The association of vitamin D, cathelicidin, and vitamin D binding protein with acute asthma attacks in children

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ABSTRACT

Background: *Recent evidence about the various effects of vitamin D (vit D) on innate and adaptive immunity has led to a search for the role of vit D in asthma. It is postulated that a decrease in cathelicidin, a multifunctional host defense molecule, production due to low vit D status may predispose to infectious complications in children with asthma.*

Objective: *The aim of this study was to determine the association of vit D, vit D-binding protein (VDBP) and cathelicidin with acute asthma attacks among children with allergic asthma.*

Methods: *This prospective study included 35 patients with acute asthma attack and 32 children with controlled asthma, and all were matched by sampling season, sensitization to mites, and previous severity of asthma. A comprehensive questionnaire about risk factors, blood sampling for 25-hydroxyvitamin D vit D, VDBP, and cathelicidin levels; spirometric indices were used. Factors that influence serum vit D and cathelicidin levels and the development of asthma attacks were evaluated with multivariate analysis.*

Results: *The mean serum vit D levels of the attack group was significantly lower than that of the controlled asthma group ($p < 0.001$). The mean cathelicidin level was significantly higher in the acute asthma group than with the controlled subjects with asthma ($p = 0.002$). There was no difference between the acute and controlled asthma groups in terms of markers of allergy and serum VDBP levels. Risk factors that may influence vit D levels revealed that body mass index (BMI) ($p = 0.038$), duration of sun exposure ($p < 0.001$), and amount of dietary vit D ($p < 0.001$) independently affected serum vit D levels. Risk factors that may result in acute asthma showed that low serum levels of vit D were significantly related to the risk of asthma attacks ($p < 0.001$, adjusted odds ratio 16.11). Cathelicidin levels showed a significant positive association with asthma attacks and BMI.*

Conclusions: *Vit D deficiency showed a significant relationship to the development of asthma attacks independent of cathelicidin deficiency and other factors associated with the severity of chronic asthma.*

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Asthma represents one of the most common chronic diseases and is a major public health problem worldwide.[1] A significant number of patients with asthma do not achieve optimal asthma control even with high dose treatment and are at high risk of severe exacerbations and asthma-related mortality.[2] Vitamin D (vit D) deficiency is highly prevalent among children and adolescents around the world.[3] In addition to rickets, there is growing evidence that indicates that vit D deficiency may play a key role in the development of cancer, autoimmune disease, recurrent wheezing, and asthma.[4,5] In many studies, a negative correlation between vit D levels and markers of asthma severity was demonstrated.[6,7] Vit D is suggested to affect the development and/or severity of asthma by its anti-inflammatory effect, prevention of respiratory tract infections, inhibition of remodeling, and reduction of steroid resistance.[8,9] Vit D axis has been linked to pulmonary disorders, including asthma.[10,11] Vit D-binding protein (VDBP), which is involved in the vit D axis, has immunomodulatory functions that may be important in pulmonary infections and inflammation.[10,12]

Vit D has several effects on the innate and adaptive immune systems that might be relevant in the primary prevention of asthma, reduction of asthma morbidity, and modulation of the severity of asthma attacks.[8,9,13] Asthma exacerbations, most often caused by respiratory tract infections, are the leading cause of asthma morbidity.[14] The active form of vit D has local effects in response to infections and might reduce the inflammation that is the consequence of these infections.[14,15] Results of two studies showed that vit D increased the transcription of the innate immune protein, human cathelicidin antimicrobial peptide (camp).[13,16] Cathelicidin is a multifunctional host defense molecule essen-

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tial for normal immune responses to infections.[17] A decrease in cathelicidin levels postulated to be due to low vit D status may predispose to infectious complications in healthy children and in children with asthma.[13,14,16]

Vit D deficiency is found to be associated with higher odds of severe asthma exacerbations, and it is emphasized that having sufficient vit D levels conferred protection against severe attacks.[6,18] Uysalol *et al.*,[19] demonstrated the correlation between plasma 25-hydroxyvitamin D levels and childhood asthma. A decrease in vit D level was shown to increase the severity of asthma and decrease the frequency of controlled asthma. However, Hughes *et al.*,[20] reported that there was no association between vit D–related measures and childhood asthma. In another study, no difference could be demonstrated between the effect of vit D on airway reactivity and inflammation in children with asthma.[21] The aim of this study was to determine the role of the vit D pathway and cathelicidin in the development of acute asthma attacks among 7- to 17-year-old children with allergic asthma.

