Original article

Vitamin D level in preschool children with recurrent wheezy chest, and its relation to the severity of the wheezing episodes

***Background**: Recurrent wheezy chest is a common complaint in pediatric practice. Vitamin D is a potent immunomodulator in allergic diseases as wheezy chest and asthma. The prevalence of vitamin D deficiency has been increasing in Egypt leading to significant morbidities. **Objectives**: This study aimed to assess serum 25 hydroxy (OH) Vitamin D level in preschool children with recurrent wheezy chest, and to assess its relation to the recurrence, severity, and level of control of the wheezing episodes. **Methods**: The study included 100 preschool children (aged 2 to 5 years), of both sexes, recruited from the Emergency department, Allergy and Pulmonology units at Assiut University Children Hospital, Egypt. They should have at least 3 documented episodes of wheeze, cough, and difficulty breathing in the last year with clinical improvement on inhaled short-acting beta 2 agonists. Patients were subjected to questionnaire-based history, clinical examination, and laboratory investigations (complete blood count (CBC) with the absolute eosinophil count, serum total IgE level, and serum 25 hydroxy (OH) Vitamin D level). Pediatric Respiratory Assessment Measure (PRAM score) for assessment of the severity of the wheezing episodes and Global Initiative for Asthma (GINA) based level of asthma control for children 5 years and younger were applied. The patients were grouped according to PRAM score to mild, moderate and severe episodes and according to vitamin D level as sufficient and below-sufficient groups (including deficient and insufficient patients). **Results**: 25(OH) Vitamin D level was below-sufficient in 53% of the studied patients (deficient in 32% and insufficient in 21%). PRAM score was significantly lower in patients with sufficient 25(OH) Vitamin D level versus those with below-sufficient level ($p < 0.025$). There was a significant negative correlation between PRAM score and 25 (OH) Vitamin D level ($r = -0.334$, $p = 0.001$). **Conclusion**: There is an inverse relationship between 25(OH)vitamin D level and parameters of asthma severity, as well as with the level of asthma control in preschool children with recurrent wheezy chest.*

Keywords: Vitamin D, recurrent wheezy chest, preschool children.

Naglaa S. Osman, Hanaa A. Mohammad, Kotb A. Metwalley, Mostafa Embaby, Tarek T. ElMelegy*

*Department of Pediatrics, and *Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.*

Correspondence:
Naglaa S. Osman
Department of Pediatrics, Assiut University Children's Hospital, Assiut, Egypt
***E-mail:** naglaaosman84 @yahoo.com*

INTRODUCTION

Recurrent wheezy chest is one of the chronic childhood disorders[1].The prevalence of wheezy chest ranges from 1 to 18 % in different countries[2]. Worldwide, the prevalence is increasing especially among the preschool children[3]. It represents a common cause of Pediatric Emergency department admission, with more sleep disturbance, more limitation to the daily activities and more healthcare expenditure[4].

The term "recurrent wheezy chest" is preferable to the term asthma in preschool children as demonstration of airflow limitation and reliable pulmonary function testing cannot be done in this age group[3]. However, the last edition of the Global Initiative for Asthma (GINA), still used to describe recurrent wheezy chest in children younger than 5 years as asthma[5].

Identification of recurrent wheezy preschool children who are at risk for persistent asthma has mainly focused on simple clinical and laboratory parameters. The Asthma Predictive Index[6] (API) has become the most used tool to predict persistent asthma beyond the age of 6 years. It is an index based on the presence of ≥3 wheezy episodes per year and 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 %). A modified API was introduced in 2004.[7] This index differs from the original API because it requires ≥4 wheezing episodes with at least one diagnosed by a physician, whereas allergic rhinitis is replaced by sensitization to aeroallergens (major criterion) and allergic sensitivity to milk, eggs or peanuts (minor criterion).

