Cord serum 25-hydroxyvitamin D correlates with early childhood viral-induced wheezing

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KEYWORDS
Vitamin D; Cord blood; Wheezing; Food allergy; Atopic dermatitis; Mother-child cohort

Summary
Background: There are investigations concluding that reduced vitamin D status in pregnancy, may be a risk factor for the development of allergic outcomes in offspring.
However, studies on the relationship between cord levels of 25-hydroxyvitamin D (25[OH]D) and risk of early childhood wheezing and early-onset atopic dermatitis/food allergy are very limited.

Objective: To assess the associations between cord blood concentration of 25[OH]D and occurrence of the incidence of wheezing, atopic dermatitis, food allergy, during the first two years of life.

Methods: We evaluated 240 children by the age of 2 years from the Polish Mother and Child Cohort Study. Women were interviewed during pregnancy to collect demographic and socio-economic data, the medical and reproductive history. At delivery, umbilical cord blood plasma was sampled. The child’s health status were examined at approximately 2 years. In the analyses multivariable model was used.

Results: Data from 190 participants were included into the analysis. The median value and quartile range of 25[OH]D in cord blood [ng/ml] were as follows: 6.33, 4.16 e 8.53.

25[OH]D in cord blood below lower quartile increases the risk of multi-triggered wheezing (MTW) in children during first 2 years of life (OR: 2.81; 95% CI: 1.13–7.00). Higher cord serum 25[OH]D levels in cord blood were associated with lower risks of atopic dermatitis, food allergy, and early childhood wheezing.

Abbreviations: 25[OH]D, 25-hydroxyvitamin D; GPx3, glutathione peroxidase; ISAAC, International Study of Asthma and Allergies in Childhood; LINA study, lifestyle and environmental factors and their Influence on Newborns Allergy Risk Study; MTW, multi-triggered wheezing; REPRO_PL cohort, The Polish Mother and Child Cohort Study; RSV, respiratory syncytial virus; RTIs, respiratory tract infections; VIW, viral induced wheezing.

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Introduction

As the main pathways of the vitamin D metabolism in the body have been well known from years, the recently reported and investigations emphasize the role of this vitamin as a new potential immunoregulator of biological functions with a particular role in immune tolerance and further, as a risk factor to the development of allergic diseases. Never the less prospective and randomized studies on the Vitamin D influence and allergic disorders are lacking. The body of epidemiological evidence in relation to nutrients and dietary factors for the prevention of asthma and allergic diseases is overall weak, never the less, suggestive in relation to vitamins A, D, vegetables, microelements and Mediterranean diet, particularly in relation to asthma [1,2]. There are also investigations concluding that reduced vitamin D status in pregnancy, respectively in umbilical cord plasma of the newborn, may be a risk factor for the development of atopic dermatitis in the first year of life [3].

It is known that innate immunity and especially regulatory T cells are presumed to play an essential role in the development of adaptive immunity, allergy or asthma in early life [4] but in the other hand recent researches have highlighted that these processes are strongly influenced by serum vitamin D level [5]. It has been also proved in LINA study an association between the number of cord blood regulatory T cells and the occurrence of sensitizations against food allergens later in life [6]. Until this date there are limited studies deliberating the parental vitamin D status and its impact on the newborn immune status and laterinfantile outcomes. Concerning the importance of the vitamin D level in newborns and subsequent immune changes, it appears that more reliable investigations are required. Over the last few years there were multiple studies investigating the effects of maternal vitamin D intake and cord blood 25(OH)D level in newborn on the inception of asthma and allergy diseases, never the less the results are contradictory.

Our aim was to assess the associations between cord blood 25(OH)D level and occurrence of the incidence of wheeze, atopic dermatitis, food allergy, frequency of infections during the first two years of life. In a previous report on this cohort [1], we found associations between zinc/cooper concentrations in cord blood and prevalence of wheezing during the 1st year of life and between lower activity of glutathione peroxidase (GPX3) in cord blood and atopic dermatitis in studied children; consequently these variables are included as a confounders in the current analysis.