METHODS

Patients with asthma who were admitted to the emergency department because of an exacerbation of asthma and to the pediatric allergy outpatient clinic for routine control visits between November 2011 and February 2012 were included in this study. Eighty-eight children with asthma were asked to participate in the study, and 67 of them accepted the diagnostic workup. The response rate was 76.1%. Classification of asthma severity and control was assessed according to the criteria of the Global Initiative for Asthma.[1] Controlled asthma was defined as the absence of daytime symptoms, the limitation of activities, nocturnal symptoms, and the need for rescue treatment with normal lung function results for the past month. Asthma attack was defined as the presence of progressive dyspnea, cough, wheezing, or chest tightness accompanied by low forced expiratory volume in 1 second (FEV_1) values in a patient with previously diagnosed asthma.[1] Regular medications were recorded for all the patients. Asthma attack and controlled asthma subgroups were matched by mild-to-moderate severity in the previous 3 months, and they were all sensitized only to house-dust mites. The exclusion criteria were the following: (1) the presence of chronic lung disease or severe asthma, (2) sensitization to allergen other than mite, (3) use of drugs or a chronic disease that can affect the levels of serum vit D, and (4) use of vit D preparations.

Asthma is considered mild persistent if the symptoms occur more than 2 days a week but do not occur every day, nighttime symptoms occur three to four times a month, symptoms interfere with daily activities, or peak expiratory flow values are 80% or more of the expected value and may vary 20% to 30% in a day. Asthma is considered moderate persistent if symptoms occur daily, nighttime symptoms occur more than one time a week; symptoms interfere with daily activities; and peak expiratory flow values are >60% and <80% of the expected value, and may vary more than 30% in a day.[1]

In all the groups, a comprehensive questionnaire about the factors that affect the level of vit D (daily exposure to sun, the amount of dietary vit D per day, inhaled corticosteroid (ICS) usage, the presence of systemic illness that leads to vit D deficiency, use of sunscreens, and vit D preparations) was used. The duration of daily exposure to the sun was grouped as <1 hour per day or > 1 hour per day on most days of the previous month. Vit D content of nutrients has been defined according to reports in the literature.[22] In the questionnaire, the children were also asked about the presence of symptoms of upper respiratory tract infection (URTI). Criteria for a clinically manifested URTI was defined as two or more of the following symptoms: fever; cough; headache; sneezing, runny nose, and nasal congestion; pharyngeal hyperemia; and sore throat.[23] Body mass index (BMI) was calculated as weight in kilograms divided by the height in squared meters and was recorded. Skin color was grouped as light- and dark-skin color.

Lung functions were conducted, just before blood sampling, in accordance with American Thoracic Society/European Respiratory Society guidelines, by using a spirometry device (Jaeger, Germany).[24] The values of FEV_1 and forced vital capacity were recorded for data analysis. After completing baseline spirometry, the subjects were given 3 puffs (90 μg per puff) albuterol by metered-dose inhaler and spirometry was repeated after 15 minutes. Sensitization was ascertained by skin testing. It was performed according to the International Study of Asthma and Allergies in Childhood protocol.[25] The following antigens were applied to the volar surface of the forearm in addition to histamine and saline solution controls: *Dermatophagoides pteronyssinus, Dermatophagoides farinae,* cockroach, cat and dog dander, *Alternaria alternata*, and mixed grass and tree pollen. A test was considered positive if the maximal diameter of the wheal was 3 mm after subtraction of the maximal diameter of the negative control.

Serum 25-hydroxyvitamin D levels were analyzed by high-performance liquid chromatography system tandem mass spectrometry (Chromsystems-Agillent 1100 device, Betamed Medical, Germany). A serum 25-hydroxyvitamin D level is considered to be the best circulating biomarker of vit D metabolic status because this form has a longer half-life (2–3 weeks) than 1,25-dihydroxyvitamin D (4 hours).[26] The VDBP and hu-

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Table 1. **The comparison of demographic characteristics, clinical variables, serum vit D, VDBP, and cathelicidin levels between study groups**

