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ORIGINAL ARTICLE

Effect of Vitamin D Adjuvant and Allergen Specific Immunotherapy on Serum IL-10 and IL-17 Levels in Childhood Asthma: A Controlled Clinical Trial

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ABSTRACT

Background: *1, 25-dihydroxy vitamin D3 (VitD3) can improve the effect of allergen-specific immunotherapy (SIT). Few data is available about its role in childhood asthma.* ***Objective:*** *To assess the immunological and clinical efficacy of VitD3 as an adjuvant to allergen specific immunotherapy in pediatric asthma.* ***Methodology:*** *Sixty nine children with atopic asthma were divided into three groups: a group received subcutaneous immunotherapy (SCIT) in combination with VitD3 (n=23), another group received SCIT alone (n=23), and the last group VitD3 alone (n=23). All children were assessed at baseline, and six months for rate of inhaled corticosteroid (ICS) discontinuation, and serum levels of IL-10, and IL-17A.* ***Results:*** *In the SCIT + vitD3, ICS discontinuation rate was higher compared to VitD3 alone group and SCIT alone group at the end of 6th month (P=0.555 and 0.016 respectively). The combined SCIT+ VitD3 group showed significant increase of serum IL-10 level in comparison to SCIT alone group and VitD3 alone group (P=0.000) and significant decrease in serum IL-17A level compared to VitD3 alone group (P= 0.011)* ***Conclusion:*** *VitD3 enhance the clinical and immunological outcomes of SIT in pediatric asthma. Further investigation is needed to evaluate this effect in a larger scale to confirm its role as an adjunct to SIT.*

INTRODUCTION

Childhood asthma is a common health problem. It is a chronic inflammatory respiratory disorder characterized by airway limitation and hyper-responsiveness[1].

Allergen-specific immunotherapy (SIT) is a special form of therapy that can provide long-term relief of symptoms in allergen-sensitive asthma[2]. This form of treatment can change the course of allergic disease mainly by induction of T regulatory cells (Tregs) with subsequent peripheral tolerance manifested by clinical improvement[3,4].

Adjuvants were investigated for enhancing the effect of SIT aiming to decrease the allergen dosing and, therefore, the cost[5, 6]. Furthermore, this can allow patients to be more adherent to the treatment, with subsequent improve in the efficiency of the SIT[7].

Among those adjuvants is 1, 25-dihydroxy vitamin D3 (VitD3) that acts as an immunomodulator and regulates the innate and adaptive immunity[8]. VitD3 promote the migration of dendritic cells (DC) and Tregs cells development with subsequent suppression of allergen-specific T helper (Th) 2 cells[3,4]. VitD3 suppress Th17 cells in different diseases[9, 10, 11]. The regulatory effect of VitD3 on Th17 cells occurs through the reduction of retinoic acid-related orphan receptor (ROR) expression [10].

Increased risk of asthma and allergic disease is correlated with VitD3 defeciency[4]. Some murine models proved the role of VitD3 in enhancing SIT efficiency [12].

Combination of VitD3 with subcutaneous immunotherapy (SCIT) showed better outcomes in asthmatic children sensitized to house dust mite[2]. VitD3 adjuvant effect on SIT in human needs further evaluation. Therefore, our aims were to assess the value of VitD3 on efficacy of SIT in pediatric asthma through estimating inhaled corticosteroids (ICS) discontinuation rate and the serum levels of IL-10 and IL-17.

METHODOLOGY

Study setting:

The present study was conducted at Allergy and Immunology Unit, Departments of Medical Microbiology & Immunology, Chest, Pediatrics and toxicology, Faculty of Medicine, Zagazig University Hospital, Zagazig University, Zagazig, Egypt. The study was carried out over a period of six months, from May 2020 to October 2020.

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Study subjects:

The study included 69 children (less than 18 years) diagnosed as asthma, according to Global Initiative for asthma consensus report (GINA) [13]. Inclusion criteria were: 1) atopic asthma, with or without allergic rhinitis, proved by positive skin prick test (SPT) for at least one inhalant allergen (± elevated total IgE); 2) mild to moderate asthma (assessed by GINA) [13].

