

Report

Association of antioxidants and vitamin D level with inflammation in children with atopic dermatitis

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Abstract

Background Changing the resources of vitamin D and antioxidant nutrients may affect the course of allergic diseases. The aim of the study was to investigate the association between CoQ10, vitamin D, retinol, and α -tocopherol serum levels and severity of atopic dermatitis (AD) in children.

Methods Twenty-nine children with AD aged from 1 to 15 years were enrolled into the study. The severity of AD was categorized into mild or moderate (≤ 50 points in SCORAD – Scoring Atopic Dermatitis index) and severe (> 50 SCORAD points). The control group was comprised of 22 children with negative history of allergy aged from 2 to 15. The serum measurements included vitamin D, retinol, α -tocopherol, CoQ10, C-reactive protein (CRP), complete blood count (CBC), and total immunoglobulin E (IgE).

Results Low vitamin D concentration (< 20 ng/ml) was observed mainly in patients with severe AD (77.8%), compared to children with mild or moderate AD (25%) or the control group (31.8%). Concentration of retinol was decreased significantly in patients with severe AD (median $1.32 \mu\text{mol/l}$), compared to children with mild and moderate AD (median $1.66 \mu\text{mol/l}$), but not to the control. Among inflammatory markers, only the group with severe AD demonstrated significantly elevated platelet count (PLT), red blood cell distribution width (RDW), and eosinophil count (EO). Retinol level correlated with PLT ($R = -0.7$; $P = 0.003$), white blood count (WBC) ($R = -0.54$; $P = 0.01$), total IgE ($R = -0.51$; $P = 0.016$), mean platelet volume (MPV) ($R = 0.51$; $P = 0.02$), and also with a disease severity index, SCORAD ($R = -0.55$; $P = 0.007$), whereas vitamin D level correlated only with MPV ($R = 0.61$; $P = 0.003$). No significant changes were found in tocopherol and CoQ10 levels between groups.

Conclusions Children with AD should be routinely tested for vitamin D deficiency, especially during disease exacerbation. Our results confirmed correlation of serum inflammatory markers with decreased concentration of vitamin A in children with AD. This finding, however, might be an effect of severe stage of disease and not only of inadequate intake of retinol in the diet.

Introduction

Atopic dermatitis (AD) is a chronic inflammatory skin disease. The pathogenesis of AD is not completely known, but it involves the genetic predispositions, altered immunity, and disrupted skin barrier function. The environmental factors, like diet low in vitamins or antioxidants, may be also responsible for skin inflammation, since the oxidative stress is an important factor of pathogenesis of AD.^{1,2} On the other hand, some of these nutrients are lost due to severe inflammatory process independently of body's vitamin stores.³ Vitamin A, vitamin E, and CoQ10 are

known to be important antioxidants, engaged in immune defense systems and epithelial proliferation.^{4,5} Those nutrients have been suggested to protect against atopy and allergic diseases, however the results of the studies with adult patients turned out to be inconsistent.^{5,6} Vitamin D, apart from other properties, is involved in immunoregulatory processes, but conflicting data regarding its levels in patients with AD have been reported.^{7–11}

This prospective study was conducted to investigate the association between CoQ10, vitamin D, retinol, and tocopherol serum levels and severity of atopic dermatitis in children.

Materials and methods

Patient selection

The study included children with atopic dermatitis and children with negative history of allergy diagnosed and treated in the Department of Paediatrics, Gastroenterology and Allergology. The severity of AD was evaluated using the objective components of Scoring Atopic Dermatitis (SCORAD) index ranging from 0 to 83 points (European Task Force on Atopic Dermatitis, 1993). The severity of AD was subsequently categorized into mild or moderate (≤ 50 SCORAD points) and severe (> 50 SCORAD points). The study protocol was approved by the Bioethics Committee of Medical University in Białystok (R-I-002/365/2012). The parents of all children provided written consent before being included in the study.