	Asthma Attack, $n = 35$	Controlled asthma $n = 32$	p Value
Age, y	10.0 ± 3.7	11.9 ± 2.3	**0.005**
Male sex (%)	19 (54.3)	24 (75)	0.077
Daily taken vit D in diet, μg/day	4.89 ± 1.18	5.89 ± 1.27	**0.003**
Exposure to sun <1 hr (%)	20 (57.1)	3 (9.4)	**<0.001**
Dark skin color (%)	19 (54.3)	9 (28.1)	**0.030**
ICS usage (%)	9 (25.7)	14(43.8)	0.120
BMI, kg/m^2	19.26 ± 4.22	19.09 ± 3.83	0.985
Serum log IgE	2.72 ± 0.54	2.62 ± 0.45	0.435
Serum eosinophil count, $cells/mm^3$	516.63 ± 362.97	495.53 ± 229.66	0.779
Prebronchodilator lung function FEV_1, %	77.99 ± 18.29	99.37 ± 11.31	**<0.001**
FEV_1, absolute	1.59 ± 0.82	2.43 ± 0.72	**<0.001**
FVC, %	92.95 ± 15.93	100.23 ± 13.19	0.047
Serum VDBP, mg/dL	25.05 ± 18.77	19.15 ± 14.33	0.205
Log cathelicidin, ng/mL	3.88 ± 0.78	3.25 ± 0.72	**0.002**
Serum vit D, μg/L	14.09 ± 5.75	28.47 ± 13.88	**<0.001**
Serum vit D, <20 μg/L deficient, %	85.7	31.3	0.153
Serum vit D, 20–29 μg/L, %	11.4	28.1	0.311
Serum vit D ≥30 μg/L, %	2.9	40.6	**<0.001**

Values are no. (%) for binary variables or mean (SD) for continuous variables. Bold data indicates statistically significant figures.
ICS = inhaled corticosteroid.

man camp levels in serum were measured by using an enzyme-linked immunosorbent assay (camp; Uscn Life Science Inc, China and VDBP; İmmundiagnostik AG, Bensheim, Germany); values are expressed in mg/dL and ng/mL, respectively. Also, total immunoglobulin (Ig) E levels and eosinophil count measurements were performed as markers of allergy. In the asthma attack group, the blood samples were taken at the time that the patients were admitted to the hospital with asthma exacerbation. The study was approved by the clinical research ethics committee of Mersin University. All the patients provided written informed consent before taking part in the study.

Statistical Analysis

A descriptive analysis of univariate predictors was performed by using categorization of vit D as sufficient (≥30 μg/L), insufficient (20–29 μg/L), or deficient (<20 μg/L), based on previous recommendations.[26] A power analysis of the study was performed, and a minimum of 24 patients was needed in each group with 5% type 1 error and 20% type 2 error. By taking the logarithm of cathelicidin and total IgE values, parametric tests could be performed. Data description was primarily based on means and standard deviations for continuous end points and on frequencies for categorical end points. Unadjusted comparisons were made by using the t-test or the Mann-Whitney U test for continuous end points and the χ^2 test for categorical end points. Correlations were assessed by using the Pearson or the Spearman correlation. The possible factors identified with univariate analysis ($p < 0.1$) and the factors that were thought to be clinically important were further entered into the multivariate regression analysis to determine independent predictors. A multiple linear regression model was used to identify independent predictors of serum vit D levels. Risk factors that may influence the development of asthma attacks were evaluated for all the patients with asthma by using multivariate logistic analysis. Receiver operating characteristic curve analysis was performed to find the cutoff values for age, vit D, and log camp to be used in multivariate analysis. A p value < 0.05 was considered statistically significant. Data analysis was performed with 17.0 SPSS software (SPSS for Windows, SPSS Inc, Chicago, IL).

RESULTS

A total of 67 children with asthma (35 patients with asthma attack and 32 patients with controlled asthma) sensitive only to house-dust mites were enrolled. Demographic characteristics and clinical variables that may influence vit D levels are presented in Table 1. The