Children who suffered from acute illness, any chronic diseases, any associated bronchopulmonary disease (assessed by chest x-ray), severe persistent asthma (assessed by GINA) [13] were excluded. Children received previous biological therapy or immunotherapy were also excluded.

Study design:

Our randomized, single blinded, controlled clinical trial included 69 children with atopic asthma who were randomly assigned to one of three groups; interventional group which included 23 children that received combined SCIT and VitD3 supplementation (Vidrops®, Medical Union pharmaceuticals, Egypt; 600 U/day); a control group which included 23 children that received SCIT alone; and another control group which included 23 children that received VitD3 supplementation alone.

All children were allowed to continue their pharmacotherapy in the form of ICS, bronchodilators and antihistamines. All cases and controls were assessed at the baseline and six months later. The primary efficacy outcomes were measuring the change in serum level of IL-10. Rate of ICS discontinuation and serum levels of both IL-17A and VitD3 were our secondary outcomes.

Ethical approval:

The study was approved by the institutional review board (IRB) no #6482/8-4-2020, Faculty of medicine, Zagazig University. An informed written consent was obtained from all parents at time of recruitment. This study was conducted in accordance with the Declaration of Helsinki.

Skin prick test (SPT):

Skin testing was performed according to *Bernstein et al.,2008* [14]. Children were asked to stop antihistamines a week before skin testing. A panel of locally encountered inhaled allergens was used including mixed mites, cockroach, cotton dust, mixed molds, hay dust, wool, house dust, mixed pollens. Histamine dihydrochloride (10 mg/ml) was used as a positive control, while saline was used as a negative control. The largest diameter of the wheal was measured and it was considered positive if it was ≥ 3 mm[14].

Allergen extracts of skin testing were locally prepared at Allergy and Immunology Unit, Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University. Aqueous allergen extracts (1:100 wt/vol) preparation was done according to allergen extract preparation guidelines developed by the AAAAI/ACAAI/ JCAAI and all aqueous allergen extracts were stored at 4 °C [15].

Sample collection:

Five ml blood were collected by venipuncture under complete aseptic conditions. Samples were allowed to clot then centrifuged at 1000 xg for 15 minutes. Sera were collected and stored at -20 °C. Sera were used to measure serum levels of total IgE, IL-10, IL17A, and VitD3.

Serum level of total IgE:

Quantitative measurement of serum level of total IgE was done using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) Kit supplied by IMMUNOSPEC Corporation (Canoga park, CA 91303, USA) according to the manufacturer's instructions. The results were expressed in IU/mL. Absorbance of standards and samples were measured at 450 nm using a microtiter plate ELISA reader (Biotek, USA).

Serum level of IL-10:

IL-10 level was measured by commercially available quantitative sandwich ELISA Kit supplied by Bioassay Technology Laboratory (Shanghai Korain Biotech Co., LTD. Shanghai, china) according to the manufacturer's instructions. The results were expressed in pg/mL. Absorbance of standards and samples were measured at 450 nm using a microtiter plate ELISA reader (Biotek, USA).

Serum level of IL-17A:

IL17-A level was measured by commercially available quantitative sandwich ELISA Kit supplied by Thermo Fisher Scientific (Bender MedSystems gmbH/Campus Vienna Biocenter 2/1030 Vienna, Austria) according to the manufacturer's instructions. The results were expressed in pg/mL. Absorbance of standards and samples were measured at 450 nm using a microtiter plate ELISA reader (Biotek, USA).

Serum level of 25-dihydroxyvitamin D3:

25-hydroxyvitamin D3 was measured by Cobas 6000/e601 autoanalyzer (Roche diagnostics/ Mannheim, Germany), according to the manufacturer's instructions. The device applied electrochemiluminescence technique and the results were expressed in ng/ml.

