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A large scale multicentre randomized, placebo-controlled subcutaneous house dust mite allergen immunotherapy (HDM SCIT) in allergic rhinitis: MITAR Study

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Abstract

Background: Previous house dust mite subcutaneous immunotherapy (HDM SCIT) placebo-controlled trials have small sample sizes and lack a consensus on baseline treatment.

Objective: To determine the efficacy of HDM SCIT in moderate-to-severe allergic rhinitis (AR) patients treated with an intranasal corticosteroid at baseline.

Methods: We conducted a randomized, placebo-controlled trial comparing HDM SCIT against placebo in *Dermatophagoides pteronyssinus* (Der p) sensitized. All patients received standard of care according to Allergic Rhinitis and its Impact on Asthma (ARIA) guideline, including an intranasal steroid (INCS) at baseline. The primary endpoint was the comparison of a composite score, combining the total nasal symptom score and medication score, assessed at the twelfth month post-treatment.

Results: Of the 144 subjects, 108 received HDM-SCIT and 36 received a placebo. The median age was 30 years (range 11-61), with 60% being female. The mean Der p wheal diameter was 9.4 mm (SD 4.4). After one year of treatment, the composite score median (IQR) in the HDM SCIT group and the placebo group was 0.75 (0.50-1.13) and 0.63 (0.50-1.25), respectively ($p > 0.05$). Both groups exhibited a significant mean change in the composite score from baseline ($p < 0.001$), but there was no significant difference between the groups. The median (IQR) serum Der p-specific immunoglobulin G4 level significantly increased only in the HDM SCIT arm ($p \leq 0.001$).

Conclusion: One-year HDM SCIT significantly reduced both symptoms and medication use in HDM-allergic rhinitis patients. However, the changes were not significantly different from those in the placebo group, who also received an INCS at baseline. A longer-term study is warranted to assess disease modification factors.

Key words: *Dermatophagoides pteronyssinus*, dust mite allergy, allergic rhinitis, subcutaneous allergen immunotherapy, aeroallergen

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Abbreviations:

HDM	house dust mite
AIT	allergen immunotherapy
SCIT	subcutaneous injection immunotherapy
SLIT	sublingual immunotherapy
PAR	perennial allergic rhinitis
AR	allergic rhinitis
AD	atopic dermatitis
SD	Standard deviation
IQR	interquartile range
AU	allergen units
DBRPC	double-blind, randomized, placebo controlled
RCT	randomized controlled trial
INCS	intranasal corticosteroid
TNSS	total nasal symptom score
CNMS	composite score (Combined Symptom and Medication Score),
MS	medication score
TNSSS	total non-nasal symptoms score
VAS	visual analogue scale
SPT	skin prick test
Der p	*Dermatophagoides pteronyssinus*
Der f	*Dermatophagoides farinae*
FEV1	forced expiratory volume in 1.0 second
MWD	mean wheal diameter
mm	millimeters
QoL	Quality of Life
IgG4	immunoglobulin G4
IgE	immunoglobulin E
ARIA	Allergic Rhinitis and its Impact on Asthma
WAO	World Allergy Organization
AE	adverse event
SAE	serious adverse event
SR	systemic reactions
LR	local reaction

Introduction

House dust mite (HDM) is the most common cause of perennial allergic rhinitis (PAR) worldwide. Allergen immunotherapy (AIT) has significantly reduced allergic symptoms and medication use. It has also been proven to modify the underlying pathological mechanisms, offering sustained long-term efficacy in allergic rhinitis (AR) even after treatment cessation.[1-6] Additionally, AIT may prevent the development of asthma and new allergen sensitizations.[6,7] While sublingual immunotherapy (SLIT) is available in several middle- and high-income countries, its main limitations are poor compliance and unaffordability.[8] Consequently, subcutaneous immunotherapy (SCIT) remains commonly used in some regions. Moreover, considering long term medical cost, SCIT remains a more cost-effective choice particularly in regions where economic considerations play a significant role in healthcare decision-making.

The American Academy of Allergy recommends a maintenance dose for HDM SCIT ranging from 500 to 2000 allergen units (AU) of the aqueous antigen extract.[9] The incidence of systemic reactions (SR) in the conventional schedule of SCIT is approximately 0.2%, with a range of 0.01 to 0.3%.[9] A survey reported near-fatal events at a rate of 5.4 per million injections, identifying uncontrolled asthma as a predictive factor for these events.[10] Calderon et al. highlighted the lack of consensus on basic treatment in HDM SCIT, which limits the reliability of comparing studies. Consequently, there is a need for rigorous, well-designed double-blind, randomized, placebo controlled (DBRPC) trials.[11]

This study aims to evaluate the efficacy of HDM SCIT compared to a placebo after one year of treatment in patients with moderate-to-severe PAR. These patients were treated with an intranasal corticosteroid (INCS) as the standard of care, following the Allergic Rhinitis and its Impact on Asthma (ARIA) guidelines.[1,3]

Methods

Study Design and Study Participants

This study is a multicentered, randomized, placebo-controlled trial comparing HDM SCIT with a placebo. The inclusion criteria are patients aged over 10 years with a well-documented history of moderate-to-severe PAR for at least a year before enrolment. The subjects needed to exhibit strong monosensitization to Der p or strong Der p sensitization with weak positive responses to other allergens (except *Dermatophagoides farinae*; Der f) in cases of polysensitization. The subjects would be asked to stop all AR medications for 2 weeks as a washout period. A total nasal symptom score (TNSS) exceeding 6 for at least three consecutive days during the washout period was required to be eligible for the study. The description of skin prick test and TNSS score is provided in the following section. The exclusion criteria include moderate-to-severe asthma with a forced expiratory volume in 1.0 second (FEV1) or peak expiratory flow below 70% of the predicted value, severe nasal obstruction (TNSS obstruction score = 3), and any contraindications for allergen immunotherapy. A basic endonasal examination was performed to rule out any pathological defects, particularly severe deviated nasal septum, nasal polyps, or nasal masses. We made referrals to ENT specialists in case the diagnosis was uncertain. For detailed inclusion and exclusion criteria, refer to **Table S1**. The subjects were in the study for 12 months.

