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The build-up phase outcome of subcutaneous immunotherapy for pediatric allergic asthma: A retrospective cohort study from Surabaya, Indonesia

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ABSTRACT

Background: Subcutaneous immunotherapy (SCIT) is an established recommended treatment for allergic asthma. SCIT provides symptomatic relief, and it is potentially curative. Its build-up phase represents vital information to improve patient compliance and treatment outcomes.

Aim: This study aims to assess the build-up phase outcomes of subcutaneous immunotherapy (SCIT) for pediatric allergic asthma.

Methods: The retrospective cohort study comprised 65 children with allergic asthma due to house dust mites at the end of initial build-up phase of SCIT (3 months) from 2009 until 2012 at one hospital in Surabaya, Indonesia. Pre-SCIT clinical evaluation included skin prick tests for Der p 1 and Der f 1, eosinophil counts test, and the reversibility of forced expiratory volume in a second. The serum level of IFN-γ (TH_1), IL-4 (TH_2), TGF-β (T_{regs}), and IL-17 (TH_{17}) were measured via enzyme-linked immunosorbent assays (ELISA) at the end of the initial build-up phase of SCIT.

Results: Of the 65 samples, 48 (73.8%) exhibited significant improvement after three months, including 37 (56.9%) partially controlled asthma as against 11 (16.9%) controlled one, respectively. Eosinophil counts, IL-4, and IL-17 levels were higher among subjects who did not have an improvement, whereas they showed lower IFN-γ levels and smaller wheal diameters for either Der p 1 or Der f 1. Total IgE and TGF-β levels were not significantly different according to the asthma refinement.

Conclusion: At the end of the build-up phase, SCIT facilitated improvement in most subjects with allergic asthma, and the outcome was associated with eosinophil counts, wheal diameters for Der p 1 and Der f 1, as well as IFN-γ, IL-4, and IL-17 levels.

Keywords: Pediatric asthma, SCIT, allergic mediator

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INTRODUCTION

House dust mite (HDM) allergen exposure is increasingly recognized as an essential cause of allergic asthma in children, particularly in Indonesia. The prevalence of asthma in Indonesia ranges from 3–16%. HDM allergens induce sensitization in 77% of asthmatic patients.[1,2] Subcutaneous immunotherapy (SCIT) is an established recommended treatment for allergic asthma with a broad and potential evidence-based treatment.[3-6] Although data are limited, the efficacy of allergen-specific immunotherapy against pediatric asthma in Surabaya, Indonesia has been reported in a small number of placebo-controlled studies.[7-10]

SCIT provides symptomatic relief and is potentially curative. The immunologic mechanisms of SCIT include all parts of the immune system. Regulatory T cells (T_{reg}) have a major pivotal role in the immunotherapy triumph. In addition to T_{regs}, elevated suppressor cytokine levels (TGF-β), suppression of TH_2 cells, increased titers of IgG_4 as well as gradual declines in the number and function both of basophils and mast cells also contribute to the successful treatment.[11] The aforementioned immune mechanisms are connected and related to each other, acting at different times during SCIT. Allergen-specific immunotherapy reduces health care costs within three months of initiation[12] and produces sustained clinical benefits after the completion of a treatment course, approximately 3–5 years.[13] Clinically successful SCIT is accompanied by altered allergen-specific T cell responses such as a decreased TH_2/TH_1 ratio, enhanced TGF-β secretion, and T_{reg} induction.[14]

SCIT is administered at a physician's office at least every six weeks for 3–5 years,[12] but it does not usually confer immediate symptom relief. Successful clinical outcomes require a strong patient commitment to treatment adherence. Available data suggest that patient demographics, illness, and insurance characteristics may substantially

12. Komentar untuk karya penelitian : 'Judul Artikel: Distribution of iNOS Expression of iNOS Expressions and TNF Neutrophil Cells as well as PGE2 and S100 Schwann Cell Dermal Nerves in The Erythema Nodosum Leprosum Patients , Penulis: I Gusti Nyoman Darmaputra, Nanny Herwanto, Luh Mas Rusyati, Wibi Riawan, Anang Endaryanto, Cita Rosita Sigit Prakoeswa, Nama Jurnal: Bali Medical Journal (Mali Med J), Volume Jurnal: 7, Nomor Jurnal: 1, Tahun Terbit Jurnal: 2018, Halaman: 262-266, ISSN: 2089-1180, 2302-2914, Penerbit: Fakultas Kedokteran Universitas Udayana ': journal internasional penulis7 simtest 105 peer review ada Penelitian pada kasus leprosi dengan peranan prostaglandin pertahanka sistm syaraf penelitian cukup baik tulisan baik dan bermanfaat untuk pengobatan kedepan

