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Original Article

A comparative study of sublingual and subcutaneous immunotherapy in mite-sensitive asthmatic children: a single center experience of 90 Chinese patients

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Abstract: Objective: Asthma is a heterogeneous disease with the geographic and ethnic diversity. Present study aimed to compare between sublingual (SLIT) and subcutaneous immunotherapy (SCIT) in Chinese asthma children with respect to the clinical efficacy, adverse effects and immunological mechanisms. Methods: The prevalence of pediatric asthma in the single center of China was investigated. Ninety patients were recruited and randomized into 3 groups to receive SLIT, SCIT, or pharmacotherapy alone. Asthma control test (SCT), asthma symptom, and medication score were collected. Total and specific IgE were determined, as well as allergen-specific IL-4 and IFN-γ. Results: 1648 cases of pediatric asthma were screened out, 42.2% (696) were allergen positive. There was a slight gender difference with boy predominance. Patients aged 0-3 years showed much lower allergen positivity ratios than other age groups. Both the SLIT and SCIT caused a significant reduction in asthma symptom and medication score and an increase in ACT score and lung functions. No statistical difference was found between the two groups. Less and negligible adverse effects were observed in SLIT group when compared with SCIT group. The decreased productions of IL-4 and sIgE with an increased IFN-γ production were detected in both the SCIT and SLIT groups. Conclusion: Both SLIT and SCIT demonstrated the favorable effects on pediatric asthma treatment, with no significant difference in asthma symptom, medication use, and immune response. However, SCIT caused much more and severe adverse effects, suggesting that SLIT may be a favorable therapy for asthma in Chinese children.

Keywords: Asthma, subcutaneous immunotherapy (SCIT), sublingual immunotherapy (SLIT), clinical efficacy, adverse effects, immunological mechanisms

Introduction

Asthma is a multifactorial chronic inflammatory disorder of airways with recurrent episodes of respiratory symptoms and has been reported to affect approximately 300 million of world population [1]. It is one of the most prevalent respiratory diseases in children with increasing incidence and mortality and has created a substantial impact on modern society, especially in China [2]. According to the statistical study, more than 25 million Chinese populations suffered from asthma, among which almost 10 million were children [3]. The mainstay treatments for asthma patients included: education of patients or their caregiver, allergen-avoidance strategy, pharmacotherapy for symptoms relief, and allergen-specific immunotherapy when appropriate [4].

Specific allergen immunotherapy is the specific treatment modality with the capacity of modifying the natural course of the allergic disease. Subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT) are two generally recognized approaches for immunotherapy [5]. SCIT has been accepted as a therapeutic option for management of respiratory allergic disorders with early onset of action and favorable clinical efficacy [6-9]. However, SCIT injection is inconvenient and may cause the discomfort after repeated injections and other unwanted

Table 1. Medication scores

Medication		Score
Budesonide (μg/d)	0	0
	0-200	1
	200-400	2
	400-800	3
	>800	4

side effects like systemic reactions in pediatric patients [10, 11]. The incidence of systemic reactions of SCIT has been reported to vary from 0.06% to 1.01% [12]. SLIT, a safer alternate modality by delivery of allergens through the sublingual area, has been proved to be a convenient and effective therapeutic strategy for modulation of the ongoing immunopathologic response in patients with allergic disorders [13]. The safety of SLIT seems better than that of SCIT and most associate with the local side effects, especially in the early phase of SLIT [14]. However, the persistent long-term effect of SLIT has also been reported following the discontinuation [15]. According to the recent systematic review, only four randomized controlled trials with head-to-head comparison of SCIT and SLIT in treatment of allergic disease in 171 participants have been reported [14, 16].

Asthma is a heterogeneous disease that may be influenced by geography and ethnicity [17]. The prevention and treatments for asthma have not been well established in China. Even in the urban areas, nearly one third of asthmatic patients was not early or correctly diagnosed [18]. Few such comparative studies have been carried out and the clinical efficacy and immunological mechanisms of SCIT and SLIT have not been well-characterized in Chinese populations. Therefore, in the present study, the prevalence of pediatric asthma in the single center of China was investigated, as well as the distribution of their allergen positivity. Pediatric patients met the inclusion criteria were recruited to compare between SLIT and SCIT with respect to the clinical efficacy, adverse effects and immunological mechanisms.