Measurement of CRP, CBC, vitamin D, total immunoglobulin E

Serum CRP (mg/l) concentration was determined by immunoturbidimetry (Roche, Hitachinaka, Japan). The vitamin D level was assessed by measuring the serum level of 25OHD (D3 [25-OH] and D2 [25-OH]) by electro-chemiluminescence immunoassay (Roche, Hitachinaka, Japan) in all patients. The complete blood count was measured using a Hematology Analyzer (Sysmex, Kobe, Japan). The total IgE concentration was determined using fluoro-enzyme immunoassay – FEIA (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Measurement of coenzyme Q10, tocopherol, and retinol

Blood samples for analyses of antioxidants were collected from 22 patients with AD and 17 control patients. The serum was stored at -80°C before further analysis. Retinol, α -tocopherol, and CoQ10 were extracted from serum samples with hexane and

analyzed by high performance liquid chromatography method with UV detection at 292 nm for α -tocopherol, 324 nm for retinol, and 272 nm for CoQ10 (chromatographic system Thermo Separation, Waltham, USA). The following chromatographic conditions were used: chromatographic column LiChrospher100 RP-18 125 mm $\times$ 4 mm (5 μm with a guard column 4 mm $\times$ 4 mm (5_μ) (Merck, Germany), a mobile phase of methanol – n-hexane 72:28 (v/v). The flow rate was set at 1 ml/min.¹²

Statistical analysis

Data were analyzed using Statistica 10 (StatSoft Inc., Tulsa, OK, USA). Since coenzyme Q10, α -tocopherol, retinol, and vitamin D were not normally distributed, the significance of the difference was evaluated by the Mann-Whitney *U*-test, and a value of $P < 0.05$ was considered as statistically significant.

Results

Clinical characteristics of the study groups

The investigation involved 29 children with AD aged 1–15 years (mean age 6 years). The control group was comprised of 22 children without symptoms of allergy and admitted to the hospital for GERD diagnosis or accidental consumption of a foreign body, aged 2–15 years (mean age 5.5 years). The background data of study groups are presented in Table 1. The mean age was similar in both groups, however more girls were affected with AD (58.62% vs. 36.4%). The mean SCORAD score was 35.2. Nine patients (31.03%) were assessed as severe AD (SCORAD > 50) and 20 (68.97%) as mild or moderate AD (SCORAD ≤ 50). Among patients with AD, seven (24.1%) reported the coexistence of asthma and three (10.3%) the coexistence of allergic rhinitis. Cow's milk elimination diet was reported in 14 (48.3%) children with AD compared to five

Table 1 Background data of patients with AD and control group

	AD		AD (total)	Control group
	SCORAD > 50	SCORAD ≤ 50		
Number (%)	9 (31.03%)	20 (68.97%)	29	22
Girls (%)	5 (55.6%)	12 (60%)	17 (58.6%)	8 (36.36%)
Boys (%)	4 (44.4%)	8 (40%)	12 (41.4%)	14 (63.64%)
Age median years (min–max)	9 (3–13)	5.5 (1–15)	6 (1–15)	5.5 (2–15)
SCORAD median (min–max)	64 (52.5–81.4)	21.85 (8–49.5)	28 (8–81.4)	0
General diet	5 (55.6%)	11 (55%)	16 (55.2)	17 (77.3%)
Cow's milk elimination diet	3 (33.3%)	9 (45%)	12 (41.4%)	5 (22.7%)
Gluten-free diet	1 (11.1%)	0	1 (3.4%)	0
Co-existing allergy				
Asthma	3 (33.3%)	4 (20%)	7 (24.1%)	0
Allergic rhinitis	1 (11.1%)	2 (10%)	3 (10.3%)	0
Vitamins' measurement time				
Mar–Oct	9 (100%)	15 (75%)	24 (82.8%)	18 (81.2%)
Nov–Feb	0	5 (25%)	5 (17.2%)	4 (18.8%)

SCORAD, Scoring Atopic Dermatitis index; AD, atopic dermatitis.

