



## Status of vitamin D levels in bronchial asthmatic Children at Tertiary Care Hospital, Tirupathi

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Received: 20<sup>th</sup> Jan 2019; Revised: 13<sup>rd</sup> Feb 2019; Accepted: 20th Feb 2019

### Abstract

The current study was carried out to estimate the levels of vitamin D in asthmatic children and to know the relation between the two. A total of 75 asthmatic children were included between 6-12 years of age based on symptoms and divided into mild, moderate, severe persistent asthmatic. Standard laboratory tests which include Absolute eosinophil count (AEC), Vitamin D levels, calcium, phosphorous, alkaline phosphatase (ALP) for asthmatic children. Blood samples were collected and Vitamin D levels were estimated using high performance liquid chromatography (HPLC) technique. The results of the current study has shown significant correlation between vitamin D and severity of asthma with male to female child ratio is 1.5:1. The total of 94.67% asthmatic children had vitamin D levels <30 ng/ml. 72% of asthmatic children had deficient levels of vit D i.e. <20 ng/ml. Mean serum Vitamin D is highest in mild persistent asthmatic group with value 23.73 +/- 8.03 ng/ml and lowest in severe persistent group with value 11.09 +/- 4.26 ng/ml. The ALP levels are raised in highest in severe persistent asthmatic group which is 252.36 +/- mg/dl. Lowest levels of ALP in mild persistent asthmatic group which is 163.44 +/- 36.6 mg/dl. 64% (48) of asthmatic children had AEC <400 and 36% (27) had AEC >400. The present study concludes that vitamin D deficiency is more common in asthmatic children (94.67%) who are attending the department of pediatrics, S.V.R.R.G.G.H., Tirupati and confirms that there is significant inverse correlation between severity of asthma and vitamin D levels.

**Key words:** Children, Asthma and Vitamin D.

### 1. Introduction

Asthma is a chronic inflammatory disease effecting an estimated number of 300 million individuals throughout the world. It has a significant social and financial burden on public health (Brown *et al.*, 2012). The prevalence of asthma is increasing in the developed and developing countries (Chambers *et al.*, 2012). The increased prevalence of asthma, as a heterogeneous disease, is not only due to genetics, but also may be influenced by a number of environmental factors associated with urban lifestyles or dietary habits.

Among the numerous dietary proposed hypotheses associated with asthma, the decrease in serum concentration of vitamin D due to increased time spent indoors, decreased exposure to sunlight, less exercise, obesity, and inadequate calcium intake are of particular interest (Finklea *et al.*, 2011). Recent epidemiologic studies have shown an association

between vitamin D deficiency and asthma 3-6. Besides the central role of vitamin D in calcium and bone physiology, the protective effects of vitamin D in asthma could be attributed to its immunomodulatory properties (Yin *et al.*, 2014). In addition, vitamin D has effects on epithelial cell, T and B lymphocytes, and antigen presenting cell functions. Moreover, by induction of regulatory T (T<sub>reg</sub>) cells to produce interleukin (IL)-10, vitamin D modulates inflammatory processes and could help to control asthma severity (Maalmi *et al.*, 2012). Many studies observed that vitamin D deficiency is more prevalent in asthmatic children. Also some studies showed that vitamin D supplementation may increase the risk of allergy.

So, the current study was undertaken to estimate the levels of vitamin D in asthmatic children to provide an observation of current epidemiologic and experimental evidence of association between

vitamin D status and asthma or asthma exacerbations at Sri Venkateswara Ramnarain Ruia Government General Hospital, Tirupathi.

## 2. Materials and Methods

### 2.1 Study area

Paediatric Department, Tertiary care hospital, Sri Venkateswara Ramnarain Ruia Government General Hospital (S.V.R.R.G.G.H.), Tirupati. The study was approved by the Institutional Ethical Committee, S.V. Medical College, Tirupathi, A.P, India.

### 2.2 Study subjects

75 asthmatic children of 6 to 12 years of age attending the Department of Pediatrics with bronchial asthma.

