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25-hydroxyvitamin D insufficiency in children with newly diagnosed asthma

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ABSTRACT

Background. 25-hydroxyvitamin D [25(OH)D] deficiency seems to be related to the development of asthma. Any evaluation of the relationship between asthma and 25(OH)D deficiency must consider the association between increased airway responsiveness, eosinophil counts and serum immunoglobulin E (IgE), and 25(OH)D as a potential player in airway remodelling.

Objective. We assessed the association of 25(OH)D with markers of atopy and eosinophilic inflammation in children with newly diagnosed asthma.

Methods. The study included 165 children aged 2–12 years. The diagnosis of asthma was performed by an experienced paediatric pulmonologist. Allergic asthma was diagnosed in 106 children, and non-allergic asthma in ten; in 49 children, asthma was excluded. Fasting blood was collected for cell counts, and serum was obtained to measure lipids, C-reactive protein (hsCRP), 25(OH)D and IgE.

Results. Children with asthma had significantly lower 25(OH)D ($p < 0.001$). Both groups had similar lipid values. Elevated total IgE concentration and eosinophil counts were found in asthmatics; neutrophils were similar in asthmatic and reference groups. There was a strong tendency to higher eosinophil counts in 25(OH)D-deficient children (< 20 ng/mL) with atopic asthma ($p < 0.08$).

Conclusion. In children with asthma, 25(OH)D insufficiency/deficiency is associated with higher eosinophil counts and IgE. 25(OH)D monitoring is important in the prevention and management of children with asthma.

Key words: immunoglobulin E, 25-hydroxyvitamin D, eosinophils, asthma

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Introduction

In recent years, a significant rise in the prevalence of childhood asthma has been widely observed [1]. Bronchial asthma is a chronic inflammatory disorder of the airways that is related to their hyperresponsiveness and remodelling, both contributing to variable degrees of airflow obstruction [1]. The common host and environmental factors that affect the development and expression of asthma in children include the exposure to allergens and respiratory infections, chemical irritants and drugs, changes in lifestyle conditions, e.g. dietary habits, high prevalence of vitamin D insufficiency/ deficiency, and decreased outdoor and indoor physical activity leading to excessive body weight [1–4].

Asthma is a heterogeneous disease with multiple, overlapping phenotypes. The primary classification of the disease into immunoglobulin E (IgE)-mediated allergic asthma and non-IgE-mediated non-allergic asthma has recently been extended [5, 6]. A major role is played by T helper type 2 (Th2) cells that mediate airway allergic inflammation by increased production of proinflammatory cytokines [interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-13 (IL-13)], of which IL-4 and interleukin-13 (IL-13) are related to increased IgE concentrations [4]. Several clinical asthma phenotypes have been distinguished, including cellular and molecular phenotypes [5]. Cellular phenotypes differ according to the distribution of eosinophils and neutrophils in the sputum of asthma patients, whereas molecular phenotypes vary according to the overex-

pression of specific factors such as periostin, serpin peptidase inhibitor-B2 (SerpinB2), Transforming Growth Factor-β2 (TGF-β2), Vascular Endothelial Growth Factor (VEGF) and others [5, 7]. Asthmatic patients are heterogeneous on the molecular level: those with high Th2 response may have a higher airway hyperresponsiveness, higher blood eosinophil count, higher serum IgE and biomarkers of airway remodelling, and benefit from different types of treatment, whereas these with low Th2 response and neutrophilia are more resistant to treatment [6, 8, 9]. Biomarkers are currently being investigated to more effectively characterise the disease phenotypes and to identify the responders to specific targeted therapies. Very recently, blood eosinophilia and fractional exhaled nitric oxide (FeNO) have been described as potential surrogate markers associated with distinct asthma phenotypes related to Th2-driven inflammation [5].