The schedule of SCIT in this study follows a cluster schedule in which the maintenance dose is reached within three months. The duration of SCIT in this study was set at "one year" in accordance with the practice parameters of AAAACI.[9] and WAO guidelines.[10] The protocol of managing local and systemic reaction from SCIT has been implemented according to the practice parameter.[9] In brief, large local reaction > 12 centimeters occurred within 30 minutes after injection, nasal symptoms related to injection and mild urticaria were managed by oral antihistamine and observed for minimum 60 minutes. Asthma symptoms were managed by β-2 agonist inhalation and corticosteroids according to severity. Systemic reactions were managed according to WAO Anaphylaxis guidelines.[10]

The study received approval from the Central Research Ethics Committee (CREC) (reference Number CREC002/57). All participants gave their full consent in accordance with the Helsinki Guidelines. Written informed consent was obtained from each participant, and for those under the age of 18, consent was secured from their parents or guardians. The clinical trial registry number is Clinicaltrials.gov ID: NCT 01115595.

Skin prick testing (SPT) to aeroallergens and definition of strong sensitization

SPT to aeroallergens included *Dermatophagoides pteronyssinus* (Der p), *Dermatophagoides farinae* (Der f), American cockroach, German cockroach, cat hair, dog hair, Bermuda Grass, Johnson grass, *Aspergillus spp*., and *Cladosporium spp*. Histamine dihydrochloride (1 mg/ml) and sterile 50% v/v glycerin were used as positive and negative controls, respectively. All extracts were sourced from ALK-Abello (Hørsholm, Denmark). SPT was performed on the volar side of the forearm and read at fifteen minutes for allergens and ten minutes for positive and negative controls. The mean of the largest and midpoint orthogonal diameters was determined as the mean wheal diameter (MWD). A positive skin response to an allergen was defined as an MWD greater than 3 millimeters (mm) compared to the negative control, while a strong positive SPT was indicated by an MWD greater than 5 mm compared to the negative control.

Total nasal symptom score (TNSS)

The TNSS is comprised of four symptoms: nasal obstruction, nasal itching, sneezing, and rhinorrhea. Each symptom is rated on a scale where 0 indicates absent symptoms, 1 indicates mild symptoms (sign/symptom clearly present, but minimal awareness; easily tolerated), 2 indicates moderate symptoms (definite awareness of sign/symptom that is bothersome but tolerable), and 3 indicates severe symptoms (sign/symptom that is hard to tolerate; causes interference with activities of daily living and/or sleeping). The subjective symptoms of pediatric subjects using diary cards with a smiling face symbol are determined by their severity. The scores were evaluated by the subjects weekly.

Sample size calculation and randomization

The sample size calculation was based on the findings of a double-blind, placebo-controlled trial (DBPCT) of HDM SCIT by Varney V.A., et al., which reported a 58% reduction score in the active group versus a 20% reduction score in the control group. Our hypothesis posits that HDM SCIT will benefit at least 60% of patients with HDM-related PAR by achieving a 30% reduction in the composite score, compared to the score of the control group. Given the tendency for HDM SCIT to be effective in previous studies, we decided to allocate more subjects to the active group to ensure fairness. With a 3:1 randomized controlled trial (RCT) design, 80% statistical power, and a 20% anticipated dropout rate, the estimated sample size is 145 participants (115 for HDM SCIT and 30 for placebo). A blocked randomization list was created using Sealed Envelope Ltd. (https://www.sealedenvelope.com/simple-randomizer/v1/lists), with block sizes of 4 by the statistician who was not involved in the study. Study coordinator who was not aware of the code group assigned participants to interventions. To ensure balance in key confounding parameters, the study was stratified based on Der p sensitization status (Der p monosensitized or polysensitized). Regarding the 'blinding method', the coding of subject allocation had been put in the sealed envelope. The subjects and investigators were not aware which group of subjects were.

Baseline and rescued medications

At the first month of the study, all participants received standard AR treatment, following the ARIA guidelines. This included INCS (Nasacort AQ®) spray, loratadine (10 mg) taken once daily, and other rescue medications as needed, such as pseudoephedrine (30-60 mg) or a topical nasal decongestant. After that, subjects could self-adjust their allergic medications.

House dust mite Der p extract for SCIT

The Der p allergen extract used for SCIT in this study is from ALK-Abello (Hørsholm, Denmark). The extracts are stored at a temperature range of 2 to 8 degrees Celsius. For dilution of the HDM allergen to the required concentration, the ALK diluent was utilized in accordance with the study protocol.

Placebo

The placebo group was administered a histamine solution from ALK-Abello (Hørsholm, Denmark)., with a final concentration of 1 μg/ml. The placebo solution was prepared by mixing 1 ml of histamine (10 μg/ml) with 0.4% phenol, and then diluting this mixture with 9 ml of 0.03% albumin. This process resulted in a final histamine concentration of 1 μg/ml.

Assessment of clinical outcome and immunologic parameters:

Primary outcome assessment

The primary outcome of this study is the composite score (combined symptom and medication score; CNMS) at month 12. The composite score is the average of the weekly TNSS and medication score (MS). The medication score (MS) is calculated based on the use of oral antihistamines and oral pseudoephedrine (each scored as 1), topical decongestants (scored as 2), and INCS (scored as 2). CNMS is determined by adding the TNSS and MS, then dividing the sum by two, and it is assessed weekly to track clinical outcomes.

Secondary outcomes assessments

The secondary outcomes of the study include: 1) comparing the percentage difference in the mean score of weekly TNSSS, total non-nasal symptoms (such as itching eyes, tearing, dyspnea, chest tightness, and sleep disturbance), daily MS, visual analogue scale (VAS), SF-36 Quality of Life (QoL), and Rcq-36 QoL. The SF-36 QoL and Rcq-36 are Thai versions of generic and specific-disease questionnaires, respectively, which have been validated and tested for reliability. 2) comparing the proportion of subjects who are medication-free or who achieve a 50% reduction in medication use between the treatment group and the control group.