Patients detailed personal medical histories and complete physical examination and analysis of information were recorded. Socio-demographic characteristics such as age, sex and place of residence and morphometric measurements such as height and weight were recorded during meetings with the children's family.

Children with disease known to effect bone metabolism (e.g. renal disease and parathyroid dysfunction) and other severe disease (e.g. cancer, gastrointestinal disorder etc), those with malnutrition, those on vitamin D supplementation and on oral steroid were excluded from the study.

From the selected subjects, all aseptic measures were taken and blood samples (3ml) were collected after obtaining consent from parents in a standard pro-forma for appropriate laboratory tests which include WBC count, AEC, Vitamin D levels, calcium, phosphorous, ALP. Chest X-rays were also carried out to eliminate cases of disease other than asthma.

Total leukocyte count and % of eosinophils are analyzed by using an automated analyzer (SYSMEX XN-1000, 6 part analyzer). AEC was calculated as a product of eosinophil percentage and total leukocyte count, using a special cell counting fluid i.e Dungers fluid. Serum calcium, phosphorous and ALP were measured using automated analyzer (ERBA CHEM 7 semi auto analyzer).

After these parameters estimation, samples were stored at  $-20^{\circ}\text{C}$  in MRU laboratory. Samples are then centrifuged at 3500rpm for 5 min and the serum is analyzed by using HPLC technique. The HPLC kit was purchased from EAGLE Biosciences, 20A Northwest Blvd., Suite 112, Nashua, NH 03063. A single measurement of vitamin D3 and vitamin D2 were made in all subjects. Vitamin D3 level are classified as Sufficient (30-100ng/ml), insufficiency (20<30ng/ml) and deficiency (<20 ng/ml) (Reichel *et al.*, 1989; Wicherts *et al.*, 2007).

### 2.3 Sample preparation for estimation of Vit-D using HPLC

The protein precipitation extraction method was used to extract the vit-D from plasma samples. The sample preparation is as follows, pipette out 400 $\mu\text{l}$  in 5ml vial plasma to 400  $\mu\text{l}$  ice cold then mix the sample by vortex. Then to the sample add 500 $\mu\text{l}$  PREC from HPLC kit, mixed the sample for 2min on a vortex mixer and incubated them the tubes for 1 min at  $2-8^{\circ}\text{C}$  and centrifuge afterwards at 10g for 5 min. After centrifugation the sample shows 2 liquid phases and a solid disc (precipitated proteins). 50 $\mu\text{l}$  of the supernatant was used for injection in HPLC.

## 3. Results and Discussion

Bronchial asthma is a chronic inflammatory condition of airways and it is one of the common disease of childhood. Now a days, with increasing awareness of people the diagnosis of asthma is dramatically increasing. Vitamin deficiencies are also common in children in developing countries. Out of these vitamin D has its own importance. Vitamin D deficiency causes skeletal problems like rickets and also it is linked to many immunological and respiratory diseases which are nonskeletal effects of vitamin D deficiency. The immunomodulatory effects of vitamin D plays an important role in allergic diseases and asthma too. In India, vitamin D deficiency is also very common as per the data published previously which is affecting all the ages and both sex. Study conducted by Eleazar suchiang *et al.*, 2016 at Jaipur S.P.M.C.H.I. hospital related to S.M.S. medical college pediatric department observed that vitamin D deficiency is relatively

frequent in asthmatic children and there was a significant inverse relationship between vit D levels and severity of asthma. But there is no such reports in South India. So, the present study was conducted in the department of pediatrics, S.V.R.R.G.G.H, Tirupati to know Status of vitamin D levels in children of age 6-12years with bronchial asthma..

In our study, total of 75 asthmatic children of age 6-12 yrs are observed after satisfying both inclusion and exclusion criteria. The mean age of children in the present study 8.64. Out of 75, 45 are male children and 30 are female children. Male and female ratio is 1.5:1 as shown in Table 1 and the severity of asthma against gender was showed in Table 2.