Vitamin D, due to its immunomodulatory properties, exerts actions on the cells of innate and adaptive immune system functions; it may be associated with respiratory infections and possibly with airway remodelling. In epidemiological studies, 25-hydroxyvitamin D [25(OH)D] deficiency has been suggested as being related to the development of asthma and the frequency of asthma symptoms [10–12]. Vitamin D deficiency may weaken pulmonary defences against respiratory infections, and this would contribute to the triggering of asthma exacerbations. In an observational study, 25(OH)D insufficiency was shown to be associated with an increased risk of asthma exacerbation in children [13]. The role of vitamin D as a predictor of atopy and asthma associated-phenotypes has also been investigated in children [14].

An evaluation of the relationship between asthma and vitamin D deficiency must take into consideration aspects such as the association with increased airway responsiveness, higher eosinophil count and serum IgE levels, and the potential role of 25(OH)D in airway remodelling. The available data strongly suggests an independent association between vitamin D deficiency and asthma [15–18]. In view of the widespread 25(OH)D insufficiency/ deficiency [14, 19], we aimed to elucidate the possible association of serum 25-hydroxyvitamin D level with traditional and novel markers of atopy and eosinophilic inflammation in children with newly diagnosed asthma.

Methods

Study participants

The study included 165 children aged 2–12 years referred to the Outpatient Clinic at the Department of Paediatrics, Pneumonology and Allergology of the Children's Hospital (Bydgoszcz, Warsaw, Poland) due to asthma-like symptoms and who agreed to participate in the study. Written informed parental consent for each participant was obtained, and the study protocol was approved by the Bioethics Committee at the Collegium Medicum of the Nicolaus Copernicus University. The diagnosis or exclusion of asthma was performed by an experienced paediatric pulmonologist on the basis of the patient's clinical symptoms and medical history according to the Global Initiative for Asthma (GINA) [20]; in children < 5 years, the diagnosis was based on family history and clinical symptoms; additional tests such as skin tests and/or total IgE/specific IgE measurement and AP Index were performed. In children > 5 years, the diagnosis was based on the medical history, clinical symptoms and spirometry, if necessary. None of the children included in the study received any additional vitamin D supplementation beyond their regular dietary intake.

Allergic asthma was diagnosed in 106 children, and non-allergic asthma in ten; in 49 children, asthma was excluded. The latter group was considered the reference group. In all subjects, anthropometric measurements were performed and body mass index (BMI) was calculated. Overweight or obesity were considered on the basis of BMI-for-age percentiles for girls or boys [21]. Children with BMI values between 85^{th}–95^{th} percentiles were considered overweight, while these with BMI > 95^{th} percentile were considered obese.

Sample collection and laboratory measurements

In all subjects, fasting blood was collected in the early morning (7.00–9.00 am). Blood cell count was performed and serum was obtained within less than one hour and stored deep-frozen (–80°C) in small aliquots until assayed within eight months. Serum was assayed for total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), and high-sensitivity C-reactive protein (hs CRP), (PENTRA 400, Horiba ABX, Montpellier, France). Serum 25(OH)D total (EIA, ids-isys, IDS Ltd, Boldon, UK), total IgE and specific IgE (ImmunoCap-100, Phadia AB, Uppsala, Sweden) were also determined.

25-hydroxy Vitamin D EIA test kit is aligned to the ID-LC-/MS/MS Reference Measurement Procedure. The detection limit for 25(OH)D total was 2.5 ng/mL(5 nmol/L). Antibodies used in this assay cross-reacted with $25(OH)D_3$ in 100%, with $25(OH)D_2$ in 75%, with $24,25(OH)_2D_3$ in > 100%. Blood cell count was performed with the use of a haematology analyser Sysmex XE-2100 (Sysmex Corporation, Kobe, Kansai, Japan).

