Original paper

Molecular profiling of allergen-antibody IgE might decide about the efficacy of allergen immunotherapy in a patient with atopic dermatitis and allergy to house dust mites

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Adv Dermatol Allergol 2023; XL (4): 542–547
DOI: https://doi.org/10.5114/ada.2023.129456

Abstract

Introduction: Allergen immunotherapy (AIT) has no clear recommendation for atopic dermatitis (AD).
Aim: To evaluate the effect of AIT on house dust mites (HDM) in AD patients sensitised to HDM with different baseline molecular profiles of antigens.
Material and methods: In this placebo-controlled study, 61 patients with moderate-to-severe AD allergy symptoms and HDM allergy were included. They received a 12 months' AIT with the use of HDM allergen extract or placebo. The authors adopted their AD improvement criterion after 1 year of AIT as a reduction of all examined indicators by at least 50% from the baseline for %BSA, TMS, and EASI scores. Additionally, the influence of individual HDM molecules on the final AIT effect was analysed.
Results: Finally, from the 24 desensitised patients, 15 achieved a positive expected effect after 12 months of HDM AIT. None of the patients who received a placebo had an improvement in AD of at least 50% after 1 year of follow-up. Patients with polysensitisation less frequently achieved the expected HDM AIT effect than patients monosensitised to mites ($p < 0.05$). The presence of sensitisation to rDer p 1 (odds ratio = 4.35, 95% CI: 4.01–4.56) and/or rDer p 2 (OR = 2.16, 95% CI: 1.98–2.33) and/or rDer f 2 (OR = 1.41, 95% CI: 1.55–1.78) molecules significantly increased the efficacy of AIT. HDM AIT could be helpful for patients with moderate-to-severe AD and sensitised to HDM as an add-on therapy. Various HDM molecules may affect the effectiveness of the expected AIT effect. The presence of sensitisation to rDer p 1 (OR = 4.35, 95% CI: 4.01–4.56) and/or rDer p 2 (OR = 2.16, 95% CI: 1.98–2.33) and/or rDer f 2 (OR = 1.41, 95% CI: 1.55–1.78) molecules significantly increased the efficacy of AIT.
Conclusions: HDM AIT could be helpful for patients with moderate-to-severe AD and sensitised to HDM as an add-on therapy. Various HDM molecules may affect the effectiveness of the expected AIT.

Key words: immunoglobulin E, mites, atopic dermatitis.

Introduction

Atopic dermatitis (AD) is an IgE-dependent, allergic disease with complex pathogenesis, which affects children in particular, but is also found in adults. Depending on the data, its occurrence is estimated at 0.5–20% of the population. In most patients, AD is transient but prone to relapse and requires periodic treatment [1–3]. In some patients, AD is a form of moderate or severe disease accompanying the patients throughout their life and often requires intensive treatment [1, 3]. In patients with AD, allergic rhinitis and asthma frequently coexist [1].

As a result, the local and systemic treatment methods of this disease are constantly being improved and based on: local steroid therapy or calcineurin inhibitors, antihistamines, PUVA, periodic immunosuppression, and now primarily biological treatment [4, 5].

The position of allergen immunotherapy in AD therapy still needs to be stronger, which results from the lack of clear evidence of its effectiveness. Several studies in-

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Received: 5.03.2023, **accepted:** 4.04.2023.

dicate the usefulness of allergen immunotherapy (AIT) in alleviating the disease, especially in patients allergic to house dust mites (HDM) [6–8].

Patients with an allergy to mites: *D. pteronyssinus* and *D. farinae*, have different molecular allergy profiles. The number of discovered antigenic components of these mites continues to increase, but their clinical significance is confirmed for some [9–12]. According to the EAACI recommendation, AIT is used in allergic rhinitis and HDM-driven asthma, but its use in AD is not recommended [6, 13, 14]. However, the possibility of the variety of HDM molecular profiles may suggest that some AD patients achieve clinical success after AIT.