Immunological parameters

Serum levels of specific immunoglobulin E (sIgE) and serum immunoglobulin G subtype 4 (sIgG4) to Der p were measured using ImmunoCAP (Thermo Fisher Scientific Inc., Massachusetts, United States) at baseline and Month 12. Samples from all sites were collected, transferred, and stored in a -80-degree Celsius freezer at a single site (Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand). All samples were analysed simultaneously after all samples were collected.

Assessment of side effects

Definition of Adverse Event (AE) and Serious Adverse Event (SAE)

Adverse events (AE) were categorized as local reaction (LR) and systemic reaction (SR). SR was defined as AE occurring beyond reactions at injection sites classified according to the World Allergy Organization (WAO) Subcutaneous Immunotherapy Systemic Reaction Grading System.[10] Serious adverse events (SAE) were defined SR more than WAO grade 3 or events in which subjects were hospitalized.

All reaction events will be record and reported to ethical committee (EC) by site manager and study coordinators within one month. Severe systemic reactions must be reported within 24 hours. Local reaction (LR) and mild systemic reaction will guide a particular physician/ investigator to adjust the next dose of SCIT.

Statistical analysis

Baseline group characteristics are summarized as mean (SD), median (IQR), and min-max for each group for continuous covariates, and as N (%) for categorical covariates. Comparisons of primary and secondary endpoints for the intention-to-treat analysis and per-protocol analysis, are reported as mean differences with 95% confidence intervals, tested using Student's t-test. In cases of non-normal data, a Wilcoxon rank-sum (Mann-Whitney) test is used to derive a *p*-value for formal comparisons. Differences in proportions between randomized arms are summarized as n (%) in each group, with Pearson 's chi-square or significant at a two-sided significance level of 0.05.

Results

A total of 343 participants were screened, out of which 146 were randomized. However, 2 later refused to participate, leaving 144 participants who received at least one dose of the injection (intention-to-treat); among them, 108 received HDM SCIT and 36 received a placebo. The CONSORT flow diagram and details on participant disposition are illustrated in **Figure 1**. Baseline characteristics were well balanced between the groups and are summarized in **Table 1**. The age of the subjects ranged from 11 to 61 years, with a median age of 30 (IQR 23-36), and 40% of them were male. The median (IQR) baseline TNSS, MS, and CNMS were 7.30 (6.55-8.20), 3.68 (2.0-6.0), and 1.25 (1.0-1.5), respectively. The mean (SD) size of the Der p SPT wheal size was 9.4 (4.4) mm. Notably, 86% of participants used an INCS as their primary treatment at baseline. One hundred and thirty-three out of 144 participants (92%) in the HDM SCIT group achieved the targeted maximal tolerated dose of 500 AU of the HDM Der p extract. The median time to reach this target dose was 14.7 weeks (IQR 14-17).

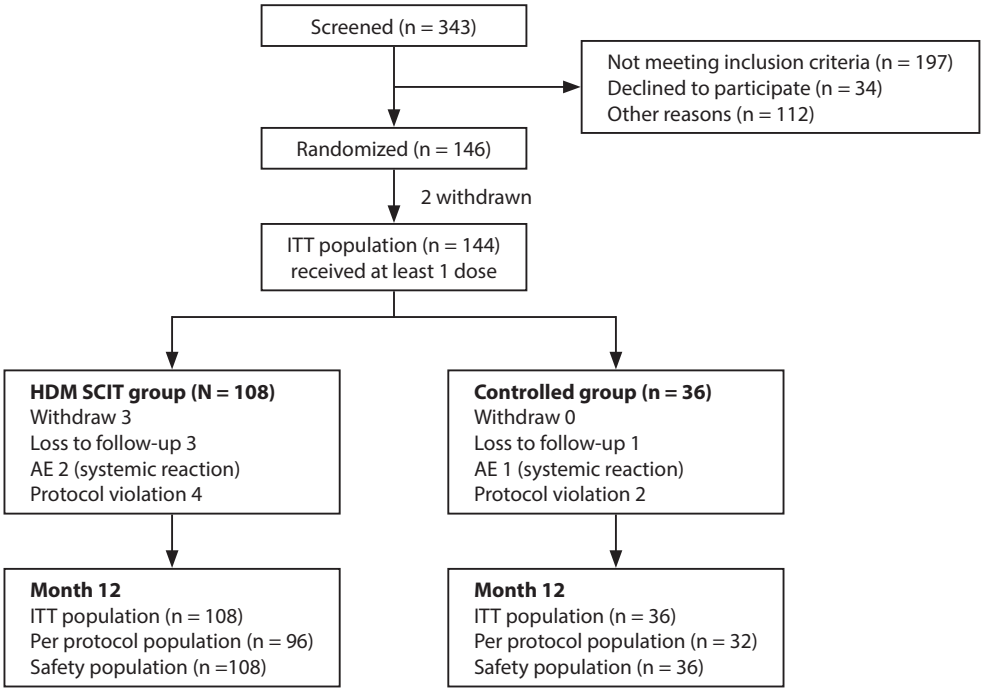

Figure 1. Randomized double-blind-placebo control CONSORT flow diagram.

ITT; Intention-to-treat, HDM SCIT; house dust mite subcutaneous immunotherapy

Table 1. Baseline characteristics.

