DIMT CLASSIFICATION OF HAIR REGROWTH PATTERNS



ANNEXURE-VI

INFORMED CONSENT FORM (ENGLISH)

All India Institute of Medical Sciences Jodhpur, Rajasthan

Title of Thesis/Dissertation: **Comparative study of efficacy of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata -randomised single blinded clinical trial**

Name of PG Student : **Dr. Thoyyib Parammal Karat**

Tel. No. : **9650945497**

Patient/Volunteer Identification No. :

I, ……. S/o or D/o …….

R/o ……give my full, free, voluntary consent to be a part of the study "**Comparative study of efficacy of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata -randomised single blind clinical trial**", the procedure and nature of which has been explained to me in my own language to my full satisfaction. I confirm that I have had the opportunity to ask questions.

I understand that my participation is voluntary and I am aware of my right to opt out of the study at any time without giving any reason.

I understand that the information collected about me and any of my medical records may be looked at by responsible individual from _____ (Company Name) or from regulatory authorities. I give permission for these individuals to have access to my records.

Date: _____ _____

Place: _____ Signature/Left thumb impression

(If minor, Parent/Guardian signature)

This to certify that the above consent has been obtained in my presence.

Date: _____ _____

Place: _____ Signature of PG Student

1. Witness 1

_____

Signature

Name: _____

Address: _____

2. Witness 2

_____

Signature

Name: _____

Address: _____

ANNEXURE-VII

INFORMED CONSENT FORM (HINDI)

अखिल भारतीय आयुर्विज्ञान संस्थान जोधपुर, राजस्थान

शोध का शीर्षक: "**एलोपेसिया अराटा के उपचार में इंट्रालेशनल ट्रायमिनोलोन एसेटोनाइड के साथ सामयिक कैल्सिपोट्रीओल बनाम इंट्रालेशनल ट्रायमिनोलोन एसिटोनाइड अकेले का प्रभावकारिता का तुलनात्मक अध्ययन: एक एकल ब्लायंडेड राँडोमिज़ेड इंटेरवेंशनल अध्ययन**"

पीजी छात्र का नाम: डॉ. थोयिब परमाल कराट

मोबाइल नंबर: **9650945497**

रोगी / स्वयंसेवी पहचान संख्या: _____

मैं, _____ पुत्र/ पुत्री_____

_____ का निवासी उपरोक्तअध्ययन "एलोपेसिया अराटा के उपचार में इंट्रालेशनल ट्रायमिनोलोन एसेटोनाइड के साथ सामयिक कैल्सिपोट्रीओल बनाम इंट्रालेशनल ट्रायमिनोलोन एसिटोनाइड अकेले का प्रभावकारिता का तुलनात्मक अध्ययन: एक एकल ब्लायंडेड राँडोमिज़ेड इंटेरवेंशनल अध्ययन" का एक हिस्सा बनने के लिए मेरी पूर्ण, स्वतंत्र, स्वैच्छिक सहमति देता हूँ।

जिस प्रक्रियाऔर प्रकृति को मुझेअपनी पूरी संतुष्टि के लिए अपनी भाषा में समझाया गया है मैं पुष्टि करता हूं कि मुझे प्रश्न पूछने का अवसर मिला है।

मैं समझता हूं कि मेरी भागीदारी स्वैच्छिक है और मुझे किसी भी कारण दिए बिना किसी भी समय अध्ययन से बाहर निकलनेका मेराअधिकार है।

मैं समझता हूं कि मेरेऔर मेरे मेडिकल रिकॉर्ड के बारे में एकत्रित की गई जानकारी को याविनियामक प्राधिकरणों से जिम्मेदार व्यक्ति द्वारा देखा जा सकता है।मैं इनलोगों के लिए मेरे रिकॉर्डों तक पहुंचकी अनुमति देता हूं।

तारीख : _____ _____

जगह: _____ हस्ताक्षर / बाएंअंगूठेकाछाप

(नाबालिगकि, माता-पिता / अभिभावक हस्ताक्षर)

यह प्रमाणित करने के लिए कि मेरी उपस्थिति में उपरोक्त सहमति प्राप्त की गई है

तारीख : _____ _____

जगह: _____ पीजीछात्रकेहस्ताक्षर

1. गवाह 1 2. साक्षी 2

_____ _____

हस्ताक्षर हस्ताक्षर

नाम: _____ नाम: _____

पता: _____ पता: _____

ANNEXURE-VIII

PATIENT INFORMATION SHEET (ENGLISH)

All India Institute of Medical Sciences Jodhpur, Rajasthan

This document has been given to provide more information about the disease and this research related to alopecia areata.