Aim

The study aimed to verify the hypothesis that the effect of HDM AIT in AD patients may be derived from the baseline molecular profile of mite allergy. A secondary aim of the study was to evaluate the efficacy of AIT in monosensitised mite patients compared to patients with polysensitisation to other inhalant allergens.

Material and methods

The study was a prospective, observational, randomised, double-blind, placebo-controlled study on AIT. Sixty-one patients with moderate-to-severe AD allergy and house dust mite allergy were included in the study. All included patients have received a 12 months' allergen injection immunotherapy with the use of HDM allergen extract or placebo.

The inclusion criteria were: diagnosis of AD with a minimum of 1 year of therapy before inclusion, moderate-to-severe AD symptoms: according to Eczema Area and Severity Index (EASI), at least 7.1 points, and %BSA (Body Surface Area) scale at least 16 points, a positive skin prick test (SPT) and positive for specific immunoglobulin E (sIgE) to extract of *D. pteronyssinus* and *D. farinae*.

The exclusion criteria were: other active skin diseases, immunosuppressant treatment including oral corticosteroids, other chronic diseases, and allergen immunotherapy.

The characteristics of the patients are presented in Table 1.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee of the Medical University of Silesia in Katowice, Poland (KNW-1-131/N/9/K). Informed consent was obtained from all subjects involved in the study.

The study was registered at ClinicalTrials.gov under no. NCT03209245.

Treatment

The patients received Purethal Mites (20,000 AUeq/ml, HAL Allergy BV, Leiden, Netherlands) with extract of allergens: *D. pteronyssinus and D. farinae* (50/50%) or a placebo. Purethal was administered as perennial therapy using the following regimen: 1 dose – 0.1 ml, 2 doses – 0.2 ml, 3 doses – 0.5 ml every week, and 0.5 ml every 4 weeks. The average cumulative dose was 375,000 BAU (Bioequivalent Allergy Units) administered to each patient undergoing active treatment [15].

During therapy, patients with bacterial infection were allowed to receive treatment with topical Bactroban (mupirocin ointment) and/or a 7-day course of amoxicillin and/or prednisolone 0.5 mg kg during 10 days was administered on any occurrence of skin exacerbation including superinfection.

Depending on individual diseases, the placebo group had oral antihistamines added such as desloratadine and topical medications.

Symptomatic treatment was scored as medication score: one point for desloratadine, mometasone furoate cream, and mupirocin ointment use every day, 7 points for every course of amoxicillin and also 10 points for the course of encortolon. The patients were required to record symptomatic drug use in the diary card [16].

Efficacy of AIT

The authors adopted their AD improvement criterion after 1 year of AIT treatment as a reduction of all examined indicators by at least 50% from the baseline for %BSA and TMS, and EASI scores.

Diagnostic procedures

Careful examination of the eyes, ears, nose, and dermatological examination was performed on all patients.

Scales

The EASI assesses the extent of the disease on a scale of 0 to 6 in 4 defined body regions plus an assessment of erythema, infiltration and/or population, excoriation, and lichenification, each on a scale of 0 to 3. A formula is then used to calculate the total score for each of the 4 regions, which are then added together. Interpretation of the EASI result: 0 = no change, 0.1–1.0 = almost no change, 1.1–7.0 = mild intensity, 7.1–21.0 = moderate intensity, 21.1–50.0 = high intensity, 50.1–72.0 = very severe.

In this analysis, %BSA was categorised according to severity bands: clear (0%), mild (> 0 to < 16%), moderate (16 to < 40%), and severe (40–100%)

Skin prick tests

The SPT was performed with inhalant allergens (HAL Allergy B.V, Leiden, Netherlands) from the following panel: *D. pteronyssinus, D. farinae,* mixed five types of grass (*Phleum pratense, Dactylis glomerata, Anthoxanthum odoratum, Lolium perenne,* and *Poa pratensis*), mixed tree, mugwort, *Alternaria, Cladosporium,* and dog and cat allergens. Positive (10 mg/ml of histamine) and negative