Methods

Study design and population

The Polish Mother and Child Cohort Study (REPRO_PL cohort) was established in 2007; the cord blood plasma was analyzed in 240 participants. A complete description of the methodological assumptions has been published elsewhere [1,7,8]. Women were recruited at maternity units during the first trimester of pregnancy if they fulfilled the following inclusion criteria: a single pregnancy of up to 12 weeks of gestation, no assisted conception, no pregnancy complications, and no chronic diseases, as specified in the study protocol. Women were interviewed three times during pregnancy to collect demographic and socioeconomic data, the medical and reproductive history. Exposure to tobacco constituents was assessed based on questionnaire data (full description of assessment of children’s exposure to tobacco smoke was published elsewhere) [7,8]. At delivery, infant (umbilical cord blood) plasma were sampled. One year after the child’s birth, an invitation letter was sent to mothers participating in the REPRO_PL cohort, proposing to have the child’s exposure and health status examined. Within the next two weeks, a telephone call was made to schedule a mother-and-child visit at the medical centre for an examination by a pediatrician/allergologist.

The current analysis is restricted to 240 children by the age of 2 years, in whom cord blood plasma was previously analyzed. All parents or guardians of the patients gave their written consent before the study. The study was approved by the Ethical Committee of the Nofer Institute of Occupational Medicine, Łódź, Poland (Decision No. 7/2007 and 3/2008). The study was registered on: www.ClinicalTrials.gov, NCT01861548.

Child health assessment

Children’s health status was assessed at approximately 24 months of age (range, 23–30 months). For the appropriate recognition of children’s health status, a questionnaire was administered to the mothers and supplemented with information from the medical chart of each child. The testing
was performed by a pediatrician/allergologist in the presence of the mother or a relative.

The first part of the questionnaire covers certain sociodemographic information (i.e., family size, material status of the family, and parental educational level). The second part of the questionnaire investigates the child’s health and condition. The incidence of upper and lower respiratory tract infections (RTIs) and any symptoms of allergy to food and inhalant allergens are noted. Infections were defined as episodes of cold without wheezing. The data provided by the mothers is supplemented with the use of information from the medical chart of each child. The duration of each infection and disease, medications taken, and hospitalizations, if any, are identified. Special attention is paid to the identification of any signs and symptoms of allergy and asthma. This part of the questionnaire has been developed by an allergologist, based on recommendations from the International Study of Asthma and Allergies in Childhood (ISAAC) [9]. Patients were defined as having food allergy or atopic dermatitis if they had ever been diagnosed by a physician. In addition, the occurrence of allergy among family members was noted.

Viral-induced wheezing (VIW) in childhood was defined as wheeze ever appearing during infection.

Multi-triggered wheezing (MTW) was defined as wheezing triggered by two or more factors (e.g., viral infection, weather, activity).

**Cord serum 25(OH)D measurement**

Immediately after delivery, cord blood serum samples were collected by midwives. Blood samples were centrifuged within 24 h of collection. The serum was separated it was stored at −80°C.

25(OH)D level was determined in cord plasma using HPLC commercial kit produced by Chromosystems® (no 38038). This method applies proteins precipitation and solid phase extraction of interfering components in simple HPLC isocratic method. Chromatograms are detected by an UV detector. The analyses are quantified by the inclusion of a stable internal standard and results are calculated by the integration of the peak area using external standard method.

