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ORIGINAL ARTICLE

Vitamin D Status in Infants with Two Different Wheezing Phenotypes

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Abstract
Objective To investigate vitamin D levels in patients with recurrent wheeze at early ages of childhood.
Methods In the present cross-sectional study, serum 25-hydroxy vitamin D [25 (OH)D], levels which is known as an indicator of vitamin D adequacy, was examined in infants with three or more wheezing attacks.
Results A total of 186 infants with recurrent wheezing were included in the study along with 118 healthy control peers. The recurrent wheezing study participants were classified into two groups according to Asthma Predictive Index (API) positivity and compared to control subjects regarding their serum vitamin D status. The API negative group had the lowest mean serum 25 (OH)D level ($n = 121$; 22.71 ± 10.76 ng/ml) followed by API positive group ($n = 65$; 24.08 ± 9.02 ng/ml) compared to healthy group (26.24 ± 11.88 ng/ml) ($p < 0.05$). In addition, higher vitamin D deficiency was observed in infants in API negative group (52.1 %; $p < 0.01$) and API positive group (38.5 %; $p < 0.05$) than control group (31.4 %).
Conclusions Low levels of 25 (OH)D were detected in infants with two different phenotypes of recurrent wheeze. Vitamin D deficiency may play a role in the pathogenesis of infants with recurrent wheezing.

Keywords Recurrent wheezing · Asthma predictive index · Vitamin D · Allergic sentisization

Introduction

Recurrent wheezing or asthma-like respiratory disorder is one of the challanging problems in infancy that has morbidity and mortality worldwide. Although respiratory syncytial virus (RSV) is the most common pathogen associated with increased severity of respiratory illness, other pathogens may also have a stronger association with recurrent wheezing [1]. Among these, rhinovirus is the most intriguing virus which is linked with recurrent wheezing and asthma. Risk factors for recurrent wheezing episodes in infants include smaller airways at birth (associated with male gender, small size for gestational age and tobacco exposure beginning in utero) and reduced interferon-γ (T-helper 1 type) response in early life [2].

Three distinct recurrent wheezing phenotypes are described in early childhood period; transient early wheeze (present in the first year of life, resolving by early school years), late-onset (non-atopic) wheeze (present in the first 3 y of life, resolving in early adolescence), and persistent (atopic) wheeze (onset in mid-preschool years with persistence into adolescence) [3]. Recurrent wheezing illnesses continuing beyond infancy and the development of asthma can be determined by an index, "Asthma Predictive Index" (API), if a child has one major criteria (physician diagnosed parental asthma and/or eczema) or two minor criteria [physician diagnosed allergic rhinitis, wheezing apart from colds and peripheral eosinophilia (≥ 4 %)] [4]. Later, this index was modified by adding two more determinants; inhalant allergen sensitization as a major criteria and food allergen sensitization as a minor criteria [5]. A positive index is associated with increased risk of continued frequent wheezing in late childhood.

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Data from both animal models and humans suggest that vitamin D (vit D) directly and indirectly induces production of antimicrobial proteins, and has antimicrobial effects and may protect against inflammatory reactions to environmental pollutants aside from its well-known role in calcium/phosphorus homeostasis and bone physiology [6]. Most immune system cells, including B- and T-lymphocytes, macrophages and antigen-presenting cells, have been shown to express the vit D receptor, which is the key mediator of vit D action [7, 8]. Furthermore, all these immune cells express the enzyme 1α-hydroxylase, which converts the circulating pro-hormone, 25-hydroxy vitamin D [25 (OH)D], into active form 1,25(OH_2)D. Active form of vit D has been demostrated to contribute to innate immunity by expressing genes encoding antimicrobial peptides, such as cathelicidin [9]. All these evidences show that vitamin D may play a broad role in regulating chronic inflammation in the lungs. Finally, there is also evidence that suggests a role of vit D in the expression of several genes in human bronchial smooth muscle cells including genes involved in smooth cell growth, proliferation and morphogenesis [10, 11].

Some recent studies have shown that low levels of vit D have been associated with increased risk of lower respiratory tract infections, atopy and asthma-associated phenotypes in early childhood [12–14]. Moreover, low intake of vit D during pregnancy or low umblical cord blood levels of 25 (OH)D is a risk factor of future wheezing episodes in infancy [15, 16]. Additionally, low levels of 25 (OH)D have been linked to asthma related emergency department visits or hospitalization in children [17]. In contrast, higher levels of 25 (OH)D significantly reduce odds of any hospitalization or need for asthma medication [18]. Vit D status is not well established among infants with different recurrent wheezing phenotypes. The aim of the present study was to determine the vit D status in two different recurrent wheezing phenotypes in infants less than 2 y of age. Recurrent wheezers were classified as API positive or negative group according to the modified API criteria, and 25 (OH)D levels of each group were compared with healthy infants of the same age.

