Original Article

Association Between the Oxidative Status, Vitamin D Levels and Respiratory Function in Asthmatic Children

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Abstract

Aim: We studied the relationship between plasma concentrations of oxidative system markers, vitamin D, and respiratory functions in children with asthma. **Materials and Methods:** Ninety one children aged 6–17 years with stable asthma seen in the clinic had the serum concentrations of oxidative system markers [total antioxidant capacity (TAC), total oxidative status (TOS), paraoxonase-1 activity (PON-1), and 25-hydroxyvitamin D3] and respiratory functions were measured. **Results:** There was no statistical correlation between TAC and age and FEV1. There was a significant positive correlation between TAC and 25(OH)D3 (r = 0.214, P = 0.021), TAC and TOS (r = 0.218, P = 0.007), TAC and PON-1 (r = 0.230, P = 0.028), TAC and IgE (r = 0.194, P = 0.033), and inverse correlation between TAC and PEF (r = −0.208, P = 0.024). In the backward multiple regression analysis, 25(OH)D3 (t = 2.613, P = 0.011), age (t = −2.158, P = 0.034), TOS (t = 2.158, P = 0.000), and OSI index (t = −13.859 P = 0.000) maintained an independent relationship with TAC (r = 0.858, $r2$ = 0.737, F = 21.436, P = 0.000). **Conclusion:** Oxidative stress correlates with the serum vitamin D concentrations. Clinical trials are required to confirm that increasing serum 25OHD may improve asthma control, as measured by clinical and oxidative stress markers.

Keywords: *Asthma, children, vitamin D, oxidative stress, respiratory functions*

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Introduction

Asthma, a disease characterized by chronic airway inflammation and hyper-responsiveness, is a common disease that affects all age groups. Asthma may be manifested as irreversible airflow obstruction in some patients.[1] Although the pathogenesis of asthma is not well-understood, increased oxidant stress due to an imbalance of oxidants and antioxidants has been found to be associated with asthma.[2,3]

In asthma, inflammation-related oxidative stress is driven by exposure to a variety of triggers, including allergens and viruses, which activate components of both the innate and acquired immune responses. Protection by escaping from triggering factors or standardization of asthma medication is difficult and usually is not enough for effective treatment.[4] On the other hand, correction of antioxidative systems may be more efficacious in the control of asthmatic inflammation and asthma symptoms.[5] In different studies, it has been proposed that vitamin D deficiency is also associated with an increased incidence of asthma symptoms.[6-9]

One of the possible explanations is that vitamin D may be a potent antioxidative.[10] However, a definitive role for vitamin D in the pathogenesis of asthma has not been determined. The aim of this study is to assess the possible interrelation among the plasma concentrations of oxidative system markers total antioxidant capacity (TAC), total oxidative status (TOS), particularly antioxidative ones, Paraoxonase 1 (PON1), serum 25-hydroxyvitamin D3 [25(OH)D3] and respiratory functions in children with asthma.

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Materials and Methods

A cross-sectional study was conducted in children 6–17 years of age who were admitted to Samsun Training and Research Hospital, Samsun, Turkey. A cross-sectional study was conducted in children 6–17 years of age who were seen on outpatient basis at Samsun Training and Research Hospital, Samsun, Turkey. Patients with a diagnosis of asthma were followed up monthly on outpatient basis.

A total of 108 of the 344 patients were recruited using a random number table. One hundred and eight patients with asthma (60 males, 48 females, mean age 9 ± 3 years) formed our study group.

Children were considered eligible for inclusion if they were diagnosed with regular asthma and did not have an acute asthma attack during the visits. Children were diagnosed and classified into clinical categories according to Global Initiative for Asthma (GINA) criteria.[11] Only patients with intermittent-mild asthma were recruited for the study to avoid the influence of regular treatment on bronchial hyperresponsiveness and, consequently, on the results of the measured vitamin D levels. We also measured the blood eosinophil and total IgE levels. Peripheral blood eosinophilia could be correlated with clinical severity.[12] High total IgE could be correlated with diseases that mimic asthma, such as allergic bronchopulmonary aspergillosis.[13] The main exclusion criteria were an inability to perform spirometry, presence of complaints or diseases other than asthma, presence of acute asthma attack, mild asthma, or history of acute illness or febrile episode in the preceding 7 days. The local ethical review board approved the study, and the parents of all participants provided written informed consent for study participation.