Immunotherapy protocol:

Allergen Immunotherapy extracts were locally prepared in Allergy and Immunology Unit, Department of Medical Microbiology and Immunology, Faculty of Medicine, Zagazig University. Aqueous allergen extracts (1:100 wt/vol) preparation was done according to allergen extract preparation guidelines developed by the AAAAI/ACAAI/ JCAAI and all allergen extracts were stored at 4 °C [15].

The selected allergens for allergen specific immunotherapy were based on the clinically relevant allergens identified for each patient according to both history and SPT results.

Immunotherapy was administered for each patient with two fold increase in concentration of each vial (1/1000, 1/500, 1/250, 1/125). Increasing volumes (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml of each vial) of allergen extracts were injected subcutaneously twice weekly for the first four vials for five months (Build up phase). After which, one ml of vial (1/125) was administered once /week (maintenance phase) for a month. Patients who could not tolerate higher doses, due to systemic or local reactions, were maintained on the highest dose they could tolerate.

Statistical analysis:

Data were analyzed using the Statistical Package of Social Science (SPSS) program for Windows (Standard version 20). The normality of data was first tested with Shapiro-Wilk test. Qualitative data were described using number and percent. Association between categorical variables was tested using Chi-square for trend test while Fischer exact test was used when expected cell count less than 5. Continuous variables were presented as mean ± SD (standard deviation). Median was used for non-parametric data. For non-parametric data, one group before and after were compared with Wilcoxon signed-ranks test and the two groups were compared with Mann-Whitney U. The results was considered Significant when the probability of error is less than 5% ($p \leq 0.05$).

RESULTS

Baseline characteristics

We recruited 69 children with atopic asthma in the study. The mean age among children was 8.53+ 2.48. Thirty nine (56.5 %) were male while 30 (43.5 %) were female. Thirty three (47.8%) of the asthmatic children had positive family history. Forty five (65.2%) of the children had associated allergic rhinitis. Majority of the children (52.2%) suffered from mild persistent asthma. Majority of the asthmatic children were polysensitized, most of them were sensitized to mixed pollens (56.5%), followed by house dust (49.3%) (table1). The characteristics of each group was illustrated in table 2.

Table 1: Baseline characteristics of the studied children

Variables	Study children (n=69)
Age, years	8.53± 2.48
Sex, male, no (%)	39 (56.5)
Total IgE (IU/ml)	90.53±72.001
Positive family history, no (%)	33 (47.8)
Associated allergic rhinitis, no (%)	45 (65.2)
Asthma severity, no (%)	
Mild Intermittent	15 (21.8)
Mild persistent	36 (52.2)
Moderate persistent	18 (26)
Positive SPT, no (%)	
Cotton dust	5 (7.2)
Wool	6 (8.7)
Cockroach	18 (26.1)
Hay dust	11(15.9)
Mixed molds	20 (29)
Mixed pollens	39 (56.5)
House dust	34 (49.3)
Mixed mites	24 (34.8)

Data was expressed as mean± SD otherwise mentioned, SPT, skin prick test; SD, standard deviation

Table 2: Characteristics of the studied three groups

Variables	Combined SCIT+VitD3 group (n=23)	SCIT alone group (n=23)	VitD3 alone group (n=23)
Age(years)	8.87±2.46	8.96± 2.619	7.76± 2.266
Sex, male, no (%)	10 (43.5)	14 (60.9)	15 (65.2)
IgE (IU/ml)	115.11±77.36	77.88±73.3	78.59±60.93
Positive family history, no (%)	8 (34.8)	14 (60.9)	11 (34.8)
Associated allergic rhinitis, no (%)	12 (52.2)	18 (78.2)	15 (65.2)
Asthma Severity, no (%)			
Mild Intermittent	7 (30)	4 (17.4)	4 (17.4)
Mild persistent	12 (52.2)	10 (43.5)	14 (60.9)
Moderate persistent	6 (26.1)	5 (21.7)	7 (30.4)
Positive SPT, no (%)			
Cotton dust	3 (13.04)	1 (4.35)	1 (4.35)
Wool	3 (13.04)	3 (13.04)	0 (0.00)
Cockroach	7 (30.43)	5 (21.74)	6 (26.1)
Hay dust	3 (13.04)	6 (26.1)	2 (8.7)
Mixed molds	9 (39.1)	8 (34.8)	3 (13.04)
Mixed pollens	14 (60.9)	15 (65.2)	10 (43.5)
House dust	10 (43.5)	15 (65.2)	9 (39.1)
Mixed mites	11 (34.8)	4 (17.40)	9 (39.1)