Table 1. Characteristics of the study patients at baseline

Parameter	SCIT group (*n* = 26)	Placebo group (*n* = 35)	*P*-value
Women	13	18	NS
Age [years] mean ± SD	19.9 ±8.2	22.6 ±7.3	NS
EASI, mean ± SD	39.8 ±10.1	42.6 ±12.1	NS
%BSA, mean ± SD	69 ±14	73 ±18	NS
Duration of AD [years]	15.9 ±7.1	17.7 ±9.1	0.05
Number of subjects with asthma	7	10	NS
Number of patients with allergic rhinitis	16	23	NS
Number of smokers	12	18	NS
Total IgE	6572 ±1240	8101 ±2456	0.05
Specific IgE to D. pter [kU/l] mean ± SD	19.4 ±5.05	23.1 ±4.9	NS
specific IgE to D. far [kU/l] mean ± SD	17.4 ±8.1	14.9 ±6.31	NS
Co-sensitization, *n* (%)	9 (35)	14 (40)	NS
Grass	6 (23)	10 (29)	0.04
Tree	4 (15)	6 (17)	NS
Weed	3 (12)	4 (11)	NS
Mould	2 (8)	3 (9)	NS
Cat/dog	2 (8)	5 (14)	0.03
Treatment before study:			
Antihistamines	25 (96%)	33 (94%)	NS
Topical glucocorticosteroids	19 (73%)	29 (82%)	NS
Calcineurin inhibitors topically	17 (65%)	29 (82%)	NS
Systemic glucocorticosteroid	5 (19%)	8 (23%)	NS
Cyclosporine	15 (57%)	23 (66%)	NS
Methotrexate	2(8%)	4 (11)	NS
Dupilumab	2 (8%)	2 (6%)	NS
PUVA	8 (31%)	6 (17%)	0.03

(saline) controls were included. A positive skin test for allergens was defined when a minimum wheal diameter of 3 mm greater than the negative control was noticed. Patients with negative tests for histamine sensitivity were excluded from further analyses [17].

Clinical assessment

In all included patients, clinical assessments with the use of EASI and %BSE scales and medication score were performed at the start and at 12 months of treatment.

Laboratory tests included: sIgE assay: Serum-specific IgE levels to *D. pteronyssinus, D. farinae*, and to rDer p 1, rDer p 2, rDer p 5, rDer p 7, r Der p 10, rDer p 11, rDer p 21, rDer p 23, rDer f 1, rDer f 2 were determined by Immuno CAP (ThermoFisher Scientific, Uppsala, Sweden), following the manufacturer's instructions at the start of the study and at 12 months. Values were expressed in kU/l.

New sensitisations

A new sensitisation was defined as a rise of IgE levels against analysed HDM antigen molecules from < 0.15 kU/l to > 0.35 kU/l. An assessment of the occurrence of new sensitisations was performed in the 12th month of the study.

Statistical analysis

The statistical analysis was performed using Statistica version 8.12 (SoftPol, Cracow, Poland). The non-parametric tests were used because the data are not normally distributed. The Wilcoxon test was used to analyse differences between the groups. The odds ratio (OR) and 95% confidence interval (CI) were estimated to assess the impact of the presence of individual HDM molecules on the effect obtained after AIT. The ANOVA test was used to compare scale scores. Differences were considered significant at $p < 0.05$ [15].

Results

Efficacy of AIT

Finally, 24 patients completed AIT and 33 placebo-completed observations. The remaining patients discontinued treatment due to failure to return to the following appointments; 1 patient was infected with COVID-19 and stopped therapy for this reason.

The data are presented in Table 2. Of the 24 desensitised patients, 15 achieved the positive expected effect according to the agreed improvement criteria after 12 months of HDM AIT. The other desensitised patients did not reach an effect, but 4 had a noticeable improvement at the level of the assessment of about 30%. None of the patients who received a placebo had an improvement in AD of at least 50% after 1 year of follow-up. Patients with polysensitisation less frequently achieved the expected HDM AIT effect than patients monosensitised to mites ($p < 0.05$).