13. Komentar untuk karya penelitian : 'Judul Artikel: Home Humidity Increased Risk of Tuberculosis in Children Living With Adult Active Tuberculosis Cases, Penulis: Pudji Lestari, Florentina Sustini, Anang Endaryanto, Retno Asih, Nama Jurnal: Universa Medicina, Volume Jurnal: 30, Nomor Jurnal: 3, Tahun Terbit Jurnal: 2011, Halaman: 138-145, ISSN: 2407-2230, 1907-3062, Penerbit: Universa Medicina by Faculty Medicine Trisakti University': ISSN online penulis 3 peer review ada Penelitian membuktikan humiditas rumah lebih 75% anak akan terkena TBC diperlukan tempat dengan huminiditas yang baik Penelitian baik tulisan baik bermanfaat cegah tb

- 14. Komentar untuk karya penelitian : 'Judul Artikel: Proteksi Probiotik pada Mukosa Ileum Mencit yang Terpanjang Lipopolisakarida Eschericha coli, Penulis: Alpha Fardah Athiyyah, Ariani Setiawati, Andy Darma, Anang Endaryanto, I Ketut Sudiana, Reza Ranuh, Subijanto MS, Nama Jurnal: Jurnal Medika Indonesiana , Volume Jurnal: 46, Nomor Jurnal: 2, Tahun Terbit Jurnal: 2012, Halaman: 80-85, ISSN: 5125-1762, Penerbit: FK UNDIP Semarang': ISSN online penulis4 peer review ada Penelitian pa mencit yg erpajan LPS E Colo ternata probiotik memberikan pelindungan pada mucosa usus ileumnya Penelitian baik tulisan cuku dan bermanfaat

- 15. Komentar untuk karya penelitian : 'Judul Artikel: Studi Observasional Pasca-Pemasaran Formula Isolat Protein Kedelai pada Bayi dengan Gejala Sugestif Alergi Terhadap Protein Susu Sapi, Penulis: Zakiudin Munasir, Dina Mukriarti, Anang Endaryanto, Ketut Dewi Kumarawati, Budi Setiabudiawan, Smadiono, Johannes Hudyono, Melva Louisa, Arini Setiawan, Nama Jurnal: Sari Pediatri, Volume Jurnal: 15, Nomor Jurnal: 4, Tahun Terbit Jurnal: 2013, Halaman: 237-243, ISSN: 0854-7823, 2338-5030, Penerbit: Badan penerbit Ikatan Dokter Anak Indonesia': ISSN online penulis 3 peer review ada Penelitian formula proteinsusu kedele cukupaman dan dapat mnggantikan anak yg alergi dgn susu sapi-penelitian baik tulisan baik dan bermanfaat

- 16. Komentar untuk karya penelitian : 'Judul Artikel: Kesesuaian Gejala Klinis dengan Hasil Uji Tusuk Kulit dan Uji Provokasi Makana pada Reaksi Simpang Terhadap Makanan, Penulis: Azwin Lubis, Wisnu Barlianto, Anang Endaryanto, Ariyanto Harsono, Nama Jurnal: Berkala Ilmu Kesehatan Kulit dan Kelamin, Volume Jurnal: 31, Nomor Jurnal: 1, Tahun Terbit Jurnal: 2019, Halaman: 106-116, ISSN: 2450-4082, 1978-4082, Penerbit: Bag/SMF Ilmu Kesehatan Kulit dan Kelamin FK UNAIR-RSUD Dr. Soetomo': ISSN online penulis3 peer review ada Penelitian alergi makanan terkait IgE tidak terdapat kesesuaian antara hasil uji tusuk kulit dengan hasil uji provokas IgE penelitian sangat baik tulisan baik dan bermafaat utk kedepan

- 17. Komentar untuk karya penelitian : 'Judul Artikel: Enahnced Efficacy of Sublingual Immunotheraphy in Chilhood Allergic Asthma By Probiotics, Penulis: Anang Endaryanto, Mira Irmawati, Nama Jurnal: Majalah Folia Medica Indonesiana, Volume Jurnal: 54, Nomor Jurnal: 1, Tahun Terbit Jurnal: 2018, Halaman: 64-74, ISSN: 2355-8393, 2599-056X, Penerbit: GRAMIK FK UNAIR, DOI: http://dx.doi.org/10.20473/fmi.v54i1.8055': ISSN online penulis 1 peer review ada Penelitian pengobatan alergy asthma pada anak dengan imunoterapi sub lingual sangat baik tulisan baik khusus utk penyebab alergi bermanfaat