Data was expressed as mean± SD otherwise mentioned; SCIT, subcutaneous immunotherapy; SPT, skin prick test; SD, standard deviation

Immunological outcomes

After six months of follow up, the combined SCIT + VitD3 showed a significant increase in serum levels of VitD3 and IL-10, while serum IL-17A was significantly decreased (P = 0.000) (table 3). The SCIT alone group showed a significant increase in serum levels of IL-10 and a significant decrease in serum IL-17A (P = 0.000) with insignificant change of serum VitD3 level (P = 0.388) (table 4). The VitD3 alone group showed significant increase in VitD3 (P = 0.000), while no significant change in serum IL-10 and IL-17A (P=0.127; P=0.067 respectively) (table 5).

No significant difference was found among the three studied groups at baseline regarding age, sex, total IgE, IL-10, IL-17A, VitD3 (P <0.05). On comparing post intervention immunological parameters between the combined SCIT+ VitD3 group and the SCIT alone group, significant differences in favor of the combined SCIT+ VitD3 group were detected in serum levels of VitD3 and IL-10 (P=0.000)(table 6). On comparing post intervention immunological parameters between the combined SCIT+ VitD3 group and the VitD3 alone group, significant differences in favor of the first group were detected in all parameters including serum levels of IL10, IL-17A, VitD3 (P= 0.000, 0.011, 0.012 respectively) (table 7).

Clinical outcomes

On comparing post intervention clinical parameters among the studied three groups, ICS discontinuation rate was higher in the combined SCIT +VitD3 group (n=13; 56.5%) compared to SCIT alone group (n=5; 21.74%; P=0.016) and VitD3 alone group (n=11; 34.8%; P=0.555) (table 6& 7).

Table 3: Comparison between baseline and six month immunotherapy levels of IL-10, IL-17A, VitD3 and ICS discontinuation in combined SCIT+VitD3 group

Variables	At baseline (n =23)	After six month (n =23)	*P*-value
IL-10 (pg/ml)	44 (27-69)	73 (60-94)	0.000
IL-17A (pg/ml)	42 (30-62)	29 (20-38)	0.000
25(OH)D3 (ng/ml)	10.3 (10-25)	30 (19-35)	0.000
Discontinuation of ICS, no (%)	0 (0)	13 (56.5)	0.000

All data expressed as (median, Min-Max) otherwise indicated; 25(OH) D3, 25-Dihydroxyvitamin D3; ICS; inhaled corticosteroids

Table 4: Comparison between baseline and six month immunotherapy levels of IL-10, IL-17A, VitD3 and ICS discontinuation in SCIT alone group

Variables	At baseline (n =23)	After six month (n =23)	*p*-value
IL10 (pg/ml)	42 (24-64)	58 (38-73)	0.000 0.000
IL17A (pg/ml)	48 (32-64)	30 (24-38)	0.388
25(OH)D3 (ng/ml)	12 (10-26)	15 (10-25)	0.186
Discontinuation of ICS, no (%)	0 (0)	5 (21.74)	

All data expressed as (median, Min-Max) otherwise indicated; 25(OH) D3, 25-Dihydroxyvitamin D3; ICS; inhaled corticosteroids

Table 5: Comparison between baseline and six month immunotherapy levels of IL-10, IL-17A, VitD3 and ICS discontinuation in VitD3 alone group