Table 2 Biomarkers' profiles in children with AD and control group

Biomarker, median (min–max)	AD			AD (total)	Control group	P value
	SCORAD > 50	SCORAD ≤ 50	P value			
Coenzyme Q10 $\mu\text{g/ml}$	0.156 (0.04–0.25)	0.16 (0.06–0.44)	NS	0.16 (0.04–0.25)	0.2 (0.06–0.25)	NS
Tocopherol $\mu\text{mol/l}$	9.85 (5.75–13.14)	7.5 (4.76–11.84)	NS	8.71 (4.76–13.14)	11.52 (2.58–17.13)	NS
Retinol $\mu\text{mol/l}$	1.32 (0.65–1.9)	1.66 (1.17–2.12)	<0.05	1.62 (0.65–2.12)	1.5 (1.08–1.9)	NS
<50 centile <i>n</i> (%)	5/8 (62.5%)	1/14 (7.14%)	<0.05	6/22 (27.3%)	6/17 (35.3%)	NS
Vitamin D3 ng/ml	15 (4–28)	26 (10–44)	<0.05	24 (4–44)	24 (8–49)	NS
<20 ng/ml <i>n</i> (%)	7/9 (77.8%)	5/20 (25%)	<0.05	12/29 (41.4%)	7/22 (31.8%)	NS
CRP mg/dl	0.05 (0.01–0.3)	0.035 (0.01–0.92)	NS	0.04 (0.01–0.92)	0.04 (0.01–0.51)	NS
WBC $\times 10^3/\mu\text{l}$	11.14 (5.9–17.45)	7.52 (4.37–20.9)	NS	7.94 (4.37–20.9)	6.98 (4.3–16.4)	NS
PLT $\times 10^3/\mu\text{l}$	459 (289–659)	320 (182–490)	<0.05	342 (182–659)	306 (220–434)	NS
>350 $\times 10^3/\mu\text{l}$ <i>n</i> (%)	6/9 (66.7%)	7/20 (35%)	NS	13/29 (44.8%)	4/22 (18.2%)	NS
RDW <i>fl</i>	39 (36.8–42.3)	37.5 (34.2–41.6)	<0.05	37.8 (34.2–42.3)	37.6 (33.8–40.9)	NS
MPV <i>fl</i>	9.7 (8.4–10.9)	10.5 (8.5–11.7)	NS	9.9 (8.4–11.7)	10.1 (9.3–11.2)	NS
EO $\times 10^3/\mu\text{l}$	1.2 (0.4–4.6)	0.3 (0.1–1.4)	<0.05	0.55 (0.1–4.6)	0.2 (0.1–0.6)	<0.05
>0.4 $\times 10^3/\mu\text{l}$ <i>n</i> (%)	8/9 (88.9%)	8/19 (42.1%)	0.053	16/28 (57.1%)	1/21 (4.8%)	<0.05
Total IgE IU/ml	5001 (435–5001)	113 (2–5001)	<0.05	442 (2–5001)	26 (3–378)	<0.05
>100 IU/ml <i>n</i> (%)	9/9 (100%)	10/19 (52.6%)	<0.05	19/28 (67.8%)	5/22 (22.7%)	<0.05

AD, atopic dermatitis; CRP, C-reactive protein; WBC, white blood count; PLT, platelet count; RDW, red blood cell distribution width; MPV, mean platelet volume; EO, eosinophil count; IgE, immunoglobulin E; NS, not significant.

(22.7%) from the control group (lactose intolerance was the indication for cow's milk elimination diet). Both in the AD group (82.8%) and control group (81.2%), the measurement of vitamins was performed in the season of the year with a stable number of sunny hours (mean 9 hours per day between March and October in Poland based on the www.weatheronline.pl).