Molecular profile and AIT

The patients enrolled in the study had 63 different variants of molecular allergy profiles to HDM. The percentage of allergies to individual antigens is presented in Table 3. The presence of sensitisation to rDer p 1 (OR = 4.35, 95% CI: 4.01–4.56) and/or rDer p 2 (OR = 2.16, 95% CI: 1.98–2.33) and/or rDer f 2 (OR = 1.41, 95% CI: 1.55–1.78) molecules significantly increased the effectiveness of immunotherapy in AD patients if they were only simultaneously monosensitised against mites.

There was no correlation between the presence of the individual HDM molecules and the effect of AIT in polysensitised patients ($p > 0.5$) and also in the entire analysed group of patients (mono- + poly-sensitised; $p > 0.05$).

Table 2. The obtained effects of AD treatment after AIT or placebo in the study groups

Criteria of efficacy	After SCIT	Placebo	*P*-value	Monosensitised HDM SCIT	Polysensitised SCIT	*P*-value
≥ 50% improvement in all parameters tested Mean reduction (%):	15	0	0.0001	13	2	0.001
EASI	78	10	0.0001	86	51	0.03
BSA	64	18	0.002	75	67	NS
TMS	59	9	0.001	68	59	NS
< 50% improvement in all parameters tested Mean reduction (%):	9	33	0.001	3	6	0.04
EASI	23	19	NS	32	28	NS
BSA	39	21	0.03	38	33	NS
TMS	19	11	NS	41	24	0.01

EASI – Eczema Area and Severity Index; %BSA – body surface area, TMS – total medication score, NS – not significant, HDM – house dust mites, SCIT – injection allergen immunotherapy.

Table 3. Prevalence of IgE reactivity in patients with success or not after SCIT

Component	Success after AIT (n = 15)		No success after AIT (n = 9)		*P*-value prevalence (mean value)
	Prevalence (%)	Value, mean (SD) [kIU/l]	Prevalence (%)	Value, mean (SD) [kIU/l]	
rDer p 1	61.5	28.08 (20.1)	29.1	21.09 (19.14)	0.01 (NS)
rDer p 2	56.6	26.21 (23.21)	42.1	23.1 (11.08)	0.02 (0.01)
rDer p 5	8.1	0.98 (0.34)	9.6	1.09 (0.51)	NS (NS)
rDer p 7	4.8	5.88 (4.12)	7.1	4.14 (2.23)	NS (0.01)
rDer p 10	31.5	1.02 (0.78)	39.9	4.13 (4.91)	NS (0.001)
rDer p 11	5.3	0.78 (0.55)	11.93	1.98 (2.19)	0.02 (0.01)
rDer p 21	16.1	2.61(1.14)	19.4	3.51(2.09)	NS (NS)
rDer p 23	39.1	19.8 (11.9)	41.1	15.1(9.43)	NS (NS)
rDer f 1	21.6	9.12 (5.62)	17.9	10.11(8.2)	NS (NS)
rDer f 2	43.2	21.12 (19.5)	36.1	17.9 (11.03)	0.04 (NS)

AIT – allergen immunotherapy, SD – standard deviation, NS – not significant (p > 0.05).

New sensitisations

Three new sensitisations to HDM molecules were noticed only in the placebo group: 2 cases for the Der p 10 antigen and one for Der p 11 (conversion from negative to negative result > 0.35 kIU/l). No new sensitisations were observed in the HDM-AIT group during the year.

