Assessment of 25 (OH) Vitamin D3 in Asthmatic Children in S.P.M.C.H.I, Jaipur

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Abstract
Objective: To determine the difference in mean of 25-OH-D in different groups of asthmatic children (i.e. mild, moderate and severe).

Study design: A hospital based observational, descriptive and comparative analysis.

Setting: This study was conducted in the Department of Pediatrics, S.P.M.C.H.I, attached to S.M.S Medical college, Jaipur, from May 2014 to April 2015.

Methods: This cross-sectional study included 75 (25 severe, 25 moderate and 25 mild asthmatic children) children aged between 6 and 15 years. Serum 25-hydroxy vitamin-D levels were determined and compared between the three groups. The association between vitamin-D levels and lung function was studied in the asthmatic children.

Results: Serum vitamin-D level was significantly lower in severe asthmatic children than in mild asthmatic children and vitamin-D levels had a significant positive correlation with FEV1%.

Conclusion: Vitamin-D deficiency is highly prevalent in asthmatic children and there was a significant inverse relationship between vitamin D levels and severity of asthma symptoms. Vitamin D is also associated with airway limitation.

Keywords: bronchial asthma, vitamin-D, FEV1

I. Introduction
Asthma is one of the most common chronic respiratory disease of childhood; in up to half of people with asthma, symptom begin during childhood, often early in life. School absence, emergency department visit and hospitalisation make asthma the leading cause of childhood morbidity from chronic disease. Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. It is defined by the history of recurrence respiratory symptoms such as wheeze, shortness of breath, chest tightness and cough that vary over time and in intensity, together with variable expiratory airflow limitation. Asthma is classified as intermittent, mild persistent, moderate persistent and severe persistent, according to Global Initiative for asthma. Although the role of vitamin D in bone health is well known, recent studies have described new non-skeletal roles for vitamin D in human health, including a role in preventing chronic diseases such as cardiovascular disease, diabetes and cancer. In addition to these chronic diseases, vitamin D which is a potent modulator of immune system and is involved in regulating cell proliferation and differentiation, may also be linked to bronchial asthma.

Asthma is a common, chronic respiratory disease affecting 1–18% of the population in different countries. Its prevalence is increasing in many countries, especially among children. Although some countries have seen a decline in hospitalizations and deaths from asthma, asthma still imposes an unacceptable burden on health care systems, and on society through loss of productivity in the workplace and, especially for pediatric asthma, disruption to the family.

Many previous studies showed the hypovitaminosis D was more prevalent with patient of bronchial asthma, this study try to establish the correlation between the severity of asthma and vitamin D levels.

II. Material and method
This cross-sectional study was carried out on 75 children aged 6 to 15 years, visited Pulmonology Clinic or admitted in Department of Pediatrics, Sir Padampat Maternal and Child Instituted, S.M.S Medical colleges, Jaipur, India, from May 2014 to April 2015. All the families filled out a written consent form. The Ethics committee of the institute approved of the study.
The subjects included 75 asthmatic patients, diagnosed according to the Global Initiative for Asthma (GINA) criteria: 1) a physician’s diagnosis of asthma, 2) symptoms of recurrent (i.e. more than two) episodes of wheezing, cough, shortness of breath, or a combination of these, 3) documented reversibility with bronchodilators and 4) symptoms of and/or use of medication for asthma in the previous six months[1]. 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.

680 patients of asthma were screened, out of which 25 eligible severe asthmatic children was included on first cum first serve basis and 25 moderate, 25 mild age matched asthmatic children reporting just next to each severe asthmatic children were included in the study. Patients’ detailed personal medical histories were recorded and complete physical examination and analysis of information was done. 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.

Appropriate laboratory tests were conducted to establish white blood cell count, eosinophil count, plasma vitamin D level, plasma calcium (Ca), phosphorus (P) and alkaline phosphatase (ALP). Chest X-rays were also carried out to eliminate cases of disease other than asthma.

