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A controlled trial of house dust mite eradication using natamycin in homes of patients with atopic dermatitis: effect on clinical status and mite populations

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SUMMARY

In a controlled clinical trial, patients with atopic dermatitis carried out house dust mite eradication procedures on their mattresses, using natamycin or a matched placebo spray, with or without vacuum cleaning, for 4 months. For the two groups that used vacuum cleaning, mite numbers fell significantly both by comparison of mean initial and final numbers and by calculating mean rates of reduction from regression analyses ($P < 0{\cdot}01$ in all cases). There were no differences between the groups in the magnitude of the decreases. For the two groups that did not use vacuum cleaning, mite populations remained virtually unchanged. These results indicate vacuum cleaning not natamycin had the major effect on mite eradication.

Mean symptom scores of patients in all four groups were slightly reduced by the end of the trial, but there was a greater reduction rate ($P < 0{\cdot}01$) in the combined scores of the groups that did not use vacuum cleaning, demonstrating a lack of correlation between improvement in clinical score and lowered mite numbers. No patients entered remission and the maximum improvement in clinical score was only 47%. Natamycin and vacuum cleaning neither alone nor in combination proved valuable in reducing mite numbers sufficiently to provide clinical benefit.

There is strong circumstantial evidence that house dust mite allergens are an important precipitating factor for atopic dermatitis. This includes mite-specific IgE immunoglobulinaemia in patients with atopic dermatitis[1] and correlation between clinical severity and specific serum IgE levels;[2] clinical improvement after mite avoidance;[3] effective immunotherapy with mite extracts;[4] and development of eczematous reactions following percutaneous challenge with

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mite allergens.[5,6] In view of such compelling evidence it is surprising that the present study is, to our knowledge, the first controlled double-blind clinical trial of mite eradication in homes of patients with atopic dermatitis.

Natamycin is an antifungal antibiotic used for topical mycoses. It works as an acaricide by killing the house dust fungi which digest lipids in human skin scales, the scales being the food of house dust mites. Without fungi the lipids remain at levels toxic to the mites.[7,8] Natamycin is available as an aerosol spray under the brand name 'Tymasil' (Brocades (Great Britain) Ltd. West Byfleet, Surrey, U.K.). It has been used in several clinical trials for control of mites in homes of mite-hypersensitive patients with asthma and/or rhinitis, both with clinically beneficial results[9] and with no benefit.[10]

METHODS

Design of trial, patient eligibility and clinical assessment

Twenty-three patients living in and around Greater Glasgow (Scotland) and attending the Dermatology Clinic at the Western Infirmary were entered into the study. Two left the study during the pre-trial period (week −2) and one left during week +2. Their data were not included in the analyses. All patients had the classical clinical features and chronic relapsing course of atopic dermatitis and their diagnostic features are shown in Table 1. They showed positive immediate skin prick tests to house dust mite extract, confirmed by positive RAST for anti-house dust mite (*Dermatophagoides pteronyssinus*) IgE, as well as elevated total serum IgE levels. Patients were seen at the Dermatology Clinic at −2, 0, +4, +8 and +12 weeks. At each visit clinical severity of eczema was scored on a scale of 0–3 for six features (extent, erythema, pustulation, excoriation, dryness and cracking) at 16 sites on the body. At the initial and final

TABLE 1. Atopic history, sexes and ages of patients

Treatment group	NV	Nv	nV	nv	Total
Number of males:females	3:3	3:1	1:4	4:1	11:9
Mean age (years)	24·8	30·0	31·0	24·6	
Age range	12–46	13–47	18–43	14–40	
Age of onset of AD (years)					
<2	6	3	3	4	16
2–5	—	—	—	1	1
>5	—	1	2	—	3
Personal history of atopy					
Asthma	4	2	4	2	12
Rhinitis	4	2	4	4	14
Urticaria	1	—	2	—	3
Conjunctivitis	1	—	—	—	1
None	—	1	—	1	2
Family history of atopy					
Eczema	—	2	3	2	7
Asthma	3	1	1	2	7
Rhinitis	1	—	1	1	3
None	2	2	1	1	6

visits, 10 ml of venous blood was taken for serological investigation (see below) and they were also skin prick tested using extracts of house dust mite (*D. pteronyssinus*), grass pollen, cat and dog dander and mould (*Aspergillus fumigatus*) (Bencard Ltd., Brentford, U.K.).