Material and Methods

The study group consisted of infants with recurrent wheezing which were followed by Pediatric Pulmonology and Pediatric Allergy & Immunology clinics or referred by primary care physicians from September 2014 through January 2015. Inclusion criteria included; patients aged 9 to 24 mo with normal growth and development appropriate to age, three or more wheezing attacks diagnosed by a physician prior to participating in the study.

Exclusion criteria included; prematurity (birth before 36th gestational week), history or presence of other chronic lung diseases (bronchopulmonary dysplasia, cystic fibrosis, tuberculosis, *etc*), congenital heart disease or heart failure, presence of immunodeficiency, neurologic, genetic or any metabolic diseases.

The control group consisted of healthy infants of same age with appropriate growth and development. The group was aimed to be selected with male predominance because of the knowledge that wheezing is common in male gender [3]. Any known previous history of lower respiratory illness and chronic disease status were exclusion criteria from the study.

Then, the parents were asked to complete the questionnaire including questions about history of recurrent wheezing, allergic rhinitis, atopic dermatitis, food allergy, exposure to indoor smoking, breast feeding and regular vit D supplementation at least 6 mo in the first year of life.

Finally, infants with recurrent wheezing were classified into two groups: API positive or negative, according to the previously explained features.

Analyses for eosinophil counts in the peripheral blood, total sIgE levels, prick skin testing for common inhalant allergens (house dust mite, cat, dog, alternaria, aspergillus, tree and grass pollen) and food allergens (cow milk, egg, soy, peanut and wheat), and 25 (OH)D levels were performed at the Mersin Children's Hospital laboratory. All serum 25 (OH)D samples were obtained in the morning and measured at the same day using RIA (radioimmunoassay kit) method (Biokit S.A., 08,186 Barcelona, Abbott Laboratories). It is well established that serum 25(OH)D concentration is the best indicator for vit D adequacy [18]. In children, there is no absolute consensus as to what a normal range for 25(OH) should be. In general, the 25 (OH)D level above 30 ng/ml is accepted sufficient; the level between 20 and 30 ng/ml insufficient; <20 ng/ml deficient and under 10 ng/ml is extremely deficient.

The study protocol was approved by the Regional Ethics Committee, University of Mersin, and written informed consent was obtained from parents of infants. The SPSS software program (Version 17.0, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. If continuous variables were normally distributed, they were described as mean ± standard deviation ($p > 0.05$ in Kolmogorov-Smirnov test or Shapira-Wilk ($n < 30$), and if the continuous variables were not normally distributed, they were described as median. Comparisons among groups were determined using Student T test or One Way ANOVA for normally distributed data and Mann Whitney U test or Kruscall Wallis test were used for the data not normally distributed. The catagorical variables among groups was analyzed by using the Chi square or Fisher's Exact test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Demographic characteristics and laboratory results are presented in Tables 1 and 2. The study population included a total

of 186 infants with recurrent wheezing (121 infants in API negative group and 65 infants in API positive group) and 118 healthy control subjects. The mean age of API negative participants was 17.16 ± 4.49 mo (81 males and 40 females), API positive participants was 17.24 ± 4.79 mo (51 males and 14 females), and control group was 17.36 ± 4.26 mo (86 males and 32 females). There were no significant difference among groups regarding age, weight, gender, parental smoking, and breast feeding and vit D supplementation for at least 6 mo in the first year of life (Table 1).

As expected, parental history of asthma, inhalant allergen sensitization, atopic dermatitis, eosinophilia and IgE levels (mean: 241.72 ± 379.95 IU, median: 105 IU) in API positive group were significantly higher than in API negative group (mean: 31.82 ± 24.46 IU, median: 16.5 IU) ($p = 0.00$; Table 2). The medications at the time of enrollment to the study were not significant among two groups.

The mean 25(OH)D level was 22.71 ± 10.76 ng/ml [median (minimum-maximum): 19.90 ng/ml (3.4–48.2)] in API negative group, 24.08 ± 9.02 ng/ml [median (minimum-maximum): 22.40 ng/ml (11.3–54.6)] in API positive group, and 26.24 ± 11.88 ng/ml [median (minimum-maximum): 25.70 ng/ml (7.6–87.2)] in healthy control subjects. The mean and median vit D levels of both recurrent wheezing groups did show statistical significance when compared to control group ($p < 0.05$) (Table 1 and Fig. 1). At the level of 25(OH)D below 20 ng/ml, there was also statistical significance between API negative group [$n = 63$ (52.1 %)] and control group [$n = 37$ (31.4 %)] ($p = 0.001$), and API positive group [$n = 25$ (38.5 %)] and control group ($p = 0.004$).

