Vitamin D Serum Levels and Markers of Asthma Control in Italian Children

Iolanda Chinellato, MD, Michele Piazza, MD, Marco Sandri, MD, Diego Peroni, MD, Giorgio Piacentini, MD, and Attilio L. Boner, MD

Objective To establish the relationship between vitamin D serum levels, pulmonary function, and asthma control in children.

Study design We studied the relationship between 25-hydroxy cholecalciferol [25(OH)D] concentrations and baseline spirometry and levels of asthma control, assessed according to Global Initiative for Asthma guidelines and the Childhood Asthma Control Test, in 75 children with asthma (age range 5-11 years; 43 males) in a cross-sectional study carried out during the winter and early spring.

Results Only 9.4% of our children had a sufficient serum 25(OH)D (at least 30 to 40 ng/mL). A significant positive correlation was found between forced vital capacity percent predicted and serum 25(OH)D (r = 0.25, *P* = .040). This was true also for forced expiratory volume in 1 second, even though it was not statistically significant (r = 0.16, *P* = .157). Subjects with well-controlled asthma had higher serum levels of 25(OH)D than children with partially controlled or non-controlled asthma, with values of (median [Q1; Q3]) 22.2 (16.3; 25.4), 17.8 (11.8; 22.1) and 18.1 (15.0; 18.5), respectively (*P* = .023). Furthermore, a positive correlation was found between 25(OH)D and the Childhood Asthma Control Test (r = 0.28; *P* = .011).

Conclusions Our results indicate that hypovitaminosis D is frequent in children with asthma living in a Mediterranean country. In those children, lower levels of vitamin D are associated with reduced asthma control. *(J Pediatr 2011;158:437-41)*.

Vitamin D deficiency has been shown to be a risk factor for several chronic diseases, in addition to the classic deleterious effects on bone.[1] Recent data suggest that vitamin D deficiency could be related to onset of asthma,[2,3] as well as be a marker of disease severity in children with asthma.[4] Furthermore, a significant relationship (with an apparent dose-response effect) between higher percent-predicted forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) values and the increased circulating concentrations of 25-hydroxyvitamin D has been demonstrated in a large study of approximately 14 000 subjects in the United States.[5] All these findings suggest a possible role of vitamin D in respiratory health outcomes.[6] We therefore investigated the hypothesis that vitamin D serum levels could have an influence on different components of asthma health status by studying this relationship in consecutively enrolled patients.

Methods

We selected Italian children with asthma, diagnosed according to the American Thoracic Society (ATS) guidelines,[7] who consecutively attended the outpatient clinic of the Department of Paediatrics at the University of Verona hospital between November 2008 and March 2009. The Hospital Ethics Committee approved the study protocol, and parents and patients provided written, informed consent to participate.

A single measurement of vitamin D [measured as 25-hydroxy cholecalciferol, 25(OH)D] was made in all subjects, using a chemiluminescent method (Liaison 25-OH Vitamin D Total; Diasorin, Saluggia, Italy). Values were used as continuous variables and vitamin D was also categorized, in descriptive analyses, as being desirable (or sufficient) when at least 30 to 40 ng/mL (75 to 100 nmol/L), insufficient between 20 and 30 ng/mL (50 and 75 nmol/L), and deficient when <20 ng/mL.[1,8]

Measurements of pulmonary function were performed according to ATS criteria,[9] considering the best of 3 efforts starting with full force vital capacity (FVC) maneuvers with an electronic spirometer (Master Screen IOS; Jaeger, Höchberg, Germany). The spirometer was calibrated for each subject with a 3-L syringe (Cardinal Health, Dublin, Ohio). The FVC maneuvers were carried out with the child

25(OH)D	25-hydroxy cholecalciferol
ATS	American Thoracic Society
C-ACT	Childhood Asthma Control Test
CAMP	Childhood Asthma Management Program
FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FP	Fluticasone propionate
FVC	Forced vital capacity
GINA	Global Initiative for Asthma
IgE	Immunoglobulin E

From the Department of Paediatrics University of Verona, Verona, Italy

The authors declare no conflicts of interest.