Patients were assigned randomly to one of four treatment groups (figure in brackets: number of patients who completed the trial): active natamycin spray and vacuum cleaning (NV; 6); active natamycin spray and no vacuum cleaning (Nv; 4); inactive matched placebo spray and vacuum cleaning (nV; 5); and inactive matched placebo spray and no vacuum cleaning (nv; 5). This scheme allows for the combination of data from any two groups for comparison with that of the remaining two groups.

Patients were entered into the trial between 23 November 1984 and 21 February 1985. The entire trial lasted from 23 November 1984 to 18 June 1985.

Serology

Serum samples were stored at -20°C. All sera from each patient were assayed in one session by RAST for IgG and IgE against house dust mite (*D. pteronyssinus*,) and IgE against mould (*A. fumigatus*) and grass pollen (*Poa pratensis*). Total IgE was assayed by IgE radioimmunoassay. Any non-specific binding of high serum IgE (expected in patients with atopic dermatitis) to the allergen-coated discs was assessed by binding to discs coupled with human serum albumin (HSA) (Lot no. 29805). All assays were carried out using commercial kits (Pharmacia Ltd., Uppsala, Sweden) following the instructions of the manufacturer.

Mite eradication measures

Mite eradication was restricted to the patients' bedrooms. Preliminary measures included the removal of house plants, soft toys, cushions and, wherever possible, upholstered furniture other than beds. The number of pillows was standardized to one per bed occupant. Spraying of the beds with natamycin or placebo was done by the patients and followed the manufacturer's instructions. Briefly, the upper surfaces of the mattresses and both sides of the pillows were sprayed every 2 weeks and aired for half an hour afterwards. One aerosol can containing 625 mg of natamycin was used for spraying a single bed and two cans for a double bed. Actual natamycin dosage was checked in the laboratory by discharging 20 cans and calculating the mean dry weight of the contents.

Sprayings were recorded on a diary card by the patients. They received full written instructions and a demonstration of the procedure. Patients using vacuum cleaning all possessed hose-type vacuum cleaners (carpet beater-type vacuum cleaners are not suitable for mattresses). They cleaned the upper surface of the mattess and both sides of the pillows just before each spraying.

Monitoring of house dust mite population change

An initial visit for dust sampling was made (-2 weeks) with four subsequent visits at weeks 0, $+4$, $+8$ and $+12$, coinciding as near as possible with the patients' attendance at the Dermatology Clinic.

Methods of dust sampling, and mite extraction followed those used previously.[11] Mites were mounted on microslides, using the frozen block technique,[12] in Hoyer's medium and identified using a compound microscope. Mites were also recorded as 'damaged' or 'intact' as an estimate of whether they were alive or dead at the time of sampling.

Statistics

House dust mite counts and clinical scores for weeks 0–12 were converted into ratios of those for week −2. After square root transformation, regression analysis was used to obtain the lines of best fit and the values of the slopes (rate of change) and intercepts were calculated. Having checked that the intercepts were on or around 1, the rates of change were treated with two-way unbalanced analysis of variance. The effects of the two treatment types were analysed using *F*-tests.

Data on skin prick tests and serology were analysed using paired *t*-tests.

RESULTS

House dust mite eradication

Dermatophagoides pteronyssinus and *Euroglyphus maynei* accounted for 97% of the total mite specimens recovered from the dust samples.

The geometric mean numbers of total house dust mites in mattresses fell by 1·9 times by week +12 in group NV and by 3·1 times in group nV (Table 2) but by only 1·08 and 1·02 times in the groups that used natamycin only and placebo spray only. Numbers of intact mites increased in both these groups by week +12.