Vitamin D has new emerging protective roles in extra-skeletal disorders, as inflammatory, allergic, infectious and autoimmune disorders[8]. Vitamin D has a significant role as an immunomodulator of the innate and adaptive immune systems. Nearly every immune cell express vitamin D receptors and 1α-hydroxylase, both are the key regulators for vitamin D action[9]. Worldwide, vitamin D insufficiency is still a growing problem, even in countries where there is abundance of sunshine that could help vitamin D synthesis all over the year[10]. There is a limited data about the prevalence of vitamin D deficiency and its relationship with wheezy chest in the preschool age group in our community.

It has been claimed that vitamin D deficiency and insufficiency may be linked to poor function of the lung, more severe wheezing episodes, and more emergency department visits in young children with recurrent wheezy chest[11]. On the other hand, conflicting findings have been reported from other studies, that denied any relationship between vitamin D status and asthma[12,13].

METHODS

Study design and patients

This cross-sectional study was conducted on 100 consecutive preschool children with recurrent wheezy chest, attending the Emergency Department, Allergy and Pulmonology units at Assiut University Children's Hospital, in Egypt, in the period from Dec 2014 to May 2016. The study was approved by the institutional ethical committee of Assiut University Faculty of Medicine, Egypt. The study was funded by Faculty of Medicine, Assiut University. An informed consent was obtained from the parents of the patients before their recruitment in the study.

The inclusion criteria were: children of both sexes, aged 2 to 5 years, fulfilling the GINA criteria of asthma diagnosis in children 5 years and younger which is 3 or more documented episodes of wheeze, cough, and difficulty breathing in the year preceding the study with clinical improvement on inhaled short-acting beta 2-agonist[5]. Children on vitamin D supplements in the last three months, those with manifestations of rickets, pneumonia or chronic respiratory diseases, and children with any chronic CNS, hepatic, metabolic, or renal diseases were excluded from the study.

Methodology

All patients enrolled in this study were subjected to complete medical history taking, comprehensive physical examination, chest X-ray, and laboratory investigations.

Phase I International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire[14] was used, it included:

- Age, sex, the season of birth, residence, mode of delivery, and socioeconomic position according to the modified classification of socioeconomic position in Egypt[15].
- Description of the wheezy episodes in the last year, whether it is **episodic** *(episodes at a specific time periods, more with infections, with free intervals in between the episodes)* or **multiple trigger wheeze** *(episodes with persistent symptoms in between, as during sleep or exercise, laughing, crying or exposure to cold air or smoke)*[16], and emergency department visits, and the number of in-hospital stays for every patient.

The Pediatric Respiratory Assessment Measure (PRAM score), that can be used for assessment of asthma severity in children aged 2-17 years was applied. This 12-point score includes oxygen saturation (0-2), suprasternal retraction (0-2), scalene muscle contraction (0-2), air entry (0-3), and wheeze (0-3). Accordingly, the patients with the wheezing episodes were classified into mild episodes (score 1-3), moderate episodes (score 4-7), severe episodes (score 8-12)[5,17].

Assessment of level of asthma control in children ≤5 years, was done by asking the parents 4 questions regarding the daytime and nighttime symptoms, activity limitation and the use of inhaled bronchodilator more than once a week. Patients were grouped as well controlled (no question was answered by yes), partially controlled (1-2 questions were answered by yes) or uncontrolled (3-4 questions were answered by yes)[5].

Laboratory investigation:

- Laboratory investigations were performed in the laboratories of Assiut University Hospitals.
- Collection of blood samples: venous blood (5 ml) sample was collected from each patient under aseptic precautions into an EDTA tube (2 ml; for complete blood count) and into a plain Wassermann's tube (3 ml). Plain tubes were placed in water bath (37°C) for 15 minutes and sera were separated by centrifugation at 3000 rpm for 15 minutes. Grossly haemolysed or lipaemic sera were rejected and new samples were collected.
- **Complete blood count**, with special attention to eosinophil count, was done on Celltac Es (MEK-7300, Nihon Kohden, Japan) and peripheral blood smear was prepared and stained by Leishman's stain[18].
- **Serum total IgE level** was measured using VIDAS Total IgE test kits (Cat. no. 30419,

BioMérieux S.A., France) according to manufacturer's instructions. VIDAS Total IgE assay is an automated quantitative test using the Enzyme-Linked Fluorescent Assay (ELFA) technique which combines an enzyme immunoassay sandwich method with a final fluorescent detection. The reference range recommended by the manufacturer is up to 46 KIU/L in children below 3 years and up to 280 KIU/L in children from 3-16 years old.

