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Comparative trail on efficacy of topical contact immunotherapy with dinitrochlorobenzene and diphenylcyclopropenone in alopecia areata

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Abstract

Alopecia areata is a common chronic inflammatory disorder of hair characterized by non-scarring type of hair loss involving scalp, face and other areas of the skin. It can be classified as localized alopecia areata with patchy areas of hair loss, alopecia total is involving the diffuse scalp and alopecia universal is involving the entire body. Extensive and recalcitrant type of AA can cause significant challenge to treating physician. Alopecia areata is a self-limiting disorder. It can be managed with topical corticosteroids, anthralin and minoxidil. Active and extensive cases of AA can be managed with oral corticosteroids, cyclosporine, azathioprine, methotrexate and photochemotherapy. Topical immunotherapy is an effective modality yet underexplored treatment option available for managing resistant and recalcitrant cases of AA. In this study we have compared two topical immunotherapeutic agents; dinitrochlorobenzene (DNCB) and diphenylcyclopropenone (DPCP) in the treatment of alopecia areata. A total of 30 patients with alopecia areata who had given written consent to undergo therapy with DNCB and DPCP were included in the study. They were randomly categorized into two groups namely DNCB group received DNCB and the second group was called DPCP group received DPCP. Initial sensitization testing was done over on inner arm with 2% solution which induces inflammatory changes of allergic contact dermatitis. After positive inflammatory changes at the sensitization site, DNCB/DPCP was applied to patients in increasing serial concentrations on weekly basis starting from 0.001% upto 2%. Inflammatory changes usually occurred after 24 to 48 hours. The patients were followed every week for six months. Treatment outcome of both the groups were compared at the end of six months. The desirable regrowth of hair (patchy or complete regrowth of terminal hairs; grade III and IV) was found to be 86.7% in DPCP group when compared to 33.3% in DNCB group. DPCP is found to be more effective in managing chronic treatment failure and recalcitrant cases of alopecia areata than DNCB from our study. Thick pigmented anagen hairs which were more persistent were achieved in DPCP group than DNCB group. To conclude DPCP is more effective in the management of AA with lesser side effects when compared to DNCB. Overall both topical immunotherapeutic agents can produce hair growth but DPCP is more tolerated with more response rates.

Keywords: Alopecia areata, topical immunotherapy, dinitrochlorobenzene, diphenylcyclopropenone

Introduction

Alopecia Areata (AA) is a common form of chronic inflammatory non scarring hair loss characterized by patchy localized or diffused hair loss on scalp or any other area of the body [1, 2].

The prevalence of AA is 0.7% of all dermatology cases in India [3]. Lifetime risk for occurrence of AA is 2%. Most of the cases develop AA before 40 years of age. Alopecia areata is further classified as localised alopecia areata, alopecia total is and alopecia universalis, representing patchy, full scalp and full body hair loss respectively [4]. Comorbid conditions that are commonly associated with AA are atopy, thyroid autoimmunity, vitamin D deficiency [5, 6]. Although most of the cases of AA are self limiting, few cases are extensive and recalcitrant. They cause significant challenge to the treating physician by their poor response to conventional treatment.

The pathogenesis of AA is still incompletely understood. Genetic, environmental and autoimmune are commonly associated with AA. Immune privilege of hair follicle is lost in AA [7].

Peribulbar inflammation which affects the hair follicles results in thin dystrophic hair with miniaturization [8].

Histopathology of AA is variable. In acute stage, peribulbar lymphocytes surround the terminal hair with 'swarm of bees appearance'. In sub-acute stage, there is an increase in catagen and telogen hairs with decrease in anagen hairs. In the chronic stage, there are decreased terminal and increased miniaturized hairs. In the recovery stage, there is no inflammation and the number of terminal anagen hairs are increased gradually [3].

Clinically, based upon site; alopecia areata is classified into AA of beard, bodyhairs, eyebrows, eyelashes, nails. Based upon pattern they are divided into diffuse, ophiasis, patchy, perinevoid, reticular and sialopho. Based on extent they are classified into circumscripta, diffuse, incognito, subtotalis, totalis and universalis.