 10.1016/j.jpeds.2010.08.043

standing and wearing a nose clip. Subjects were instructed to avoid the use of short-acting bronchodilators for at least 6 hours before testing.

Fractional exhaled nitric oxide (FeNO) was measured by the NIOX system (Aerocrine, Stockholm, Sweden) by use of a single-breath on-line method according to European Respiratory Society/American Thoracic Society guidelines.[10] Briefly, the children inhaled NO-free air to total lung capacity and exhaled through a dynamic flow restrictor with a target flow of 50 mL/sec for at least 6 to 7 seconds. No nose clip was used. The NIOX system was calibrated according to the manufacturer's instructions.

Serum total immunoglobulin E (IgE) was determined by high-sensitivity nephelometry (BN II System; Siemens Healthcare Diagnostics, Deerfield, Illinois) and expressed as kIU/L. Peripheral blood eosinophil count was measured by automatic flow cytometer (Advia 2120 Hematology System; Siemens Healthcare Diagnostics) and expressed as cells per cubic millimeter.

Skin testing was done following standard methods.[11] In addition to histamine and saline solution controls, the following antigens were applied to the volar surface of the forearm: *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* cat dander, dog dander, mixed grass pollen, mixed tree pollen, *Parietaria officinalis*, and *Alternaria tenuis*. A test result was considered positive if the maximal diameter of the wheal was ≥3 mm after subtracting the maximal diameter of the negative control. Mean wheal dimensions were calculated by measuring the greater diameter plus its perpendicular diameter and dividing by two. All the wheal mean diameters were measured and added together to obtain a total allergy score expressed in millimeters as previously described.[12]

Asthma control was categorized as controlled, partly controlled or uncontrolled in agreement with Global Initiative for Asthma (GINA) guidelines.[13] In particular, levels of asthma control were defined depending on the presence/absence of daytime symptoms, limitations of activities, nocturnal symptoms/awakening, need for reliever/rescue treatment, and FEV_1 results. Furthermore, the children were asked to complete the Childhood Asthma Control Test (C-ACT) questionnaire[14] before their clinical evaluation. C-ACT is a self-administered questionnaire with 7 questions on asthma symptoms and the effects of asthma on daily functioning. The first 4 questions are answered by the children and the last 3 by their parents. Each question had 4 response options for children, from 0 (worse) to 3 (best), and 6 response options for parents, from 0 (worse) to 5 (best). The lowest and highest possible scores were thus 0 (totally uncontrolled asthma) to 27 (total asthma control), respectively; and a score of 19 or less was an indication of poorly controlled asthma.[14] Permission was obtained to use the C-ACT questionnaire.

Statistical Analysis

The degrees of association between vitamin D and baseline FVC percent-predicted, FEV_1 percent predicted, and C-ACT were estimated by use of partial correlation,[15] that is, linear correlation after removing the effects of other variables/confounders (treatment, season in which levels were obtained, sex, and age).

Because of the skewed distributions of total IgE, eosinophil count, mean total allergy scores, and FeNO, median values and interquartile ranges (first to third quartiles) were calculated in place of mean and standard deviation. The strength of the associations between these variables and vitamin D serum levels was measured by use of Spearman rank correlation.

Similarly, median and interquartile ranges of vitamin D serum levels in children were grouped according to treatment and GINA classification of control. Medians of these subgroups were compared by use of the nonparametric k-sample test on equal medians.

P values <.05 were considered statistically significant. All calculations were performed with the Stata 11.0 statistical package (Stata Statistical Software: Release 11; StataCorp, College Station, Texas).

Results

Seventy-five children with asthma (mean ± SD age of 9.6 ± 1.7 years [range 5-11 years], 43 [59.7%] male) were included in the study. Forty-four patients had intermittent asthma and therefore were receiving treatment only when needed. Of the remaining 31 children who had persistent asthma, 21 were receiving fluticasone propionate (FP) 200 μg/d, and 10 were treated with Salmeterol + FP 25/50 μg (two puffs two times daily) plus daily Montelukast 5 mg. Only two patients had a clinical history of emergency department visits and prednisone treatment in the previous 6 months, and both were in the group with uncontrolled asthma.