- **Serum Vitamin D** (25-OH-D) level was measured using 25-OH Vitamin D3/D2 test strips (cat. no. ORG 270, ORGENTEC Diagnostika GmbH, Germany) on Alegria Random Access Analyser (ORGENTEC Diagnostika GmbH, Germany) according to manufacturer's instructions. The Alegria® Random Access Analyser is an enzyme linked immunosorbent assay (ELISA) based test system. The Measurement range of this Alegria assay is 5 - 200 ng/ml.

  Reference range of serum (OH) vitamin D levels were as follow[19]

  Deficient: (< 20 ng/ml),

  Insufficient: (≥ 20ng/ml - < 30ng/ml) and

  Sufficient: (≥ 30ng/ml)

The studied patients were grouped into 2 groups: **sufficient group** when 25(OH) vitamin D level is ≥ 30 ng/mL and **below-sufficient** when 25(OH) vitamin D level is <30 ng/mL (including patients with deficient and insufficient 25(OH) vitamin D level).

Statistical analysis

Data entry and statistical analysis were done into SPSS version 19 (Statistical Package for Social Sciences). The quantitative data was presented as mean ± SD, median and interquartile range (IQR). For comparison, the Student's *t*-test and ANOVA were used in the normally distributed data and Mann-Whitney U-test and Kruskal-Wallis test were used with data not normally distributed.

The qualitative data was presented as number and percentage. For comparison, Chi-square (X^2) test was used and Fisher's exact test was used instead when the expected frequency is less than 5. Pearson and Spearman correlation tests were used for correlation. For all tests, a probability value (*p* value) less than 0.05 was considered statistically significant.

RESULTS

Tables 1 and **2** show the clinico-demographic and laboratory characteristics of the enrolled patients. **Table 3** shows the variation of the clinical and laboratory parameters of the enrolled patients according to their PRAM score. Vitamin D levels were significantly lower among patients with severe episodes. Furthermore, the PRAM score was significantly lower in the Vitamin D sufficient group in comparison to the below-sufficient group (*p*=0.025) **(Table 4)**. There was a negative significant correlation between PRAM score and 25(OH) vitamin D level (r = -0.334, *p* = 0.001) **(Figure 1)**.