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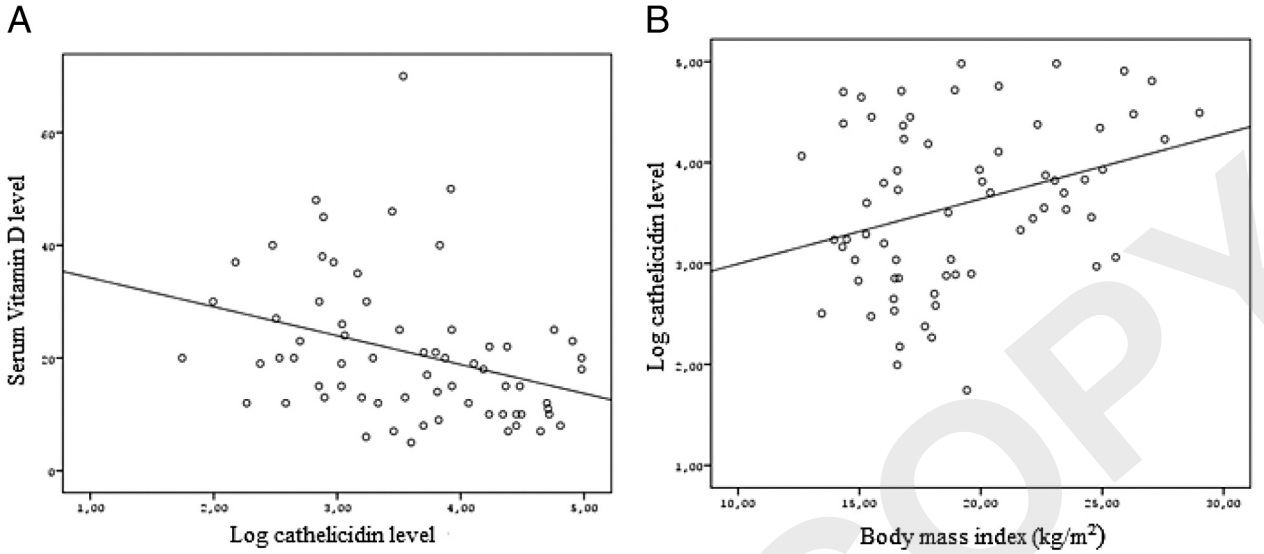

Figure 1. *(A) The relationship between serum vit D and cathelicidin levels in patients with asthma. (B) The relationship between serum cathelicidin levels and BMI in patients with asthma.*

mean age of the asthma attack group was significantly lower than that of the other group. In the attack group, the percentage of dark skin color and daily exposure to the sun <1 hour were significantly higher and daily taken vit D in foods was significantly lower when compared with patients with controlled asthma. There was no difference between the groups in terms of ICS usage, BMI, markers of allergy (total IgE, serum eosinophils), and forced vital capacity values. The mean FEV_1 values were significantly higher in the controlled asthma group (Table 1).

Serum vit D and related protein levels are presented in Table 1. Among 67 study participants, 53 (79.1%) had vit D levels <30 μg/L. The mean (standard deviation) serum vit D level was 14.09 $\pm$ 5.75 μg/L in the asthma attack group and 28.47 $\pm$ 13.88 μg/L in the controlled asthma group. The difference between the attack and the controlled asthma groups was highly significant ($p < 0.001$). The mean serum VDBP levels were not different between the attack and the controlled asthma groups. The mean log camp levels were significantly higher in the acute asthma group than in the controlled asthma group ($p = 0.002$) (Table 1).

Serum vit D and VDBP levels were not significantly different between the patients in the attack group with and without URTI symptoms. However, serum cathelicidin levels were found to be higher in the patients with URTI, significant at a border level ($p = 0.056$). Also, there was no difference between the patients in the attack group with and without URTI symptoms in terms of allergy markers (serum IgE, absolute eosinophil count).

In the attack group, 16 patients had symptoms of URTI. Serum vit D levels showed a significant inverse correlation with serum cathelicidin (Fig. 1 *A*) (Pearson correlation coefficient, −0.331; $p = 0.006$) and VDBP levels (Pearson correlation coefficient, −0.289; $p = 0.018$). No significant correlation was found between vit D levels and total IgE, absolute eosinophil counts, BMI, and spirometric indices among all the patients with asthma when using univariate analysis. There was a significant positive correlation between serum cathelicidin levels and BMI (Pearson correlation coefficient, 0.316; $p = 0.009$) (Fig. 1 *B*) and serum VDBP levels (Pearson correlation coefficient, 0.375; $p = 0.002$). We found no association between cathelicidin and markers of allergy and spirometric indices among all the patients with asthma.