Comparison vitamin D, CoQ10, retinol, and tocopherol levels between study groups

The median values of vitamin D, CoQ10, retinol, tocopherol, and CRP concentration and parameters of CBC are presented in Table 2. There were no differences in median concentration of vitamin D3, CoQ10, tocopherol, and retinol among children with AD and control group. Only patients with severe AD demonstrated the lowest median value of vitamin D3 and retinol comparing to children with moderate or mild AD (respectively 15 ng/ml vs. 26 ng/ml and 1.32 $\mu\text{g/ml}$ vs. 1.66 $\mu\text{g/ml}$; $P < 0.05$) (Table 2). Low concentration of vitamin D3 (<20 ng/ml) was observed in 77.8% patients with severe AD, compared to 25% children with mild and moderate AD or to 31.8% of the control group (Table 2, Fig. 1). Among patients with low vitamin D3, the cow's milk elimination diet was used by three (42.8%) children with severe AD, one (20%) patient with moderate AD, and two (28.6%) subjects from the control group. However, analysis of all study groups demonstrated the same percentage of children on cow's elimination diet and with both low (<20 ng/ml) and normal (>20 ng/ml) vitamin D3 level (26.3% vs. 31.3%, respectively). The measurements of vitamin D3, in the majority of the studied children, were performed between March and October (Table 1), and the median vitamin D3 level was not

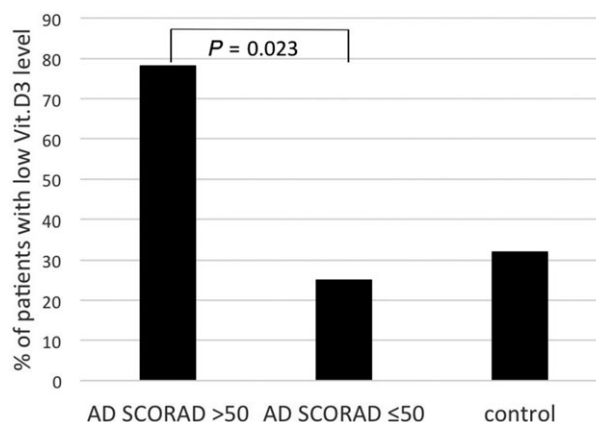


Figure 1 Percentage of patients with low vitamin D3 (<20 ng/l) in study groups. SCORAD, Scoring Atopic Dermatitis index

dependent on the year's season (respectively: winter – 24 ng/ml ; spring – 24.5 ng/ml ; summer – 29 ng/ml , autumn – 24 ng/ml). Low vitamin A concentration (<50 centile for age and sex according to NHANES III references values¹³) was found not only in 62.5% of patients with severe AD but also in 35.3% of the control group (Table 2); however, only one patient with severe AD had extremely low vitamin A (0.65 $\mu\text{mol/l}$). No significant differences in CoQ10 and tocopherol concentration were found among those groups.

Comparison of inflammatory markers between study groups

In the majority of patients with severe AD (SCORAD > 50), significant increase of platelet count, eosinophil count, and total

IgE were found compared to other groups (Table 2). Extraordinarily high total IgE (>5000 IU/ml) was detected in 75% of subjects with severe AD and only in 5.3% of children with $\text{SCORAD} \leq 50$. The median value of RDW was higher in patients with severe AD however never exceeded upper normal limit (47 fl). There were no differences in values of other tested inflammatory markers (CRP, WBC, MPV) between the groups (Table 2).

Correlation analysis between vitamins concentrations and levels of selected inflammatory markers

Analyzing the data of the AD group, we found the significant negative correlations of the retinol with some inflammatory markers like PLT ($R = -0.7$; $P = 0.003$), WBC ($R = -0.54$; $P = 0.01$), total IgE ($R = -0.51$; $P = 0.016$), and positive correlation with MPV ($R = 0.51$; $P = 0.02$) (Fig. 2). Interestingly, the retinol level was also associated with a disease severity index, SCORAD ($R = -0.55$; $P = 0.007$). Considering the vitamin D value, we noted its correlation only with MPV ($R = 0.61$; $P = 0.003$). Although tocopherol concentration did not significantly differ among groups, the positive correlation with PLT ($R = 0.59$; $P < 0.006$) was observed. No associations between CoQ10 and inflammatory markers were found.