Diagnosis is primarily based on the clinical appearance. Biopsy is necessary in doubtful cases to differentiate AA from early scarring alopecia [2]. Potassium hydroxide mount, fungal culture, serology for lupus erythematosus and syphilis maybe necessary in difficult diagnosis. Trichogram reveals a mixed telogen dystrophic pattern. Presence of exclamation mark hairs at periphery, positive hair pull test (>6 hairs), daily hair count (>100hairs) and hair pluck test (more telogen hairs) suggest active disease [9]. Trichoscopy in alopecia areata reveal black dots, exclamation hairs, coudability hairs, broken hairs, clustered vellus hairs, hort vellus hairs, yellow dots, zig-zag hairs, Pohl-pinkus constrictions and tulip hairs [10].

Treatment options for AA include topical, systemic and photo-chemotherapy. Topical corticosteroids like fluocinolonone acetonide cream, betamethasone valerate foam, betamethasone dipropionate lotion, clobetasol ointment/foam are commonly used with a success range of 28.5% - 61% [3]. Other topical agents are anthralin (0.5-1% cream) as short contact therapy for three to six months, minoxidil 5% solution with twice-daily application, topical immunotherapy with contact sensitizers like dinitrochlorobenzene, diphenylcyclopropenone and squaric acid dibutylester; Prostaglandin analogues like latanoprost and bimatoprost as they cause hypertrichosis [3]. Intralesional corticosteroids are very effective in managing localised cases of AA.

Systemic therapy for AA [11] include oral corticosteroids either as daily dosing or as oral mini pulse therapies. Other drugs include sulfasalazine, azathioprine, cyclosporine and methotrexate. Photo-chemotherapy options include all types of PUVA (oral PUVA, topical PUVA, local or whole body UVA irradiation) [3, 11].

Topical immunotherapy is defined as induction and periodic elicitation of allergic contact dermatitis by applying potent contact allergens into the affected skin in cases of AA. A contact sensitisation is induced and a mild contact eczema is maintained by weekly applications of potent contact allergens. Topical sensitizers serve as haptens for allergic contact dermatitis. An ideal therapeutic topical sensitizer should be safe to use, it should be absent from natural environment and it should have little potential to induce cross sensitization with other substances. The contact sensitizers used in the treatment of AA include dinitrochlorobenzene (DNCB), diphenylcyclopropenone (DPCP) and squaric acid dibutylester (SABDE).

Topical immunotherapy is being considered as a first option for patients with severe AA (50 % scalp involvement) or

chronic relapsing AA. In alopecia areata, there is decrease in CD4 to CD8 lymphocyte ratio; which changes from 4:1 to 1:1 after contact immunotherapy. Suppressor T cells are produced by allergic reaction that non-specifically inhibit the autoimmune reaction against a hair follicle [14]. After initial sensitization in the first week at a distant site, weekly applications of the contact sensitizer is applied to the diseased area starting with lowest concentrations. Aim is to obtain mild to moderate eczema for which we need to apply the sensitizers in increasing concentrations in the successive weeks. Once after obtaining eczema, it should be maintained by applying the last lowest dose applied. This immune deviation which occurs after application of topical contact sensitizers help in hair regrowth. Hence we thought to consider the less looked treatment for alopecia areata and to bring its usefulness in treating and obtaining desirable results in AA.

Aim and Objectives

To evaluate the efficacy of topical dinitrochlorobenzene (DNCB) and diphenylcyclopropenone (DPCP) in the treatment for alopecia areata.

Materials and Methods

Our study was an open labeled prospective interventional comparative study conducted in the department of dermatology in a tertiary care centre in Tamilnadu, India. The study was done between October 2019 and September 2021 after obtaining institutional ethical committee clearance. Thirty patients who presented with alopecia areata attending dermatology out-patient department were included in the study. The patients were divided into 2 groups randomly, comprising of 15 patients in each group and the therapy was initiated. Patients in group A were with topical dinitrochlorobenzene and those in group B with topical diphenylcyclopropenone.