Vitamin D levels (deficient, insufficient, and sufficient) are reported in relation to the different study measurements (**Table I**). Only 7 (9.4%) patients had sufficient vitamin D levels (at least 30 to 40 ng/ml), 28 (37.3%) had insufficient levels (between 20 and 30 ng/mL), and the remaining 40 (53.3%) showed deficient levels (<20 ng/mL).

The analysis of the relationship between serum levels of vitamin D and C-ACT scores showed a moderate, statistically significant positive correlation (r = 0.28; 95% CI = 0.06 to 0.49, *P* = .011) (**Figure 1**), indicating that higher serum levels of vitamin D correspond to a better perception of disease control by the patients and their parents.

The distribution of vitamin D serum levels in the children grouped according to the GINA classification of control is shown in **Figure 2** and median values with interquartile ranges are reported in **Table II**. The serum levels of vitamin D were significantly different among the 3 groups (*P* = .023). As can be observed, serum vitamin D was significantly higher in the group of controlled patients as compared with the partially-controlled group (*P* = .034). The lack of a statistically significant difference between controlled and uncontrolled patients (*P* = .054) was probably due to the low number of subjects (n = 5) in the latter group. There were no significant differences by treatment.

Table I. Investigated parameters stratified according to deficient, insufficient, and sufficient vitamin D serum levels

Patient characteristics	Vitamin D levels			Spearman's correlation (*P* value)
	Deficient (< 20 ng/mL)	Insufficient (20-30 ng/mL)	Sufficient (≥ 30 ng/mL)	
Absolute and relative (%) frequency distribution of patients	40 (53.3%)	28 (37.3%)	7 (9.4%)	
Total IgE (IU/mL)	197 (102-554)	425 (100-788)	332 (124-495)	0.04 (0.722)
Eosinophil count (cells/mm^3)	409.3 (217.7-645.9)	350.0 (251.0-651.0)	254 (223.0-441.0)	−0.05 (0.646)
Mean total allergy score (mm)	22.8 (7.8-42.3)	23.3 (11.0-38.5)	12.0 (10.0-35.0)	−0.07 (0.526)
FeNO (ppb)	18.9 (8.5-30.6)	17.5 (8.8-41.1)	16.1 (10.1-22.8)	0.02 (0.880)

Median with interquartile range (1st quartile - 3rd quartile) and Spearman rank correlation with *P* values.

Considering the whole population, serum levels of 25(OH) D were moderately associated with FVC percent predicted (partial correlation r = 0.25, 95% CI = 0.12 to 0.49, $P = .04$). A lower and statistically non-significant correlation between FEV_1% predicted and serum levels of 25(OH)D was found (r = 0.16, 95% CI = −0.06 to 0.39, $P = .16$). Similarly, no correlation between vitamin D levels and FEV_1/FVC was observed (r = −0.15, 95% CI = −0.35 to 0.05, $P = 0.14$). No statistically significant differences were observed between total IgE, eosinophil count, total allergy scores and FeNO and vitamin D levels (Table I).

Discussion

Our study shows that there is a positive association between vitamin D levels and asthma control as defined by the C-ACT and GINA parameters. It is tempting to speculate that this correlation is based on the effect that vitamin D has on immune function. In fact, a number of studies have established that vitamin D is a principal controller of innate immunity, with the production of antimicrobial peptides able to kill viruses, bacteria and fungi[16] and that it exerts a inhibitory effect on the inflammatory response to viral infections.[17] Vitamin D deficiency has been shown to predispose children to respiratory infections,[18,19] and this predisposition is much stronger in children with asthma.[20] Conversely, it has been shown that vitamin D supplementation decreases the incidence of respiratory infections,[21] which are a major trigger of asthma exacerbation.[22,23] It is difficult to ascertain from cross-sectional studies whether vitamin D deficiency is responsible for reduced asthma control or whether uncontrolled asthma associated lifestyles, such as less outdoor exercise with decreased exposure to sunlight, are responsible for lower serum levels of vitamin D. Interventional trials aimed at evaluating the effect of vitamin D supplementation on asthma exacerbations and double-blind, placebo-controlled trials in vitamin D–deficient children with asthma are warranted to explore whether there is a causal relationship or a simple association between the two events.