Discussion

The obtained results indicate the effectiveness of HDM AIT in patients with advanced AD allergy, but mainly in patients with monosensitised HDM. Not many studies evaluate the effectiveness of HDM allergen injection immunotherapy in adult AD patients [18–21]. Zhou *et al.* confirmed that 3 years of injection allergen immunotherapy to HDM significantly reduced the severity and pruritus of moderate-to-severe AD [19]. Research by other authors also indicates that such desensitization may be effective in treating AD during 1 year using similar criteria of improvement by 50%, however, with the use of the SCORAD skin assessment scale [18]. This last scale dominates as a parameter assessed in similar studies; however, the combination of EASI, %BSA and TMS scales seems more reliable and accurate [19–21]. In many studies, similar AD patients had the presence of allergic rhinitis with or without allergic asthma, which was significantly improved after HDM AIT [19–21]. These observations are consistent with the statements in the presented study. However, the results concerning improving allergic rhinitis and asthma were not presented due to the assumed research goals. It should be emphasized that some patients with severe AD were not qualified for the study due to their inability to withdraw from immunosuppressive treatment, which could weaken or exclude the effects of AIT. In these patients, the AD mechanism is usually very complex, and apart from the dependent IgE mechanism, autoimmune and infectious inflammation coexist [1, 22].

On the other hand, some authors are critical of the effectiveness of HDM AIT in treating AD and emphasize that a weak effect after AIT occurs in patients with a severe and persistent form of AD [23].

In each of the analysed studies, there were different criteria for including patients, which led to problems with appropriate comparing results of AIT. The observed lack of AIT effects in most patients with polysensitization indicates a significant limitation of eligibility for this treatment. It was compatible with previous observations where patients with polysensitization also obtained the worse effect after desensitization, even after 3 years of AIT [19]. It is worth adding that such a relationship between the clinical severity of AD and exposure to mite allergen is more difficult to confirm than in respiratory allergic diseases.

A recent observation is a link between the efficacy of HDM AIT in treating AD on the mite molecular profile. In this study, 63 variants of HDM molecular profiles were confirmed in patients diagnosed with AD, the most common ones being rDer p 1, rDer p 2, rDer p 23 and Der f 2: 61.5%, 56.6%, 39.1% and 43.2%, respectively.

Gonzales-Perez *et al*. observed a similar, large number (n = 72) of HDM molecular profile variants in AD patients, but a significantly higher frequency of the same tested molecules, reaching even 80% in the case of rDer p 1 and 60% for rDer p 5 [24]. A similar prevalence of profile molecules of HDM was noticed in another Spanish observation [25]. This may be because there is a significant variety of allergies in different populations depending on the geographical region (area of Spain compared to Poland).

In the presented study, the presence of major allergens, such as rDer p 1, rDer p 2 or rDer f 2, a positive predictor of AIT efficacy, was confirmed. It is similar to HDM AIT in the therapy of allergic rhinitis and allergic asthma [25]. At the same time, it was impossible to establish negative predictors of effectiveness depending on individual molecules, which may result from a too small group of subjects. It is one of the main limitations of the study. It results from the restrictive inclusion criteria, which shows that AIT can only be useful in a small group of patients. Other limitations are the short observation time, consistent with similar studies, and the lack of *in vitro* biomarker evaluation during desensitization.

In particular, it was impossible to confirm the pessimistic prediction of rDer p 10 for no AIT effect, according to earlier data [26]. However, it should be remembered that other HDM principal molecules dominate AD patients' images, which may be the key to AIT effectiveness [25]. It requires further research.

The analysis of new allergies in the scope of the analysed molecular profile of HDM indicates a protective effect of AIT. It is consistent with other observations confirming new allergy inhibition in desensitized patients. Because the groups are too small, this requires further research.

Conclusions

HDM AIT could be helpful in patients with moderate-to-severe AD and sensitized to HDM as an add-on therapy. The presence of various HDM molecules may affect the effectiveness of the expected AIT, just as in the case of allergic rhinitis, it may inhibit new sensitization of individual HDM molecules; however, this requires further research.

Acknowledgments

The Medical University of Silesia, Katowice, Poland funds this research work.

The study was conducted in the Clinical Department of Internal Medicine, Dermatology and Allergology in Zabrze, Medical University of Silesia in Katowice, Poland.

Conflict of interest

The authors declare no conflict of interest.

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