Characteristic	HDM SCIT (n = 108)	Placebo (N = 36)	Total (N = 144)
Age at screening (years), mean (SD), (min-max)	31.1 (9.8), (11-61)	28.5 (9), (11-51)	30.5 (9.6), (11-61)
Age group, n (%)			
Children	4 (3.7)	3 (8.33)	7 (4.86)
Adult	104 (96.3)	33 (91.67)	137 (95.14)
Sex (N, %)			
Male	47 (43.52)	11 (30.56)	58 (40.28)
Female	61 (56.48)	25 (69.44)	86 (59.72)
Baseline clinical score (at the end of month 1)			
Nasal symptom score (TNSS), median (Q1-3)	7.30 (6.60-8.20)	7.15 (6.35-8.15)	7.30 (6.55-8.20)
Non-nasal symptom score (NNSS), median (Q1-3)	3.7 (2.0-6.0)	3.6 (2.2-6.6)	3.68 (2.0-6.0)
Composite score, median (Q1-Q3)	1.25 (0.88-1.5)	1.31 (1.13-1.63)	1.25 (1.0-1.5)
VAS score, median (Q1-Q3)	6 (5-7)	6.15 (5-7)	6 (5-7)
Type of Der p sensitization, n (%)			
Der p monosensitization	35 (32.41)	11 (30.56)	46 (31.94)
Der p predominant polysensitization	73 (67.59)	25 (69.44)	98 (68.06)
Duration of allergic rhinitis			
Less than 5 years	14 (12.96)	5 (13.89)	19 (13.19)
5-10 years	27 (25)	8 (22.22)	35 (24.31)
More than 10 years	67 (62.04)	23 (63.89)	90 (62.5)
Family history of atopy, n (%)	69 (63.89)	24 (66.67)	93 (64.58)

Table 1. (Continued)

Characteristic	HDM SCIT (n = 108)	Placebo (N = 36)	Total (N = 144)
Co-morbidities of allergic diseases, n (%)			
Food allergy	11 (10.19)	5 (13.89)	16 (11.11)
Atopic dermatitis	19 (17.59)	5 (13.89)	24 (16.67)
Asthma	10 (9.26)	8 (22.22)	18 (12.5)
Positive skin prick test to aeroallergens⁺, n (%)			
House dust Mite- Der p	108 (100)	36 (100)	144 (100)
House dust Mite- Der f	82/89 (92.13)	31/31 (100)	113/120 (94.17)
Cat	28 (25.93)	8 (22.22)	36 (25)
Dog	15/106 (14.15)	6/36 (16.67)	21/142 (14.79)
Cockroaches	52/104 (50)	18/34 (52.94)	70/138 (50.72)
Bermuda grass	19/98 (19.39)	7/35 (20)	26/133 (19.55)
Mean wheal diameter of Der p in mm, mean (SD)	9.8 (4.6)	8.4 (3.5)	9.4 (4.4)

HDM SCIT; house dust mite subcutaneous immunotherapy, Der p; *Dermatophagoides pteronyssinus*, Der f; *Dermatophagoides farinae*, VAS: visual analogue scale, ⁺positive skin prick test is defined as mean wheal diameter > 3 mm compared to negative control

After one year of treatment, median (IQR) composite score of the HDM SCIT group was 0.75 (0.5-1.13), and of the placebo group was 0.63 (0.5-1.25). Compared to the pre-intervention score, the mean changes in both groups were statistically significant by ITT analysis ($p < 0.001$) (**Figure 2A**). However, the post-intervention composite score and the mean change from baseline between the groups was not statistically significant. Subgroup analysis according to the pattern of sensitization had been done. There was no statistically significant difference of the composite score between Der p monosensitization and placebo groups, (**Table 2**). As the number of children included in our study is small. We performed sub analysis only on the adult population (**Table S4**). However, the difference of the composite score at Month 12 between those who received active drug and placebo could not be determined.

There was a significant improvement of TNSS, VAS, and MS in both groups, the comparison between pre- and post-intervention ($p < 0.001$). When comparing between HDM SCIT and placebo groups, there was no statistically significant difference (**Table 3**, **Figure 2B-E**). Five months into the HDM SCIT, 82 subjects (78.85%) reached the maintenance dose. By month 12, 89% (96 out of 108) of the participants remained in the study, with 86% receiving the maintenance dose of 500 AU of HDM Der p extract.

Approximately 23% of patients in both the overall study and in each individual arm did not require any anti-allergic medication after 12 months following enrolment. The difference in medication requirement between the two groups was not statistically significant, as shown in **Table S3**.

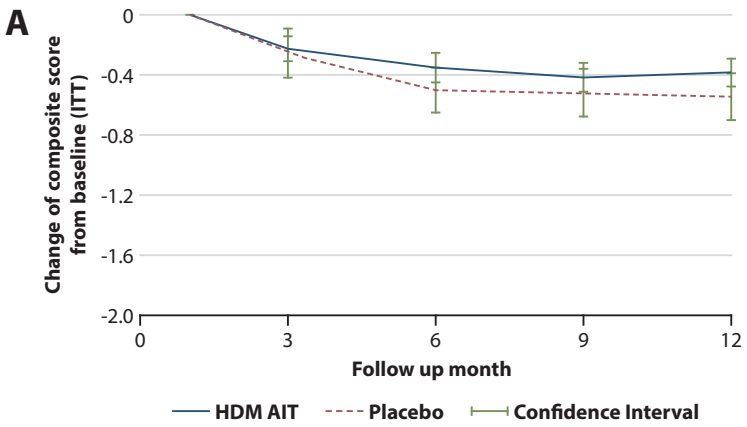

Figure 2. Mean Changes of clinical outcomes from baseline. A. Composite score, B. Nasal symptom score, C. Non-nasal symptom score, D. Visual analogue score (VAS), E. Daily medication score.
HDM SCIT; house dust mite subcutaneous immunotherapy