- 18. Komentar untuk karya penelitian : 'Judul Artikel: Gambaran Klinis Steven Johnson Syndrome dan Toxic Epidermal Necrolysis pada Pasien Anak, Penulis: Annisa Fitriana, Anang Endaryanto, Afif Nurul Hidayati, Nama Jurnal: Berkala Ilmu Kesehatan Kulit dan Kelamin, Volume Jurnal: 30, Nomor Jurnal: 2, Tahun Terbit Jurnal: 2018, Halaman: 102-110, ISSN: 1978-4279, 2549-4082, Penerbit: Bag/SMF Ilmu Kesehatan Kulit dan Kelamin FK UNAIR-RSUD Dr. Soetomo, DOI:

influence treatment adherence.[15] Patient education regarding the treatment course and the slow effect are important factors for improving compliance and treatment outcomes.[16] One-third of treated patients fail to complete SCIT. Among those who complete SCIT, another one-third (34.6%) did not believe that the therapy was effective.[17] The SCIT satisfaction scores at the end of treatment were higher than those in the first year of therapy among adherent patients.[18] The duration of SCIT may be prolonged (5 years or more) depending on the clinical response of the subjects. Currently, no specific laboratory tests or biomarkers are available to distinguish patients who will relapse from those who will enjoy prolonged clinical remission after discontinuing SCIT.[19] The duration of SCIT should be individualized from the patients' clinical response, disease severity, adverse events (AEs), and preferences.[20] The build-up phase (3 months) clinical outcomes of SCIT represent valuable information for patient education to improve patient compliance and treatment outcomes. Those could be according to disease severity, clinical response, and immune response to SCIT.

METHODS

Subjects

The retrospective cohort study comprised 65 children with allergic asthma due to HDM allergens who finished the initial build-up phase of SCIT (3 months) from 2009 until 2012 at Dr. Soetomo General Hospital in Surabaya, Indonesia. The subjects were 6–17 years old and they had a diagnosis of asthma as defined by the American Thoracic Society (i.e., "a disease characterized by increased responsiveness of the trachea and bronchi to various stimuli and manifested by widespread narrowing of the airway that changes in severity either spontaneously or as a result of therapy") for ³ 3 months. The inclusion criteria were as follows: pre-bronchodilator forced expiratory volume in 1 s (FEV1) ³ 60% and £ 90% of the Polgar predicted normal value, reveals the reversibility criteria defined as an increase in FEV1 ³ 12% from the pre-bronchodilator value 15–30 min after two actuation of salbutamol pMDI (100 mg/actuation), and fits them with the GINA criteria for uncontrolled asthma.

A skin prick test (SPT) was applied to examine sensitivity to the following HDM allergens (Allergopharma, Reinbek, Germany): *Dermatophagoides pteronyssinus* (Der p 1) and *Dermatophagoides farinae* (Der f 1). Reactivity to HDM allergens in the SPT was identified via allergen-induced wheals with an equal or larger diameter mean than histamine-induced wheals. Total IgE levels were measured using the ImmunoCAP system (Thermo-Fisher, Uppsala, Sweden).

Informed consent was obtained from either each patient or his or her legal guardians before study inclusion. The ethics committee of Dr. Soetomo Hospital approved this study. General data – including name, address, age, gender, body weight, body height, and telephone number – were collected and recorded for all participants. A comprehensive medical and allergy history was obtained for all subjects, especially on allergic asthma and its duration, as well as details about allergies and controller medications for family members.

Clinical evaluations

Based on Dr. Soetomo General Hospital guidelines for SCIT, the authors reviewed subjects' diaries, medical records on drug prescriptions, the presence of exacerbation, AEs during immunotherapy, and the results of lung function tests and serum total IgE levels over the treatment period. The subjects' diary cards recorded symptoms and medication scores, including daytime symptoms, limitation of activities, nocturnal symptoms or awakening, need for reliever or rescue treatment, lung function, and exacerbation history.

The level of asthma control was recorded in each patient's standardized hospital medical record based on GINA guidelines. Controlled asthma was defined as daytime symptoms no more than two types per week, no limitation of activities, no nocturnal symptoms or awakening, use of reliever or rescue treatment no more than two types per week, normal lung function, and no exacerbation history. Second, partially controlled, the presence of at least one criterion among daytime symptoms more than twice per week, any limitation of activities, any nocturnal symptoms or awakening, need for reliever or rescue treatment more than twice per week, less than 80% predicted or normal lung function result and one or more exacerbations per year. At least, uncontrolled asthma, the presence of at least three of the criteria for partial control.

As for each patient, the total number of medications taken daily (systemic antihistamines, nasal cromoglycate, ocular cromoglycate, beta-2 agonist) was recorded on the daily diary cards. Symptoms and medication scores were determined as the monthly cumulative values obtained by summing the daily scores. The clinical evaluation also included SPTs for Der p 1 and Der f 1, eosinophil counts, and assessment of the reversibility of FEV1. The immunological assessment included an examination of the plasma levels of IFN-γ (TH_1), IL-4 (TH_2), TGF-β (T_{regs}), IL-17 (TH_{17}), and total IgE.