The current research project is titled: **"Comparative study of efficacy of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide alone in the treatment of alopecia areata -randomised single blinded clinical trial".**

Alopecia areata is a non-infectious disease affecting scalp or beard leading to hair loss. Various treatment options are available, but none of them is found to be fully effective. In this research, we would like to see the result of intralesional triamcinolone acetonide with topical calcipotriol versus intralesional triamcinolone acetonide only. The average duration of the study is likely to be 3 months. During this time, the patient is expected to come every 4 weeks and meet the doctor for evaluation. The patient is also expected to take his injections from the doctor every 4 week.

Intralesional Triamcinolone acetonide may have some side effects as thinning of the skin & appearance of fine blood vessels in the skin. Topical Calcipotriol also may have some side effects like drying of skin and itching at the site of application. Most of these effects are transient and reversible.

All the information given by you will be kept confidential. You also reserve the right that during this research, you can withdraw the consent & can be out of this research without explaining the reasons.

Principle investigator: **Dr.Thoyyib Parammal Karat** Contact number: **9650945497**

ANNEXURE-IX

PATIENT INFORMATION SHEET (HINDI)

अखिल भारतीय आयुर्विज्ञान संस्थान जोधपुर, राजस्थान

यह दस्तावेज़ रोग के बारे में अधिक जानकारी और एलोपेसिया अराटा से संबंधित इस शोध को प्रदान करने के लिए दिया गया है।

वर्तमान रीसर्च प्रोजेक्ट का शीर्षक है: **"एलोपेसिया अराटा के उपचार में इंट्रालेशनल ट्रायमिनोलोन एसेटोनाइड के साथ सामयिक कैल्सिपोट्रीओल बनाम इंट्रालेशनल ट्रायमिनोलोन एसिटोनाइड अकेले का प्रभावकारिता का तुलनात्मक अध्ययन: एक एकल ब्लायंडेड राँडोमिज़ेड इंटेरवेंशनल अध्ययन"**

एलोपेसिया अरेटा एक गैर संक्रामक रोग है, जो खोपड़ी या दाढ़ी को प्रभावित करता है, जिससे बाल झड़ने लगते हैं । विभिन्न उपचार विकल्प उपलब्ध हैं । लेकिन उनमें से कोई भी पूरी तरह से प्रभावी नहीं पाया जाता है । इस शोध में, हम केवल इंट्रालेशनल ट्रायमिनोलोन एसेटोनाइड के साथ सामयिक कैल्सिपोट्रीओल बनाम इंट्रालेशनल ट्रायमिनोलोन एसिटोनाइड अकेले का परिणाम देखना चाहेंगे। अध्ययन की औसत अवधि 3 महीने होने की संभावना है । इस समय के दौरान, मूल्यांकन के लिए रोगी हर 4 सप्ताह में आने और डॉक्टर से मिलने की उम्मीद है । रोगी को हर 4 सप्ताह में डॉक्टर से अपने इंजेक्शन लेने की उम्मीद है । आपको प्री ट्रीटमेंट ब्लड जांच कराने की भी उम्मीद है ।

इस इंजेक्शन के कुछ दुष्प्रभाव होते हैं, जैसे त्वचा की पतली और त्वचा में ठीक रक्त वाहिकाओं की उपस्थिति। सामयिक कैल्सिपोट्रीओल के भी कुछ दुष्प्रभाव होते हैं, जैसे आवेदन स्थल पर त्वचा का सूखना और खुजली। इनमें से अधिकांश प्रभाव क्षणिक और प्रतिवर्ती हैं।

आपके द्वारा दी गई सभी जानकारी को गोपनीय रखा जाएगा। आप इस अधिकार को भी सुरक्षित रखते हैं कि इस रीसर्च के दौरान, आप बिना कारण बताए सहमति को वापस ले सकते हैं और इस रीसर्च से बाहर हो सकते हैं।