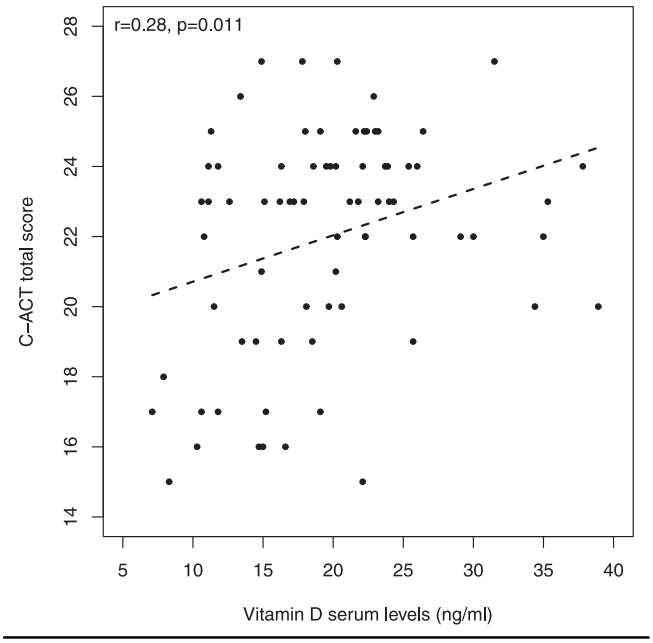

Figure 1. Scatter plot shows the relationship existing between vitamin D serum levels and C-ACT scores. Partial correlation analysis is reported.

However, some recent findings suggest a possible cause-effect relationship. In vitro studies have shown that 1.25 (OH)2D3 has a direct inhibitory effect on passively sensitized airway smooth muscle cells[24] and that it increases glucocorticoid bioavailability in bronchial smooth muscle cells.[25] Moreover, decreased serum vitamin D levels in children with asthma have been observed to be associated with increased corticosteroid use[26] and an investigation with T cells from patients with steroid-resistant asthma showed that vitamin D supplementation could potentially increase the therapeutic response to glucocorticoids by restoring the impaired steroid-induced interleukin-10 response.[27]

Our finding are in agreement with recent studies showing that insufficient vitamin D status is associated with an increase in the risk of asthma exacerbations in patients of the Childhood Asthma Management Program (CAMP) cohort[28] and with augmented airway responsiveness and increased risk of asthma hospitalization in children with asthma living in Costa Rica.[4]

In the study of children with asthma living in Costa Rica a significant relationship was found between serum levels of vitamin D and both circulating eosinophil counts and serum IgE levels.[4] This was not observed in either the CAMP cohort[28] or in our patients. The fact that, in our study group,

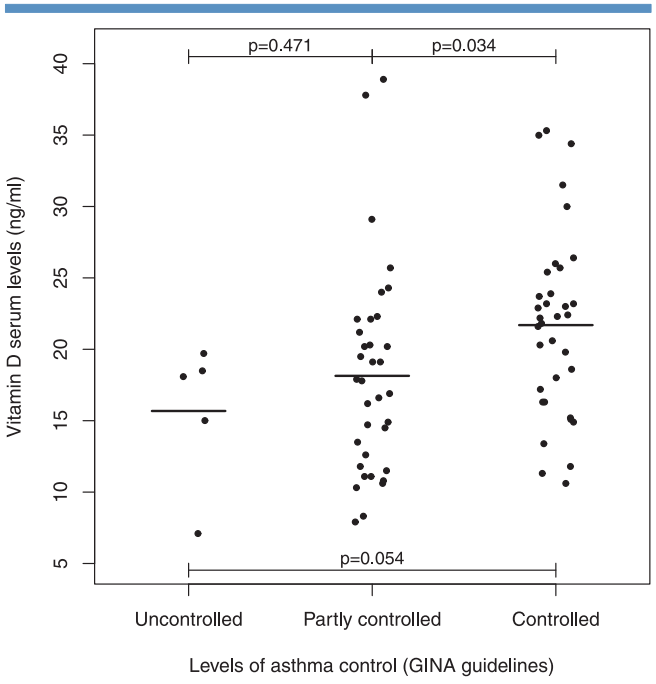

Figure 2. Distribution of vitamin D serum levels in children grouped according to GINA classification of control. *Horizontal lines* represent mean values.

