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Subnormal levels of vitamin D are associated with acute wheeze in young children

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Abbreviations: 25(OH)D = 25 hydroxyvitamin D, RSV= Respiratory syncytial virus,

OR = Odds Ratio, CI=Confidence Interval

ABSTRACT

Aim: This study evaluated risk factors for acute wheeze in preschool children and investigated if subnormal levels of vitamin D were associated with increased risk for acute wheeze, atopy or viral/bacterial respiratory infections.

Methods: We recruited 130 children with acute wheeze, aged six months to four years, from paediatric emergency departments in Stockholm, Sweden, and 101 age-matched controls with no history of wheeze or sensitisation to airborne allergens. Parents answered standardised questionnaires and blood samples were analysed for specific IgE to airborne and food allergens and levels of 25 hydroxyvitamin D (25(OH)D). Nasopharyngeal virus samples were collected during the emergency department visit in the group of children with wheeze and a subset were also tested for bacteria.

Results: Vitamin D insufficiency (25(OH)D <75nmol/L (30 ng/mL) was associated with an odds ratio of 2.7 (95% confidence interval 1.1-6.2) for acute wheeze. However, no association was found between vitamin D insufficiency and atopy, presence of virus or bacteria or recurrent infections. Children older than 24 months were particularly at risk of subnormal vitamin D levels, irrespective of wheezing history.

Conclusion: Our findings support the hypothesis that subnormal levels of vitamin D are associated with acute wheeze in young children.

Key Words: Acute wheeze, atopy, infections, preschool children, 25-hydroxyvitamin D, virus

Key Notes:

- This Swedish study shows that preschool children admitted to hospital with acute wheeze (n=130) had significantly lower levels of vitamin D than children with no wheeze in an age-matched control group (n=101).
- No association was found between vitamin D insufficiency and atopy or presence of virus/bacteria.
- Children older than 24 months were particularly at risk of subnormal vitamin D levels irrespective of wheezing history.

INTRODUCTION

Respiratory illness with wheeze is very common in preschool children and 30 to 40% will continue to wheeze with viral infections at school age (1, 2). It is currently not known why respiratory infections trigger obstructive symptoms in some children, but not others. Parental asthma/allergy, early sensitisation, exposure to tobacco smoke, specific characteristics of the immune system and small airways are some of the important risk factors discussed (3-5). Some studies indicate that there are critical time periods in early life when certain virus infections, like rhinovirus and respiratory syncytial virus (RSV), may change the pattern of the immune reaction to a more harmful inflammatory response (6, 7). Others suggest that this might only be true in a subgroup of susceptible children with allergic sensitisation (8) or vitamin D deficiency/insufficiency (9). Vitamin D is recognised as an important modulator of both the innate and adaptive immune system and deficiency is associated with increased susceptibility to infections (10), development of asthma and atopy (11, 12) and reduced lung volume (13). The optimal level of 25-hydroxyvitamin D (25(OH)D) for the function of the immune system is not known. Generally, a level of ≥ 75 nmol/L (30 ng/mL) or > 50 nmol/L (20 ng/mL) is considered sufficient in adults and children, but this is estimated from studies on bone health (14, 15). UVB radiation, which is important for the production of 25(OH)D in the skin, is too

weak during September to April above the 35° latitude (16). In Sweden, which is situated above the 57° N latitude, vitamin D deficiency is a potential health concern and reports from the northern parts of Europe indicate a high prevalence of deficiency in adolescents (17, 18). Children in Sweden are recommended to take daily supplements of 10 ug (400 IU) of vitamin D from birth until the age of two years and children with dark skin should take it until the age of five years.

The aim of this study was to compare two selected hospital based study groups with respect to hereditary and life-style factors, atopy and 25(OH)D levels. The groups were Swedish children presenting with acute wheeze before the age of four and age-matched controls without a history of wheeze or sensitisation to airborne allergens. We also investigated if levels of 25(OH)D <75 nmol/L were associated with colonization with certain viruses and/or bacteria in acute and recurrent infections during a two to four month period, until a follow-up visit in the group of children with acute wheeze.