The geometric mean rates of change of total mite numbers in mattresses, as calculated from lines of best fit, were −25·8% in 12 weeks for group NV, ($P < 0{\cdot}01$), −50·3% for group nV ($P < 0{\cdot}01$), +15·1% for group Nv and −24·9% for group nv (*P* not significant in both cases) (Fig. 1). There was no significant difference between the rate of reduction in group NV and group nV. Analysis of the separate and combined effects of vacuum cleaning and natamycin demonstrated that vacuum cleaning and not natamycin had the major effect in reducing house dust mite numbers ($P < 0{\cdot}01$).

TABLE 2. Geometric means of total house dust mites (all spp., damaged plus intact)/0·25 m^2/min from mattresses of each patient

	Week of sampling				
	−2	0	+4	+8	+12
Treatment group					
Natamycin, vacuuming (NV)	63·6	45·6	76·7	54·8	34·2
Natamycin, no vacuuming (Nv)	385·3	270·2	419·8	302·8	357·4
Placebo, vacuuming (nV)	47·5	42·7	33·1	36·9	15·0
Placebo, no vacuuming (nv)	144·1	225·6	211·2	154·4	141·1

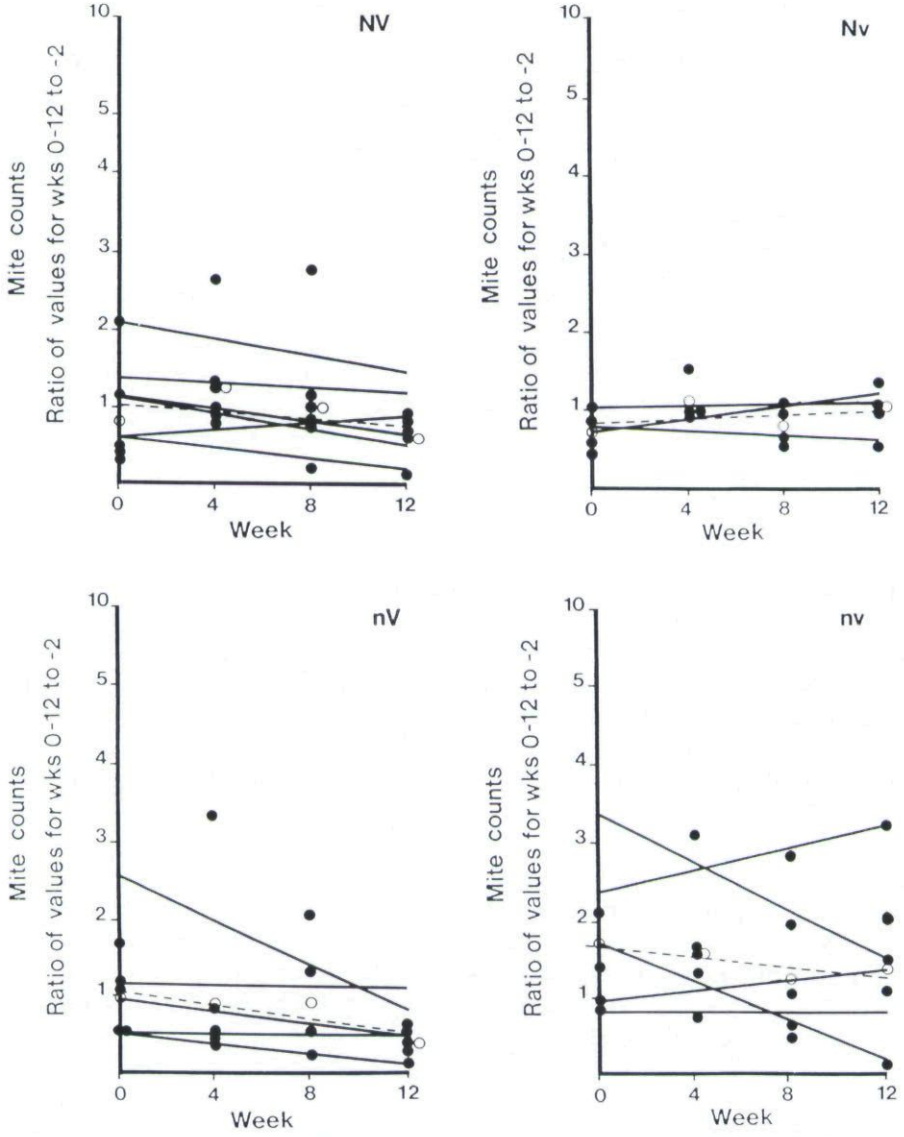

FIGURE 1. Rates of reduction of total house dust mite numbers represented by lines of best fit derived from regression analysis of ratios of scores for weeks 0–12 to scores for week −2. ●, individual values; ○, geometric mean of individual values. ----, line of best fit for geometric means.