सिद्धांत अन्वेषक: डॉ. थोयिब परमाल कराट संपर्क नंबर: ९६५०९४५४९७

ANNEXURE-X

MASTER CHART WITH IMPORTANT KEYWORDS

Sl.No	VARIABLE	CODING
1	**Site of lesion**	Scalp-1 Beard-2
2	**Sex**	Male-1 Female-2
3	**Occupation**	Student-1 Housewife-2 Business-3 Farmer-4 Professional-5 Govt. employee-6 Skilled worker-7
4	**Total duration of illness**	<6 months- 1 6month to 1 year- 2 1 to 2 years- 3 >2 years- 4
5	**Presence of autoimmune disease**	Yes-1 No-2
6	**History of atopy**	Yes-1 No-2
7	**History of scalp or beard infection**	Yes-1 No-2
8	**Presence of comorbidities**	Yes-1 No-2
9	**Presence of DM**	Yes-1 No-2
10	**Presence of HTN**	Yes-1 No-2
11	**Presence of TB**	Yes-1 No-2
12	**Presence of thyroid illness**	Yes-1 No-2
13	**History of any immediate illness**	Yes-1 No-2
14	**Immediate illnesses**	Covid 19-1 Not applicable-99
15	**Family history of cutaneous illness**	Yes-1 No-2
16	**Family history of AA**	Yes-1 No-2
17	**Family history of atopy**	Yes-1 No-2
18	**Family history of AI diseases**	Yes-1 No-2

19	**History of other cutaneous illness**	Yes-1 No-2
20	**Details of other cutaneous illness**	Tinea-1 Psoriasis-2 Dermatofibroma-3 Verruca-4 Not applicable-99
21	**BMI category**	<18.5 -1 18.5 to 22.9 -2 23 to 24.9 -3 25 to 29.9 -4 >30 -5
22	**Treatment history**	Yes-1 No-2
23	**Treatment history with oral steroids**	Yes-1 No-2
24	**Treatment history with intralesional steroids**	Yes-1 No-2
25	**Treatment history with topical steroids**	Yes-1 No-2
26	**Treatment history with minoxidil**	Yes-1 No-2
27	**Presence of nail changes**	Yes-1 No-2
28	**Nail pitting**	Yes-1 No-2
29	**Longitudinal ridges**	Yes-1 No-2
30	**Trachonychia**	Yes-1 No-2
31	**Leukonychia**	Yes-1 No-2
32	**splitting**	Yes-1 No-2
33	**Onycholysis**	Yes-1 No-2
34	**Onychomadesis**	Yes-1 No-2
35	**Red lunula**	Yes-1 No-2
36	**Presence of treatment activity at baseline**	Yes-1 No-2
37	**Presence of treatment activity at 4 weeks**	Yes-1 No-2
38	**Presence of treatment activity 8 weeks**	Yes-1 No-2
39	**Presence of treatment activity 12 weeks**	Yes-1 No-2
40	**Presence of yellow dotes at baseline**	Yes-1 No-2
41	**Presence of yellow dotes at 4 weeks**	Yes-1 No-2
42	**Presence of yellow dotes at 8 weeks**	Yes-1 No-2
43	**Presence of yellow dotes at 12 weeks**	Yes-1 No-2
44	**Presence of black dotes at baseline**	Yes-1 No-2