All fresh untreated cases of alopecia areata irrespective of age, sex were included in the study. Those with treatment failure and refractory cases were also included. All patients who were on regular treatment for AA, were advised to stop treatment for eight weeks and then were included in the study. Patients who had given written consent and willing for follow-up were included. The exclusion criteria were patient those who were not willing to sign the consent or failed to come for regular follow-up, patients who developed severe allergic and irritant reaction to DNCB and DPCP; pregnant and lactating females and those who were on immunosuppressive therapy.

During the initial visit, after obtaining relevant history, thorough general and dermatological examination was done. Detailed explanation about the procedure was done in the regional language, Patient was explained about the sensitivity testing, frequency, duration, possible side effects and prognosis of the treatment. An informed consent was obtained and routine blood investigations and dermoscopic evaluation of the lesions were done.

Preparation of various concentration of DNCB and DPCP were done by dissolving DNCB and DPCP powder in acetone and diluting it to appropriate concentrations of 0.001%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5% and 2% solutions. Solution were stored in dark bottles until to be used in room temperature. Sensitization was done by applying 2% DNCB/DPCP in acetone solution to a 1×1cm area over upper arm using cotton buds during the first visit.

Weekly applications of DNCB/DPCP was be done starting with lowest concentration (0.001%) to the affected area of hair loss. Patients were be advised to avoid washing the area and protect it from sunlight for 48 hours. Applications of DNCB/DPCP were repeated weekly with increasing concentrations. Aim was be to produce moderate eczema. Mild to moderate eczema will be maintained by titrating the DNCB/DPCP concentrations. The patients were advised to inform if any side effects like erythema, blister, lymphadenopathy, severe burning, urticaria, hyperpigmentation during the treatment period. If there was no sensitivity after 12 weeks, it was considered as treatment failure and the patient was withdrawn from the study.

Dermoscopic and trichoscopic assessments were carried out to assess the hair growth. The clinical response after 6 months of treatment was rated as per grading system proposed by McDonald Hull and Norris. Grade 1 - Regrowth of vellus hair; Grade 2 – Regrowth of sparse pigmented terminal hair; Grade 3 – Regrowth of terminal hair with patches of alopecia and Grade 4 – Regrowth of terminal hair on scalp.

Statistical analysis was performed with the treatment outcome after 6 months using SPSS version 23 software. Statistical significance was assessed using Fisher’s exact test and the results with $p < 0.05$ were considered to be significant.

Results

Among all patients, those treated with DPCP showed very good response than DNCB. Grade 3 and grade 4 regrowth of hair (desirable regrowth) was better achieved in DPCP group (86.7%) than DNCB group (33.3%). Out of 30 patients included in the study, 29 patients successfully completed the treatment and one patient was lost to follow-up from DNCB group. Alopecia areata was commonly observed in age group of less than 30 years (53%).

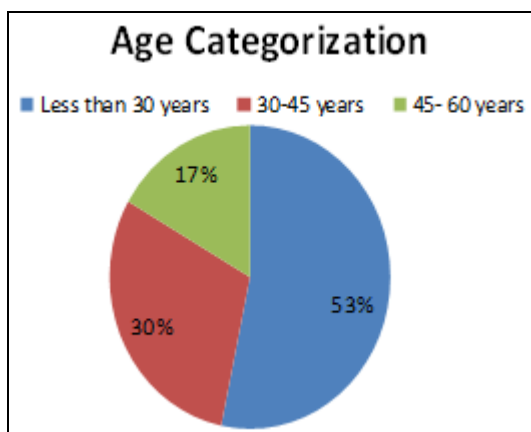


Fig 1: Age Categorization

Males (53.3%) were more affected when compared to females (46.7%). The mean duration of the illness was 12 months in our study. Family history of atopy was present in 50% of cases. Personal history of allergic rhinitis was present in 23.3% of cases. 16.6% of the cases had history of chronic urticaria. Bronchial asthma was present in 16.7% of the cases. 10% of the cases had hypothyroidism. Co-existing dermatosis like vitiligo was present in 6.6% of the total cases. Diabetes mellitus was present in 16.6% of our cases. Around 16.6% of cases had family history of alopecia areata. Recurrence of AA was present in 16.7% of the cases

with atleast one previous episode in the past. In our study, the alopecia patches were predominantly present over the occipital and parietal areas constituting 61.9% and 51.6% respectively. This was followed by frontal area and temporal areas. Among the nail changes observed in our cases, 10% had nail pitting and 6.7% had nail dystrophy.