Methods

Patients

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional committee and informed consent was obtained from patients and their caregivers. The patients were selected from the pediatric asthmas referred to the pediatric outpatient of the Eighty-eight Hospital of People's Liberation Army during 2009 October to 2014 October. A total of 1684 asthma children aged below 14 years were diagnosed according to the guidelines for childhood asthma diagnosis and prevention made by Chinese medical association in 2008 [19]. Ninety children presenting with allergic asthma were included into this study. Recruitment criteria were as follows: (1) age between 5 and 14 years; (2) positive for skin prick test (SPT) and house dust mite (HDM) specific IgE (sIgE) and have HDM-related asthma symptoms; (3) a clinical history of asthma for at least 1 years while without other chronic diseases; (4) presence of symptoms despite optimal treatment and avoid allergens but without uncontrolled asthma; (5) no prior immunotherapy; (6) bronchial provocation test or exercise test positivity.

Clinical assessment

A skin prick test (SPT) screening was performed with commercial allergens from Alk-Abelló (Hørsholm, Denmark) according to the manufacture's instruction. Histamine was used as positive control, and normal saline as negative control. A ratio of the allergen wheal to the histamine wheal ≥0.25 was considered to be a positive reaction (+: ≥0.25/<0.5; ++: ≥0.5/<1.0; +++: ≥1.0/<1.5; ++++: ≥1.5/<2.0), and no response or the same response as to the saline control was considered as negative. sIgE were analyzed using the allergens obtained from Zhejiang Meidikang Ltd. (Zhejiang, China) according to the manufacturer's instructions. sIgE>0.35 kU/L was considered positive (grade 0: sIgE≤0.35 kU/L; grade 1: 0.35<sIgE≤0.7; grade 2: 0.7<sIgE≤3.5; grade 3: 3.5<sIgE≤17.5; grade 4: 17.5<sIgE≤50.0; grade 5: 50.0< sIgE≤100; grade 6: 100<sIgE).

Asthma symptom score (ASS) was calculated according the previously proposed method [20]. Asthma control test (ACT) was performed to evaluate disease control using a standardized form as described previously [21]. ACT score of less than 20 defines uncontrolled asthma, score ranging from 20 to 24 defines controlled asthma, while score equal to 25 defines full asthma control. Medication scores

Table 2. Doses and duration of immunotherapy

	SLIT	SCIT
Induction	Day 1-7: 1 μg/mL (1, 2, 3, 4, 6, 8, 10 drops/day)	Week 1-3: 100 SQ-U/mL (0.2, 0.4, 0.8 mL/week)
	Day 8-14: 10 μg/mL (1, 2, 3, 4, 6, 8, 10 drops/day)	Week 4-6: 1000 SQ-U/mL (0.2, 0.4, 0.8 mL/week)
	Day 15-21: 100 μg/mL (1, 2, 3, 4, 6, 8, 10 drops/day)	Week 7-9: 10000 SQ-U/mL (0.2, 0.4, 0.6, 0.8 mL/week)
		Week 10-15: 100000 SQ-U/mL (0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mL/week)
Maintenance	Day 22-: 333 μg/mL (3 drops/day)	Week 16-: Maximum tolerated dose (every 4th week)

SLIT: Sublingual immunotherapy; SCIT: Subcutaneous immunotherapy.

were calculated according to the scale shown in **Table 1**. Lung function was evaluated by peak expiratory flow rate measurement using a peak expiratory flow-rate meter. Forced expiratory volume in one second (FEV1)/forced vital capacity (FVC) ratio (FEV1%) was calculated by dividing FEV1 by FVC and then multiplying by 100 to express the value as a percentage. Fractional exhaled nitric oxide (FeNO) was measured using a NiOX MINO device (Aerocrine, Sweden), and reference range of FeNO was: low <5 ppb; normal 5-20 ppb; increased 20-35 ppb; high >35 ppb. Serum IL-10 and IFN-γ levels were measured by double-antibody sandwich enzyme-linked immunosorbent assay according to the instruction.

Immunotherapy

The patients were classified into three groups according to their therapeutic regimens (n=30), and were treated with SLIT and seretide (SLIT + seretide group), SCIT and seretide (SCIT + seretide group), or with seretide alone (seretide group). All patients in SLIT and SCIT groups received the protocol as recommended by the manufacturers (**Table 2**).

SCIT was administered in the clinic using allergen extracts from ALK-Abelló (Hørsholm, Denmark) and included a 15-week induction phase (weekly injections) followed by a monthly maintenance phase. A dose of 0.2-0.8 mL of 100 SQ-U/Ml was administrated in week 1-3, 1000 SQ-U/mL in week 4-6, and of 10000 SQ-U/mL in week 7-9. During the 10-15 weeks, a dose of 0.1-0.8 mL of 100000 SQ-U/Ml (0.1, 0.2, 0.4, 0.6, and 0.8 mL) was administrated. The maximum tolerated dose obtained during the induction phase was the maintenance dose and was administrated every 4th week. The patients were allowed to use rescue medications (epinephrine, bronchodilator drug, antihistamines, oxygen inhalation equipment and etc.).