Discussion

Epidemiologic studies have demonstrated that low levels of vit D are associated in increased risk of atopy and allergic lower respiratory illnesses in young children [19]. Lately, these findings have led to the interest to examine the possible role of vit D in infants with recurrent wheezing. A few published studies revealed conflicting results about the status of vit D in this group of patients. In this regard, Özaydin et al. investigated 25(OH)D vitamin levels in a small sample of infants less than 2 y of age with recurrent wheezing [20]. They were not able to demontrate significant association between 25(OH)D vitamin levels and recurrent wheezing in their limited study population. There was no information regarding the atopic status of their patients, possibly due to small sample size. In a cross sectional study, the clinical characteristics of children less than 2 y of age with recurrent wheezing were classified as API positive, episodic or multi-trigger wheezing [21]. The serum levels of vit D along with trace elements (copper and zinc) were compared among groups. The investigators reported a correlation of low levels of vit D and zinc, and high serum copper and copper/zinc ratio in patients with recurrent wheezing, API positive and multi-trigger temporal pattern of wheeze compared with non-recurrent wheezing, API negative and episodic temporal pattern of wheeze.

More recently, previously conducted US based multicenter MIST (Maintenance versus Intermittant Inhaled Steroids in wheezing Toddler) clinical trial data has addressed the association between vit D status and the rate of exacerbations requiring oral steroids in young children with recurrent wheezing [22]. Baseline serum 25(OH)D levels were measured in 284 children aged between 12 and 32 mo. The low level of 25(OH)D was

Table 1 Demographic characteristics and vitamin D results of the study groups

	API Negative ($n = 121$)	API Positive ($n = 65$)	Control ($n = 118$)	p value
Age (months) [1]	17.16 ± 4.49	17.24 ± 4.79	17.36 ± 4.26	NS
Weight (kg) [1]	10.87 ± 87	11.13 ± 1.42	11.07 ± 1.05	NS
Male gender (%)	66.9	78.5	72.9	NS
Breast milk [2] (%)	80.2	70.8	82.2	NS
Vitamin D supplementation [2] (%)	61.2	60.0	64.4	NS
Indoor smoking (%)	37.2	41.5	33.1	NS
25(OH)D level (ng/ml)				
Mean ± SD	22.71 ± 10.76*	24.08 ± 9.02**	26.24 ± 11.88	$p < 0.05$
Median (minimum-maximum)	19.90 (3.4–48.2)*	22.40 (11.3–54.6)**	25.70 (7.6–87.2)	$p = 0.014$*; $p = 0.03$**
25(OH)D level (<20 ng/ml, %)	52.1*	38.5**	31.4	$p = 0.01$*; $p = 0.04$**

API Asthma predictive index; *NS* Not significant among groups

[1] Results are given mean ± SD

[2] At least 6 mo

*API negative *vs.* Control

**API positive *vs.* Control

Table 2 Clinical and laboratory findings of the study groups

	API Negative $n = 121$	API Positive $n = 65$	p value
Parental asthma (%)	0.0	58.5	$p < 0.001$
Inhalant allergen positivity (%)	0.0	52.3	$p < 0.001$
Atopic dermatitis (%)	0.0	13.8	$p < 0.01$
Total IgE (IU) [1]	16.5 (5.0–137.0)	105 (10.5–1620.0)	$p < 0.001$
Eosinophilia (%)	5.8	56.9	$p < 0.001$
Allergic rhinitis (%)	0.0	4.6	$p = 0.04$
Food allergy (%)	0.0	3.0	NS
Treatment [2]			
Inhaled steroids (%)	2.5	3.1	NS
Montelukast (%)	26.4	27.7	NS
Inhaled steroids & montelukast (%)	9.1	20.0	NS
None (%)	62.0	49.2	NS

API Asthma predictive index; *NS* Not significant

[1] Results are given as median (minimum-maximum)

[2] At the time of enrollment

considered <20 ng/ml. Vitamin D-deficient participants had significantly higher mean rate of exacerbations requiring oral steroids compared with non-deficient participants. Limitations of the study included relatively small number of participants with vit D deficiency and ethnic heterogenity (black race defined as a risk for low vit D levels). Because the data was historical with an absence of control group, the investigators were not able to compare the vit D status of recurrent wheezers with healthy subjects. Most recent study investigated the severity of vit D deficiency and its association with recurrent wheezes in children less than 3 y of age [23]. The authors found that each 10 mg/ml decrease in vit D level was associated with 7.25 % greater odds of wheezing.