Discussion

The resources of vitamins in the human body depend on their intake in the diet and state of health. During infection decreased synthesis of retinol binding protein and enhanced urinary loss of retinol determine transient low vitamin A level.^{3,14} It is also possible that inflammation leads to sequestration of plasma retinol. These findings might support our observation of decreased level of serum retinol in children with severe AD as an effect of excessive skin inflammation. The concentration of retinol also correlated with some inflammatory markers like PLT, WBC, MPV, and clinical activity of the disease measured by SCORAD. The negative association of serum retinol with severity of AD in children has already been reported, in contrast to adult patients with AD, in whom reduced concentration of retinol was detected only in skin biopsies.¹⁵ Moreover, the expression of retinoic acid synthesizing enzyme in the skin was also decreased, suggesting disturbed retinoid-mediated signaling in the skin. Also, other authors reported decreased retinol concentration in serum and skin of children with AD compared to the control group.¹⁶ Unfortunately, none of these studies referred to disease activity score.^{15,17} In this analysis, we did not test the vitamin A intake, which could be inadequate due to restricted diet used by

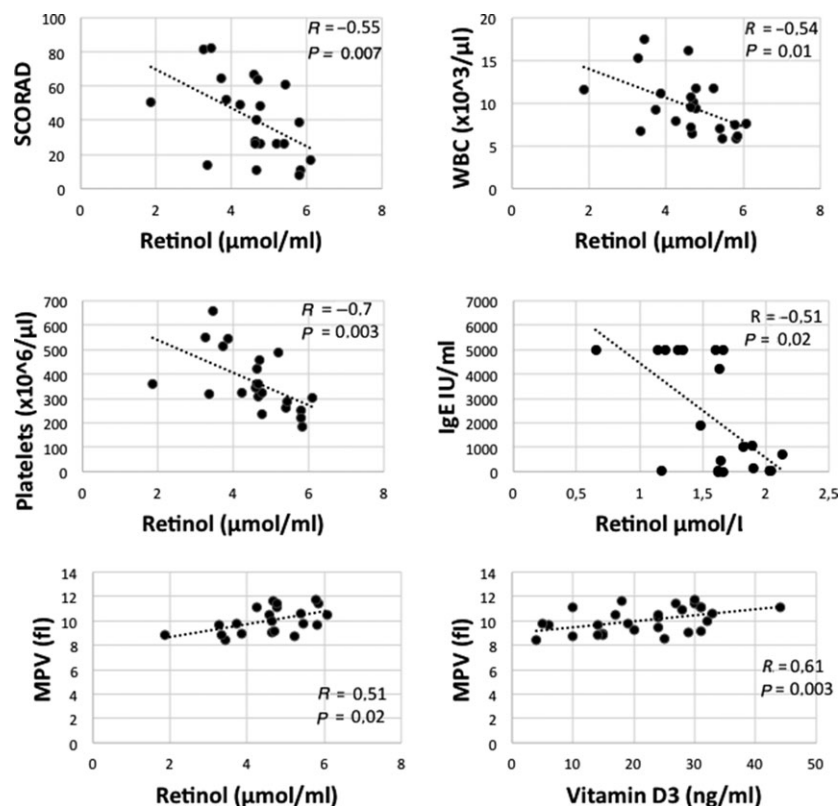


Figure 2 Correlations between retinol and vitamin D3 concentration and levels of inflammatory markers in children with AD (Spearman's rank correlation coefficient). WBC, white blood count; MPV, mean platelet volume; IgE, immunoglobulin E; SCORAD, Scoring Atopic Dermatitis index