SLIT was self-administered at home using a standardized dust mite allergen drops (Zhejiang Wowu Biotech Co., Ltd., Hangzhou City, China). The initial dose was 1 drop of 1 μg/mL up to 10 drops on day 7 (1, 2, 3, 4, 6, 8, and 10 drops, respectively), 1 to 10 drops of 10 μg/mL on days 8-14, and 1 to 10 drops of 100 μg/mL on days 15-21. The maintenance dose was 3 drops of 333 μg/mL daily on days 22-27. The patients were allowed to use bronchodilator drug and antihistamines provided in a stepwise fashion according to the persistence and severity of the symptoms as recommended. Clinical data of the patients, including age, gender, body mass index (BMI), score of asthma control test (ACT), medication scores, lung function, FeNO, and immunological parameters (HDM-sIgE, total IgE, IL-4, and IFN-γ), were evaluated.

Adverse events

The adverse events including time of onset and resolution, severity, action taken were recorded. Systemic side effects were classified and graded according to World Allergy Organization recommendations [22].

Statistical analysis

Data were presented as mean ± SD unless otherwise specified. Statistical analysis was performed using SPSS version 19.0 (SPSS Inc., Chicago, IL, USA). Differences between the groups were analyzed by Student's t-test or chi-square test in appropriate. A *P* value of less than 0.05 was considered statistically significant.

Results

A total of 1648 cases of pediatric asthma were screened from the Eighty-eight Hospital of People's Liberation Army alone during the October 2009 to October 2014. The 1274 patients had a history of allergic disease,

Table 3. Clinical data of 1648 cases of pediatric asthma diagnosed in a single center of Shandong, China

Age distribution (years)	Cases (male/female)	Allergen positive (male/female)	Allergen positive (%)	HDM positive (male/female)	HDM positive (%)
0~3	216/120	50/30	23.8	25/15	60.0
3~6	328/200	183/114	53.0*	131/78	71.5*
6~14	472/312	222/114	42.8*	153/79	69.0*
Total	1016/632	455/258	42.3	309/172	68.9

HDM: Home dusty mites; *P<0.05 vs. the patients between 0-3 years old.

Table 4. Demographic characteristics of the patients

	Seretide	SCIT + seretide	SLIT + seretide
Age (years)	7.07±1.21	7.57±1.46	7.37±1.26
Gender (male/female)	19/11	17/10	18/12
Duration of asthma (months)	1.57±0.6	1.66±0.633	1.60±0.63
Basal drug consumption (n)	25	24	25
Inhaled steroids	0.31±0.12	0.30±0.12	0.29±0.11
Antihistamines	0.21±0.20	0.19±0.19	0.22±0.21
β_2 *agonists*	0.18±0.06	0.17±0.05	0.19±0.04
Epinephrine	0.23±0.21	0.24±022	0.22±0.20
Bronchodilator drug	0.14±0.03	0.13±0.03	0.15±0.04
Treatment duration (months)	2.57±0.9	2.66±0.933	2.60±0.93

SLIT: Sublingual immunotherapy; SCIT: Subcutaneous immunotherapy.

accounting for about 75.7% of the asthma children, 702 patients (41.7%) with eczema or atopic dermatitis, and 36.9% of asthma children (621) complicated with allergic rhinitis. A familial history of allergy was found in 842 patients, and 16% of pediatric patients (269) had a family history of asthma. About 696 pediatric patients (42.2%) were found to be allergen positive, among which, 69.0% of patients (n=480) were positive for HDM. There was a slight gender difference with more boys than girls experiencing asthma at all age groups. No significant gender differences were observed in allergen and HDM positivity. Patients aged 0-3 years showed much lower positivity ratios of allergen and HDM when compared with that of patients between 3-6 or 6-14 years old (P<0.05), while there was no significant difference between the lateral two groups (**Table 3**).

Out of 90 children recruited in this study, 87 patients completed the study, and 3 patients in the SCIT + seretide group quitted the therapy (1 had systemic urticarial, 1 had asthma exacerbations, and the other one due to the discomfort of repeated injections). The number of patients evaluable for efficacy, safety and immunological comparison were 27 for SCIT + seretide group, and 30 for SLIT + seretide and seretide group each. The demographic characteristics of the patients were summarized in **Table 4**. There was no significant difference among the three groups with respect to age, gender, BMI, duration of asthma symptoms, medication score, ASS, ACT score, lung function, FeNO, eosnophils, and immunological parameters (HDM-sIgE, total IgE, IL-4, and IFN-γ).

