# Efficacy of dinitrochlorobenzene in the treatment of alopecia areata

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**Background:** Various kinds of sensitizers are administrated for alopecia areata treatment. The aim of this study was to evaluate treatment response to Dinitrochlorobenzene (DNCB) in alopecia areata patients.

**Method:** In this study, 117 patients were treated with DNCB under a specific checklist. All patients were sensitized with a 2% DNCB and then treated with ascending DNCB concentrations (0.001%-2%). Response to treatment was categorized as none, mild, moderate and marked improvement.

**Result:** Thirty three (27.5%) patients showed no response, 49 (40.8%) had relapse 6 months after improvement, 29 (24.2%) had no relapse 6 months after the treatment and 6 patients were excluded because they did not return for follow-up visits. Response to treatment in patients without eyelash and eyebrow involvement increased significantly (P=0.01). We did not observe any side effects except for localized dermatitis seen in 5% of the patients.

**Conclusion:** With respect to the suitable response to DNCB application and its availability, the authors suggest that DNCB be reconsidered in alopecia areata. However, attention must be paid to its mutagenicity.

**Keywords:** alopecia areata, contact sensitizer, dinitrochlorobenzene

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## INTRODUCTION

Local immune therapy by contact sensitizers is administrated in many benign and malignant diseases like universal alopecia areata (AA), resistant viral warts, melanoma, and recently for HIV patients [1-4]. For AA treatment, various kinds of the sensitizers are used like squaric acid dibutylester (SADBE), diphenyl cyclopropenon (DPCP), and dinitrochlorobenzene (DNCB) [5]. DNCB was the first sensitizer but today, it is not used for its mutagenicity [5]. Although DPCP is the most promising local sensitizer used for AA treatment [6,7], it is unfortunately not available; therefore, it can not be used in routine treatments in all parts of our country. Mohan et al [8] motivated the authors to perform a retrospective study to evaluate the efficacy of DNCB in AA patients. The aim of this study was to evaluate the efficacy of DNCB treatment in AA patients.

## PATIENTS AND METHODS

In this cross sectional clinical study which was conducted from August 1998 to August 2006, the profiles of AA patients who were treated with DNCB were evaluated. A special profile including information on background and previous treatments

was created for each patient. All patients signed an informed consent form prior to participation. Finally, 117 patients with AA (57 male ranged 2- 45 years) were enrolled. Our inclusion criteria were as following:

a) Not receiving any other treatments like phototherapy, systemic steroids and other immune suppressive therapies during the treatment period or in the last three months.
b) Being treated only with DNCB

The following patients were excluded:

a) Patients without special information on the purpose of the study.
b) Patients who did not return to our center for follow up visits.

The DNCB powder (Merck Company) was dissolved in acetone at appropriate ascending concentrations of 0.001%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 1.5% and 2% solution. The solutions were stored in dark bottles at room temperature. At the first visit, sensitization was carried out by applying the 2% DNCB solution. After two weeks, DNCB solutions were applied weekly on the affected area starting with the minimum concentration (0.001%). Then, the concentration was increased gradually until slight dermatitis and erythema was developed; this concentration was considered for continuation of the treatment. If no response to treatment was seen after six months, therapy was discontinued. Collected information included age, sex, extent of the involved site (head<0.25%, 25-50%, 50-75% and >0.75%, and eyebrow and eyelash), relapse and treatment response. Treatment response was categorized s none (without any hair growth), mild (terminal hair growth up to 25% of the area), moderate (terminal hair growth 25% to 50% of the area) and marked hair growth (terminal hair growth over 50% of the area).

Statistical analyses were performed using SPSS Software (Version 11.5). Data were expressed as Mean ± SD. Chi-square ($\chi 2$) test was used to evaluate association between relapse and sex and other dummy variables. The relation between continuous or ordinal variables with relapse and other dummy variables was determined by T-test and Mann- Whitney test. A two tailed P value of $P<0.05$ was considered statistically significant for all calculations.

## RESULTS

We studied 117 patients (57 male and 60 female) with an age range of 2-45 years old (15.2±9.2 and 14.8± 9.0 for males and females, respectively). Six patients were excluded from our study for insufficient information. The distribution of the involved sites in AA patients is summarized in Table 1. DNCB treatment was discontinued in 33 (27.5%) patients after 6 months because they did not respond to DNCB treatment. As for response to treatment in other patients, 21 (7.5%), 20 (16.7%)

**Table 1.** Distributions of involvement sites in patients

| Scalp Involvement Site | Frequency (patients %) |
|---|---|
| head <25% | 27 (22.5%) |
| head 25-50% | 34 (28.3%) |
| head 50-75% | 33 (27.5%) |
| head>75% | 23 (19.2%) |
| Eyebrow | |
| - | 20 (16.7%) |
| + | 97 (80%) |
| Eyelash | |
| - | 50 (41.7%) |
| + | 66 (55%) |