Symptom scores

Arithmetic mean symptom scores in all four treatment groups were slightly reduced by week +12 compared with week −2 scores (Table 3). The mean rate of change of symptom scores, as calculated from lines of best fit (Fig. 2), showed only group Nv had a greater percentage improvement rate (24·0% in 12 weeks) than the control group, nv (20·4%). Improvement rates for groups NV and nV were 8·4% and 0·7% respectively.

TABLE 3. Arithmetic means of symptom scores ± 95% confidence intervals

	Week of clinical examination				
	−2	0	+4	+8	+12
Treatment group					
Natamycin, vacuuming (NV)	55·2 ± 12·5	46·2 ± 25·8	47·3 ± 43·9	42·3 ± 41·2	38·6 ± 37·1
Natamycin, no vacuuming (Nv)	55·3 ± 49·3	52·0 ± 38·5	53·8 ± 53·4	45·0 ± 41·7	43·8 ± 41·3
Placebo, vacuuming (nV)	57·4 ± 20·6	47·8 ± 33·4	62·0 ± 42·0	58·8 ± 52·5	51·0 ± 44·5
Placebo, no vacuuming (nv)	45·2 ± 18·6	46·6 ± 35·6	43·0 ± 33·9	40·2 ± 27·2	35·8 ± 26·4

When the effect of vacuum cleaning and natamycin on symptom scores was analysed, a significantly greater rate of reduction ($P < 0.01$) was found in groups that did not use vacuum cleaning.

No patients went into remission. Four (67%) in group NV had positive improvement rates at week 12; four (100%) in group Nv; two (40%) in group nV and five (100%) in group nv. The greatest rate of increase in clinical score, as calculated from lines of best fit, was only 16% for a patient in group nV whilst the greatest decrease was 47% for a patient in group nv.

Skin prick tests

Skin prick testing was completed for 16 of the patients (Table 4). All four groups showed no significant change in geometric mean area of weals raised to any of the test extracts by week + 12 even though three patients in group NV had > 2-fold reductions in weal area to *D. pteronyssinus* by then. All patients were skin prick test positive to house dust mite, 15 (94%) to grass pollen and to cat dander, 12 (75%) to mould and 11 (69%) to dog dander. All but one patient showed a positive reaction to at least four preparations.

Serology

Levels of total serum IgE, and anti-*D. pteronyssinus* IgG and IgE were elevated in all 20 patients (Table 5). Geometric mean levels declined in all four groups by week 12. There were statistically significant reductions for anti-*D. pteronyssinus* IgE in group NV ($P < 0.05$), and for anti-*D. pteronyssinus* IgG and total IgE in group nV ($P < 0.01$ and < 0.05 respectively). The only other statistically significant changes were reductions in mean anti-*Poa pratensis* IgE in groups nV ($P < 0.02$) and nv ($P < 0.05$).

Natamycin dosages

Arithmetic mean surface areas of the upper side of a single bed plus one pillow and a double bed plus two pillows were 2·24 and 4·21 m^2 respectively. Arithmetic mean delivered dosage (dry weight) of natamycin was 574 mg per can (range 369–688 mg). Representative doses were thus 256 mg/m^2 for single beds and 273 mg/m^2 for double beds.