As shown in **Table 5**, pharmacotherapy with seretide alone showed some promise in asthma treatment as demonstrated by better symptom (ASS, ACT) and medication scores, lower FeNO concentration, eosnophils, and total IgE (P<0.05). As for SCIT + seretide and SLIT + seretide treatment groups, there was a statistically significant difference in asthma symptom and medication scores, lung function, sIgE, and allergen-induced IL-4 and IFN-γ responses in comparison to the baseline.

Compared with the seretide group, the increase in PEFR and INF-γ, and the decreasein ASS, FeNO, sIgE, IL-4, and eosnophils were found significant in the SCIT and SLIT groups (P<0.01 and P<0.05, respectively). Although SCIT performed better than SLIT in pediatric asthma therapy, no statistical difference was found in terms of the reduction in asthma symptoms or in medication scores between the two groups.

Four cases of local reaction and one case of systematic reaction were observed in the seretide and SLIT groups, three being grade 1 and the other grade 2. Two cases in the SLIT group occurred in the induction phase and the other in the maintenance phase. All were remit-

Table 5. Primary outcomes of SCIT, SLIT and pharmacotherapy

	Seretide		SCIT + seretide		SLIT + seretide	
	Before	After	Before	After	Before	After
ACT score	18.74±3.33	23.01±2.66*	18.84±3.11	24.75±1.82*	19.06±3.51	23.35±2.13*
Medication score	0.80±0.66	0.40±0.23*	0.81±0.50	0.10±0.06*	0.85±0.36	0.34±0.11*
FEV1%	75.66±4.06	79.63±7.05	77.25±6.60	89.79±9.55*	77.66±5.71	87.35±9.96*
PEFR	79.69±8.02	86.95±5.59	81.79±8.60	89.56±4.21*,##	80.65±8.60	88.77±6.42*,#
FeNO	31±12	26±17*	30±11	19±6*,##	32±12	21±11*,#
sIgE	17.89±8.78	16.07±9.35	17.02±9.25	11.12±8.27*,##	18.62±8.32	13.07±9.15*,#
Total IgE	655.21±70.65	556.58±123.12*	684.99±67.78	95.99±86.92*,##	675.28±69.25	112.99±95.46*,#
IL-4	18.62±8.15	17.66±9.33	17.99±7.55	10.62±5.11*,##	19.00±8.17	11.98±6.01*,#
INF-γ	2.86±3.17	2.93±3.42	3.44±2.88	6.14±2.56*,##	2.95±3.02	5.63±3.17*,#
ASS	3.49±1.26	2.08±0.43*	3.42±1.32	0.74±0.13*,##	3.54±1.25	0.84±0.38*,##
Eosnophils	0.73±0.38	0.77±0.16	0.77±0.26	0.12±0.1*,##	0.76±0.23	0.30±0.26*,##

SLIT: Sublingual immunotherapy; SCIT: Subcutaneous immunotherapy; ACT: Asthma control test; FEV1%: Forced expiratory volume in one second/forced vital capacity ratio; FeNO: Fractional exhaled nitric oxide; sIgE: Specific IgE; Ass: Asthma symptom score; *P<0.05 vs. the group before therapy, #P<0.05 and ##P<0.01 vs. the seretide group.

ted by oral antiallergics or inhaled β_2 agonists. In the SCIT group, 11 patients had local reaction and 2 had systematic reaction, 10 being graded 1 and the other grade 2. Three patients in the SCIT group quitted because of the systemic urticarial, aggravated asthma, and discomfort of repeated injections.

There was no significant difference between any of the groups in terms of sIgE, IL-4, and INF-γ levels and levels seen in the pharmacotherapy group after therapy. A significant decrease in sIgE, IL-4, and INF-γ levels was observed in SCIT and SLIT groups following the immunotherapy when compared with pharmacotherapy alone. When comparing SLIT with SCIT, the immune response was generally similar although SCIT induced much larger changes (**Table 5**).

Discussion

Asthma is the most common chronic respiratory disorder among children, and the prevalence of childhood asthma has increased dramatically during the last few decades [23]. An increased risk of death has been reported in individuals with asthma [24, 25]. According to the Third nationwide survey of childhood asthma in urban areas of China, 13992 of 463982 urban children were diagnosed with asthma, among which 10143 patients (72.5%) had a personal history of allergy, and asthma prevalence was significantly different among the different regionsandcities, and different age and gender groups. Approximately one third of asthma patients were not well diagnosed, asthma therapy and management was still poor [18]. Allergen-specific immunotherapy has been used for almost a century in human medicine and was clinically effective in treatment of atopic asthma. However, it has long been considered as a controversial treatment for asthma [26]. SCIT has long been established as clinically effective in patients with asthma. However, such method of allergen administration was inconvenient and invasive, and caused severe adverse effects. Emerged as an alternative route for SCIT, SLIT has been reported to induce fewer systemic side effects althoughlocal side effects may be encountered [27]. SCIT was well documented to be more beneficial than SLIT regarding reduction of asthma symptoms. However, the utilization of SLIT in clinical practice was controversial, with both favorable and unfavorable outcomes reported [14, 16].