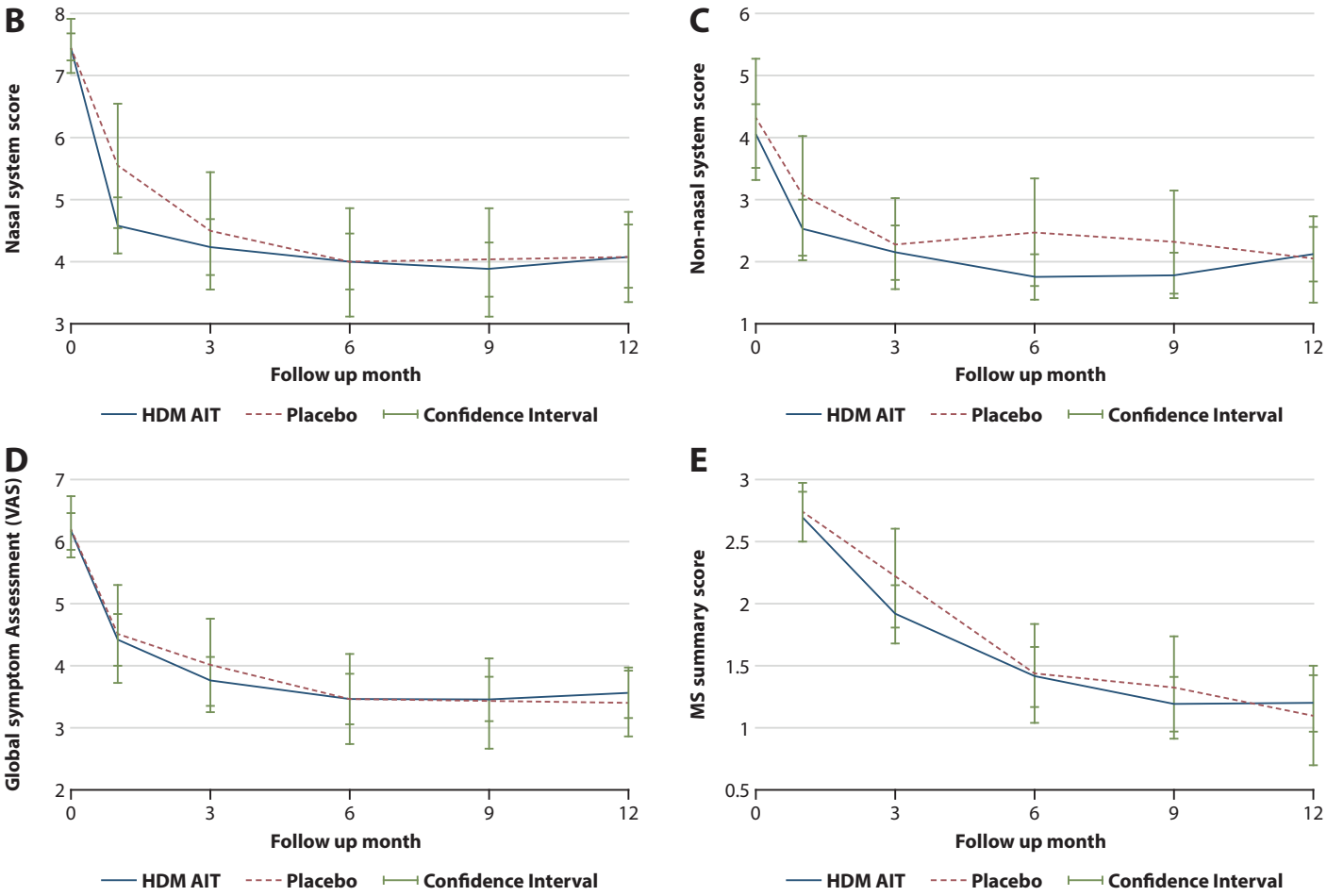

Figure 2. (Continued)

Table 2. Comparison of the composite score at month 12 (primary outcome) between the treatment arms.

Analysis population	Treatment arm	N	Median (Q1-Q3) at month 12	Mean difference (95%CI)	*P*-value
Intention-to-treat analysis	HDM SCIT	108	0.75 (0.50-1.13)	0.03 (-0.14 to 0.21)	0.564
	Placebo	36	0.63 (0.50-1.25)	Ref	
Per protocol analysis	HDM SCIT	96	0.75 (0.50-1.13)	0.01 (-0.17 to 0.19)	0.746
	Placebo	32	0.63 (0.50-1.19)	Ref	
Subgroup analysis					
Der p monosensitization	HDM SCIT	35	0.75 (0.38-1.13)	-0.11 (-0.47 to 0.26)	0.501
	Placebo	11	0.88 (0.38-1.38)	Ref	
Der p predominant polysensitization	HDM SCIT	73	0.75 (0.50-1.13)	0.10 (-0.11 to 0.30)	0.263
	Placebo	25	0.63 (0.50-0.88)	Ref	

HDM SCIT; house dust mite subcutaneous immunotherapy, Der p = *Dermatophagoides pteronyssinus*,
*p-value by Wilcoxon rank-sum test, < 0.05 is considered as significant

Table 3. Comparison of mean change of secondary clinical outcomes at month 12 from baseline.

Parameter	Treatment arm	N	Mean change from baseline	SD	Lower 95%CI	Upper 95%CI	*P*-value
Nasal symptom Score (TNSS)	HDM SCIT	99	- 3.34	2.65	-3.87	-2.82	< 0.001*
	Placebo	34	-3.36	2.29	-4.15	-2.56	< 0.001*
	Difference		0.01	0.51	-1.00	1.02	0.982**
Non-nasal symptom (NNS) score	HDM SCIT	99	-1.87	2.47	-2.37	-1.38	< 0.001*
	Placebo	34	-2.17	2.51	-3.05	-1.30	< 0.001*
	Difference		0.30	0.49	-0.68	1.27	0.546**
Visual analogue scale (VAS) score	HDM SCIT	99	-2.52	2.80	-3.08	-1.96	< 0.001*
	Placebo	34	-2.85	2.18	-3.61	-2.08	< 0.001*
	Difference		0.33	0.53	-0.72	1.38	0.534**
Medication score (MS)	HDM SCIT	99	-1.45	1.29	-1.71	-1.19	< 0.001#
	Placebo	34	-1.63	1.29	-2.08	-1.18	< 0.001#
	Difference		0.18	0.26	-0.32	0.69	0.353##

HDM SCIT; house dust mite subcutaneous immunotherapy, *p-value was from t-test, **p-value was from pair t-test, #p-value was from Wilcoxon rank-sum, ##p-value was from Wilcoxon signed-rank test, < 0.05 is considered as significant.