children with severe AD, however controversial results were reported from interventions involving retinol.^{18,19} Some studies on vitamin A supplementation showed its association with increased risk of atopy.^{19,20} However, in *in vitro* study, the inhibitory effect of retinoic acid on the production of IgE was documented.²¹ This phenomenon could be associated with high total IgE and low retinol observed in our patients with severe AD. Although this explanation is too simplified and inadequate, intake of vitamin A in the children's diet is more likely. Our similar observation applied to vitamin D, in which decreased level was detected mainly in children with severe AD, confirming previous reports.^{7,10,11} Moreover, in other studies, the correlations of low vitamin D with SCORAD, allergic sensitization, and high IgE were noted, but we did not find such associations. The recent publications also denied the thesis, that serum vitamin D concentrations were significantly related to AD severity.^{8,22} The role of vitamin D in immunoregulation, involving pro-inflammatory cytokine synthesis, has been well described.²³ Among inflammatory markers tested in our patients, only MPV correlated with vitamin D, although its level below the norm was not detected in any patient. Decreased MPV reflecting inflammation has already been reported in rheumatic diseases, in contrary to cardiovascular disorders, in which high MPV has been described with low vitamin D association.^{24,25} Since serum vitamin D level is associated with dietary supplementation and sunlight exposure, it is still unclear whether low vitamin D concentration in children with severe AD, observed in our study, is an effect of vitamin deficit in their restricted diet or impaired vitamin synthesis in the disrupted skin due to chronic inflammation. On the other hand, the keratinocytes are not only a source of vitamin D but also a responder to its active form through vitamin D receptor (VDR). Moreover, it has already been published that the polymorphisms of VDR increased the risk of AD in some populations.^{26,27} Mice lacking the VDR or the enzyme (CYP27B1) producing its ligand 1,25(OH)₂ D show defective epidermal differentiation.²⁸ Another gene associated with AD is responsible for synthesis of filaggrin, one of the structural components of the skin, which expression is regulated by vitamin D.²⁹ It is worth pointing out that more than 30% of healthy children from control group presented with laboratory vitamin D deficiency, despite the recommendation to supplement this vitamin in pediatric population. Moreover, the measurements of vitamin D3 in the majority of the studied children were performed between March and October, so there was no seasonal variation on vitamin D levels. Some reports have demonstrated clinical improvements in AD after vitamin D supplementation, especially when combined with a well-known antioxidant – vitamin E.^{30,31} The published data concerning the relation of vitamin E with inflammatory process of allergic diseases are inconsistent.^{6,32,33} Kompauer *et al.* found that allergic sensitization was negatively associated with plasma γ -tocopherol concentration, but only in patients over 50 years old, not in children.⁶ Other studies revealed significant negative association between α -

tocopherol and atopic eczema in children.^{17,32} Our results stay in line with the PASTURE study reporting no association between vitamin E concentration and AD.³³ Also, in our study we have not noted any difference in CoQ10 measurements between groups, although its decreased level in allergic diseases has already been shown.^{34,35} Moreover it has been suggested that children with food intolerance and allergies might acquire CoQ10 deficiency with disease progression.³⁵

The limitation of the present study was the small number of participants. Therefore, the results may not be relevant to the general pediatric population. Also, we did not evaluate the intake of antioxidant nutrients.

In conclusion, children with AD should be routinely tested for vitamin D deficiency, especially during disease exacerbation. However, the results of this study have revealed vitamin D deficiency in more than one-third of examined children (including control group) not only with AD. These findings point to the need to improve compliance with the recommendations of vitamin D supplementation in children, especially those with chronic inflammation. Our results confirmed correlation of serum inflammatory markers with decreased concentration of vitamin A in children with AD. This finding, however, might be an effect of severe stage of disease and not only due to inadequate intake of retinol in the diet. Currently there is no recommendation to supplement vitamin A in AD, probably due to the lack of routine testing of serum vitamin A and inconsistent reports on the effect of this vitamin supplementation on the course of the disease.

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