**Statistical analysis**

The associations between presence of: i) atopic dermatitis, ii) food allergy, >1 episode of respiratory tract infection, iii) at least 1 episode of multi-triggered wheezing (MTW), iv) at least 1 episode viral-induced wheezing (VIW) (defined as dependent variables) and 25(OH)D level (defined as independent variable) were assessed by logistic regression. Vitamin D level in cord blood was included as continues variable (log-transformed before analysis) or as nominal variable (defined in two different cut-offs < median and <lower lower quartile). The effect of vitamin D was corrected for the effects of other independent risk factors of dependent variable, defined previously in this cohort [1]. First, logistic regression was used to assess the relationship between dependent variables and each of the independent variables (presented in Table 1). A stepwise forward procedure was then used to select variables. Predictors with p-levels of at least 0.1 estimated in univariate models were included into multivariate regression analyses. All of the statistical analyses were performed using SPSS 11.5. The null hypothesis was rejected if \( p < 0.05 \).

**Results**

Data from 190 participants were included into the analysis. Description of the study cohort is given in Table 1. Among all studied children 39 (24.5%) had at least one curse of antibiotic; 6 (5.9%) were hospitalized. Median value and quartile range of 25(OH)D in cord blood [ng/ml] were as follows: 6.33, 4.16–14.9%. The effect of vitamin D was corrected for the effects of other independent risk factors of dependent variable, defined previously in this cohort [1].

**Table 1** Description of the study cohort.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical profile: ( n ) (%)</td>
<td></td>
</tr>
<tr>
<td>Atopic dermatitis</td>
<td>24 (12.6)</td>
</tr>
<tr>
<td>Food allergy</td>
<td>36 (22.4)</td>
</tr>
<tr>
<td>Multi-triggered wheezing</td>
<td>22 (11.7)</td>
</tr>
<tr>
<td>Viral-induced wheezing</td>
<td>13 (8.2)</td>
</tr>
<tr>
<td>Inhaled corticosteroid use</td>
<td>35 (22.8)</td>
</tr>
<tr>
<td>Participants with infection</td>
<td>62 (39)</td>
</tr>
<tr>
<td>Infection rate( e )</td>
<td>1.4</td>
</tr>
<tr>
<td>Family status:</td>
<td></td>
</tr>
<tr>
<td>Parental atopy, ( n ) (%)</td>
<td>71 (41.8)</td>
</tr>
<tr>
<td>Mother’s age [year] mean ± SD</td>
<td>29.5 ± 4.1</td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
</tr>
<tr>
<td>Primary, ( n ) (%)</td>
<td>9 (4.8)</td>
</tr>
<tr>
<td>Secondary, ( n ) (%)</td>
<td>52 (27.8)</td>
</tr>
<tr>
<td>University, ( n ) (%)</td>
<td>126 (67.4)</td>
</tr>
<tr>
<td>Father’s age [year] mean ± SD</td>
<td>32.1 ± 5.4</td>
</tr>
<tr>
<td>Paternal education</td>
<td></td>
</tr>
<tr>
<td>Primary, ( n ) (%)</td>
<td>32 (17.2)</td>
</tr>
<tr>
<td>Secondary, ( n ) (%)</td>
<td>68 (36.6)</td>
</tr>
<tr>
<td>University, ( n ) (%)</td>
<td>86 (46.2)</td>
</tr>
<tr>
<td>Worse financial situation, ( n ) (%)</td>
<td>22 (13.1)</td>
</tr>
<tr>
<td>Children’s environment tobacco smoke:</td>
<td></td>
</tr>
<tr>
<td>Maternal smoking, ( n ) (%)</td>
<td>28 (15)</td>
</tr>
<tr>
<td>Paternal smoking, ( n ) (%)</td>
<td>71 (37.8)</td>
</tr>
<tr>
<td>Parental smoking, ( n ) (%)</td>
<td>80 (43.0)</td>
</tr>
<tr>
<td>Cotinine in saliva during pregnancy [ng/ml], median (quartile range)</td>
<td>0.91 (0.57–1.52)</td>
</tr>
<tr>
<td>Children’s cotinine in urine [ng/ml], median (quartile range)</td>
<td>3.49 (1.78–8.89)</td>
</tr>
</tbody>
</table>

\( a \) Total number of infection/participant/12 months.