Various studies have been carried out to compare the clinical efficacy and immunological outcome between SCIT and SLIT. However, the number of patients included was comparatively small, and most studies only included less than 20 pediatric patients in each arm [16]. To our best knowledge, there are few such research work performed on Chinese population, and differing standardization of potency between countries caused the dose translation extremely hard. In our study, we screened the prevalence of asthma in the pediatric outpatient of the Eighty-eight Hospital of People's Liberation Army during 2009 October to 2014 October. A total of 1684 cases of pediatric asthma were identified, and about 75.7% (n=1274) of patients had a personal allergic history. About

696 patients (42.2%) were found to be allergen positive, among which, 69.0% (n=480) of patients were HDM positive, suggesting that sensitization to allergens, especially HDM, may be a dominant risk factor for asthma.

Ninety pediatric patients with asthma were then screened and recruited to further compare the clinical efficiency of SLIT and SCIT using the pharmacotherapy (seretide) alone as the control, and the immunologic outcome of the patients was also evaluated. The results of our study showed that both SLIT and SCIT demonstrated clinical improvement in pediatric asthma patients when compared with pharmacotherapy group, as demonstrated by lower medication scores, better asthma symptom control and lung functions. The more favorable results were obtained in patients treated with SCIT, while the difference was not significant enough for us to come to a definitive conclusion. The findings were partially consistent with the results reported by Eifan et al, which indicated a differentbut no significant benefit between the SLIT and SCIT groups, while lung functions were not found to be significantly improved by either SLIT or SCIT in their study [28]. However, the other studies reported also a significant reduction of asthma symptom in only SCIT group, while treatment with SLIT yielded no obvious effect when compared with the control group [7, 28-30]. These conflicting results can be partially explained by different dose used and time treatment, as well as the general condition of the patients.

Regarding the safety, our study showed few adverse effects in patients treated with SLIT, and most effects were local reactions, corroborating the results of previous studies, which showed negligible or no side effects in SLIT group [7, 28-30]. However, SCIT resulted in 11 cases of local reactions and 2 cases of systematic reactions, and 3 patients dropped out because of severe systematic reaction, enhanced asthma symptom, or discomfort of repeated injections. These results demonstrated SLIT as a safer therapy for patients with asthma.

Previous study indicated that clinical and immunologic benefits from immunotherapy were slightly different, and different immune mechanisms may be involved [7]. However, other study also indicated that there are no clear-cut qualitative differences between SCIT and SLIT, and they may have similar mechanisms [31]. Allergic asthma was associated with IL-4, IgE, and INF-γ production [32, 33]. In our study, a decrease in sIgE was detected in both the SCIT and SLIT groups when compared with pharmacotherapy alone, consistent with the results of previous study [28, 29]. However, unlike the results reported by their studies, which indicated no change in IL-4 and/or IFN-γ levels, our study showed a decreased production of IL-4 while an increased IFN-γ production in both the SLIT and SCIT groups, and SLIT group showed a small change in the data, suggesting that immune mechanisms involved could be quite similar, the clinical efficacy was less in SLIT group and resulted in less immune response. Future studies should be further performed to better clarify the potential effects.

To summary up, our study showed favorable effect of both SCIT and SLIT in treatment of the pediatric patients with asthma, and no statistical difference in asthma symptom, medication use, and immune response were found between the SLIT and the SCIT group. However, there were much more and severe adverse effects in the patients treated with SCIT. Put these all altogether, we believed that SLIT may be a favorable therapy for Chinese children with asthma. Further studies are still needed to address the time-coursed and long-term effect of the two treatment modes.

Disclosure of conflict of interest

None.

Abbreviations

ASS, Asthma symptom score; BMI, Body mass index; ACT, Asthma control test; FEV1, Forced expiratory volume in one second; FVC, forced vital capacity; FEV1%, FEV1/FVC ratio; FeNO, Fractional exhaled nitric oxide; HDM, House dust mite; SCIT, Subcutaneous immunotherapy; sIgE, Specific IgE; SLIT, Sublingual immunotherapy; SPT, Skin prick test.

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