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Table 1. Demographic and clinical parameters of pre-SCIT patients

Demographic and clinical parameters	Mean (SD)
Age (years)	9.3 (1.99)
Body Weight (kgs)	29.4 (10.80)
Body Height (cm)	131.7 (12.77)
Male/Female (n)	33/32
Daytime symptoms/week (n)	38.1 (25.54)
Limitation of activities/week (n)	17.8 (7.24)
Nocturnal symptoms or awakening/month (n)	4.0 (2.48)
Need for reliever or rescue treatment/week (n)	3.3 (1.45)
Lung function, FEV_1 (% predicted)	60.2 (14.45)
Exacerbations/month (n)	1.8 (1.18)
Blood eosinophil count ($\times 10^6$/L)	739.9 (408.31)
Total IgE (kU/L)	238.4 (219.86)
Skin prick test for Der p 1, wheal (mm)	8.5 (3.44)
Skin prick test for Der f 1, wheal (mm)	7.3 (2.07)

Abbreviations: SCIT, subcutaneous immunotherapy; FEV_1, forced expiratory volume in 1 s.

Table 2. Clinical parameters after the build-up phase (after 3 months) of SCIT

Clinical parameters	Controlled asthma (n = 11)	Partially controlled asthma (n = 37)	Uncontrolled asthma (n = 17)	p-value
Daytime symptoms (week)	0.4 (0.67)	6.0 (4.37)	16.9 (8.22)	0.000
Limitation of activities (week)	0.0 (0.00)	0.0 (0.00)	0.5 (0.51)	0.000
Nocturnal symptoms/ awakening (month)	0.0 (0.00)	1.6 (1.79)	3.8 (2.61)	0.000
Need for reliever/rescue treatment (week)	0.0 (0.00)	1.0 (0.70)	3.1 (1.65)	0.000
FEV_1 % predictive	87.2 (7.99)	75.0 (14.23)	61.4 (15.36)	0.001
Exacerbations history	0.5 (0.52)	1.3 (0.80)	1.8 (1.19)	0.001

Notes: Data are presented as the mean (SD).
Abbreviations: SCIT, subcutaneous immunotherapy; FEV_1, forced expiratory volume in 1 s.

In vitro cytokine measurements

Plasma cytokines levels were measured via ELISA using a human $TH_1/TH_2/T_{reg}$ cytometric bead array kit according to the manufacturer's instructions (BD Biosciences). All samples were examined in duplicate.

Immunotherapy

All subjects conducted SCIT with HDM allergen extract produced by the Dr. Soetomo General Hospital Pharmaceutical Unit. The treatment period was divided into two phases: an initial build-up phase and a maintenance phase. In the initial build-up phase, subjects received subcutaneous injections of gradually increasing doses of allergen extract every week for 14 weeks, followed by once-monthly maintenance doses. Outcomes were evaluated after the initial build-up phase.

Statistical analysis

The sample size of 65 patients provided sufficient power (90%) to detect a difference of 10% among 3 groups ($\alpha = 0.05$). Statistical analysis was performed via multivariate testing, as well as used SPSS software. Data are presented as the mean ± SD, and p-values of 0.05 or less were considered statistically significant.

RESULTS

Table 1 showed the demographic and clinical parameters of the patients (n = 65) prior to the initial treatment. The standardized hospital medical records revealed that all subjects were sensitive to HDMs, as evaluated via skin test (wheal ≥ 3 mm), and all met the GINA criteria for uncontrolled

Symptoms and lung function

The clinical parameters of the subjects after the initial build-up phase are shown in Table 2. Symptoms and lung function significantly differed among subjects with controlled, partially controlled, and uncontrolled asthma. Improvements of symptoms and lung function were observed in 48 of 65 subjects (73.8%) after three months of SCIT, including partial and complete control in 37 (56.9%) and 11 (26.2%) subjects, respectively (Table 2).

Laboratory parameters

Table 3 presented data for various laboratory parameters after the initial build-up phase of SCIT. The results illustrated that subjects with uncontrolled asthma had significantly higher eosinophil counts than those with controlled or partially controlled asthma (p = 0.029). Additionally, IL-4 (p = 0.008) and IL-17 levels (p= 0.000) were also significantly elevated among subjects with uncontrolled asthma, whereas these subjects exhibited significantly lower IFN-γ levels (p= 0.002) and significantly smaller wheal diameters for Der p 1 (p = 0.000) and Der f 1 (p = 0.000). Total IgE and TGF-β levels did not significantly differ according to the improvement of asthma.