**Table 1.** Gender distribution of subjects

Sex	No of patients	Percentage
Male	45	60%
Female	30	40%
Total	75	100%

**Table 2.** Gender Vs Severity of asthma

Severity of asthma	Male [N]	Female [N]
Mild persistent	14	11
Moderate persistent	14	11
Severe persistent	17	8
Total	45	30

Family history of asthma in study group was found to be 37.3% i.e. 28 children had the positive family history and the remaining does not have family history. The AEC was carried for study subjects, 27(36%) out of 75 children had an AEC of >400 as compared to 48(64%) children who had AEC <400.

The mean ALP level is calculated for all the 3 groups. ALP levels are highest in severe persistent asthmatic groups i.e 252.36 +/-38.57mg/dl. In moderate persistent asthmatic group, 200.52 +/- 33.45mg/dl. Mild persistent asthmatic group showing 163.44 +/- 36.60mg/dl which is lowest when compared with other two groups (Table 3).

**Table 3.** Serum Alkaline Phosphatase Vs Severity of asthma

Severity of asthma	Mean±SD
Mild persistent	163.44±36.6
Moderate persistent	200.52±33.45
Severe persistent	252.36±38.57

The results of estimated vit-D showed variations in its levels depends on asthma severity i.e. 94.67% of asthmatic children have vit-D levels <30ng/ml, 72% of children are having vit-D deficiency with levels <20 ng/ml and 22.67% have insufficient levels of vit D (Table 4).

**Table 4.** Serum Vitamin D3 levels Vs severity of asthma

Severity of asthma	Vitamin D levels ( ng/ml )		
	<20	20-30	>30
Mild persistent	9 (36%)	12(48%)	4(16%)
Moderate persistent	23(92%)	2(8%)	0(0%)
Severe persistent	22(88%)	3(12%)	0(0%)
Total	54(72%)	17(22.67%)	4(5.33%)

There is gender variation is also observed in vit-d deficiency i.e. 31(68.89%) out of 45 males and 23(76.67%) out of 30 females had vit D deficiency. 10(22.22%) males and 7(23.33%) females had vit-D insufficiency. There was no statistically significant difference between gender and vit D deficiency.

Many studies previously showed that vitamin D deficiency is a marker of severity of asthma. There is an inverse relationship between vitamin D levels and severity of asthma.

Study conducted by Eleazar suchiang *et al.*, 2016 in Jaipur showed that mild persistent asthmatic group had mean vitamin D level of 21.54 +/- 11.05 ng/ml, moderate persistent group had 15.43 +/- 4.60 ng/ml, severe persistent group had 10.60 +/- 5.57 ng/ml with a P value < 0.001 which is highly significant.

Study conducted by Elnadya *et al.*, 2013 in Egyptian children observed that there is highly significant inverse correlation between vitamin D levels and severity of asthma.

Study conducted by Avinash kumar *et al.*, 2014 observed that vitamin D level of < 10 ng/ml in 7.69% of intermittent asthma, 3.5% of mild persistent asthma, 35% of moderate persistent asthma, 100% of severe persistent asthma. P value is <0.001 which is significant and concludes that severity of asthma is inversely related to vitamin D deficiency.

In our study, mild persistent asthmatic children have vitamin D mean value of 23.73 +/- 8.03 ng/ml, moderate persistent asthmatic group have mean of 15.92 +/- 2.88 ng/ml, severe persistent asthmatic group have mean of 11.09 +/- 4.26 ng/ml. P value < 0.05 which is significant. Out of 75 asthmatic children, 54 children have vitamin D levels <20 ng/ml. No mild persistent asthmatic child have vitamin D <10 ng/ml.

#### 4. Conclusion

The present study concludes that vitamin D deficiency is more common in asthmatic children (94.67%) who are attending the department of pediatrics, S.V.R.R.G.G.H., Tirupati. There is significant inverse correlation between severity of asthma and vitamin D levels.

#### Acknowledgments

The authors are thankful to faculty members, Department of Pediatrics, S.V.R.R.G.G.H., Tirupati for their help and providing the necessary facilities in completion of research work.

#### Conflict of Interest

Authors declare none.

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