We accepted the following cut-off values for normal lipids and hsCRP: TC ≤ 170 mg/dL (4.4 mmol/L), HDL-C ≥ 45 mg/dL (1.16 mmo/L), LDL-C ≤ 110 mg/dL (2.85 mmol/L), TG ≤ 75 mg/dL up to 9 years of age

(0.85 mm/L), ≤ 90 mg/dL from 10 years of age (1.02 mmol/L), hsCRP ≤ 1 mg/L [22]. Serum 25(OH)D total concentration ≥ 30 ng/mL (≥ 75 nmol/L) was accepted as sufficient, 21–29 ng/mL as insufficient, and ≤ 20 ng/mL (≤ 50 nmol/L) as deficient according to the recommendations for the Polish population [19]. The cut-offs for total IgE (kU/L) were accepted in reference to age according to the manufacturer's recommendations. The following cut-offs for blood eosinophil values were used: less than 300 cells/μL to define normal values, 300–500 cells/μL for intermediate, and > 500 cells/μL for high values (eosinophilia). The normal range for neutrophil count was accepted as 1.5–8.0 × 10^3/μL.

Statistical analysis

We used descriptive statistics to obtain the characteristics of the study and reference groups. Continuous variables were expressed as mean ± standard deviation (Gaussian distribution) and median with 25th and 75th percentiles (non-Gaussian distribution). Data was compared using the Student-t test for normally distributed variables and the Mann-Whitney-U-test for non-normally distributed variables. Natural-log transformed total IgE was used for comparisons. The Spearman correlation was used to analyse the associations between variables. A p value < 0.05 was considered statistically significant. The statistical analysis was performed using Statistica 10.0 for Windows (StatSoft, Tulsa, OK, USA) and MedCalc 12.7.0 (MedCalc Software, Ostend, Belgium).

Results

The demographic and biochemical characteristics of the study participants are presented in Table 1. Children with asthma had significantly lower 25(OH)D. Both groups had similar median age and BMI, yet the proportion of overweight and obesity was significantly higher in the asthma group. The same median lipid values were observed in both groups, although the percentage of hypertriglyceridemia was higher in the reference group. Median CRP was only slightly higher in children with asthma, but we observed a significantly higher proportion of children with elevated CRP. Substantially elevated total IgE (log10 IgE) concentration and eosinophil count were other characteristic features in children with asthma. Neutrophil counts were not significantly higher in asthmatics.

Mean concentration of 25(OH)D in children with asthma was 27.9 ± 8.4 ng/mL, and was within the range of insufficiency (21–29 ng/mL). 60.7% of children with asthma were found to be insufficient or deficient, with the level below the accepted sufficient cut-off of 30 ng/mL, 19.1% were deficient with serum 25(OH)D levels ≤ 20 ng/mL, and 6.4% were severely deficient (≤ 15 ng/mL). In the reference group, only 2.1% were severely deficient. The proportion of 25(OH)D insufficient and deficient subjects was considerably higher in asthmatics than in the reference group (Tab. 1).

The number of children with non-atopic asthma was very small (ten cases), which made it reasonable to perform further analysis only in the group diagnosed with atopic asthma. Very similar results were observed in subjects with atopic asthma (n = 106) compared to the reference group. The average 25(OH)D concentration was lower (28 ± 8.3 vs 33.8 ± 12.7; p = 0.002) but the eosinophil count (290/μL vs 140/μL; p < 0.001) and the total IgE (log IgE 2.10 ± 0.80 vs 1.18 ± 0.56; p < 0.000001) were considerably higher in subjects with atopic asthma. The proportion of children with atopic asthma, 25(OH)D insufficient and deficient, was 63.5% compared to 40.4% in the reference group.

Table 2 presents a comparison of selected biochemical and anthropometric indices in relation to 25(OH)D concentration in children with atopic asthma. Interestingly, we found that eosinophil counts showed a strong tendency to higher values in 25(OH)D-deficient children with atopic asthma (p < 0.080). Also neutrophil counts were slightly higher in 25(OH)D-deficient atopic asthma children. A weak but significant inverse correlation of 25(OH)D with neutrophil count was observed (R = –0.288; p = 0.011). Moreover, in asthmatic children, a tendency to higher CRP at lower vitamin D concentrations was found (R = –0.216; p = 0.067). It is worth noting that 25(OH)D did not correlate with serum IgE .