Laboratory variables at the end of the build-up phase of SCIT

Figure 1 and 2 illustrated that the non-improved group had significantly higher eosinophil counts (p = 0.009) than the improved one (both partially controlled and controlled asthma), as well as had significantly higher IL-4 (p = 0.045) and IL-17 levels (p = 0.000). Meanwhile, IFN-γ levels (p = 0.001)

Table 3. Laboratory parameters after the build-up phase (after 3 months) of SCIT

Laboratory parameters	Controlled asthma (n = 11)	Partially controlled asthma (n = 37)	Uncontrolled asthma (n = 17)	p-value
SPT for Der p 1 (mm)	7.6 (2.98)	12.2 (7.43)	25.4 (12.61)	0.000
SPT for Der f 1 (mm)	7.0 (2.12)	10.7 (5.92)	18.8 (8.20)	0.000
IgE (kU/L)	469.2 (537.21)	321.3 (310.99)	225.7 (344.22)	0.190
Eosinophil count (× 10^6/L)	548.9 (273.50)	321.3 (206.17)	569.2 (295.75)	0.029
IFN-γ (pg/mL)	10.0 (8.77)	11.7 (7.42)	4.5 (2.80)	0.002
IL-4 (pg/mL)	6.6 (9.04)	14.06 (8.80)	17.7 (91.47)	0.008
TGF-β (×1000 pg/mL)	66.3 (38.69)	56.9 (31.73)	47.1 (28.74)	0.302
IL-17 (pg/mL)	4.6 (0.2)	5.9(14.78)	16.6 (10.81)	0.000

Abbreviations: SCIT, subcutaneous immunotherapy; SPT, skin prick test.

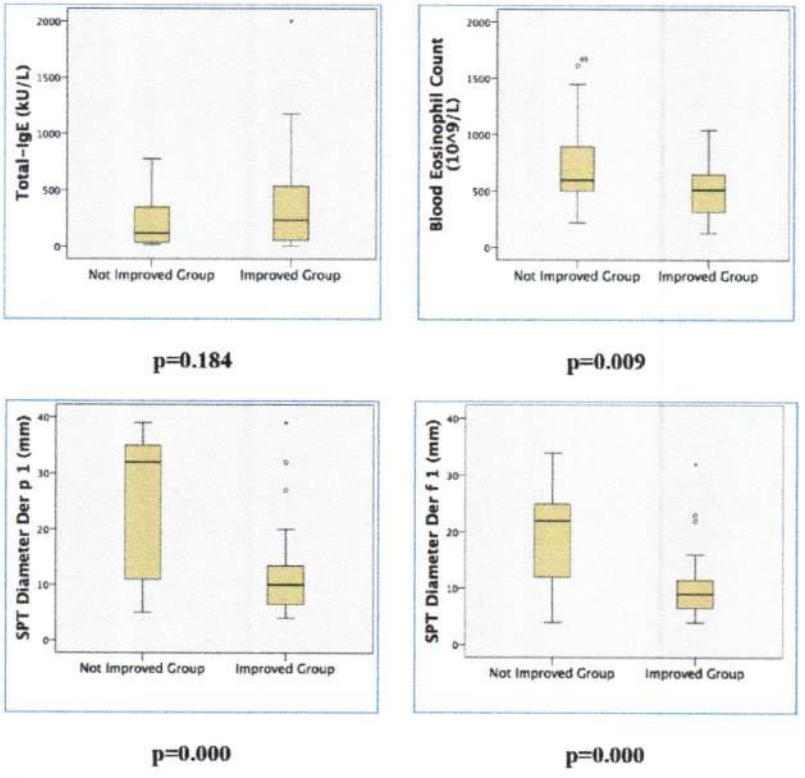

Figure 1. Laboratory data for the non-improved (uncontrolled asthma) and improved groups (both partially controlled and controlled asthma) at the end of the build-up phase of subcutaneous immunotherapy.

and wheal diameters for both Der p 1 (p = 0.000) and Der f 1 (p = 0.000) were significantly lower in the non-improved group. Total IgE and TGF-β levels were not significantly different between the groups.

DISCUSSION

The goals of asthma therapy are to alleviate symptoms, minimize risks of adverse outcomes (e.g., hospitalization, loss of lung function), and minimize AEs associated with treatment.[21-24] In our retrospective cohort study, SCIT using HDM allergen extract improved symptoms and lung function in nearly three-fourths of subjects within 3 months. In line with this finding, the previous studies reported that the majority of patients receiving immunotherapy exhibit increased FEV_1 within 3 months.[7,25] However, these findings conflicted with those obtained by Maggie *et al.*[26]

Larger wheal diameters for Der p 1 and Der f 1 in patients with uncontrolled asthma indicates that we can create these variables to predict the clinical outcomes of SCIT.[27] The findings were different from those of other studies in children and adults, which observed significantly higher baseline levels of specific IgE to HDM allergens in responders.[28-31]

A previous study demonstrated the effectiveness of immunotherapy was in line with the increased levels of TH_1 and T_{regs}.[32] There were also some reports that IFN-γ levels were identified more increased against post-immunotherapy.[33-35] On the other hand, post-immunotherapic TGF-β levels were also turned up progressively as gained from the previous study.[32,36,37] The wheal diameters for Der p 1 and Der f 1 represent the reactivity of patients to the HDM allergens, which is caused by high levels of allergen-specified IgE. IgG_4 levels were remained low after HDM SCIT in some patients.[38] High IgG_4 levels are associated with high levels of IFN-γ as well as a previous research confirmed high IgG_4 levels were in line with high levels of IL-10 and TGF-β.[39] Higher IFN-γ levels in uncontrolled asthma