QoL outcomes are detailed in **Table S7**. Participants in both the HDM SCIT and placebo groups (with standard of care) exhibited significant improvements in all measured allergic rhinitis related QoL questionnaires (SF36: Physical, Mental; RCQ36, and EQ5D) after 6 months of treatment ($p < 0.001$). Notably, some parameters, such as SF36-Mental Health and RCQ36, showed significant mean improvements from baseline as early as 3 months into the treatment. However, the degree of QoL improvement in the HDM SCIT arm was not significantly different from that in the placebo arm.

In the HDM SCIT group, systemic reactions occurred in 40 subjects (37.04%). Of these, four subjects (3.70%) experienced serious adverse events leading to their discontinuation from the study (**Table S8** and **Table S9**). The overall incidence of systemic reactions was 77 out of 3,328 injections (2.31%), while serious adverse events occurred at a rate of 4 out of 3,328 injections (0.12%). Importantly, none of the systemic reactions in this study were classified as grade III or higher according to the World Allergy Organization (WAO) criteria. In the placebo arm, where histamine was used as a control agent to validate the randomized blinding of the study, 8.9% of participants reported histamine-related adverse effects. There were reports of systemic reactions in 0.9% of cases and serious adverse effects in 0.18% of cases.

In the analysis of specific immune responses, the serum sIgG4 to Der p levels in the HDM SCIT group showed a significant increase from 0.21 (0.19-0.25) at baseline to 1.08 (0.81-1.46) mgA/l ($p < 0.001$) by Month 12 of treatment. In contrast, the placebo group exhibited no change in serum IgG4 levels (**Figure 3** and **Table S10**). The difference in sIgG4 levels at month 12 between the HDM SCIT and placebo groups was statistically significant ($p < 0.001$). Serum sIgE to Der p levels in both the HDM SCIT and placebo groups did not significantly change following the treatment (**Table S10**).

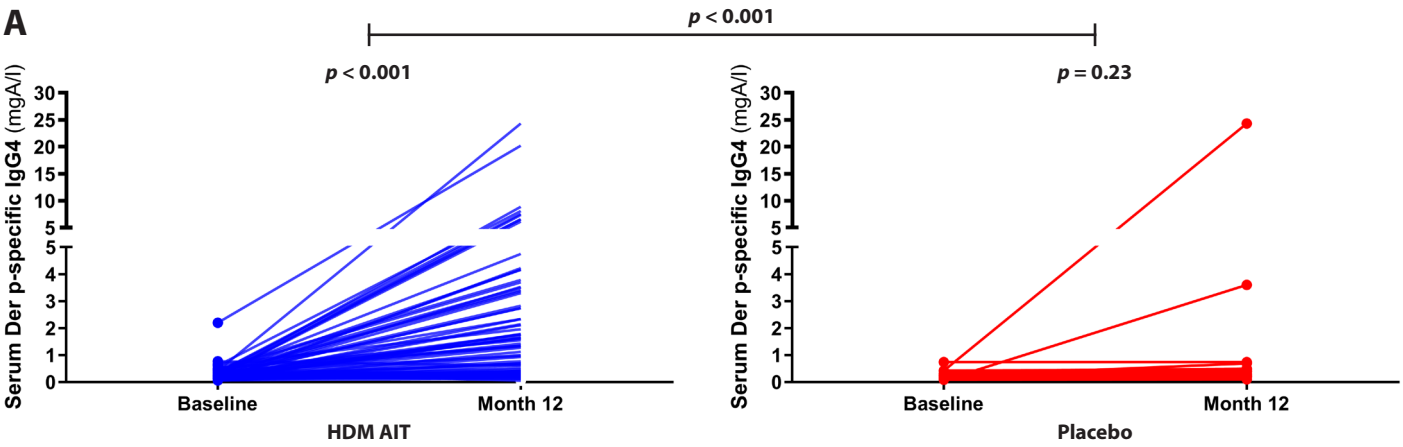

Figure 3. Serum Der p IgG4 level at month 12 Individual changed of serum Der p IgG4 at year 1 of treatment.
A. Individual changed from baseline, B. Overall results.
HDM SCIT; house dust mite subcutaneous immunotherapy

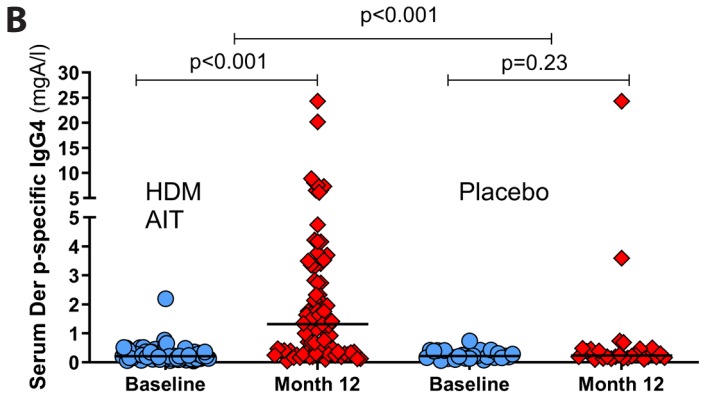

Figure 3. (Continued)

An exploratory analysis compared sIgG4 levels between responders and non-responders, the latter defined by a decrease in the CNMS of more than 30% from baseline.[12,13] Both subgroups in the SCIT arm demonstrated a significant increase in sIgG4 levels, but no difference in the median values was observed between these subgroups (**Figure S1**).