25(OH)D concentration may depend on the season, but we found that the average 25(OH)D in samples collected between May and September and between October and April did not differ in these small children (in children with asthma 28.3 vs 27.9 ng/mL; p = 0.85 in summer vs winter, or in the reference group 35.3 vs 32.5 ng/mL; p = 0.46 in summer vs winter).

Discussion

Several recent studies on 25(OH)D level in children with asthma have revealed that more than half of them were vitamin D insufficient or deficient [16, 17, 23, 24]. In our study, the majority of children with newly diagnosed asthma had significantly lower concentration of serum 25(OH)D compared to the reference group. Even at this early age (2–12 years), 25(OH)D concentrations were below the recommended value of 30 ng/mL in more than three out of five (63.5%) children with atopic asthma. In subjects without an asthma diagnosis, the prevalence of insufficiency or deficiency was much lower (40.4%). The proportion of severely deficient (≤ 15 ng/mL) was three-fold higher in asthmatics than in the reference group (6.4 vs 2.1%). This is not surprising, as according

Table 1. Biochemical and clinical characteristics of study participants

	Asthma (n = 116)	Reference group (n = 49)	P value
Gender M /F (%)	53.4/ 46.6	57.1/42.9	0.600
Age (years)	5.0 (4.0–7.0)	5.0 (4.0–7.0)	0.174
BMI [kg/m^2]	15.9 (15.0–17.0)	15.8 (14.8–17.1)	0.664
25(OH)D [ng/mL]	27.9 ± 8.4	33.8 ± 12.7	0.001
Log total IgE kU/L	2.05 ± 0.73	1.18 ± 0.56	0.000001
Eosinophil count [cells/μL]	290 (160–440)	140 (90–290)	0.001
Neutrophil count [10^3/μL]	3.77 (2.75–4.56)	3.13 (2.31–4.42)	0.127
hsCRP [mg/L]	0.32 (0.10–0.70)	0.18 (0.07–0.65)	0.220
Overweight/obesity (%)	9.0/11.2	4.1/2.0	0.05/0.04
25(OH)D ≤ 20 ng/mL (%)	19.1	12.8	0.090
25(OH)D < 30 ng/mL (%)	60.7	40.4	0.050
TC > 170 mg/dL (%)	46.1	51.0	0.390
LDL-C > 110 mg/dL (%)	33.7	36.7	0.210
HDL-C < 45 mg/dL (%)	21.3	18.4	0.866
Hypertriglyceridemia (%)	28.1	42.9	0.060
hsCRP > 3 mg/L (%)	10.1	6.1	0.050
Eosinophil count > 300 cells/μL (%)	47.7	21.7	0.030
Eosinophil count > 500 cells/μL (%)	20.9	4.3	0.020

BMI — body mass index; 25(OH)D — 25-hydroxyvitamin D; IgE — immunoglobulin E; hsCRP — high-sensitivity C-reactive protein; TC — total cholesterol; LDL-C — low-density lipoprotein cholesterol; HDL-C — high-density lipoprotein cholesterol

Table 2. Comparison of selected biochemical and anthropometric indices in relation to 25(OH)D concentration in children with atopic asthma

	25(OH)D deficiency (10–20 ng/mL)	25(OH)D > 20 ng/mL	P value
Age (years)	6.5 (5.0–9.0)	5.0 (4.0–7.0)	0.095
BMI [kg/m^2]	15.5 (14.5–15.8)	16.1 (15.0–17.1)	0.043
25(OH)D [ng/mL]	17.3 ± 2.4	30.3 ± 7.3	0.001
hsCRP [mg/L]	0.43 (0.24–2.32)	0.27 (0.10–0.70)	0.190
Eosinophil count [cells/μL]	320 (120–540)	280 (160–430)	0.080
Neutrophil count [× 10^3/μL]	4.58 (3.37–5.62)	3.64 (2.59–4.29)	0.087
Log total IgE [kU/L]	2.37 ± 0.46	2.05 ± 0.77	0.172