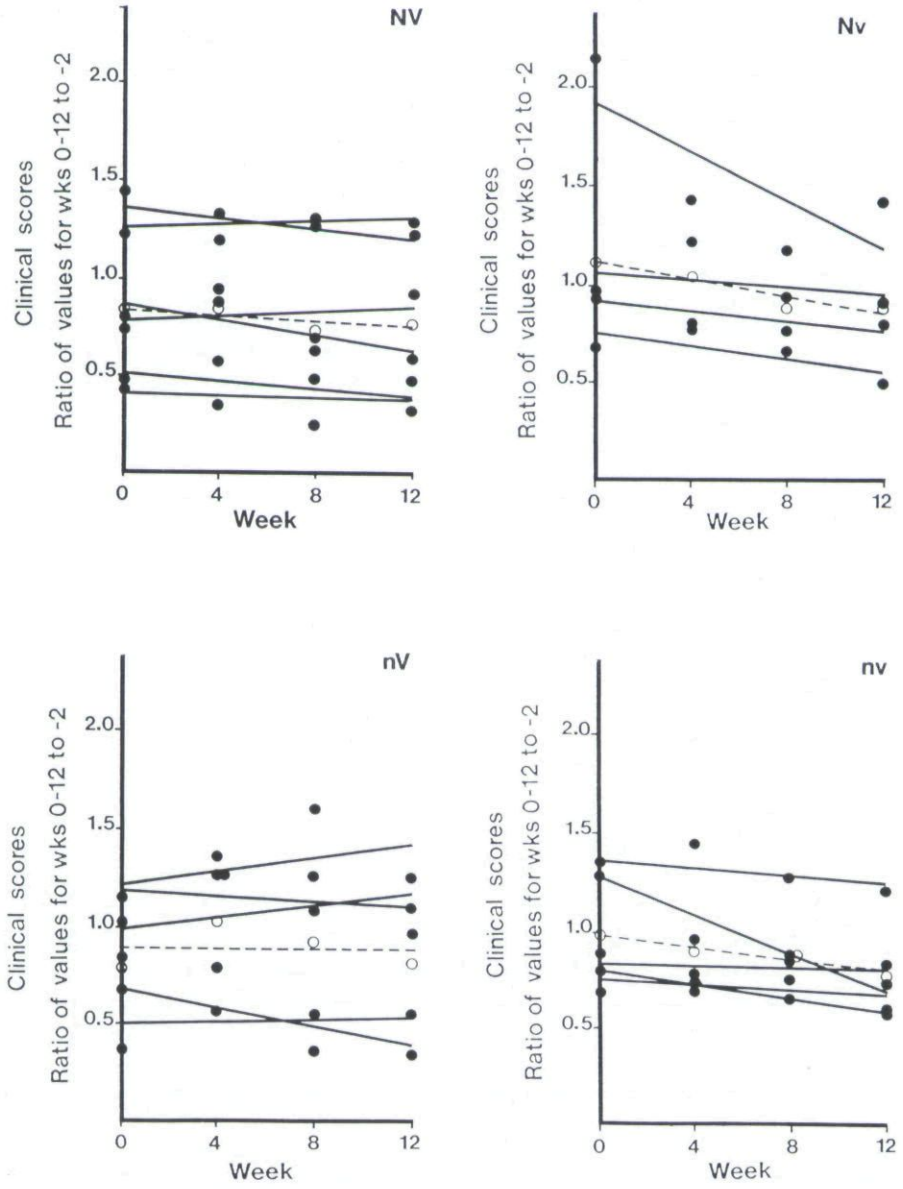

FIGURE 2. Rates of reduction of symptom scores represented by lines of best fit derived from regression analysis of ratios of scores for weeks 0–12 to scores for week −2. Symbols as in Figure 1.

DISCUSSION

In this trial the major effect on mite eradication was due to vacuum cleaning rather than to natamycin. There was no clear synergistic effect from combining the two treatments and natamycin had only a slight acaricidal effect. Recently Saint Georges-Gridelet reported that double the recommended dosage of 300 mg/m^2 was required for persistent fungicidal activity and efficient mite control[8] and suggested that inefficacy was caused by irregular distribution of

TABLE 4. Skin prick test results at weeks −2 and +12. $\bar{x}$ = geometric means of wheal area (mm^2) minus control, n = number of patients positive