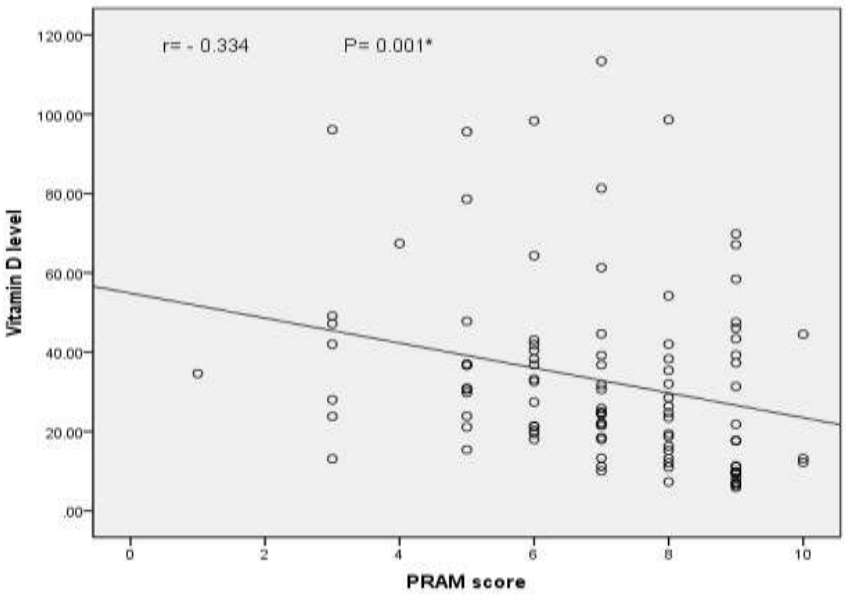

Figure 1. Correlation between 25(OH) Vitamin D level and PRAM score.

Table 1. Demographic and clinical characteristics of enrolled patients.

Item	n (%), Mean ± SD or Median (IQR)
Age (years)	
2 - < 3	35(35.0)
3 - < 4	36(36.0)
4 - ≤ 5	29(29.0)
Mean ± SD	3.20 ± 0.84
Median (IQR)	3.0(2.4-4.0)
Sex	
Male	68(68.0)
Female	32(32.0)
Residence	
Rural	75(75.0)
Urban	25(25.0)
Socioeconomic Status	
Low	70(70.0)
Medium	26(26.0)
High	4(4.0)
Pattern of wheeze	
Episodic	43 (43.0)
Multiple trigger wheeze	47(47.0)
Number of wheezy episodes in the last year Median (IQR)	6 (4-9)
Number of patients admitted to the hospital by the wheezy episode in the last year	47 (47.0)
Number of hospital admission in last year (per patient) Median (IQR)	6(2 - 8)
Duration of in-hospital stay(days) Mean ± SD	2.91 ± 0.43
Median (IQR)	3(2-4)
PRAM score	
Mild episode	7(7.0)
Moderate episode	49(49.0)
Severe episode	44(44.0)
Level of asthma control under 5 years	
Uncontrolled	50 (50.0)
Partially controlled	49 (49.0)
Controlled	1 (1.0)

IQR: Interquartile Range, n:number (n=100), PRAM: Pediatric Respiratory Assessment Measure, SD: Standard Deviation.

Table 2. Laboratory data of the enrolled patients.

Item	n (%), Mean ± SD or Median (IQR)
Total leukocytic count (cells/µ L)	
Mean ± SD	9470 ± 4350
Median (IQR)	8600(6125 - 11350)
Absolute Eosinophil count (cells/ µ L)	
Mean ± SD	392.21 ± 465.57
Median (IQR)	250(76.25 - 458.25)
Patients with Eosinophilia ≥ 4 %	41(41.0)
Serum IgE level (kIU/L)	
Mean ± SD	476.56 ± 933.26
Median (IQR)	122.2 (48.31- 428.55)
Patients with elevated serum IgE level, n (%)	50(50.0)
< 3 years	26(26.0)
3 - ≤ 5 years	24(24.0)
Serum 25(OH) Vitamin D (ng/ml)	
Mean ± SD	32.97 ± 22.81
Median (Range)	27.7 (17.65- 42)
Serum 25(OH) Vitamin D category, n (%)	
Sufficient (≥ 30ng/ml)	47(47.0)
Below-sufficient	53(53.0)
Including: Deficient(< 20 ng/ml)	32/53
Insufficient(≥ 20ng/ml - <30ng/ml)	21/53

kIU/L: International kilo-units per liter, IQR: Interquartile Range, µL: microliter, ml: milliliter, ng: nano gram, SD: Standard Deviation.

Table 3. Comparison of the characteristics of the enrolled patients according to PRAM score.