Study measurements

Pulmonary function test

Spirometry was conducted in accordance with American Thoracic Society recommendations.[14] A Survey Tach Spirometry (Warren E Collins, Braintree, USA) was used to perform spirometry in a standardized manner. The best forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and peak expiratory flow (PEF) were recorded for data analysis. However, in particular, the FEV1 value was considered because PEF measurements are more effort dependent than FEV1, and may therefore, underestimate the degree of airway obstruction.

FEV1/FVC, which is more likely to rule out restrictive diseases, was used. FEV1, FVC, and PEF were measured until three reproducible recordings (with a divergence of less than 5%) were obtained, of which the highest was used in the analysis. The reference values of FEV1, FVC, and PEF were those of the European Respiratory Society.

Blood samples

Blood samples were collected into both empty and anti-coagulated tubes containing ethylenediaminetetraacetic acid (EDTA). The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples for measurement of serum 25(OH)D3, TOS, and TAC levels and Paraoxonase-1 (PON-1) activity were stored at −80°C until they were used.

Serum 25(OH)D3: A single measurement of 25(OH) D3 concentrations was conducting using the high-performance liquid chromatography method with Agilent 1100 bio analyzer (Waldbronn, Germany, Roche).

Measurement of TAC and TOS: The TAC and the TOS of serum were determined using novel automated measurement methods, developed by Erel.[15]

Measurement of PON-1 activity: Paraoxonase 1 (PON1) is an antioxidizing enzyme that contributes to the hydrolysis of lipid peroxides into oxidized lipoproteins, and has been associated with diseases characterized by high oxidative stress such as asthma.[16] Paraoxonase-1 activity was measured using a commercially available kit (Relassay, Turkey).

The TAC, TOS, and PON-1 activity levels were evaluated spectrophotometrically using the ADVIA 2400 bio analyzer (Tarrytown, NY, Siemens). All blood sample measurements were performed at the Department of Biochemistry, Atatürk Training and Research Hospital, Ankara, Turkey.

Determination of oxidative stress index (OSI): The ratio of TOS to TAC was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAC was converted to mmol/l, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (μmol H2O2 Eq/l) / TAC (mmol Trolox Eq./L) × 100.[15]

Statistical analysis

The primary end point, with respect to respiratory function tests in asthmatic patients, was finding potential factors that influence TAC, particularly 25(OH)D3.

Statistical analyses were performed using the software packages SPSS, version 17 (SPSS, Chicago, IL, USA). The variables were investigated using visual (histograms, probability plots) and analytical (Kolmogorov–Simirnov/ Shapiro Wilk's Test) methods to determine whether they were normally distributed. The parameters, particularly the 25(OH)D3 level affecting TAC in asthmatic patients, were investigated using Spearman/Pearson correlation and Student's test where appropriate. A multiple linear regression test model was used to identify independent predictors of TAC. A 5% type 1-error level was used to

determine interstatistical significance. A *P* value of <0.05 was considered significant.

Results

One hundred and eight patients with asthma formed our study group. Eight children (3 males and 5 females) were excluded involvement due to exacerbations of acute asthma attack or febrile illness. Nine other children were also excluded due to inappropriate pulmonary function test.

The study was conducted among 91 patients with mild asthma (between 6 and 17 years of age, with a mean age of 9.4 ± 3.1 years. The serum 25(OH)D3 D levels, circulating oxidative stress markers, and lung function of the study population are presented in Table 1.