45	**Presence of black dotes at 4 weeks**	Yes-1 No-2
46	**Presence of black dotes at 8weeks**	Yes-1 No-2
47	**Presence of black dotes at 12 weeks**	Yes-1 No-2
48	**Presence of exclamation mark hair at baseline**	Yes-1 No-2
49	**Presence of exclamation mark hair at 4 weeks**	Yes-1 No-2
50	**Presence of exclamation mark hair at 8 weeks**	Yes-1 No-2
51	**Presence of exclamation mark hair at 12 weeks**	Yes-1 No-2
52	**Presence of tapered hair at baseline**	Yes-1 No-2
53	**Presence of tapered hair at 4 weeks**	Yes-1 No-2
54	**Presence of tapered hair at 8 weeks**	Yes-1 No-2
55	**Presence of tapered hair at 12 weeks**	Yes-1 No-2
56	**Presence of broken hair at baseline**	Yes-1 No-2
57	**Presence of broken hair at 4 weeks**	Yes-1 No-2
58	**Presence of broken hair at 8 weeks**	Yes-1 No-2
59	**Presence of broken hair at 12 weeks**	Yes-1 No-2
60	**Presence of short vellus hair at baseline**	Yes-1 No-2
61	**Presence of short vellus hair at 4 weeks**	Yes-1 No-2
62	**Presence of short vellus hair at 8 weeks**	Yes-1 No-2
63	**Presence of short vellus hair at 12 weeks**	Yes-1 No-2
64	**Presence of upright regrowing hair at baseline**	Yes-1 No-2
65	**Presence of upright regrowing hair at 4 weeks**	Yes-1 No-2
66	**Presence of upright regrowing hair at 8 weeks**	Yes-1 No-2
67	**Presence of upright regrowing hair at 12 weeks**	Yes-1 No-2
68	**Presence of pigtail hair at baseline**	Yes-1 No-2
69	**Presence of pigtail hair at 4 weeks**	Yes-1 No-2
70	**Presence of pigtail hair at 8 weeks**	Yes-1 No-2
71	**Presence of pigtail hair at 12 weeks**	Yes-1 No-2
72	**Presence of pohl pinkus constriction at baseline**	Yes-1 No-2
73	**Presence of pohl pinkus constriction at 4 weeks**	Yes-1 No-2
74	**Presence of pohl pinkus constriction at 8 weeks**	Yes-1 No-2
75	**Presence of pohl pinkus constriction at 12 weeks**	Yes-1 No-2

76	**Regrowth score**	<10% -0 11 to 25% -1 26 to 50% -2 51 to75% -3 >75% -4
77	**Presence of 50% regrowth score at baseline**	Yes-1 No-2
78	**Presence of 50% regrowth score at 4 weeks**	Yes-1 No-2
79	**Presence of 50% regrowth score at 8 weeks**	Yes-1 No-2
80	**Presence of 50% regrowth score at 12 weeks**	Yes-1 No-2
81	**Regrowth type- Diffuse**	Yes-1 No-2
82	**Regrowth type- Irregular**	Yes-1 No-2
83	**Regrowth type- Marginal**	Yes-1 No-2
84	**Regrowth type- Targetoid**	Yes-1 No-2
85	**Presence of side effects**	Yes-1 No-2
86	**Presence of itching at baseline**	Yes-1 No-2
87	**Presence of itching at 4 weeks**	Yes-1 No-2
88	**Presence of itching at 8 weeks**	Yes-1 No-2
89	**Presence of itching at 12 weeks**	Yes-1 No-2
90	**Presence of dryness at baseline**	Yes-1 No-2
91	**Presence of dryness at 4 weeks**	Yes-1 No-2
92	**Presence of dryness at 8 weeks**	Yes-1 No-2
93	**Presence of dryness at 12 weeks**	Yes-1 No-2
94	**Presence of burning sensation at baseline**	Yes-1 No-2
95	**Presence of burning sensation at 4 weeks**	Yes-1 No-2
96	**Presence of burning sensation at 8 weeks**	Yes-1 No-2
97	**Presence of burning sensation at 12 weeks**	Yes-1 No-2
98	**Presence of atrophy at baseline**	Yes-1 No-2
99	**Presence of atrophy at 4 weeks**	Yes-1 No-2
100	**Presence of atrophy at 8 weeks**	Yes-1 No-2
101	**Presence of atrophy at 12 weeks**	Yes-1 No-2
102	**Presence of redness at baseline**	Yes-1 No-2
103	**Presence of redness at 4 weeks**	Yes-1 No-2

104	**Presence of redness at 8 weeks**	Yes-1 No-2
105	**Presence of redness at 12 weeks**	Yes-1 No-2
106	**Vellus hair at baseline**	Yes-1 No-2
107	**Terminal hair at baseline**	Yes-1 No-2
108	**Mixed hair at baseline**	Yes-1 No-2
109	**Vellus hair at 4 weeks**	Yes-1 No-2
110	**Terminal hair at 4 weeks**	Yes-1 No-2
111	**Mixed hair at 4 weeks**	Yes-1 No-2
112	**Vellus hair at 8 weeks**	Yes-1 No-2
113	**Terminal hair at 8 weeks**	Yes-1 No-2
114	**Mixed hair at 8 weeks**	Yes-1 No-2
115	**Vellus hair at 12 weeks**	Yes-1 No-2
116	**Terminal hair at 12 weeks**	Yes-1 No-2
117	**Mixed hair at 12 weeks**	Yes-1 No-2

ANNEXURE-XI

MASTER CHART