Item	Mild (score 0-3) (n= 7)	Moderate (score 4-7) (n= 49)	Severe (score 8-12) (n= 44)	*p*-value	Test value
Age Median (IQR)	2.5(2-4.5)	3(2.5-3.8)	3(2.6-4.23)	0.463	1.53
Sex					
Male	5 (71.4%)	33 (67.3%)	30 (68.2%)	0.976	0.048
Female	2 (28.6%)	16 (32.7%)	14 (31.8%)		
Residence					
Rural	7 (100%)	38 (77.6%)	30 (68.2%)	0.166	3.594
Urban	0 (0%)	11 (22.4%)	14 (31.8%)		
Socioeconomic status					
Low	3 (42.9%)	35 (71.4%)	32 (72.7%)	0.306	4.824
Medium	4 (57.1%)	11 (22.4%)	11 (25.0%)		
High	0 (0.0%)	3 (6.1%)	1 (2.3%)		
No. of the wheezy episodes in last year Median (IQR)	5 (3-8)	7(4-10)	6(4-8.5)	0.454	1.58
Total leukocytic count (cell/µL) Median (IQR)	10.8(10-16.5)	8.6 (6.05-12.05)	7.2(5.8-10.2)	0.020*$	7.791
Absolute Eosinophil count (cell/µL) Median(IQR)	216 (145.6-343.2)	260 (77.5-452.2)	250.2(60.5-610)	0.901	0.209
Serum IgE level (kIU/L) Median (IQR)	28.3(8.2-429.6)	107.3(41.04-373.7)	171.1(69.2-542.7)	0.098	4.64
25(OH)D level(ng/ml) Median (IQR)	42(23.8-49.1)	30.9 (21.5-41.2)	19.1(11.03-39.1)	0.012*$$	8.925

kIU/L: International kilo-units per liter, IQR: Interquartile Range, µ L: microliter, ml: milliliter, n:number (n=100), ng: nano gram, SD: standard deviation.

*Significant. Chi-square, Kruskal Wallis, Mann Whitney tests were used to estimate *p* value.

$ Significant difference between mild and moderate groups (p=0.034) and between mild and severe groups (p=0.009).

$$ Significant difference between the moderate and severe groups (p=0.007).

Table 4. Comparison of the characteristics of the enrolled patients according to 25 (OH) Vitamin D level.

	25(OH) Vitamin D level			
Item	**Below-sufficient (< 30ng/ml) n=53**	**Sufficient (≥ 30ng/ml) n=47**	**Test value**	***p*-value**
Age Median (IQR)	3(2.5-4)	3(2.5-4)	0.150	0.881
Sex				
Male	36 (67.9%)	32 (68.1%)	0.000	0.986
Female	17 (32.1%)	15 (31.9%)		
Residence				
Rural	39 (73.6%)	36 (76.6%)	0.120	0.729
Urban	14 (26.4%)	11 (23.4%)		
Socioeconomic status				
Low	44 (83.0%)	26 (55.3%)	9.148	0.010*
Medium	8 (15.1%)	18 (38.3%)		
High	1 (1.9%)	3 (6.4%)		
Number of the wheezy episodes in last year Median (IQR)	6 (3-9)	6 (4-10)	1.037	0.300
PRAM score Median (IQR)	8(6.5-9)	7(5-8)	2.240	0.025*
Level of asthma control, n (%)				
Controlled	0 (0.0%)	1 (2.1%)	-	1.000
Partially controlled	22 (41.5%)	27 (57.4%)	2.53	0.112
Uncontrolled	31 (58.5%)	19 (40.4%)	3.25	0.071
Total leukocytic count (cells/µL) Median (IQR)	9.9 (6.45-12.9)	7.5 (6-10.1)	1.969	0.049*
Absolute Eosinophil count (cell/µL) Median (IQR)	260.4 (82.5-480)	216.2(66-450)	0.366	0.714
Serum IgE level (kIU/L) Median (IQR)	108.5 (42.92-326.9)	132.5 (54-588.3)	0.798	0.425

kIU/L: international kilo-units per liter, IQR: Interquartile Range, µ L: microliter, ml: milliliter, n:number (n=100), ng: nano gram, SD: standard deviation

* Significant. Chi-square, Fisher exact and Mann Whitney test were used to estimate *p* value.