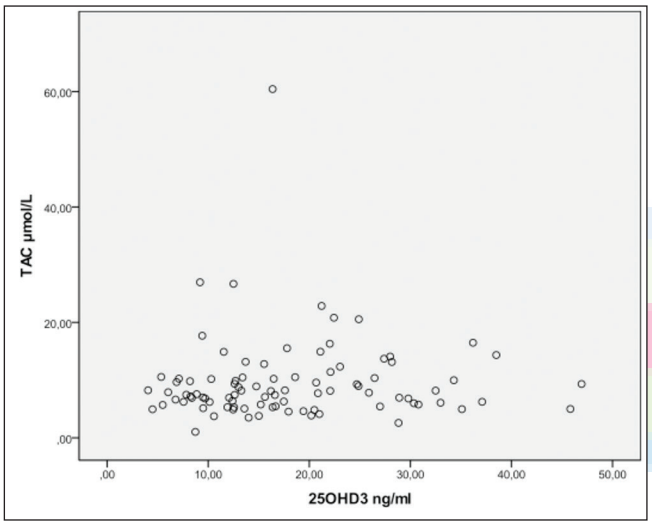

Figure 1: Relationship between 25(OH)D3 and TAC concentrations. Serum concentrations of 25(OH)D3 were correlated with TAC (t = 2.613, P = 0.011). TAC - Total antioxidant capacity

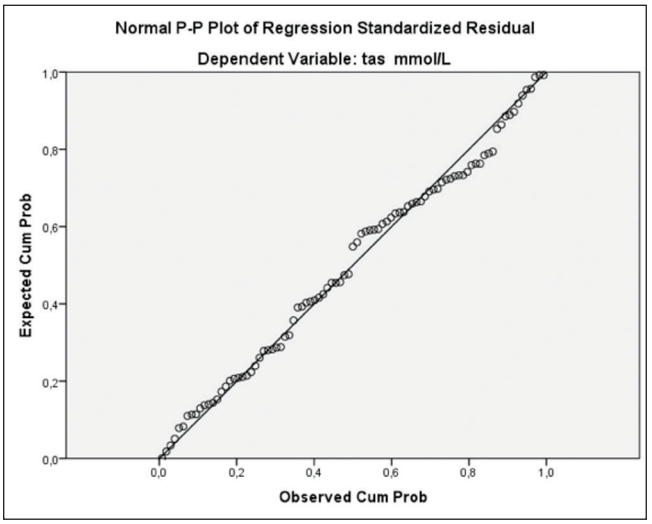

Figure 2: Regression standardized residual dependent variable: TAS

The total IgE and blood eosinophil levels, which were used to confirm the eligibility of enrolled patients, were found to be in the normal range. The mean serum 25(OH) D3 concentration among asthmatic children was 18 ng/ml

Table 1: Serum 25(OH)D3 D levels, circulating oxidative stress markers, and lung function of patients (N = 91)

	Mean (SD)/%	Min-Max
25(OH) D3 ng/ml	18 (9.4)	4.05-46.9
PON-1 U/l	165.34 (103.8)	42-460
TAC mmol/L	2.09(0.3)	1.4-2.8
TOS µmol/L	9.6 (6.99)	1.0-60.4
OSI index	455.05 (297.6)	51-2370
FVC	85.60 (19.3)	46-137
FEV1	89.4 (21.4)	32-137
FEV1/FVC	100.1 (8.3)	69-114
PEF liters/minute	102.4 (31.0)	50-224
Eos%	3.8 (3.3)	0-16.5
Total IgE IU/ml	433.9 (781.8)	5-6070

25(OH)D3 - 25-Hydroxyvitamin D3; PON-1 - Paraoxonase-1; TAC - Total antioxidant capacity; TOS -Total oxidative status; OSI index - Oxidative stress index; FVC - Forced vital capacity; FEV1 - Forced expiratory volume in one second; FEV1/FVC - Forced expiratory volume in one second/forced vital capacity; PEF - Peak expiratory flow; Eos% - Eosinophil percentage; Total IgE - Total Immunoglobulin E

Table 2: Correlation between TAC, FVC, FEV1/FVC, PEF, PON-1, FEV1, 25(OH)D3, TOS, OSI index, age, eosinophil, and IgE of asthmatic children