Multivariate linear regression analysis of risk factors that may influence vit D levels among the patients with asthma revealed that an increasing amount of dietary vit D and >1-hour duration of sun exposure daily were associated with higher serum vit D levels, whereas higher BMI values showed a significant negative relationship to serum vit D levels (Table 2). Multivariate analysis of risk factors that may influence serum cathelicidin levels revealed that higher serum vit D levels were associated with decreased serum cathelicidin levels (β coefficient, −0.018; $p = 0.016$), whereas higher BMI values showed a significant positive relationship to cathelicidin levels (β coefficient, 0.083; $p = 0.001$) (Table 3).

To evaluate the relationship of vit D levels with asthma attacks, the risk factors that may relate to acute asthma were included in multivariate analysis among all the patients with asthma. Serum vit D levels <15 μg/L were found to be significantly related to the risk of asthma attacks in logistic regression analysis ($p < 0.001$; adjusted odds ratio, 16.11), independent of age, sex, use of inhaled steroids, BMI, exposure to sun (as a

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Table 2. **Univariate and multivariate analysis of risk factors associated to serum vit D levels**

Variable	Univariate Analysis β Coefficient (95% CI)	Univariate Analysis *p* Value	Multivariate Analysis β Coefficient (95% CI)	Multivariate Analysis *p* Value
Age	0.020 (–0.048 to 0.088)	0.552	0.615 (–0.028 to 1.259)	0.061
Male sex	5.916 (0.892–10.940)	**0.022**	1.55 (–2.473 to 5.574)	0.444
BMI, kg/m^2	–0.206 (–0.826 to 0.414)	0.510	–0.590 (–1.145 to –0.034)	**0.038**
Dark skin color	–4.295 (–9.550 to 0.960)	0.108	–2.122 (–6.076 to 1.831)	0.287
Not using ICS	–1.790 (–8.326 to 4.747)	0.586	–2.170 (–6.012 to 1.673)	0.263
Daily taken vit D in diet, μg/day	6.387 (4.904–7.870)	**<0.001**	5.911 (4.336–7.486)	**<0.001**
Exposure to sun >1 hr	14.570 (10.286–18.853)	**<0.001**	9.087 (4.709–13.466)	**<0.001**
Serum VDBP level, mg/dL	–0.175 (–0.315 to –0.034)	**0.015**	-0.061 (–0.179 to 0.056)	0.300

Bold data indicates statistically significant figures. CI = confidence interval, ICS = inhaled corticosteroid.

Table 3. **Univariate and multivariate analysis of risk factors associated with cathelicidin levels**

Variable	Univariate Analysis β Coefficient (95% CI)	Univariate Analysis *p* Value	Multivariate Analysis β Coefficient (95% CI)	Multivariate Analysis *p* Value
Age	–0.003 (–0.008 to 0.001)	0.170	–0.057 (–0.119 to 0.005)	0.072
Male sex	0.125 (–0.237 to 0.487)	0.495	0.097 (–0.323 to 0.498)	0.621
BMI, kg/m^2	0.056 (0.014–0.098)	**0.010**	0.083 (0.033–0.132)	**0.001**
Not using ICS	–0.065 (–0.474 to 0.344)	0.752	0.080 (–0.314 to 0.473)	0.687
Serum IgE, IU/mL	0.298 (0.020–0.576)	**0.036**	0.177 (–0.232 to 0.586)	0.389
Absolute eosinophil count, $\times 10^3/\mu$L	<0.001 (0.000–0.001)	0.124	<0.001 (<0.001 to <0.001)	0.598
Serum vit D, μg/L	–0.015 (–0.030 to 0.000)	**0.044**	–0.018 (–0.032 to –0.003)	**0.016**
Presence of upper respiratory tract infection symptoms	0.483 (0.097–0.868)	**0.015**	0.285 (–0.129 to 0.699)	0.173

Bold data indicates statistically significant figures. CI = confidence interval, ICS = inhaled corticosteroid.