DISCUSSION

Recurrent wheezy chest in preschool children represent a challenging problem in pediatric practice, it has a higher global burden and consumes more health-care resources when compared with older asthmatic children[20]. Vitamin D deficiency and insufficiency are prevalent in Egypt.[10,21] This study aimed to assess the serum 25 (OH) vitamin D level in preschool children aged 2 to 5 years with recurrent wheezy chest in Assiut, as well as, to assess the relationship between serum 25 (OH) vitamin D level and recurrence, severity, and level of asthma control.

The results showed that 68% of the studied patients were males. The high prevalence in males is supported by Turkeli et al., 2016[22] in his study on preschool asthmatic children, showing that 60 % of them were males, and Abd El-Menem et al., 2013[10] found 63% of the studied Egyptian asthmatic children were males. This could be explained that females have a higher percentage of large airways to small ones, with a greater size corrected air flow, and more surfactant production that increases patency of the small airways. This could reduce the risk of wheeze in females[23].

Our study showed that 32% of the studied preschool recurrent wheezy children had deficient serum 25 (OH) vitamin D level and 21% had insufficient level. In consistent with our results Turkeli et al., 2016[22] stated that 25 (OH) vitamin D level was deficient in 30.4%, and insufficient in 30.4% of the studied preschool children, and Jensen et al., 2016[24] stated that approximately three-quarters of the studied preschoolers were vitamin D-insufficient. Also, AbdelKader and Nassar study in 2018[25] found that 29.8% of their studied wheezy preschool children had deficient 25 (OH) vitamin D levels while 38.4% had insufficient levels. Furthermore, Stenberg Hammar et al. 2014[26] demonstrated that subnormal levels of 25(OH)vitamin D are associated with acute wheeze in pre-school children.

Fuleihan 2009[27] studied 25 (OH) vitamin D status in Africa, she reported that its low level could be due to limited outdoor activity with inadequate sunlight exposure for cultural reasons, insufficient vitamin D supplementation during pregnancy and lactation, lack of fortification of food with vitamin D, and increased burden of infectious disease whereby utilization and turnover of vitamin D is increased. Also, Turkeli et al., 2016[22] stated that prolonged stay of the preschool children with recurrent wheezy chest at home for their protection from infections triggering wheeze leads to less sunlight exposure, which could be another important reason for their low 25(OH) vitamin D level.

On the other hand, Beigelman et al., 2014[28] found that 7% of the studied preschool aged children were vitamin D deficient, and Dogru et al., study 2014[29] showed that there was no difference in 25(OH) vitamin D level between asthmatic children and healthy control in Turkey. A similar observation was reported from the United States, where no association was found between vitamin D level and wheezy episodes in asthmatic children, although asthma was more severe in those with vitamin D insufficiency[30].

The previous studies determining the relationship between vitamin D level and childhood asthma had shown controversial results. This could be explained by the difference in sample size, age groups, and seasons during which the studies were performed. Studies conducted in winter usually had lower vitamin D level in comparison with those conducted in summer, as solar radiation is weaker, and hours of sunlight are shorter in winter compared with summer[31].

When comparing our patients according to the PRAM score, the total leukocytic count was significantly higher in the mild PRAM score group in comparison to either moderate or severe PRAM score groups. This may signify that the wheezy episodes in the mild score group could be precipitated by infections that raised the total leukocytic count. Although statistically insignificant, markers of atopy (absolute eosinophil count and serum total IgE) were observed to be higher in the moderate and severe PRAM score groups versus the mild score group. This agrees with Nagavi et al., 2007[32], and Wever-Hess et al., 2000[33] who reported that total IgE levels were inversely correlated with asthma severity. Ulrik 1995[34] showed a positive correlation between blood eosinophil count and the intensity of asthma symptoms.