	Pearson Correlation	Significance (2-tailed)
TAC mmol/L and 25(OH)D3 ng/ml	0.214	0.021*
TAC mmol/L and TOS mmol/L	0.218	0.007*
TAC mmol/L and PON-1 U/l	0.230	0.028*
TAC mmol/L and OSI index	0.086	0.417
TAC mmol/L and FEV1	−0.135	0.101
TAC mmol/L and FVC	−0.120	0.129
TAC mmol/L and PEF	−0.208	0.024*
TAC mmol/L and FEV1/FVC	−0.145	0.086
TAC mmol/L and Age	0.161	0.064
TAC mmol/L and Eos%	0.046	0.334
TAC mmol/L and IgE	0.194	0.033*

25OHD3 - 25-Hydroxyvitamin D3; PON-1 - Paraoxonase-1; TAC - Total antioxidant capacity; TOS -Total oxidative status; OSI index - Oxidative stress index; FVC - Forced vital capacity; FEV1 - Forced expiratory volume in one second; FEV1/FVC - Forced expiratory volume in one second/forced vital capacity; PEF - Peak expiratory flow liters/minute; Eos% - Eosinophil percentage; Total IgE - Total Immunoglobulin E; *A P < 0.05 was considered statistically significant

Table 3: Backward Linear regression analysis of total antioxidant capacity (TAC) as dependent variable in asthmatic children

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	2.062	0.62		33.134	0.000
	Age (year)	0.010	0.005	0.120	2.158	0.034
	25OHD3 ng/ml	0.004	0.002	0.145	2.613	0.011
	TOS μmol/L	0.143	0.05	0.120	2.158	0.000
	OSI index	−0.003	0.000	−3.725	−13.859	0.000

a. Dependent Variable: TAC mmol/L; Beta indicates the standardized partial regression coefficient 25(OH)D3 - 25-Hydroxyvitamin D3; TAC - Total antioxidant capacity; TOS -Total oxidative status

[standard deviation (SD) 9 ng/ml]. The associations among the participants' age, TAC, FVC, FEV1/FVC, PEF, PON-1, FEV1, 25(OH)D3, TOS, and OSI index are shown in Table 2. There was no statistical correlation between TAC and age, TAC and FEV1, TAC and FEV1/FVC, or TAC and FVC activity [Table 2]. There was a significant direct correlation between TAC and 25(OH)D3 (r = 0.214, P = 0.021) TAC and TOS (r = 0.218, P = 0.007), TAC and PON-1 (r = 0.230, P = 0.028), TAC and IgE (r = 0.194, P = 0.033) and an inverse correlation between TAC and PEF (r = −0.208, P = 0.024) in the study population [Table 2].

Using multiple regression analysis, only 25(OH)D3 (t = 2.613, P = 0.011) [Figure 1], TOS (t = 2.158, P = 0.000), and OSI index (t = −13.859 P = 0.000) maintained an independent relationship with TAC [Table 3] (r = 0.858, $r2$ = 0.737, F = 21.436, P = 0.000) [Figure 2]. In addition, serum concentrations of TAC were inversely correlated with age of the patients (t = −2.158, P = 0.034).

Discussion

A direct correlation was found between the total antioxidant status, 25(OH)D3 level, and total oxidative status and an inverse relationship with the PEF value. However, there was no statistical correlation between TAC and OSI index, between TAC and eosinophils, between TAC and FEV1, TAC and FEV1/FVC, or TAC and FVC activity. Furthermore, there was no correlation among vitamin D-total IgE and vitamin D-blood eosinophil levels. Applying a backward stepwise regression analysis revealed that 25(OH)D3, age, TOS, and OSI index were independent predictors of determining TAC. These results indicate that 25(OH)D3 is an important, independent determinant of TAC in children with asthma.