Variables	At baseline (n =23)	After six month (n =23)	*P*-value
IL-10 (pg/ml)	53 (32-64)	51 (32-84)	0.127
IL-17A (pg/ml)	33 (20-42)	32 (24-38)	0.067
25(OH)D3 (ng/ml)	19.4 (10-28)	32 (25-37)	0.000
Discontinuation of ICS; no (%)	0 (0)	11 (34.8)	0.001

All data expressed as (median, Min-Max) otherwise indicated; 25(OH) D3, 25-Dihydroxyvitamin D3; ICS; inhaled corticosteroids

Table 6: Comparison between combined SCIT+VitD3 group and SCIT alone group in serum IL-10, IL-17, VitD3 and ICS discontinuation after six month

Variables	Combined SCIT +VitD3 group (n =23)	SCIT alone group (n =23)	*P*-value
IL-10 (pg/ml)	73 (60-94)	58 (38-73)	0.000
IL-17A (pg/ml)	29 (20-38)	30 (24-38)	0.611
25(OH)D3 (ng/ml)	30 (19-35)	15 (10-25)	0.000
Discontinuation of ICS, no (%)	13 (56.5)	5 (21.74)	0.016

All data expressed as (median, Min-Max) otherwise indicated; SCIT, subcutaneous immunotherapy; 25(OH) D3, 25-Dihydroxyvitamin D3; ICS; inhaled corticosteroids

Table 7: Comparison between combined SCIT+VitD3 group and VitD3 alone group in serum IL-10, IL-17, VitD3 and ICS discontinuation after six month

Variables	Combined SCIT+VitD3 (n =23)	VitD3 alone group (n=23)	*P*-value
IL-10 (pg/ml)	73 (60-94)	51 (32-84)	0.000
IL-17A (pg/ml)	29 (20-38)	32 (24-38)	0.011
25(OH)D3 (ng/ml)	30 (19-35)	32 (25-37)	0.012
Discontinuation of ICS, no (%)	13 (56.5)	11(34.8)	0.555

All data expressed as (median, Min-Max) otherwise indicated; SCIT, subcutaneous immunotherapy; 25(OH) D3, 25-Dihydroxyvitamin D3; ICS; inhaled corticosteroids

Adverse effects

Three children in SCIT + VitD3 group and two children in SCIT alone group experienced mild attacks of asthma, which were managed with inhaled short acting beta 2-agonist. Four children in SCIT alone group experienced a local large induration at SCIT injection sites that did not indicate immunotherapy discontinuation. No patients in the three groups experienced a systemic anaphylactic reaction. Serum levels of parathyroid hormone, phosphorus, and calcium were within the normal values among VitD3 receiving children during the study.

DISCUSSION

In this prospective clinical trial, we investigated whether supplementation of VitD3 could enhance the efficacy of SCIT in improving the clinical and immunological outcomes of childhood asthma. To our knowledge, this is the first study to investigate the role of VitD3 adjuvant and SCIT among asthmatic children in Egypt.

In this study, the three groups of patients achieved clinical improvement of asthma indicated by the rate of ICS discontinuation at the end of six months follow up. But this improvement was most significant in the combined SCIT+ VitD3 followed by the VitD3 alone group.

These results were similar to previous studies[2,16,17,18,19] that found patients achieved clinical improvement indicated by lower ICS doses, less exacerbation, less systemic steroid need, and higher rates of ICS discontinuation in comparison to the medication alone. However, the authors did not study the effect of VitD3 supplementation alone.

These data is consistent with the finding of a Cross-section study that found higher levels of VitD3 in children with controlled asthma[20]. Additionally, VitD3 deficiency was associated with impaired pulmonary functions in asthmatic patients [21].

In other clinical studies VitD3 supplementation during pregnancy resulted in reduction of recurrent wheeze risk in early childhood life [22].

The mechanism of action is thought to include both steering of the immune system towards a more tolerogenic response, as well as reinforcing the barrier and antiviral properties of the bronchial epithelium [22].