	Allergen extract									
	HDM		Cat		Dog		Grass		Mould	
	−2	+12	−2	+12	−2	+12	−2	+12	−2	+12
Treatment group										
Natamycin, vacuuming (NV)										
n	6	6	4	6	3	3	6	6	2	3
$\bar{x}$	30·4	18·7	5·1	9·8	1·9	2·1	18·7	12·3	1·9	2·6
Natamycin, no vacuuming (Nv)										
n	3	3	2	3	1	2	3	3	1	2
$\bar{x}$	32·4	35·4	4·3	5·5	1·6	2·0	6·3	13·6	1·7	2·7
Placebo, vacuuming (nV)										
n	3	3	2	3	2	2	3	3	1	3
$\bar{x}$	35·2	28·4	3·0	7·3	2·2	6·5	9·8	18·2	1·4	4·4
Placebo, no vacuuming (nv)										
n	4	4	3	0	3	4	3	3	2	4
$\bar{x}$	29·2	28·1	6·3	10·2	3·3	8·3	7·0	6·1	1·9	2·9

TABLE 5. Serological results at weeks −2 and +12. Geometric means. Units: IgG = arbitrary units, IgE = International RAST units, Total IgE = International RAST units/ml

	Assay											
	HDM IgG		HDM IgE		Grass IgE		Mould IgE		Total IgE		HSA	
	−2	+12	−2	+12	−2	+12	−2	+12	−2	+12	−2	+12
Treatment group												
Natamycin, vacuuming (NV)	61·0	54·7	88·2	74·0	44·1	25·0	1·7	1·6	8939	5930	0·2	0·2
Natamycin, no vacuuming (Nv)	27·3	25·0	94·9	71·7	56·9	30·3	1·6	1·9	9980	4583	0·3	0·1
Placebo, vacuuming (nV)	48·5	42·7	69·1	51·7	62·9	35·2	1·1	0·9	8998	6784	0·2	0·1
Placebo, no vacuuming (nv)	74·7	82·5	63·6	48·4	6·2	4·5	0·8	0·6	3284	2061	0·1	0·1

natamycin after spraying. We observed that patients often overestimated the time it took the aerosol can to empty and this did indeed lead to patchy spraying. We also found that many of the cans had not delivered their full contents before the propellant was exhausted.

The lack of correlation between improved symptom scores and reductions in mite numbers in mattresses and pillows has three major implications. Firstly, the decrease in clinical score of patients in the control group indicates a placebo effect due to the substantial intervention involved in the trial. The encouraging reductions in symptom scores achieved by August[13] and Roberts[14] in uncontrolled trials of mite avoidance may likewise be due in part to intervention as well as reduced allergen exposure. Secondly, the greatest improvements in mean symptom scores were in the groups which did not use vacuum cleaning. This may have been because vacuum cleaning disperses fine dust particles in the outlet aircurrent.[15] Allergen-containing dust is sucked up from sub-surface locations, e.g. the insides of mattresses, dispersed into the air and then deposited onto surfaces where it may be picked up on the patients' skin. Thirdly, we have attempted to control only one source of allergens: atopic dermatitis is a multi-factorial disease and many other stimuli such as infection, stress and a variety of other allergens may all cause a flare of the disease and obviate the beneficial effects of lowered numbers of house dust mites.

Were the trial to be repeated, a longer study period with more participants, combined with more efficient acaricidal methods, would be preferable. The success of any mite eradication method is dependent on patients following the protocol correctly. Three patients admitted to single omissions of sprayings and cleanings. To prevent this problem we would suggest that all eradication procedures be carried out by a member of the clinical trial team.

It has been proposed that significant improvement in symptoms of house dust mite-mediated asthma require at least a 10-fold decrease in allergen exposure and an arbitrary hygiene standard of below 100 mites per g of dust, or 2 μg of allergen *Der p*I, per g has been set.[16] We have found that natamycin cannot achieve such reductions when used at the recommended dosage. For patients with atopic dermatitis reductions in mite exposure even greater than 10-fold may be necessary, as marked improvement in symptoms has so far only been achieved by removing patients from their normal environment, usually to hospital where house dust mite numbers are very low.[3,13,17] In the future more efficient acaricidal methods may prove to be of benefit to patients with atopic dermatitis.

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