no relationship was found between 25(OH)D concentrations and total IgE, eosinophil count and mean total allergy scores can be explained by the narrow serum range of vitamin D concentrations in our study population. In fact, no change in total IgE has been previously demonstrated in children with asthma with 25(OH)D concentrations in the range between 12.5 ng/mL and 35.7 ng/mL; and a clear reduction in eosinophil counts was only observed for levels over 30 ng/mL.[4] Beside these considerations, in our patients as in those enrolled in the CAMP study a greater variability in vitamin D levels is expected over the course of a year in comparison with equatorial Costa Rica patients. However, there are no data on the effect of seasonal variations of vitamin D levels and environmental exposures on allergic outcomes.[28]

In line with these findings, no effect of vitamin D was observed on FeNO values in our patients. This is not surprising because FeNO mirrors allergic eosinophilic inflammation,[29] which reflects allergen exposure and multiple sensitization.[30]

In agreement with a previous observation in a large cross-sectional study in randomly selected adolescents and adults,[5] the data from our study confirm that serum 25-OH vitamin D is significantly and positively associated with FVC and, to a lesser extent, with FEV_1 but not with FEV_1/FVC. However, the level of correlation between FVC and vitamin D is relatively weak (r = 0.25). It is of interest that the correlations for all 3 pulmonary function values were in the same direction.

In conclusion, deficient and insufficient vitamin D serum levels were found in most children with asthma in a study population from northern Italy. As a practical consequence, we observed that lower levels of vitamin D were associated with reduced asthma control and, to some degree, with reduced pulmonary function. ■

Table II. Median with interquartile range (1st quartile to 3rd quartile) and *P* values of the nonparametric k-sample test on the equality of medians for the investigated parameters, grouped by treatment and level of disease control

	Vitamin D serum levels	*P* value
Treatment		.665
When needed	19.6 (15.1-22.9)	
FP	18.0 (12.6-21.8)	
FP/S+M	22.4 (15.1-24.3)	
Levels of asthma control (GINA guidelines)		.023
Uncontrolled	18.1 (15.0-18.5)	
Partly controlled	17.8 (11.8-22.1)	
Controlled	22.2 (16.3-25.4)	

FP/S+M, Fluticasone propionate/salmeterol+montelukast.

We are grateful to Ms Judyth D. Benini for editing the English version of the manuscript.

Submitted for publication Mar 5, 2010; last revision received Jul 30, 2010; accepted Aug 25, 2010.

Reprint requests: Attilio L. Boner, MD, Department of Paediatrics, Policlinico GB Rossi, Piazzale L. Scuro, 37134 Verona, Italy. E-mail: attilio.boner@univr.it

References

1. Holick MF. Vitamin D deficiency. N Engl J Med 2007;357:266-81.
2. Camargo CA, Jr., Rifas-Shiman SL, Litonjua AA, Rich-Edwards JW, Weiss ST, Gold DR, et al. Maternal intake of vitamin D during pregnancy and risk of recurrent wheeze in children at 3 y of age. Am J Clin Nutr 2007;85:788-95.
3. Devereux G, Litonjua AA, Turner SW, Craig LC, McNeill G, Martindale S, et al. Maternal vitamin D intake during pregnancy and early childhood wheezing. Am J Clin Nutr 2007;85:853-9.
4. Brehm JM, Celedón JC, Soto-Quiros ME, Avila L, Hunninghake GM, Forno, et al. Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med 2009;179:765-71.
5. Black PN, Scragg R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the Third National Health and Nutrition Examination Survey. Chest 2005;128:3792-8.
6. Wright RJ. Make no bones about it: increasing epidemiologic evidence links vitamin D to pulmonary function and COPD. Chest 2005;128:3781-3.
7. American Thoracic Society. Medical Section of the American Lung Association. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987;136:225-44.
8. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25- hydroxyvitamin D for multiple health outcomes. Am J Clin Nutr 2006;84:18-28.
9. American Thoracic Society. Standardisation of spirometry: 1994 update. Am J Respir Crit Care Med 1995;152:1107-36.
10. Baraldi E, De Jongste J. European Respiratory Society/American Thoracic Society. Measurement of exhaled nitric oxide in children. 2001. Eur Repir J 2002;20:223-37.
11. Dreborg S, Holgersson M, Nilsson G, Zetterström O. Dose response relationship of allergen, histamine, and histamine releasers in skin prick test and precision of the skin prick test method. Allergy 1987;42:117-25.