Regrowth of hair was assessed using dermoscopy. In our DPCP study group, regrowth started in 8 weeks. Complete regrowth of hair (grade 4) was seen at sixth month of the therapy. Out of 15 patients in DPCP group, grade IV regrowth of hair was attained in 33.3%, grade III regrowth in 53.3%, grade II regrowth in 6.7% and grade I regrowth in 6.7%. In our DNCB study group, regrowth started in 12 weeks. Complete regrowth of hair (grade 4) was seen at sixth month of the therapy. Out of 15 patients in DNCB group, grade IV regrowth of hair was attained in 6.7%, grade III regrowth in 26.7%, grade II regrowth in 20%, grade I regrowth in 13.3% and no regrowth in 26.7% of the cases.

Table 1: Comparison of treatment outcome at 6th month of the study participants

Outcome	Group A		Group B		p-value
	N	%	N	%	
Grade 0	4	26.7	0	0	0.04
Grade 1	2	13.3	1	6.7	
Grade 2	3	20	1	6.7	
Grade 3	4	26.7	8	53.3	
Grade 4	1	6.7	5	33.3	
Loss of follow up	1	6.7	0	0	

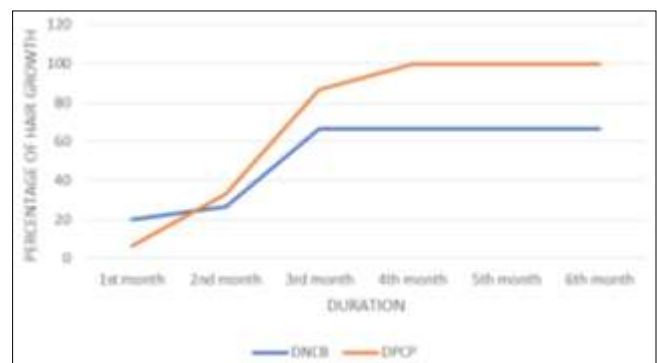


Fig 2: Comparison of minimum hair re growth rate between the study participants

With regard to the side effects, blister and occipital lymphadenopathy was present in 10% of the cases and erythema, urticaria and cervical lymphadenopathy was present in 6.6% of cases each.

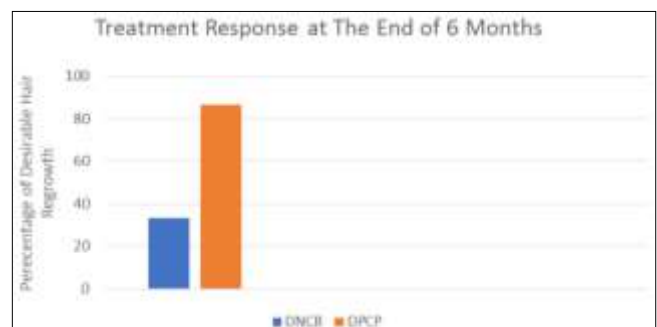


Fig 3: Comparison of desirable hair regrowth at 6th month of the study groups

Discussion

In our study, we evaluated the efficacy of topical dinitrochlorobenzene (DNCB) and diphenylcyclopropenone (DPCP) in Alopecia areata in dermatology OPD patients. A total of 30 patients enrolled in the study of which 53.3 % were less than 30 years of age. These results are consistent with the study done by Fricke *et al.* who reported that alopecia areata has historically been more prevalent in the younger age groups [12]. In our study, 53.3 % were males and 46.7% were females. A study by Mirzoyey *et al.* also observed similar results [13].

In our study, there duration of AA was ranging between 6 to 36 months with mean duration of 12 months. More severe forms of alopecia are seen in chronic patients [14]. Around 16.6% of cases had family history of alopecia areata. Patients with alopecia areata reporting a family history of the disease have been estimated between 8.6% and 16 % by other studies [14, 15]. Family history of alopecia areata was associated with reduced hair regrowth after relapses, more severe symptoms, and earlier age of onset [16].