**Table 2.** Correlation between scalp involvement sites and response to treatment and relapse

| Site | Improvement | | | | Relapse | |
|---|---|---|---|---|---|---|
| | Non | Mild | Moderate | Marked | + | - |
| head <25% | 9.1% | 9.5% | 25% | 39.5% | 30.6% | 17.2% |
| head 25-50% | 18.2% | 38.1% | 25% | 34.9% | 28.6% | 44.8% |
| head 50-75% | 36.4% | 28.6% | 30% | 20% | 30.6% | 20.7% |
| head>75% | 36.4% | 23.8% | 20% | 4.7% | 10.2% | 17.2% |
| Eyebrow | | | | | | |
| - | 45.5% | 47.6% | 50% | 73.8% | 56.3% | 75.9% |
| + | 54.5% | 52.4% | 50% | 26.2% | 43.8% | 24.7% |
| Eyelash | | | | | | |
| - | 69.7% | 85.7% | 80% | 93% | 91.8% | 89.7% |
| + | 30.3% | 14.3% | 20% | 7% | 8.2% | 10.3% |

and 43 (35.8%) patients showed mild, moderate and marked response, respectively. There was no correlation between treatment response and gender (P=0.59). Twenty (35%) male and 23 (38%) female patients showed marked treatment response. Twenty-three patients (24.2%) reported no relapse after 12 months of DNCB treatment.

Table 2 presents the variation of response to treatment and relapse in different involved sites. Response to treatment decreased with the increase in the extent of scalp involvement (P<0.01). In patients with scalp involvement over 75%, only 4.7% of the patients showed marked improvement. The correlation between relapse and eyebrow and eyelash involvement was not significant (P= 0.67, 0.74 respectively). When we divided patients into two groups of children (age<14 years old) and adults (age>14 years), we found that relapse was less common in adults (P=0.39).

## DISCUSSION

DNCB is a strong contact allergen that almost always causes hypersensitivity reaction type IV. Also, it is a useful test for the evaluation of T cell activity in immune compromised patients [5]. The mechanism of contact immunotherapy in AA is unknown. The proposed theories for diphencyprone mechanisms of action, similar to contact sensitizers like DNCB, are a) antigenic competition that causes CD4 shift from the perifollicular area toward the epidermis; b) perifollicular apoptosis; c) the CD4 ratio variations in the peribulbar area d) IL10 excretion that has inhibitory effects on T cells [5,9-12].

The mutagenicity of aromatic compounds like DNCB has been reported in previous studies [5,13]. Also, in recent years, confirmation of DNCB mutagenicity by the Ames test restricted its application, although DNCB is still used for AA treatment in some parts of the worlds [8].

In many studies with local diphencyprone immunotherapy, 50-60% of the patients have shown response rates between 9 to 87% [5,14]. The patients with extensive involvement have lower responses of up to 17.6% [15,16]. The duration of treatment in these studies were 6 month periods and if treatment did not result in any response, it was discontinued [2,6]. Happle et al, showed that response to DNCB was good in 12 out of 13 patients with severe hair loss [17], while complete hair growth was 36.6% regardless of the involved site, alopecia type and extent of scalp involvement in a study by Mohan et al [8]. Singla et al, reported that 16.25% of treated patients showed complete response to DNCB treatment [18]. In our study, we divided scalp involvement into <25%, 25-50%, 50-75% and >75% and response to treatment were 39.5%, 34.9%, 20%, and 4.7%, respectively.

Our results are similar to Gordon et al [19] study that was performed by diphencyprone. As in both of them, response to treatment decreased by increasing the involvement site. Wiseman et al [16] showed that response to diphencyprone during 6 months was 30%, and this response improved to 72% after 32 months, therefore longer treatment is more valuable.

In our study, the DNCB treatment period was at most one year. If hair re-growth was not acceptable after this period of time, the treatment was canceled. So, there is possibility that if the duration of treatment in patients with extensive involvement increased, the therapeutic results were better.

Prognosis of alopecia areata is good in 50% of cases and complete hair re-growth is seen in the one-year follow up [5] while 7- 10% of AA cases become chronic, especially in children [20]. We divided the patients into 2 groups of children (<14 years old) and adults (>14 years old) and categorized the improvement response as moderate and marked. Children's and adults' improvement response rates were 37.7% and 62.3%, respectively. The response to immunotherapy in treated case series by diphencyprone in children is 32% to 33% [21,22]; however, an improvement rate below 10% has been reported by Tosti et al in severe AA [23]. Although younger patients had lower response to treatment (P=0.57) and relapse was higher in children than adults (57.1% vs. 42.9% P=0.16) in our study, age did not affect response to treatment; this finding is congruent with a number of studies on diphencyprone [16,24]. Studies have shown that a positive family history and nail involvement affect disease severity and its prognosis [5,14]. There was no significant correlation between family history and response to treatment (P=0.79). Because nail involvement was not recorded in all patients, this factor was not statistically analyzed in our survey. We suggest that DNCB could be considered as a treatment option in AA patients in spite of its

mutagenicity, systemic absorption and recent arguments regarding its non carcinogenicity in humans [13,25,26]. The authors recommend DNCB for AA patient with attention to its mutagenicity.

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