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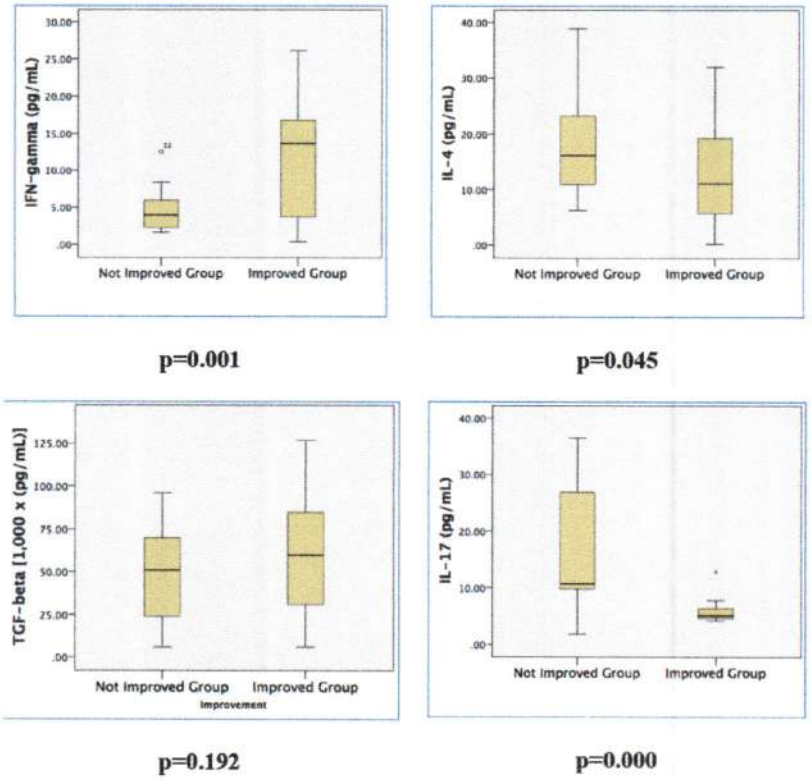

Figure 2. Serum cytokine levels in the non-improved (uncontrolled asthma) and improved groups (both partial controlled and controlled asthma) at the end of the build-up phase of subcutaneous immunotherapy.

patients indicated an opportunity to provide other immunomodulators as adjunct immunotherapies.[40]

Most clinical trials that evaluated the efficacy of SCIT had a treatment period of approximately 1–2 years.[41] The current study evaluated an early efficacy because the effects of SCIT after the build-up phase are considered as important data for a patient education program to improve compliance and treatment outcomes. Nevertheless, the endotype, phenotype, and severity of allergic diseases differ among patients. In this regard, lower eosinophil counts and stronger TH_1 and T_{reg} immune responses were significantly associated with uncontrolled asthma at the end of the build-up phase of SCIT in pediatric allergic asthma provoked by HDM allergens.

CONCLUSION

This retrospective cohort study found that SCIT improved symptoms in 73.8% of pediatric allergic asthma at the end of the build-up phase. The improved outcome was associated with lower eosinophil counts, lower IL-10 and IFN-γ levels, higher IL-4 levels, and smaller wheal diameters for Der p 1 and Der f 1.

LIMITATIONS

The present study had several limitations. In addition to its retrospective nature, the lack of a control group that did not receive SCIT made it difficult to estimate the true effectiveness of SCIT. In addition, the study covered maintenance durations of 3 months in patients at a single institution.