Discussion

The MITAR study, a double-blinded, randomized placebo-controlled trial involving 144 individuals with moderate-to-severe AR, demonstrated significant improvements in a composite of total nasal symptom scores and medication scores from baseline after one year of HDM SCIT treatment (**Figure 2A**). Secondary endpoint analyses also revealed significant improvements in TNNSS, VAS, QoL, and medication-free rate after 12 months of treatment (**Figure 2B-E**). Notably, the CNMS, TNSS, and TNNSS were dramatically improved in the HDM SCIT group after 6 months of intervention. The possible explanations are, firstly, we used a cluster protocol, enabling participants to reach the maintenance dose faster than conventional protocols. Secondly, our results align with other publications demonstrating that SCIT responders show significant improvement from 6 months onwards. This significant improvement also correlated with a significantly predicted clinical response at 3 years.[14] Furthermore, some international guidelines and publications mention that 6 months might be a critical time point for the efficacy assessment of SCIT and SLIT.[15,16]

However, an unexpected finding was that patients in the placebo arm, who received the standard of care according to ARIA guidelines, also showed notable improvement in both the composite score and the secondary endpoints. Thus, the mean change from baseline for these endpoints did not significantly differ between groups. However, a significant increase in serum sIgG4 to Der p level was observed only in the HDM SCIT treatment arm.

House dust mite (HDM) is the most common cause of perennial allergic rhinitis (PAR) worldwide. Allergen immunotherapy (AIT) has been shown to significantly reduce allergic symptoms and medication use and proven to modify the underlying pathologic mechanisms with a sustained long-term efficacy in allergic rhinitis even after treatment cessation. In addition, AIT may prevent the development of asthma and sensitization to novel allergens. Although SLIT is available in several middle- and high-income countries, the key limitations of SLIT are poor compliance and unaffordability. Therefore, SCIT remained commonly used in some settings. Moreover, considering long term medical cost, SCIT remains a more cost-effective choice particularly in regions where economic considerations play a significant role in healthcare decision-making.

In terms of baseline treatments, we adhered to the ARIA guidelines in selecting medications for AR management. Nasacort AQ® spray and loratadine were chosen as first-line therapies due to their proven efficacy in treatment of allergic rhinitis symptoms, the cost and accessibility in our country. Additionally, we included pseudoephedrine and topical nasal decongestant as a rescue medication to address nasal congestion when needed, allowing participants to self-adjust their treatment regimen based on individual symptom severity and responsiveness.

The decision to allow participants to self-adjust their allergic medications post enrolment was based on the principles of patient-centered care and treatment optimization. This approach not only promotes treatment adherence but also reflects real-world clinical practice, where patients continue to use their medications while receiving SCIT and often adjust their medications based on symptom severity and response.

However, an unexpected finding was that patients in the placebo arm, who received the standard of care according to ARIA guidelines, also showed notable improvement in both the composite score and the secondary endpoints. Thus, the mean change from baseline for these endpoints did not significantly differ between groups. However, a significant increase in serum sIgG4 to Der p level was observed only in the HDM SCIT treatment arm. Regarding the elevation of sIgG4 in 2 cases of the placebo group, allergen-specific IgG4 was associated with allergic sensitization and correlated to allergic diseases, such as allergic rhinitis (AR), asthma, atopic dermatitis (AD), and anaphylaxis. IgG4 levels may vary significantly in healthy individuals, strongly hindering its clinical application as a diagnostic tool. Nevertheless, recent findings from molecular structure and clinical investigations have brought new insights into this subset of IgG antibodies in allergic diseases.[17]

Notably, other studies have demonstrated intensive biomarkers associated with the HDM AIT response in a subgroup of adult participants from the MITAR study. These include IL-10-producing innate lymphoid cells, Der p-1-specific IgG41 B cells, plasmablasts,[18] as well as IL-10+ and dual-positive IL-10+IL1RA+ regulatory B cells.[19] These findings emphasize the potential necessity of more intensive biomarkers to monitor patients undergoing AIT. These findings emphasized the role of more intensive biomarkers might be in need to follow the patient receiving AIT.

Meta-analyses consistently show that both HDM SCIT and SLIT are efficacious compared to placebo controls in reducing AR symptoms and medication use.[20] The MITAR study's inability to confirm the superiority of HDM SCIT over placebo raises questions about its validity and implications. In terms of validity, several factors support the scientific robustness of the MITAR study: 1) Its design as a double-blinded, randomized placebo-controlled trial minimizes bias. 2) The use of a regulatory-approved, commercialized HDM Der p antigen extract from ALK-Abelló, Denmark. 3) Excellent compliance, with 90% of patients in the HDM SCIT arm receiving the maximal tolerated dose of 500 IU of the ALK aqueous HDM Der p antigen extract by Month 12. 4) Significant sIgG4 responses to Der p were elicited only in the HDM SCIT treatment group. 5) A MITAR sub-study showed significant induction of IL-10+CTLA-4+ ILCs among responders to HDM-SCIT treatment.[18,19] 6) The study was conducted in allergy clinics of various medical schools with expertise and a strong academic background. 7) The MITAR study's strength lies in its large RCT sample size, the largest compared to all previously reported HDM SCIT double-blinded, randomized placebo-controlled trials. As noted in a recent meta-analysis by Kim et al. 2021 (20), all six HDM SCIT double-blinded placebo-controlled studies had a total sample size of 60 or fewer participants, whereas the MITAR study included 144 participants.

Several factors may influence the outcomes of allergen immunotherapy studies in AR. These include the severity of AR, degree of HDM sensitization, levels and duration of HDM antigen exposure, exposure to various indoor and outdoor pollutants including PM2.5, exposure to non-HDM antigens, seasonal changes, lifestyle, adherence to AIT, nasal cleansing practices, and appropriate rhinitis medication. Although these confounding factors can be controlled or minimized in a well-designed and well-conducted DBPCT, especially with a larger sample size, comparing outcomes across different trials remains challenging. This is due to variations in baseline characteristics, eligibility criteria, primary and secondary endpoints, definitions for medication use scores, and reliance on self-subjective assessments of symptoms and medication use reported as pre-defined scores.