MTW during first two years of life was observed in 14.9% of children when 25(OH)D in cord blood was above vs below median value (Fig. 1B) and in 8.6% vs 20.8% of children when 25(OH)D in cord blood was above vs below lower quartile (Fig. 1C).
VIW during first two years of life was observed in 2.6% vs 13.8% of children when 25(OH)D in cord blood was above vs below median value (Fig. 1B) and in 4.3% vs 19.5% of children when 25(OH)D in cord blood was above vs below lower quartile (Fig. 1C).

![Figure 1](image)

**Discussion**

Our study shows that vitamin D status is inversely related to wheezing as a symptom of viral infections in early childhood. MTW, and especially VIW by the age of 2 years, were associated with low 25(OH)D level in cord blood. This association was independent from the frequency of infections and other risk factors of wheeze, including paternal smoking and exposure to smoke during pregnancy and infancy.

It seems that 25(OH)D does not prevent the infections themselves but it prevents wheezing as the symptom of viral infection. 25(OH)D has an important role in the control of inflammatory responses. Regulatory T-cells mediate this control. Studies have shown that infectious diseases occur due to dysfunction of regulatory T-cells related to 25(OH)D deficiency [10,11]. T-reg’s role in the inflammatory disease of the infancy period has been shown in different clinical trials to be strongly related to 25(OH)D deficiency (e.g. wheezy infant) [12,13]. Studies have shown that cord serum 25(OH)D levels are inversely associated with the risk of transient early wheezing [14], lower respiratory tract infections [15]. Recent findings by Łuczyńska et al. [16] suggest that vitamin D deficiency at birth is associated with increased risk of lower respiratory tract infections particularly in infants born to mothers without allergy. This was not confirmed by our study. We believe that this discrepancy could be a result of lower level of 25(OH)D in our cohort, Łuczyńska et al. studied 25(OH)D as categorized variable with cut-offs higher than ours. From statistical point of view it could explained different results. Camargo et al. have shown that increased maternal intake of 25(OH)D during pregnancy may reduce wheeze risk in offspring at 3 years of age [17]. In that study, compared to children of mothers in the lowest quartile of vitamin D intake during pregnancy (median, 356 IU/day), children of mothers in the highest vitamin D intake quartile (median, 724 IU/day) had a significant lower risk of recurrent wheeze [17,18]. Devereux et al. reported similar findings — an inverse association between maternal vitamin D intake during pregnancy and risk of recurrent wheezing in 5-year-old children [19]. In other study Balderbos et al. revealed an association between lower vitamin D status in newborn and increased risk of infant RSV-associated pulmonary infections and further wheezing [20]. According to their findings infants who contracted an RSV-associated infection had significantly lower cord blood 25(OH)D concentrations at birth than infants who did not develop infection. It should be noted that cord blood level of 25(OH)D in our cohort was different (much lower) to those of ex. Baiz et al. [14],

VIW in children during first 2 years of life. However, 25(OH)D in cord blood below median value or below lower quartile increased the risk of VIW in children during first 2 years of life (Table 2). All above results of 25(OH)D level in cord blood were corrected for the effects of other independent risk factors for MTW and VIW in this cohort. Odds ratios describing associations between MTW or VIW and 25(OH)D were adjusted for the number of infections and paternal smoking. Adjustments were allowed by multivariate logistic regression analysis.
The effect of vitamin D level in cord blood is corrected for the effects of other independent risk factors of multi-triggered wheezing. Odds ratios for each ng/ml increase in vitamin D levels are presented in Table 2.

### Table 2

Associations between presence of: i) atopic dermatitis, ii) food allergy, iii) >1 episode of respiratory tract infection, iv) at least 1 episode viral-induced wheezing and vitamin D level in cord blood (included in the model as continues variable or included in the model as nominal variable: <median or < lower-quartile).