FUNDING

None

CONFLICT OF INTEREST

None

REFERENCES

1. Moustaki M, Loukou I, Tsabouri S, Douros K. The Role of Sensitization to Allergen in Asthma Prediction and Prevention. *Front Pediatr*. 2017;5:166. Published 2017 Jul 31. doi:10.3389/fped.2017.00166
2. Johan El Hakim Siregar, Lily Irsa, Supriatmo, Sjabaroeddin Loebis, Rita Evalina. Association between bronchial asthma in atopic children and their number of siblings. *Paediatrica Indonesiana*. 2014; 54(5): 289-93
3. Elliott J, Kelly SE, Johnston A, Skidmore B, Gomes T, Wells GA. Allergen immunotherapy for the treatment of allergic rhinitis and/or asthma: an umbrella review. *CMAJ Open*. 2017;5(2): 373-385.
4. EAACI Guidelines. Muraro A, Roberts G, editors. Allergen Immunotherapy Guidelines Part 2: Recommendations; 2017: 1-168. ISBN Number: 978-3-9524815-1-6.
5. Jutel M, Agache I, Bonini S, Burks AW, Calderon M, Canonica W, Cox L, Demoly P, Frew AJ, et al. International Consensus On (ICON) Allergy Immunotherapy (AIT). *J Allergy Clin Immunol*. 2015 Sep;136(3):556-68
6. Purkey T, Smith L M, Ferguson T, Luong B, Reisacher A, Pillsbury WC, Toskala H, Elina. Subcutaneous immunotherapy for allergic rhinitis: An evidence based review of the recent literature with recommendations [Abstract]. *International forum of allergy & rhinology*. 2013; 3. 10.1002/alr.21141.
7. Endaryanto, A & Irmawati, M. Enhanced Efficacy of Sublingual Immunotherapy in Childhood Allergic Asthma by Probiotics. *Folia Medica Indonesiana* 2018; 54 (64). 10.20473/fmi.v54i1.8055.
8. Jutel M. Allergen-Specific Immunotherapy in Asthma. *Curr Treat Options Allergy*. 2014;1(2):213-219. Published 2014 Mar 12. DOI:10.1007/s40521-014-0013-1
9. Incorvaia C, Riario-Sforza GG, Incorvaia S, Frati F. Sublingual immunotherapy in allergic asthma: Current evidence and needs to meet. *Ann Thorac Med*. 2010;5(3):128-32. [Accessed on March 2019] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2930649/.
10. Harsono, A. Specific immunotherapy for allergic diseases. *Paediatrica Indonesiana* 2005; 45 (7-8): 137-44.
11. Martin H, Taube C. Regulatory T cells and regulation of allergic airway disease. *Am J Clin Exp Immunol*. 2012;1(2):166-78. Published 2012 Nov 15.
12. Hankin CS, Cox L, Bronstone A, Wang Z. Allergy immunotherapy: reduced health care costs in adults and children with allergic rhinitis [Abstract]. *J Allergy Clin Immunol*. 2013 Apr;131(4):1084-91. DOI: 10.1016/j.jaci.2012.12.662.