In our study, despite the increase in the levels of IL-10 in the SCIT alone group, the group of combined SCIT + VitD3 showed a more significant increase compared to baseline values. A finding that indicates effective role of VitD3 as SCIT adjuvant. However, VitD3 alone group did not show a significant rise in serum IL-10 level. This discrepancy might be attributed to the small sample size, duration of treatment, differences in allergen vaccine used, associated medication or degree of asthma severity. These findings raised the need for further evaluation of the role of VitD3 supplementation alone in cases of asthma. Our results were in accordant with *Majak et al* who reported elevated levels of IL-10 and Tregs cells in combined allergen SIT and VitD3 group [16].

Similar results were reported by previous murine model of atopic asthma. VitD3 could increase IL-10 in

lung tissue and reduce IL-13 and IL-5 in bronchoalveolar lavage fluid [23].

These data could be explained by tolerogenic response associated to the successful allergen SIT due to activation of Tregs cells with increased secretion of its cytokines. Although, VitD3 act as an immunomodulator, that inhibits the differentiation of DCs and enhances IL-10 secretion [24], its role as an immunotherapy adjuvant is not adequately addressed yet.

Asthma is a heterogeneous inflammatory disease with distinct types of inflammation where IL-17 is a key player. Therefore, we investigated VitD3 effect on the inflammatory nature of asthma by estimating serum levels of IL17A. In our study, we found significant reduction in IL-17 in both SCIT groups. SCIT + VitD3 showed a more significant reduction in comparison with the baseline values. A finding that indicates effective role of VitD3 as a SCIT adjuvant. Although, VitD3 inhibit IL-17 expression in many diseases [9, 10, 11], we did not report a significant reduction in serum IL-17 levels among VitD3 alone group. This discrepancy could be attributed to our sample size. Moreover, there may be a relation between serum level of VitD3 and its immunomodulatory effect on Th17. Therefore, these results need to be investigated on a larger scale.

Poor VitD3 status noticed in our study is a reflection of a global health problem which highlight the importance of effective strategies to prevent VitD3 deficiency. These preventive strategies can improve the outcome of asthma and allergic diseases.

CONCLUSION

VitD3 supplementation enhanced SCIT efficacy among asthmatic children. We strongly recommend screening of children with atopic asthma for VitD3 deficiency or insufficiency to be properly supplemented beside allergen-specific immunotherapy.

- The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.
- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Ali Niloufer S, and Kashmira Nanji. A Review on the Role of Vitamin D in Asthma. *Cureus*. 2017; 9, 5 e1288.
2. Baris S, Kiykim A, Ozen A, et al. Vitamin D as an adjunct to subcutaneous allergen immunotherapy in asthmatic children sensitized to house dust mite. *Allergy*. 2014; 69: 246–53
3. Heine G, Tabeling C, Hartmann B, et al. 25-hydroxvitamin D3 promotes the longterm effect of specific immunotherapy in a murine allergy model. *J Immunol*. 2014; 193:1017–23.
4. Julie Chesné, Carsten B Schmidt-Weber, Julia Esser von-Bieren. The Use of Adjuvants for Enhancing Allergen Immunotherapy Efficacy. *Immunol Allergy Clin North Am*. 2016; 36(1):125-45.
5. Barlan IB, Bahceciler N, Akdis M, Akdis CA. Role of bacillus Calmette-Guerin as an immunomodulator for the prevention and treatment of allergy and asthma. *Curr Opin Allergy Clin Immunol*. 2005; 5: 552–557.
6. Larchet M. Peptide and recombinant immunotherapy. *Immunol Allergy Clin North Am*. 2011; 31: 377–389.
7. Petrarca C, Clemente E, Amato V, Gatta A, Cortese S, et al. Vitamin D3 improves the effects of low dose Der p 2 allergoid treatment in Der p 2 sensitized BALB/c mice. *Clin Mol Allergy*. 2016; 14:7.
8. Tsoukas CD, Provvedini DM, Manolagas SC. 1, 25-Dihydroxyvitamin D3: a novel immunoregulatory hormone. *Science*. 1984; 224: 1438–1440.
9. Mehta S., Hunter D. J., Mugusi F. M., et al. Perinatal outcomes, including mother-to-child transmission of HIV, and child mortality and their association with maternal vitamin D status in Tanzania. *The Journal of Infectious Diseases*. 2009; 200(7):1022–1030.
10. Mus A. M., van Hamburg J. P., Asmawidjaja P., et al. Vitamin D suppresses Th17 cytokines via down regulation of RORgammat and NFATC2 and by differential regulation of GATA3. Arthritis & *Rheumatism*. 2010; 62 (supplement 10): 38.
11. Schaalan M. F., Mohamed W. A., Amin H. H. Vitamin D deficiency: Correlation to interleukin-17, interleukin-23 and PIIINP in hepatitis C virus genotype 4. *World Journal of Gastroenterology*. 2012; 18 (28):3738–3744.
12. Bakdash G, Schneider LP, van Capel TMM, et al. Intradermal application of vitamin D3 increases migration of CD141 dermal dendritic cells and promotes the development of Foxp31 regulatory T cells. *Hum Vaccin Immunother*. 2013; 9: 250–8.
13. National Institutes of Health. Global Initiative for Asthma. Global Strategy for Asthma Management