PATIENTS AND METHODS

From October 2008 to September 2011, 130 children aged six months to four years with acute symptoms of wheeze, were recruited at the Children's Emergency Wards, Astrid Lindgren Children's Hospital, Stockholm, Sweden. Exclusion criteria were prematurity (birth before 36 gestational weeks) and/or any chronic disease or simultaneous complications, such as sepsis, bacterial pneumonia or diabetes at time point of inclusion. Nasal swab tests for the presence of virus were obtained within 24 hours of hospital admission by a research nurse. All 130 children were also re-investigated after two to four months at a follow-up visit. At the emergency visit, 76 children, enrolled from September 2010 to September 2011, were also tested for bacteria. The legal guardian filled out a standardised questionnaire about disease, lifestyle and environmental factors. Blood samples were acquired for all analyses at the follow-up visit. During the same time period, 101 age-matched controls were recruited (Figure S1) at the Surgical Day-care Ward, Astrid Lindgren Children's Hospital. Exclusion criteria were prematurity (birth before 36 gestational weeks) and/or a history of bronchial obstruction/asthma or known sensitisation to airborne allergens. The legal guardians of the cases and controls filled out the same standardised questionnaire. The controls

had minor surgery performed during the day (Table S1) and blood was drawn at the same time as an intravenous line was inserted prior to surgery and anaesthesia.

Ethics

The study protocol was approved by the Regional Ethics Committee of Stockholm, Karolinska Institutet and written informed consent was obtained from the legal guardians.

Laboratory analyses

Analyses for neutrophil and eosinophil counts, levels of 25(OH)D, total and specific IgE levels against common airborne and food allergens (Phadiatop and Fx5, Thermo Fisher Scientific, Copenhagen) were performed at the Karolinska University Hospital Laboratory, Stockholm, on blood samples collected at the follow-up visit since the amount of blood possible to draw at the emergency visit was limited. The 25(OH)D was assessed using direct, competitive chemiluminescence analysis (CLIA, DiaSorin inc, USA). We applied the commonly used level of 25(OH)D <75 nmol/L (30 ng/mL) as the cut-off for vitamin D insufficiency (11). Sensitisation was defined as specific IgE ≥ 0.35 kUA/L. The nasal swabs were transported within two hours of collection in a standardised virus medium (Sigma-Virocult, Medical Wire & Equipment Co Ltd, Corsham, Wiltshire, UK) and then analysed using real time PCR assays on a 15 virus platform (see Supplemental information for details) at the Department of Clinical Microbiology, Karolinska University Hospital. Presence of bacteria was tested with a nasal nylon swab (Copan Eswab, Copan Diagnostics Ltd, Murrieta, CA, USA) transported at room temperature within two hours to the Department of Clinical Microbiology, Karolinska University Hospital, for qualitative aerobic culture on solid media with *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, beta haemolytic streptococci of groups A, C and G as target bacteria. Presence of other types of bacteria, such as *Staphylococcus aureus* was regarded as part of the upper respiratory microbiota.

Statistical analysis

The χ^2 test was used to examine significant proportional differences of risk factors between the acute wheeze and control groups. For continuous variables, an unpaired *t*-test was used to compare between group means and an independent group non-parametric test was used for comparing medians (age, total IgE). Multivariate logistic regression analyses with adjustment for age, sex, ethnicity and other potential confounders (eg. sampling month for vitamin D analyses) were also performed for all variables with unadjusted *p*-value ≤ 0.05 . SPSS (version 19) was used for all analyses.

RESULTS

Clinical presentation, life-style factors and earlier infections

Of the 130 children with acute wheeze, 80% were admitted to the ward for in-house treatment for at least 24 hours and 82% received an oral steroid burst within the first 24 hours of admission. The majority of children had experienced wheeze before, 22% were having their first wheezing episode and 52% had previously been diagnosed with asthma by a doctor. Both cases and controls were recruited throughout the year, although most children were recruited during the winter months. Detailed clinical information is given in Table S2. Parental asthma and pollen allergy, mothers' smoking during pregnancy, no exclusive breastfeeding and childcare centre attendance were factors associated with acute wheeze ($p \leq 0.01$ - 0.04 , Table 1). Children with acute wheeze had had significantly more recurrent respiratory infections, earlier reported RSV infections and bacterial pneumonias than the healthy controls ($p \leq 0.01$).