<table>
<thead>
<tr>
<th>Dependent variables: D3 continues variable</th>
<th>OR 95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atopic dermatitis</td>
<td>0.97</td>
<td>0.86–1.10</td>
</tr>
<tr>
<td>Food allergy</td>
<td>2.72</td>
<td>0.43–17.29</td>
</tr>
<tr>
<td>Infection &gt;1 episod</td>
<td>1.52</td>
<td>0.33–6.94</td>
</tr>
<tr>
<td>MTWa</td>
<td>0.20</td>
<td>0.02–1.71</td>
</tr>
<tr>
<td>Viral-induced wheezing</td>
<td>0.05</td>
<td>0.00–0.81</td>
</tr>
</tbody>
</table>

Significance for bold values is p < 0.05.

a The effect of vitamin D level in cord blood is corrected for the effects of other independent risk factors of multi-triggered wheezing (MTW) and viral induced wheezing in this cohort: number of infection and paternal smoking.

b Odds ratios for each ng/ml increase in vitamin D.

This confirms previous observation that Vitamin D deficiency is frequent among Polish population [21]. It seems that although the current Polish recommendations [22] suggest vitamin D supplementation for prophylactic in infants including pregnant and lactating women, this is not effective, probably because Vitamin D is very often given only occasionally, if at all. The evident consequence is low average 25(OH)D level among populations of Central Europe, including our cohort.

On the other hand, the most dynamic lung development is in the prenatal period. It could be that the lung development in the conditions of high concentration of vitamin D3 prevents wheezing at first two years of life. Our result is supported by other studies. Zosky et al. in their study showed that 25(OH)D deficiency during lung development may impact on postnatal lung growth and increase the risk of developing lung disease [23]. Other authors state that 25(OH)D also regulates genes involved in the inflammation, immunity, cellular proliferation, and apoptosis associated with obstructive airways disease, likely via a genetic mechanism and that 25(OH)D deficiency directly affects programming within the developing fetal lung [24–26]. It is difficult to completely assess the role 25(OH)D plays in the development of wheezing. In order to further delineate a possible correlation between 25(OH)D and asthma/wheezeing, large and prospective studies need to be conducted.

In the light of several conflicting hypothesis, 25(OH)D levels have been both positively and negatively correlated with allergic disease prevalence. For example, high asthma prevalence and increased asthma symptoms have been observed in patients with low 25(OH)D levels [18,27–34]. Recently, vitamin D deficiency has been shown to correlate with many food and environmental allergies in children [35]. Studies have shown that cord serum 25(OH)D levels are inversely associated with atopic dermatitis by the age of 5 years [14], but no association was found with asthma and allergic rhinitis [14]. Conversely, other studies have shown an association between high 25(OH)D levels and the development of allergic diseases [16,36]. What is more, Xystrakis et al. showed evidence that 25(OH)D may have a therapeutic role in asthma by enhancing responsiveness to glucocorticosteroids for induction of il-10 [37]. Chi et al. have shown that higher vitamin D levels at birth may be associated with a low number of T-regulatory cells concluding that vitamin D status in utero may influence immune regulation in early life [5].

The small sample size is one of the limitations of our study. Other limitation of the study is the reliance on a single 25(OH)D measurements per subject. More reliable results would be acquired if we could measure 25(OH)D levels during the subsequent years of life. It seems also that it is important to establish the vitamin D supplementation in the investigated population of children, the way of feeding and sun-exposure.

In conclusion, our data revealed an inverse correlation between cord serum 25(OH)D levels and multi-triggered wheezing, and especially viral-induced wheezing by the age of 2 years. This suggests that improving the 25(OH)D status of children and pregnant women must be included in the priorities of physicians and healthcare professionals. Adequate Vitamin D intake and its optimal concentration in serum may reduce the risk of multi-triggered wheezing, especially of viral-induced wheezing.

### Conflict of interest

All authors declare no conflict of interests.

### Acknowledgments

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