and Prevention Revision, 2020. http://www.ginaasthma.org

14. Bernstein IL, Li JT, Bernstein DI, Hamilton R, Spector SL, Tan R et al. Allergy diagnostic testing: An updated practice parameter. *Ann Allergy Asthma Immunol.* 2008:100: S1–S148.
15. American Academy of Allergy Asthma and Immunology. American College of Allergy, Asthma and Immunology. Joint task force on practice parameters, allergen immunotherapy: a practice parameter Ann. *Allergy Asthma Immunol*. 90 (2003): 1-40
16. Majak P, Rychlik B, Stelmach I. The effect of oral steroids with and without vitamin D3 on early efficacy of immunotherapy in asthmatic children. *Clin Exp Allergy*. 2009; 39: 1830–1841.
17. Majak P, Jerzyńska J, Smejda K, Stelmach I, Timler D, Stelmach W. Correlation of vitamin D with Foxp3 induction and steroid-sparing effect of immunotherapy in asthmatic children. *Ann Allergy Asthma Immunol.* 2012; 109(5): 329-35.
18. Jeffery LE, Burke F, Mura M, Zheng Y, Qureshi OS, Hewison M et al. 1, 25-Dihydroxyvitamin D3 and IL-2 combine to inhibit T cell production of inflammatory cytokines and promote development of regulatory T cells expressing CTLA-4 and FoxP3. *J Immunol* 2009; 183: 5458–5467.
19. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS et al. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. *J Allergy Clin Immunol*. 2010; 126: 52–58.
20. Chinellato I, Piazza M, Sandri M, Peroni D, Piacentini G, Boner AL. Vitamin D serum levels and markers of asthma control in Italian children. *J Pediatr*. 2011; 158 (3): 437-41.
21. Li F, Peng M, Jiang L, Sun Q, Zhang K, Lian F, Litonjua AA, Gao J, Gao X. Vitamin D deficiency is associated with decreased lung function in Chinese adults with asthma. *Respiration*. 2011; 81(6):469-75.
22. Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M; Drug and Therapeutics Committee of the Lawson Wilkins Pediatric Endocrine Society. Vitamin D deficiency in children and its management: review of current knowledge and recommendations. *Pediatrics*, 2008; 122: 398–417.
23. Taher YA, van Esch BC, Hofman GA, Henricks PA, van Oosterhout AJ. 1alpha, 25-dihydroxyvitamin D3 potentiates the beneficial effects of allergen immunotherapy in a mouse model of allergic asthma: role for IL-10 and TGF-beta. *J Immunol* 2008; 180: 5211–5221.
24. Penna G, Adorini L. 1 Alpha, 25-dihydroxyvitamin D3 inhibits differentiation, maturation, activation, and survival of dendritic cells leading to impaired alloreactive T cell activation. *J Immunol* 2000; 164: 2405–2411.