The present findings support the hypothesis that low levels of vit D is associated with respiratory morbidity in infants with recurrent wheezing. This finding is observed in two different phenotypes (atopic and non-atopic) of recurrent wheezing of infancy. Yet, the present results also show that vit D deficiency is more prominent in non-atopic recurrent wheezers than atopic wheezers. It is well documented that the most common trigger of wheezing in infants is viral infections. On the other hand, wheezing could be triggered not only by viral infection but also allergen exposure in atopic infants. Based on the present observation, it is plausable to speculate that more low vit D level might contribute wheezing in non-atopic infants along with other factors in the presence of viral infection.

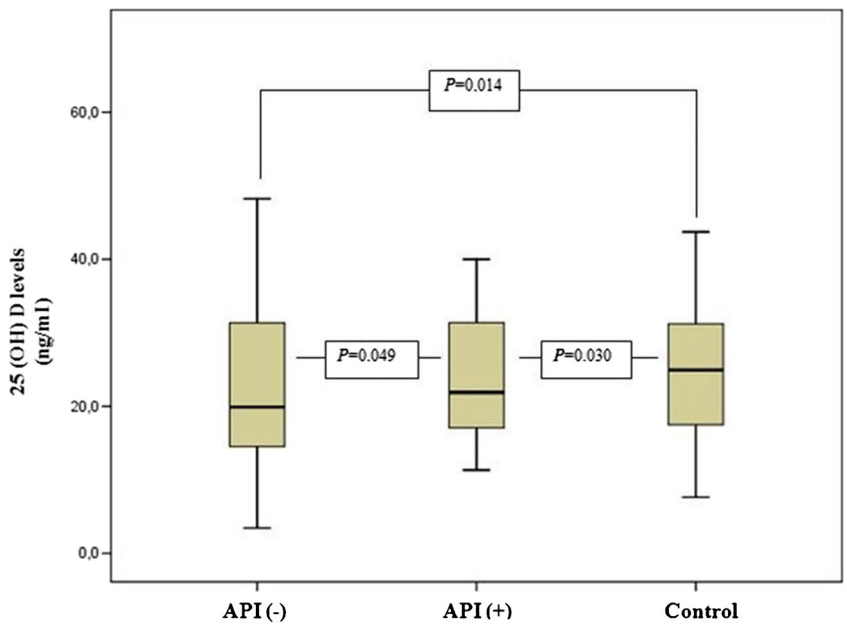

Fig. 1 The median vitamin D levels of the study groups

Therefore, it might not be surprising to find the lowest vit D levels in API negative group infants than API positive group infants. To authors' knowledge, this observation has not been reported in the literature. However it is premature to speculate that the presence of vit D deficiency is more common in the non-atopics than the atopics and this observation needs to be clarified with further studies. Moreover, authors' observed unexpectedly high percentage of subnormal levels of 25(OH)D in their control group. In Turkey, a recent study suggests that the prevalence of vit D insufficiency is 22.5 % and vit D deficiency is 14.5 % in children between 1 to 7 y of age [24]. It is important to note that high percentage of vit D deficiency (31.4 %) in the control group probably led some limitation of the index study results. Although there is sufficient sunlight intensity for cutaneous synthesis of vit D throughout the year in authors' province, different variables may influence the levels of 25(OH)D, including family compliance to supplemental use of vit D, time of exposure to the sun, diet, latitude, skin color and skin coverage. A serum 25(OH)D level of at least 20 ng/ml is recommended for the maintenance of bone health. In Turkey, the national recommendation is that all infants less than 1 y of age receive 400 IU/d vit D supplementation soon after birth. However, it seems reasonable to recommend vit D in dosage 800–1200 IU/d especially during winter months in authors' province.

In conclusion, this study investigated the possible role of vit D in recurrent wheezing at early ages of life. The present results suggest that infants with recurrent wheezing are prone to be vit D deficient when compared to healthy infants. However, it is premature to assume the importance of vit D in the etiology of recurrent wheezing. The relation between vit D and recurrent wheezing in infancy remains an area of investigation. Thus, there is a need to conduct well-designed, randomized, double-blinded controlled trials in infants with recurrent wheezing who have low levels of vit D initially to determine its role in prevention and treatment of childhood wheezing as well as define optimal vit D supplementation, optimal timing and duration of such an intervention.

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Contributions AO is the primary investigator and made substantial contribution to conception, data interpretation and design of the study. DD made substantial contribution in accessing the statistical analysis and preparation of the manuscript. OY made substantial contribution in patient enrollment to the study and preparation of the manuscript. All authors read and approved the final version of the manuscript. AO will act as guarantor for the paper.

Compliance with Ethical Standards

Conflict of Interest None.

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