In our study, 50% of the patients had positive family history pertaining to atopy which was similar to results by Kaur *et al.* in their study [17]. In our study, 16.6% of the patients had diabetes mellitus. A systematic review by Fricke *et al.* found increased insulin resistance in AA patients and Diabetes mellitus was found in 11.1% of AA patients [12].

Personal history of allergic rhinitis was present in 23.3% of cases. 16.6% of the cases had history of chronic urticaria. Bronchial asthma was present in 16.7% of the cases. Atopic disorders have been reported to occur with an incidence of 1 to 52% in patients with alopecia areata in various studies [14, 19, 20, 21]. It has also been observed that alopecia areata pursues a severe course in the presence of atopy and has an early onset of alopecia and a longer duration of disease with a poor response to treatment [17].

In our study, 10% of the cases suffered from hypothyroidism; 6.6% patients of the patients had a vitiligo as a co-existing dermatosis; The reason for vitiligo to be associated with AA is probably because both are autoimmune diseases and therefore can occur together as indicated in several other studies. Sharma VK *et al.* reported vitiligo in 1.8% patients which is in concordance with our study [14]. Thyroid disorders and vitiligo have the strongest association with AA. Our results were also in agreement with a clinical study done by Thomas *et al.* who reported hypothyroidism as the most frequent form of thyroid function abnormalities associated with AA [22].

In our study, the alopecia patches were predominantly present over the occipital and parietal areas constituting 61.9 and 51.6% respectively. This was followed by frontal area and temporal areas. Our finding was in concordance with a study done by Yogesh *et al.* who found that alopecic patches over the scalp were predominantly present over the parietal and occipital areas constituting 35.17% and 29.65% respectively [23].

10% of our cases had nail pitting and 6.7 % had onychodystrophy. A study done by Ferreria reported that nails are involved in 10–66% of all AA cases. Nail changes in AA can be an indicator of AA severity and possibly reflects a more refractory disease. Furthermore, nail dystrophy is a poor prognostic indicator of AA [25]. Thomas *et al.* reported nail changes in 16.8% of cases in his study, the commonest being nail pitting (7.2%), followed by longitudinal ridging (4.2%), brownish discoloration (2.8%)

onycholysis (1.4%) and leuconychia (1.4%) [22].

Desired hair growth was observed in 86.7% in DPCP group. Our results were consistent with several studies that evaluated the efficacy of DPCP in patients with AA; hair regrowth rate was found to be 6–77%. A systematic review had previously reported an overall hair regrowth rate of 53.75% in DPCP-treated patients [12]. Tosti *et al.* reported complete hair regrowth in 77% of patients with mild AA [25]. In our DNCB study group, at the end of six months 33.3% patients achieved desirable hair growth. Our results were consistent with study done by Zebrinati *et al.* stated that a good response to the therapy was achieved in 44.05% and hair regrowth was achieved in 50.98% of patients treated with DNCB [26].

In our study, side effects to treatment like blister and occipital lymphadenopathy was present in 10% of the cases and erythema, urticaria and cervical lymphadenopathy was present in 6.6% of cases each. As reported by various studies, the most common adverse effect was severe eczema, including scaling, exudation, and blistering. Furthermore, generalized eczema in nontreated and treated sites was common. Systemic symptoms like lymphadenopathy were relatively common. Pigmentary changes were also frequently reported. Compared with other therapeutic agents used for AA, contact immunotherapy had these treatment-related adverse effects¹⁵ which implies that it should be used after proper counselling of the patient.

Conclusion

Topical immunotherapy is a useful alternative modality more effective in treating alopecia areata especially in severe and recalcitrant cases. In our study, the desired hair growth was achieved with the DPCP group was much more than that of DNCB group. Thick pigmented anagen hairs were achieved in DPCP group than DNCB group. To conclude DPCP is more effective in the management of AA with lesser side effects when compared to DNCB. Overall both topical immunotherapeutic agents can produce hair growth but DPCP is more tolerated with more response rates and hence more effective.

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