Vitamin D, atopy and acute wheeze

Vitamin D insufficiency (25(OH)D < 75 nmol/L) was found in 39% of children with acute wheeze and in 24% of the controls ($p = 0.04$, Table 2). A significant difference between the groups was also seen using 25(OH)D levels as a continuous variable ($p = 0.03$). When we looked at the two age groups - six to 24 months and 25 to 48 months – separately, children with wheeze had lower levels

compared to controls in both groups. In the older age group, the majority of the children were vitamin D insufficient, which was not the case in the younger age group (Table 2, Figure 1 and Figure S2). In the group of children with wheeze, the atopy level with specific sensitisation or reported food sensitivity was not associated with 25(OH)D insufficiency.

Levels of vitamin D and presence of virus and/or bacteria

Viruses were detected in 67% (n=86) of the 130 children in the acute wheeze group. The rhinovirus was the most common (35%, n=46), followed by RSV (18%, n=23) (Figure S3). We tested 76 children tested for both viruses and bacteria. This showed that 74% (n=56) had bacteria in nasopharyngeal cultures from 74% (n=56), 31% (n=24) had bacteria but no virus present and 26% (n=20) only had virus present. *Moraxella catarrhalis* was the most common bacterial finding, detected in 58% (n=44) of the children, followed by *Streptococcus pneumoniae* (25%, n=19) and *Hemophilus influenzae* (22%, n=17). Children with vitamin D insufficiency were not colonised with virus or bacteria to any higher degree than vitamin D sufficient children.

Levels of vitamin D and recurrent infections

At the clinical follow-up on 130 children after approximately 12 weeks (median 11 weeks, range six to 31 weeks), 76% of the children with wheeze had had one or several episodes of respiratory infections, 38% had paid an unplanned visit to a doctor because of acute wheeze and 15% had been readmitted to the hospital because of respiratory problems since the initial emergency visit (Table S3). No association was found between 25(OH)D <75nmol/L and respiratory disease, defined as recurrent respiratory infections, unplanned visits, and/or hospitalization, between the initial admission and the follow-up 12 weeks later.

Logistic regression analyses

When the significant life-style variables (Table 1) were tested using multivariate logistic regression analysis, maternal asthma, pollen allergy in parents, childcare centre attendance and no exclusive

breastfeeding time remained significantly associated with acute wheeze (Table 3). When more than six infections a year, any reported RSV infections and levels of 25(OH)D <75nmol/L were tested together in the regression analysis, including sampling month for the 25(OH)D analysis, they all remained significant. Vitamin D insufficiency was associated with an adjusted odds ratio (OR) of 2.7 (95% confidence interval, CI 1.1-6.2) for acute wheeze.

DISCUSSION

Our findings support the hypothesis that vitamin D insufficiency is associated with acute wheeze in young children. Our results remained significant after adjustment for several potential confounders, including earlier RSV infections, ethnicity and sampling month for vitamin D analyses. We also show that subnormal vitamin D levels are common in Swedish children over 24-months-of-age, irrespective of wheezing history.

Eczema and reported food sensitivity were significantly overrepresented in the wheeze group compared to the healthy controls, but there was no difference in specific IgE levels to food or airborne allergens between the two groups. Levels of 25(OH)D <75nmol/L could not be associated with specific IgE or total IgE levels in this study. This lack of association could have been affected by the generally, relatively high levels of vitamin D compared to other studies. An earlier US study by Sharief et al on 3,136 children and 3,454 adults (19), found that allergic sensitisation was more common in children with 25(OH)D deficiency, but not in adults. However, other studies have not confirmed these associations in children (20).

In our study, vitamin D insufficiency was associated with acute wheeze with an OR of almost three, but there was no significant increase of recurrent respiratory infections between the acute visit and the follow-up in children with low vitamin D levels. A similar observation was recently reported from the US, where no association between vitamin D levels and exacerbations in asthmatic children was seen over a four-year time period, although asthma was more severe in those with 25(OH)D insufficiency (21). It should be noted that our study only included a short period of follow-up. Another

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limitation with our study was that we were only able to measure 25(OH)D levels from blood taken at the follow-up, ie. on average 12 weeks after the emergency visit. Although vitamin D levels are considered stable over weeks, or even months, depending on factors such as the vitamin source, route of administration and dose – vitamin D has an estimated half-life about two to eight weeks (22, 23) - we cannot exclude that children with wheeze had different levels of vitamin D at the emergency visit as acute illness may alter the vitamin D metabolism (24). The stress experienced prior to surgery may also have influenced the levels of vitamin D in the healthy controls (25).