Our study identified a significant positive correlation between the TAC and 25(OH)D3 levels, which supports previous studies that revealed the positive impact of 25(OH) D3 on chronic asthmatic inflammation and the protective effect of 25(OH)D3 from recurrent respiratory tract infections in asthmatic patients.[6,8,17] It may be interpreted that 25(OH)D3 plays a role in antioxidant production. By virtue of this indirect impact, 25(OH)D3 may also be important in combating oxidative stress, which is produced as a consequence of asthmatic inflammation.[18,19] We believe that characterizing the systemic antioxidant defenses is important in the pathophysiology of asthmatic inflammation. Factors that enhance the capability of antioxidant production should also eliminate the deleterious effects of inflammation in asthma.[20]

Data from an increasing number of randomized, controlled, interventional studies of vitamin D supplementation in pediatric and adult asthma are being published.[19,20,23] Asthmatic children with low blood vitamin D levels may have a greater risk of suffering severe asthma attacks. Two major cohort studies found that low Vitamin D levels in preschool-aged children were found to be associated with the exacerbation of asthma in adolescents.[20,21]

Different studies have suggested that Vitamin D has protective effects against respiratory tract infections and is associated with a decrease in episodes of wheezing in early childhood.[22] However, the relationship between the cord Vitamin D levels and the development of asthma was not demonstrated.[23,24] Because between time at which vitamin D were measured and the time of vitamin D intake is unknown, establishing a cause and effect relationship is difficult.

An oxidant-antioxidant imbalance in asthma and resultant inflammation aggravation may manifest in different ways. It might result from an increase or decrease in oxidative stress, depending on whether the changes are due to a defense response (increase) or neutralization by oxidants (decrease).[5,20] If the reserves are sufficient, there might be no change. In our study, we found a positive correlation between the TAC and TOS status of the patients. This is reasonable because we know that during asthmatic inflammation, rather than increasing oxidative stress, a

lack of compensatory TAC elevation may be one of the possible determinants of the clinical outlook.

In evaluation of TAC with various respiratory functions, a negative correlation was observed between TAC and PEF. Clinically, inflammation of the airways is directly related to the deterioration of respiratory functions and a possibly inadequate increase of TAC,[2,3] which is consistent with our results. This association may lead to a possible reverse relationship between PEF and TAC. As inflammation increases, TAC increases to balance. Because this increase is mostly insufficient to compensate for oxidative stress in asthmatics, it leads to inadequate progressive elevation in TAC, progressive respiratory insufficiency and, as a result, decreased respiratory functions. Our results support this explanation. However, we have not found any correlation between TAC and FEV1, which may be explained in two ways. First, inflammation in bigger airways may much more accurately reflect TAC status of the body, and PEF value may be much more reliable than FEV1 as a surrogate for TAC status. Second, inflammation is not always reflected by airway obstruction and may be masked. Therefore, FEV1 may be normal in aggravated inflammation, which is mostly characterized by an inadequate TAC response.[25]

In the study of Brehm *et al*., the 25(OH)D3 at levels greater than 30 ng/ml, eosinophil counts and IgE levels of asthmatic children were found to be inversely proportional.[26] However, there are other studies in which this relationship was not demonstrated.[27] In our study, there was no correlation among the vitamin D-total IgE and vitamin D-blood eosinophil levels, which are important markers of the asthmatic inflammation. It is possible that the substantial anti-inflammatory treatment prescribed for these children may have masked a relationship between vitamin and airway inflammation. Moreover, the correlation has not been fully explored because of relatively small population.

Although linear regression has shown that the effect of Vitamin D on inflammation is minimal, this relationship should be considered important. A wide spectrum of factors is important in asthma pathogenesis, and each factor is thus expected to have a limited impact on asthma pathogenesis similar to the effect of Vitamin D on asthmatic inflammation in our study.

To our knowledge, the relationship between the measured Vitamin D levels, and oxidative system markers, and respiratory functions of asthma in childhood has not been examined before. These findings are important because augmentation of the antioxidant defenses by means of Vitamin D supplementation interventions might be beneficial in the treatment of asthma.[20,28]

Conclusion

In conclusion, although our data support an association between 25(OH)D3 and the respiratory functions of asthmatic patients in a positive manner, there is no control group to confirm that our results are different from those obtained in children who do not have asthma. Clinical trials are required to confirm that increasing serum 25(OH)D3 may improve asthma control as measured by clinical markers and inflammatory/oxidative stress markers. Also relationship between Vitamin D and other possible important factors, such as gender or age group, should be evaluated in future studies.

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Conflicts of interest

There are no conflicts of interest.

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