Our study demonstrated a relationship between the deficiency of 25 (OH) vitamin D and the severity of the wheezing episodes. This came in agreement with Abd El-Menem et al., 2013[10], Brehm et al., 2009[35] and Sandhu and Casale 2010[36], who all reported that asthmatic children with low 25(OH) vitamin D level, have a greater risk of suffering from severe asthma episodes than those with higher vitamin D level. Vitamin D has been shown to alleviate respiratory diseases as recurrent wheezy chest, as it helps suppressing the inflammation, has an anti-inflammatory effect, and modulates the bronchomotor tone[37]. This could

explain why patients with below-sufficient 25(OH) vitamin D level have severe wheezy episodes.

Turkeli et al., study in 2016[22] showed that the number of wheezy episodes in preschool children within the previous year was higher in the vitamin D deficient and insufficient groups as compared to the sufficient group. Dogru et al., study 2014[29], Gupta et al., study[38] 2011, Brehm et al., study 2012[39], and El-Asheer et al., study 2016[21] showed highly significant negative correlation between 25(OH)vitamin D level and the number of wheezy episodes indicating that vitamin D deficiency has a significant role in the recurrence of wheezy chest. In our study, there was no statistically significant difference between the patients with below-sufficient vitamin D level and sufficient patients as regard the number of the wheezy episodes in the year preceding the study (p= 0.300), this could be explained by the small sample size included in this study. Similar to our study, Alysian et al., 2011[40] stated that there was no association between 25(OH) vitamin D level and the number of wheezy episodes in the previous year ($p > 0.05$).

Although statistically insignificant, 58.5 % of the patients with below-sufficient vitamin D level were uncontrolled versus 40% of those with sufficient vitamin D level. Similar observation was found by Turkeli et al., study 2016[22]. Chinellato et al., 2011[41] and Gupta et al., 2011[42] documented a significant positive correlation between 25(OH) vitamin D level and improved level of asthma control in children with asthma. On the other side, Alyasin et al., study 2011[40] did not identify any relationship between 25(OH) vitamin D level and asthma control in children.

There was an observed difference in total leukocytic count between below-sufficient and sufficient patients (p= 0.049), it can be explained in the light of absence of a significant difference in eosinophil count between the two groups that there was a higher incidence of infections in the below-sufficient groups. Vitamin D insufficiency is supposed to be an important risk factor of infection and viral respiratory tract infections are less common in children supplemented with vitamin D than those not supplemented, and that could be due to the immunomodulatory effect of vitamin D which may lead to an increased resistance to infection[43].

In the present study, there was no statistically significant difference in the atopy markers as eosinophil count and serum total IgE level (p= 0.714, and 0.425 respectively) between below-sufficient and sufficient vitamin D level groups. These results are consistent with those of Turkeli et al., study 2016[22] and Alysian et al., study 2011[38], they both concluded that there was no relationship between 25 (OH) vitamin D level and atopy markers in patients with asthma. However, other studies reported significant negative correlation between vitamin D and total serum IgE levels[21,40,44].

This study has some strengths and limitations. It is the first study conducted in Allergy unit, Children Hospital, Assiut University, which serves a large population of children especially those living in rural areas where there is limited access to medical care facilities. Most of the previous cross-sectional studies about wheezy chest and asthma in children have been conducted on school-aged children and little is known about this problem in the preschool age. However, the sample size is relatively small, which decreases the power of the study and does not allow for solid conclusions. The use of a questionnaire to parents, although it is the only validated tool, may not always be accurate due to the possible problems in understanding the questions, and over or under-evaluation of symptoms.

In conclusion, vitamin D deficiency and insufficiency are prevalent in our studied preschool recurrent wheezy children in Assiut, and there is an inverse relationship between 25(OH) vitamin D level and parameters of asthma severity.

It is recommended that the estimation of the serum 25(OH) vitamin D level can be done in the routine workup in children with recurrent wheezy chest. Larger follow up studies are required to elucidate the effect of vitamin D supplementation on different parameters of asthma severity, level of asthma control, and how it can therapeutically be beneficial in patients with recurrent wheezy chest.

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