Rhinovirus was the predominant virus and *Moraxella catarrhalis* the most frequent bacterial finding in this group of wheezing children. Bacteria were found in a large proportion of children with wheeze. A Danish prospective birth cohort study (26) found similar levels of any virus (65% vs. 67% in our study) and bacteria (86% vs. 74%) in a group of children with acute wheeze. Non-wheezing healthy controls were also investigated and showed a similar distribution of virus as the wheezing group, although bacteria were significantly more common in children with wheezy episodes. A limitation of our study is that the controls were not tested for the presence of virus or bacteria. We found no significant association between low levels of 25(OH)D and the presence of virus or bacteria in nasopharyngeal cultures at the emergency visit.

The controls admitted for minor surgery were recruited from the Surgical Day-care Ward and selection bias for this group of children related to the variables and outcomes used in this study seems unlikely in view of the inclusion criteria. Comparison with the BAMSE population-based birth cohort from the same recruitment area of Stockholm, showed that the proportion of children with parental allergy, lifestyle factors such as presence of furred pets, breastfeeding and ethnicity did not differ to any major extent between the controls and children in BAMSE (27-29). However, the children in the acute wheeze group in our study were significantly more likely to attend childcare centres than the control children, which is similar to the BAMSE study, where childcare centre attendance was 83% at two-years-of-age. This suggests that the control group differed from the general population in this respect, rather than with the children with acute wheeze. Attending

childcare centres early in life has also been associated with childhood wheeze in previous studies (30). It is striking that only three % of the control children were exposed to maternal smoking during pregnancy, compared to 11% in the wheezing group and 13% in BAMSE study (27). It is likely that the identified protective factors (non-allergic parents, moderate childcare centre attendance, non-smoking mothers during pregnancy and exclusive breastfeeding time ≥ 4 months) contributed to healthy lung development in our control children.

In summary, we found that preschool children attending the emergency ward with acute wheeze, with a large majority being admitted to the ward for in-house treatment, had significantly lower vitamin D levels than healthy controls. Children aged between 24 to 48 months with wheeze were at a particular risk of vitamin D insufficiency. We could not find a significant correlation between subnormal vitamin D levels and atopy or with infections with any particular virus and/or bacteria. Further studies on the biological effects of vitamin D levels on respiratory diseases are warranted. The results from on-going randomised clinical trials in this area are much anticipated and will hopefully provide insight about the benefit of different supplementation regimes.

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TABLE 1. Clinical characteristics of 130 preschool children with wheeze compared with 101 healthy age matched controls

	Wheeze (n=130)% ¹	Healthy controls (n=101)% ¹	p-value
Male	65	79	0.02
6-24 months ¹	75	69	
25-48 months ¹	25	31	0.32
Mean age in months ²	19	20.5	0.29
Mean birth weight (kg)	3.5	3.6	0.15
Mother Caucasian	82	76	0.22
Father Caucasian	85	76	0.09
Asthma mother	27	7	<0.01
Asthma father	12	7	0.23
Pollen allergy mother and/or father	74	26	<0.01
Breastfeeding total time ≥4 months	68	75	0.30
No exclusive breastfeeding time	28	16	0.04
Attend childcare centre or family home day-care ³	73	53	<0.01
Smoking during pregnancy mother	11	3	0.02
Current smoking	22	20	0.62
Furred pets at home	25	22	0.55
Current eczema	18	7	0.02
Reported food sensitivity	15	3	<0.01
>6 respiratory infections a year ⁴	66	19	<0.01
Reported RS infections ever ⁵	31	5	<0.01
Bacterial pneumonia ever ⁶	8	0	0.01

¹Age 6-24 months n=97 in the wheezing group of children, and n=70 in the healthy group. Age 25-48 months n=33 in the wheezing group and n=31 in the healthy group.