Table 4. **Univariate and multivariate analysis of factors that may affect the development of asthma attack**

Variable	Univariate Analysis OR (95% CI)	Univariate Analysis *p* Value	Multivariate Analysis OR (95% CI)	Multivariate Analysis *p* Value
Age, <10 y	8.30 (2.68–25.69)	**<0.001**	11.19 (2.37–52.84)	**0.002**
Male sex	0.39 (0.14–1.12)	0.081	0.54 (0.11–2.56)	0.443
BMI, kg/m^2	1.01 (0.89–1.14)	0.862	1.06 (0.83–1.35)	0.635
Serum vit D ≤ 15, μg/L	17.5 (4.87–62.86)	**<0.001**	16.11 (3.45–75.11)	**<0.001**
Not using ICS	2.24 (0.80–6.29)	0.124	2.59 (0.53–12.48)	0.235
Exposure to sun >1 hr	0.07 (0.02–0.30)	**<0.001**	0.80 (0.04–15.47)	0.885
Log cathelicidin ≥ 3.54 ng/mL	5.50 (1.93–15.67)	**0.001**	5.95 (1.36–25.95)	**0.018**
Serum VDBP, mg/dL	1.02 (0.99–1.05)	0.159	1.02 (0.97–1.08)	0.275

Bold data indicates statistically significant figures. OR = odds ratio, CI = confidence interval, ICS = inhaled corticosteroid.

marker of activity level), serum levels of cathelicidin, and VDBP (Table 4). A 1-μg/L increase in serum vit D was found to predict a 1.2-fold decrease in the risk of having an asthma attack ($p < 0.001$). Log cathelicidin levels higher than 3.54 ng/mL were found to be significantly and independently associated with asthma attacks. Although ICS usage did not show a significant association with asthma attack, combined effects of ICS

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usage and vit D levels were analyzed. Compared with the reference group of children who received regular inhaled steroids and had vit D levels >10 μg/L, the highest risk of asthma attack was found to be in the group that was not receiving ICS and vit D levels <10 μg/L (odds ratio 7.94 [95% confidence interval, 1.40–44.80]).

DISCUSSION

Vit D insufficiency in children is a major health problem in many countries, and different rates have been reported in many studies.[3,4] In our study, vit D insufficiency was found in 79.1% of all children, 97.1% of patients with acute asthma, and 59.4% of those with controlled asthma. The risk factors that affect the level of serum vit D revealed that the increase in the amount of dietary vit D and duration of sun exposure significantly augmented vit D levels, whereas the increase in BMI was significantly associated with a decrease in serum vit D level.

In our study, the mean serum vit D level was significantly lower in the asthma attack group than in the controlled asthma group. A multivariate analysis revealed that the increase in serum levels of vit D was significantly associated with a lower risk of asthma attacks, independent of age, sex, allergic markers, use of inhaled steroids, BMI, exposure to sun (as a marker of activity level), serum levels of cathelicidin, and VDBP. For the relationship between low vit D levels and the development of asthma attack, one can mention a reverse causality. It may be possible that children who have more severe and frequent attacks or more severe asthma are more likely to spend time indoors because of the limitation of activity that may lead to low levels of vit D or *vice versa.* Because our study design was cross-sectional, reverse causality cannot be ruled out. However, the groups were matched by age group, previous asthma severity, and allergic sensitization profile, which should have resulted in similar degrees of activity limitation due to asthma severity. Although a similar argument could be suggested for the relationship between BMI and vit D status, our multivariate model included both sun exposure duration and BMI, and showed that low vit D levels were significantly related to the risk of asthma attack independent of these factors.

There was no correlation between serum vit D levels and lung function variables in the present study. Larose *et al.*,[27] showed that a serum 25(OH)D level <50 nmol/L was significantly associated with a lower FEV_1 to forced vital capacity ratio in men with asthma. In another study, a significant relationship between insufficient serum vit D levels and worse lung function was demonstrated in children in the community with a suggested dose-response effect.[28]