13. Penagos M, Eifan AO, Durham SR, Scadding GW. Duration of Allergen Immunotherapy for Long-Term Efficacy in Allergic Rhinoconjunctivitis. *Curr Treat Options Allergy.* 2018;5(3):275-290.
14. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune tolerance to allergens. *World Allergy Organ J.* 2015;8(1):17. Published 2015 May 14. doi:10.1186/s40413-015-0063-2
15. Rolnick SJ, Pawloski PA, Hedblom BD, Asche SE, Bruzek RJ. Patient characteristics associated with medication adherence. *Clin Med Res.* 2013;11(2):54-65.
16. Musa F, Al-Ahmad M, Arifhodzic N, Al-Herz W. Compliance with allergen immunotherapy and factors affecting compliance among patients with respiratory allergies. *Hum Vaccin Immunother.* 2016;13(3):514-517.
17. Tsabouri S, Mavroudi A, Feketea G, Guibas GV. Subcutaneous and Sublingual Immunotherapy in Allergic Asthma in Children. *Front Pediatr.* 2017;5:82. Published 2017 Apr 21. DOI:10.3389/fped.2017.00082
18. Gelincik A, Demir S, Olgaç M, İşsever H, Khishigsuren B, Özşeker F, Çolakoğlu B, Büyüköztürk S. High adherence to subcutaneous immunotherapy in a real-life study from a large tertiary medical center. *Allergy Asthma Proc.* 2017 Nov 1;38(6):78-84. DOI: 10.2500/aap.2017.38.4091.
19. Pajno GB, Bernardini R, Peroni D, et al. Clinical practice recommendations for allergen-specific immunotherapy in children: the Italian consensus report. *Ital J Pediatr.* 2017;43(1):13. Published 2017 Jan 23. DOI:10.1186/s13052-016-0315-y
20. Tsabouri, Mavroudi S, Feketea A, Guibas G. Subcutaneous and Sublingual Immunotherapy in Allergic Asthma in Children. *Frontiers in Pediatrics* 2017; 5. 10.3389/fped.2017.00082.
21. National Asthma Education and Prevention Program, Third Expert Panel on the Diagnosis and Management of Asthma. Expert Panel Report 3: Guidelines for the Diagnosis and Management of Asthma. Bethesda (MD): National Heart, Lung, and Blood Institute (US); 2007 Aug. Section 4, Managing Asthma Long Term: Overview. Available from: https://www.ncbi.nlm.nih.gov/books/NBK7234/
22. Scichilone N, Contino A, Figlioli GB, Paglino G, Bellia V. Patient perspectives in the management of asthma: improving patient outcomes through critical selection of treatment options. *Patient Prefer Adherence.* 2010;4:17-23. Published 2010 Feb 4.
23. Braido F. Failure in asthma control: reasons and consequences. *Scientifica (Cairo).* 2013;2013:549252.
24. GINA. Difficult-to-Treat & Severe Asthma in Adolescent and Adult Patients. Diagnosis and Management. A GINA Pocket Guide for Health Professionals. Global Initiative for Asthma; 2018. www.ginasthma.org.
25. Yukselen A. Allergen-specific immunotherapy in pediatric allergic asthma. *Asia Pac Allergy.* 2016;6(3):139-48.
26. Maggie Pik-Kee Lit. Occupational Asthma [Newsletter]. *Hongkong Institute of Allergy.* May 2017: 29-30.
27. Cheng L, Chen J, Fu Q, He S, Li H, Liu Z, Tan G, et al. Chinese Society of Allergy Guidelines for Diagnosis and Treatment of Allergic Rhinitis. *Allergy Asthma Immunol Res.* 2018 Jul; 10 (4): 300-353. DOI: org/10.4168/aair.2018.10.4.300
28. Lee JH, Kim S-C, Choi H, Jung C-G, Ban G-Y, Shin YS, et al. Subcutaneous Immunotherapy for Allergic Asthma in a Single Center of Korea: Efficacy, Safety, and Clinical Response Predictors. *J Korean Med Sci* 2017; 32: 1124-1130. DOI: org/10.3346/jkms.2017.32.7.1124
29. Taketomi E.A., Silva, D.A.O., Sopelete, M.C., Gervásio, A.M., Alves, R., Sung, S.J. Differential IgE reactivity to Der p 1 and Der p 2 allergens of *Dermatophagoides pteronyssinus* in mite-sensitized patients. *J Investig Allergol Clin Immunol 2006; Vol. 16(2): 104-109*
30. Lee JH, Kim SC, Choi H, Jung CG, Ban GY, Shin YS, Nahm DH, Park HS, Ye YM. A Retrospective Study of Clinical Response Predictors in Subcutaneous Allergen Immunotherapy with House Dust Mites for Allergic Rhinitis. *Allergy Asthma Immunol Res.* 2018; 10 (1): 18-24. DOI: ORG/10.4168/AAIR.2018.10.1.18
31. Majori, M, Caminati A, Coradi M, Brianti E, Scarpa S, Pesci A. T-cell cytokine pattern at three time points during specific immunotherapy for mite-sensitive asthma [Abstract]. Clinical and Experimental Allergy: *Journal of the British Society for Allergy and Clinical Immunology* 2000; 30(3):341-347.
32. Durham SR, Kay AB, Hamid Q. Changes in Allergic Inflammation Associated with Successful Immunotherapy [Abstract]. *Int Arch Allergy Immunol* 1995; 107: 282-284. DOI: 10.1159/000237003
33. Plewako H., Holmberg, K., Oancea, I., Gotlib, T., Samoliński, B., Rak, S. A follow-up study of immunotherapy-treated birch-allergic patients: effect on the expression of chemokines in the nasal mucosa [Abstract]. *Clinical and Experimental Allergy* 2008; 38(7): 1124-31. DOI: org/10.1111/j.1365-2222.2008.03005.x
34. Meiler, F, et al. In vivo switch to IL-10–secreting T regulatory cells in high dose allergen exposure. *Journal of Experimental Medicine* 205.12 (2008): 2887-2898.
35. Francis M and Sharon M. Wahl. Dysregulation of IFN-γ Signaling Pathways in the Absence of TGF-β1. *J Immunol* 2002; 169 (10): 5941-7. DOI: https://doi.org/10.4049/jimmunol.169.10.5941
36. Martín-Muñoz, M.F., Pineda, F., Muiños, T., Fontan, M., Nevot, S., Bosque, M., Palomo, J.J., Torredemer, A., et al. Changes in IL-10 and specific antibodies associated to successful Dermatophagoides pteronyssinus immunotherapy in children during the first year of treatment [Abstract]. *Allergologia et Immunopathologia* 2013; 41 (Issue 1): 4-10. DOI: ORG/10.1016/J.ALLER.2011.12.005
37. Shamji, M. H., Kappen, J. H., Akdis, M., Jensen-Jarolim, E., Knol, E. F., Kleine-Tebbe, J., Bohle, B., Chaker, A. M., et al. Biomarkers for monitoring clinical efficacy of allergen immunotherapy for allergic rhinoconjunctivitis and allergic asthma: an EAACI Position Paper, *Allergy*, 2017; 72 (8): 1156-1173.
38. Fujita H, Soyka MB, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. *Clin Transl Allergy.* 2012; 2(1): 2. DOI:10.1186/2045-7022-2-2
39. Vitaliti G, Pavone P, Guglielmo F, Spataro G, Falsaperla R. The immunomodulatory effect of probiotics beyond atopy: an update [Abstract]. *J Asthma.* 2014 Apr; 51(3): 320-32. DOI: 10.3109/02770903.2013.862259.
40. Tosca MA, Licari A, Olcese R, Marseglia G, Sacco O, Ciprandi G. Immunotherapy and Asthma in Children. *Front Pediatr.* 2018; 6: 231. DOI:10.3389/fped.2018.00231