²Independent samples t-test for equality of means ³2% in each group had family home day-care. ⁴Of children with 25(OH)D <75 nmol/L 74% (n=32) of cases and 12% (n=3) of healthy children have >6 respiratory infections a year, p<0.01⁵

58% of children with wheeze and 100% of the healthy control children had RSV infection at the age ≤ 6 months. ⁶Definition of bacterial pneumonia: fever >38°C, CRP >50 and confluent infiltrates on X-rays and treated with antibiotics, or diagnosis made by the paediatrician at the Emergency Ward. Fishers's exact test was used to test for this variable.

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TABLE 2. Levels of 25(OH)D, specific IgE and total IgE>100 kU/L and blood counts in preschool children with wheeze compared with a healthy age-matched control group

	Wheeze	Healthy controls	p-value
25-OH-Vitamin D¹			
Mean, nmol/L (SD)	82 (23)	90 (26)	0.03
Mean Age 6-24 months ² (SD)	88 (20)	94 (20)	0.08
Mean Age 25-48 months ³ (SD)	64 (21)	79 (36)	0.05
<75 nmol/L, %	39	24	0.04
<75 nmol/L, sampling month Oct-March, %	41	24	0.05
≤ 50 nmol/L, %	6	3	0.42
Sampling quarter of vit D Jan-March, %	41	31	
Sampling quarter of vit D April-June, %	40	27	
Sampling quarter of vit D July-Sept, %	9	17	
Sampling quarter of vit D Oct-Dec, %	13	24	<0.01 ⁶
Atopy⁴			
Foodx5 ≥0.35 kUA/L, %	24	20	0.49
Phadiatop ≥0.35 kUA/L, %	9	4	0.14
Total IgE >100 kU/L, %	19	13	0.28
Blood count, mean⁵			
Neutrophils (10 ⁹ x L ⁻¹) (SD)	3.4 (1.7)	2.9(1.6)	0.03
Eosinophils (10 ⁹ x L ⁻¹) (SD)	0.5 (0.7)	0.3 (0.3)	≤0.01 ⁷

¹Children with wheeze n=114, controls n= 99

²Children with wheeze n=84, controls n=69

³Children with wheeze n=30, controls n=30

⁴Children with wheeze n=120, controls n= 80

⁵Children with wheeze n=120, controls n=95

⁶Overall chi-2 test between sampling quarter and wheeze

⁷T-test for unequal variances

TABLE 3. Multiple logistic regression analysis of risk factors for acute wheeze

	OR	95% CI	p-value
Heredity and life style factors¹⁾			
Asthma mother	4.7	1.7-12.9	<0.01
Pollen allergy mother	4.3	1.8-10.1	<0.01
Pollen allergy father	5.2	2.1-13.4	<0.01
No exclusive breastfeeding time	2.3	1.0-5.1	0.05
Attend childcare centre	9.3	3.5-23.8	<0.01
Smoking during pregnancy mother	3.0	0.6-24.2	0.16
Vitamin D and earlier infections			
Vitamin D <75 nmol/L, adjusted ²	2.5	1.2-5.2	0.02
Vitamin D <75nmol/L, adjusted ³	2.7	1.1-6.2	0.02
>6 respiratory infections a year ³	10.0	4.6-21.7	<0.01
Reported RS infection ever ³	7.9	2.6-24.1	<0.01

¹ Variables included in the regression model: age, sex, ethnicity, asthma mother, pollen allergy mother, pollen allergy father, no exclusive breast feeding time, attend childcare centre, smoking during pregnancy mother.

²Variables included in the regression model: age, sex, ethnicity, sampling month (each month separately) of 25(OH)D.

³ Variables included in the regression model: age, sex, ethnicity, sampling month (each month separately) of 25(OH)D, > 6 respiratory infections a year, reported RS infections ever, 25(OH)D levels <75nmol/L.

The unadjusted OR for Vitamin D <75 nmol/L corresponded to 1.9, 95% CI 1.0-3.4.

Bacterial pneumonia was not included in the model because too few controls (n=0) had had this condition.

Figure legend

Figure 1. Levels of 25(OH)D in preschool children with and without wheeze at different ages. One outlier in the control group with 239 nmol/L is not shown in the figure, but included in the adjusted logistic regression analyses (Table 3).