Viral respiratory infections are among the most important causes of asthma attacks.[14] Studies showed that vit D increased the transcription of the innate immune protein, human camp,[16,29] which is a multifunctional host defense molecule essential for normal immune responses to infections.[30] When we compared the attack subgroups with and without URTI symptoms, there was no significant difference for serum vit D levels and a difference in opposing direction at border level for cathelicidin levels. Our results did not support the hypothesis that vit D deficiency could result in an increased frequency of URTI-induced asthma attacks through a reduced production of cathelicidin. Levels of pH are suggested to decrease in exhaled breath condensate of children with asthma with exacerbation.[31] A decreased pH level reduces the antimicrobial activity of cathelicidin[32] and, therefore, may increase the frequency of an asthma attack independently from vit D levels. In addition to the antimicrobial effects, cathelicidin also has immunomodulatory properties, such as chemoattractant function, inhibition of neutrophil apoptosis, tissue regeneration, and cytokine release.[17,29,30] The relationship of molecules participating in the vit D receptor– vit D–camp pathway have been investigated in many infectious diseases.[33,34] These studies indicate that cathelicidin can be used as a marker for a strong systemic immune response in viral or bacterial infections[35,36]. Recently, Liu *et al.*,[37] showed that levels of vit D metabolites increased after allergen challenge in the lung and the increases correlated with the magnitude of inflammation and increases in cathelicidin in subjects with allergy. Our study revealed that, despite lower levels of vit D in the asthma attack group, serum cathelicidin levels were significantly higher in the attack group than in the controlled asthma group; also, there was a negative correlation between vit D and cathelicidin levels. Moreover, higher cathelicidin levels were found to be significantly associated with asthma attacks in multivariate analysis of our study. Based on our results and other studies, it can be hypothesized that high serum cathelicidin levels may reflect a systemic immune activation in acute attacks independent of vit D levels.

In the present study, there was a significant positive correlation between BMI and serum levels of cathelicidin in all the patients with asthma. Benachour *et al.*,[38] showed that cathelicidin messenger RNA expression was significantly positively correlated with BMI. The effects of adipose tissue on cathelicidin gen regulation (messenger RNA expression) is not clear. However, it may be that the proinflammatory cytokines secreted from adipose tissue, *e.g.*, leptin, may induce the cathelicidin production.

The majority of vit D in circulation is transported by VDBP.[10] In addition to carrying vit D, it has several

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anti-inflammatory and immunomodulatory functions.[10–12] It is postulated that, in asthma, high levels of airway VDBP drive alveolar macrophages into a more inflammatory state, which diverts them from their tolerogenic role. The VDBP level might be thought to be a marker of airway inflammation. Gupta *et al.*[11] found a negative correlation between asthma control and the level of VDBP in bronchoalveolar lavage. Chalmers *et al.*[36] observed that patients with vit D deficiency showed an increased frequency of asthma attacks, together with higher sputum inflammatory markers and VDBP levels. In our study, we found a significant negative correlation between serum vit D and VDBP levels. Although we could not evaluate sputum VDBP levels, serum VDBP levels in acute asthma were higher than in the controlled asthma group but had not reached statistical significance.

Vit D appears to be an important factor in influencing the effectiveness of treatment, thereby ensuring asthma control. Increasing serum vit D levels were found to be associated with enhanced steroid response.[39] We did not find a significant relationship between steroid use and vit D. However, when the combined effects of ICS usage and the levels of vit D were evaluated, the patients with asthma and with no ICS usage, and with serum vit D levels <10 μg/L were found to have the highest risk for asthma attacks. Hence, our study showed that vit D deficiency may also predispose to asthma attacks by blunting response to steroids.

The limitations of this study were (1) a relatively low number of patients because of the limited budget; (2) for the relationship between low vit D levels and the development of asthma attack, reverse causality could be ruled out because of the cross-sectional nature of the study; and to assess this situation, a general population of patients with asthma should be prospectively followed up; (3) the factors that affect the levels of serum vit D were evaluated based on the subjective data supplied by family reports; and (4) the inflammatory markers, VDBP, and cathelicidin levels could not be performed in sputum; therefore, the correlation between the local immune response in the lungs and vit D was not evaluated more reliably.

The strengths of this study were that it was a unique study that evaluated the roles of vit D, VDBP, and cathelicidin all together in the development of childhood asthma exacerbations. To our knowledge, the study that demonstrated the association of cathelicidin with asthmatic exacerbations was not reported in the literature before; in this regard, our study can be a premise for future studies that evaluate cathelicidin as a marker of asthma attack. In addition, the factors that affect the level of vit D and asthma attack were evaluated with the simultaneous application of a detailed questionnaire and measurement of serum 25 (OH) D, cathelicidin, and VDBP levels.

In conclusion, our study demonstrated that vit D deficiency showed a significant relationship to the development of asthma exacerbations, independent of cathelicidin levels and other factors, such as BMI and outdoor activity. VDBP and cathelicidin are rarely investigated molecules in this group of patients, so further studies are needed to evaluate the relationship of these molecules with the inflammatory response in acute asthma attacks.

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