12. Turkeltaub PC, Rastogi SC, Baer H, Anderson MC, Norman PS. A standardized quantitative skin-test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. J Allergy Clin Immunol 1982;70:343-52.
13. Global Initiative for Asthma. Global strategy for asthma management and prevention. 2008 [Accessed June 2009]. Available from: www.ginasthma.com.
14. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the Childhood Asthma Control Test. J Allergy Clin Immunol 2007;119:817-25.
15. Cohen J, Cohen P, West SG, Aiken LS. Applied multiple regression/correlation analysis for the behavioral sciences Lawrance Erlbaum Associates, Inc., New Jersey. 2003.
16. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab 2008;4:80-90.
17. Hansdottir S, Monick MM, Lovan N, Powers L, Gerke A, Hunninghake GW. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. J Immunol 2010;184:965-74.
18. Wayse V, Yousafzai A, Mogale K, Filteau S. Association of subclinical vitamin D deficiency with severe acute lower respiratory infection in Indian children under 5 y. Eur J Clin Nutr 2004;58:563-7.
19. Cannell JJ, Vieth R, Umhau JC, Holick MF, Grant WB, Madronich S, et al. Epidemic influenza and vitamin D. Epidemiol Infect 2006;134:1129-40.
20. Ginde AA, Mansbach JM, Camargo CA, Jr. Association between serum 25-hydroxyvitamin D level and upper respiratory tract infections in the Third National Health and Nutrition Examination Survey. Arch Intern Med 2009;169:384-90.
21. Aloia JF, Li-Ng M. Re: epidemic influenza and vitamin D. Epidemiol Infect 2007;135:1095-6. author reply Epidemiol Infect 2007;135:1097-8.
22. Sykes A, Johnston SL. Etiology of asthma exacerbations. J Allergy Clin Immunol 2008;122:685-8.
23. Singh AM, Busse WW. Asthma exacerbations. 2: aetiology. Thorax 2006;61:809-16.
24. Song Y, Qi H, Wu C. Effect of 1,25-(OH)2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. Respirology 2007;12:486-94.
25. Bosse Y, Maghni K, Hudson TJ. 1a,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. Physiol Genomics 2007;29:161-8.
26. Searing DA, Zhang Y, Murphy JR, Hauk PJ, Goleva E, Leung DY. Decreased serum vitamin D levels in children with asthma are associated with increased corticosteroid use. J Allergy Clin Immunol 2010;125:995-1000.
27. Xystrakis E, Kusumakar S, Boswell S, Peek E, Urry Z, Richards DF, et al. Reversing the defective induction of IL-10-secreting regulatory T-cells in glucocorticoid-resistant asthma patients. J Clin Invest 2006;116:146-55.
28. Brehm JM, Schuemann B, Fuhlbrigge AL, Hollis BW, Strunk RC, Zeiger RS, et al. Childhood Asthma Management Program Research Group. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol 2010;126:52-8.
29. Payne DN. Nitric oxide in allergic airway inflammation. Curr Opin Allergy Clin Immunol 2003;3:133-7.
30. Salo PM, Arbes SJ, Jr., Crockett PW, Thorne PS, Cohn RD, Zeldin DC. Exposure to multiple indoor allergens in US homes and its relationship to asthma. J Allergy Clin Immunol 2008;121:678-84.