25(OH)D — 25-hydroxyvitamin D; BMI — body mass index; hsCRP — high-sensitivity C-reactive protein; IgE — immunoglobulin

to recent epidemiological data on vitamin D deficiency (≤ 20 ng/mL) in the Polish population, 35% of children aged 2–4 years and 80% of those aged 5–11 years are vitamin D deficient [19].

The high proportion of 25(OH)D insufficiency or deficiency may have implications pertaining to changes in lung function, because in early childhood the respiratory system is particularly sensitive to environmental and nutritional influences [24]. Data from epidemiological studies has shown that low serum 25-hydroxyvitamin D concentration is associated with a higher risk of upper and lower respiratory infections in children and may contribute to asthmatic patients' symptoms and morbidity rates [14, 16, 25]. 25(OH)D insufficiency may be considered as an important aspect in the onset of childhood atopy and food allergies. A report from Australia showed that low 25-hydroxyvitamin D concentration in boys at age 6 predicted atopy or asthma at age 14 [14].

The relationship between vitamin D insufficiency/deficiency and asthma may be affected by factors such as ethnicity and socioeconomic status. In Costa Rican children, 25(OH)D deficiency was related to increased airway responsiveness and higher biomarkers of asthma and atopy, such as eosinophil count and IgE levels [11]. On the other hand, a very recent study performed on

a population of Asian children and adolescents aged 5–18 years provided epidemiological evidence against the association of serum 25(OH)D status with concurrent asthma, atopy and serum total IgE [26]. The authors noted, however, that the role of serum 25(OH)D as a predictor of subsequent asthma remains to be defined. Nevertheless, it seems that it is not yet clear whether vitamin D level is critical in asthma onset, and more studies in this field are needed.

The important role of vitamin D in asthma pathogenesis, based on modulation of T cell driven immune responses, has been extensively studied. The inhibitory effect of 1.25 (OH)2D on Th1 cells' cytokine production seems to be evident but the effect on Th2 response remains to be explained further [16]. Asthma is a heterogeneous disease with multiple subtypes. One of them is characterised by higher blood eosinophil count, higher serum IgE and biomarkers of airway remodelling, and shows better responsiveness to treatment, particularly with corticosteroids [6–9, 27].

We demonstrated that children diagnosed with atopic asthma with 25(OH)D deficiency presented a strong tendency to higher eosinophil counts. Like others, we used blood eosinophil count to quantify eosinophilic inflammation as this method is simple, non-invasive and economical, even though it does not anatomically localise inflammation. In our study, almost half of the children with asthma presented with elevated blood eosinophil count (> 300 cells/μL), and 21% presented with eosinophilia. The association of 25(OH)D insufficiency/deficiency in asthmatic patients with high eosinophils and total IgE observed in our study may suggest an effect of inflammation on circulating 25(OH)D.

Whether vitamin D could have a role in the prevention and treatment of asthma, allergic diseases and respiratory infections in childhood, and whether it represents a novel preventive and/or therapeutic strategy still remains to be elucidated. For this purpose, well designed clinical trials on vitamin D supplementation with the right dose are necessary.

Our study was an observational study and included a limited number of participants. However, our group of asthmatic subjects was well defined, and our study and reference groups were age- and gender-matched. Our conclusion is valid for children with allergic asthma.

We conclude that in children with asthma, 25(OH)D insufficiency/deficiency is associated with higher eosinophil counts and IgE. 25(OH)D monitoring is important in the prevention and management of children with asthma.

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