Calderon et al. (11), the authors have advocated for a rigorous, long-term DBPCT with well-defined primary outcomes.

In our attempt to understand why patients in the placebo arm of the MITAR study showed similar improvements in rhinitis symptoms and medication reduction as those in the HDM SCIT arm, we examined key parameters and outcomes from 6 other DBRPCT alongside the MITAR study, as summarized in **Table S8**. Notably, none of these 6 studies used a composite score as their primary endpoint; they focused on either rhinitis symptom scores, medication scores. Interestingly, one study did not find a significant difference in nasal symptom score changes compared to its placebo arm.

Our comparative analysis in **Table S8** reveals several heterogeneous observations among these studies: 1) Only 2 out of the 6 studies, in addition to the MITAR study, reported HDM SPT wheal sizes, showing a wide range of mean diameters (from 6.30 ± 0.61 mm to 11.25 ± 3.91 mm), with the MITAR study reporting 9.4 ± 4.4 mm. 2) The percentage of patients with HDM monosensitization varied: 0% and 50% in two studies, compared to 32% in the MITAR study. 3) Three studies included antigen provocation tests for eligibility screening: two used nasal and one used conjunctival provocation tests. 4) Different HDM antigens were used for SCIT across the studies, with ALUTARDS SQ being the most common (3 studies), other used Pharmalgen (ALK Abelló, Denmark), Novo-Helisen Depot (Allergopharma Joachim Ganzer KG, Germany), PURETHAL (HAL Allergy B.V., the Netherlands); The MITAR study used Der p aqueous extract from ALK-Abelló, Denmark. However, all studies utilized the maintenance dose according to the product recommendation. 5) Regarding placebo usage, only one study.[21] and the MITAR study used histamine, while others likely used buffer control or normal saline. 6) Five studies, but not the MITAR, used SPT and/or nasal or bronchial provocation tests as secondary endpoints. 7) The most notable factor is the variation in background rhinitis treatment or rescue medication allowance. While most patients in the 6 studies had moderate-to-severe AR, the use of INCS was not mandatory. The rhinitis treatment medications were either chosen freely by the patients as needed or administered in a stepwise approach based on the ARIA guidelines. While INCS as needed has shown effectiveness in some studies, a larger, well-designed RCT is necessary before it can be recommended in future guidelines.[22] The significant difference in approaches to rhinitis treatment medication – using INCS for all patients at baseline in the MITAR study, versus an as-needed or stepwise regimen in the other six DBPCTs – might be a key factor in the notable performance of the placebo arm in the MITAR study. This arm showed significant improvements in nasal symptoms. Additionally, the encouragement for all participants to step down their medication use at each study visit, when they experienced clinical improvement, may have contributed to the substantial reduction in medication use observed.

The MITAR study, however, has some limitations: 1) Its duration of only one year precludes assessment of longer-term disease modification compared to placebo (with standard of care). 2) It lacks other objective endpoints such as nasal provocation and/or skin prick tests.

In the MITAR study, there was no difference in the mean changes of the composite score from baseline between monosensitized and polysensitized participants. This aligns with the conclusions drawn in recent meta-analyses.[5,6]

Regarding safety and side effects, 37% of participants in the HDM SCIT group reported systemic reactions, and four subjects (3.70%) experienced serious adverse events leading to study discontinuation. The incidence of serious adverse events was 4 out of 3,328 injections (0.12%), with no events classified as WAO grade III or higher. In the study, two individuals with serious adverse events permanently discontinued HDM SCIT. Overall, HDM-SCIT was well tolerated, with no life-threatening reactions, consistent with other reports. Recent developments in allergoid SCIT, including both HDM and pollen immunotherapy, especially in Europe, have shown effectiveness and potentially more favourable safety profiles at higher tolerated doses.[23]

While AIT is the only allergy treatment proven to modify allergic diseases, it requires a long-term commitment of up to 3-5 years, and conventional SCIT is associated with more immediate local and systemic side effects. Alternatives to conventional SCIT include chemically modified allergoid immunotherapy, which minimizes the risk of immediate reactions, and SLIT, which can be self-administered at home.[8] However, a major drawback of SLIT is poor compliance.[24] Consequently, even in countries where SLIT is available, allergoid SCIT continues to be used.

In summary, the MITAR study demonstrated that one year of HDM SCIT in moderate-to-severe AR is beneficial but not superior to the placebo arm when standard care includes daily intranasal corticosteroids as a background therapy. This finding contrasts with previous DBPCTs, where the placebo arm received rescue medication, INCS as needed, or followed a stepwise regimen. The study also highlights the limitations of using subjective measures, such as symptom and medication scores, as primary outcomes. It underscores the need to include validated objective measurements and a longer follow-up period to properly assess the durability and disease-modifying effects of HDM SCIT. Future studies should aim to address the limitations identified in this study by conducting longitudinal investigations with extended follow-up periods, incorporating validated objective endpoints, and integrating a combination of subjective and objective measures such as nasal provocation or other advance tests such as component resolved diagnostic for house dust mite component specific IgE, immunological parameter such as regulatory B cells or innate lymphoid cells biomarkers. Moreover, future study should also focus on the differences and predictive phenotypes of responders and non-responders. This multifaceted approach can enhance the reliability and relevance of treatment outcomes and evaluate the long-term impact of HDM SCIT in allergic rhinitis management.

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Conflict of interest (in the past 3 years)

PT received research grants from Viatris, Abbott, AstraZeneca. Other authors have no conflict of interest.

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Author contributions

- NS, TL, PT, PC, KR: Protocol writing, grant submission, ethical protocol submission, subject recruitment, conducted the study, drafting manuscript, approved statistical analysis, and approved the final manuscript.
- TA: Statistical analyses and report, manuscript review and approval.
- JW: performed specific IgE and specific IgG4 of the samples.
- AS, TB, TT, BR, HC, PK, ST, PS, SF, WM, SC, OP, TA: Subject recruitment, conducting the study, manuscript draft and review, and final manuscript approval.

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