A single measurement of 25-OH-D₃ was made in all subjects using a chemiluminescent method in Central Laboratory of S.M.S Hospital, Jaipur. All aseptic measure was taken and blood was collected in a serum vial and allow it to stand for 30 minutes at room temperature to ensure full clotting. All samples are subsequently centrifuged at 3000rpm for 5 minutes, and the supernatant is aliquoted and analyzed by ADVIA Centaur XP immunoassay system. 25-OH-D₃ level are classified as Sufficient (30-60ng/ml), insufficiency (20-30ng/ml) and deficiency (<20 ng/ml.)[4,5]. Peripheral leukocyte analyses included total leukocyte counts and percentages of eosinophil, using an automated cell counter (Name of the machine). The absolute count of eosinophil was calculated as the product of its percentage and the total leukocyte count. Serum total calcium, inorganic phosphorus and alkaline phosphatase were measured using an automated analyser (Cobas C 311, Roche Diagnostics). Serum calcium was adjusted for albumin; corrected serum calcium= (serum calcium [measured] + 0.8 * [4-serum albumin]).

Flow chart:
III. Statistical analysis

Continuous data was summarized in form of mean ± SD. The difference in mean was analyzed using ANOVA test. Continuous data was expressed in proportion. The level of significance would be kept at 95% for all statistical analysis.

IV. Result

A total of 75 asthmatic children aged between 6 and 15 years were studied; the mean age of children was 8.56 ± 2.1 years. 46 [61.3%] of the children were male and only 29 [38.7%] were females. The male, female ratio was 1.58:1(Table 1). The mean serum Alkaline phosphatase level was calculated for all three groups of patients. It was highest in severe asthmatics 248.38 ± 97.138 mg/dl and was lowest in mild asthmatics 180.72 ± 74.982 mg/dl. This difference was statistically significant [p=0.006] (Table 2).

As shown in Table 3, 97.3% of cases have serum vitamin D level of less than 30 ng/ml; 81.3% of the patients had vitamin D deficiency (<20 ng/ml) and 16% had insufficiency (20-<30 ng/ml)

There was statistically significant negative correlation between vitamin D level and severity of asthma. The mean levels of serum 25(OH) D3 (vitamin D) in asthmatic patients are; mild persistent: 21.54 ± 11.05 ng/ml, moderate persistent: 15.43 ± 4.60 ng/ml and severe persistent: 10.60 ± 5.57 ng/ml; p value of < 0.001 which is highly significant (Table 4).

As shown in Table 5, the mean value of vitamin D3 level is 11.50 ± 4.74 ng/ml, 15.07 ± 5.9 ng/ml, and 19.79 ± 9.70 ng/ml for % predicted FEV1 of <60, 60-79% and >80% of respectively; p value is <0.001 which is statistically significant.

### Table 1: Distribution of subjects according to sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>No of patients</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>29</td>
<td>38.7</td>
</tr>
<tr>
<td>Male</td>
<td>46</td>
<td>61.3</td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>100.0</td>
</tr>
</tbody>
</table>

### Table 2 - Serum Alkaline phosphatase with severity of Asthma

<table>
<thead>
<tr>
<th>SEVERITY OF ASTHMA</th>
<th>MEAN S.ALK PHOSPHATASE(IUL/L)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild Persistent [25]</td>
<td>180.72</td>
<td>74.982</td>
</tr>
<tr>
<td>Moderate Persistent[25]</td>
<td>203.43</td>
<td>48.273</td>
</tr>
<tr>
<td>Severe Persistent [25]</td>
<td>248.38</td>
<td>97.138</td>
</tr>
</tbody>
</table>

### Table 3. Serum 25 (OH) D3 level with severity of asthma

<table>
<thead>
<tr>
<th>SEVERITY OF ASTHMA</th>
<th>Vitamin D levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;20</td>
</tr>
<tr>
<td>Mild Persistent</td>
<td>17(68%)</td>
</tr>
<tr>
<td>Moderate Persistent</td>
<td>20(80%)</td>
</tr>
<tr>
<td>Severe Persistent</td>
<td>24(96%)</td>
</tr>
<tr>
<td>Total</td>
<td>61(81.3%)</td>
</tr>
</tbody>
</table>

### Table 4. Mean serum vitamin D3 with severity of asthma

<table>
<thead>
<tr>
<th>SEVERITY OF ASTHMA</th>
<th>No of patients</th>
<th>MEAN S. VITAMIN D3 (ng/ml)</th>
<th>±</th>
<th>SD</th>
<th>MINIMUM</th>
<th>MAXIMUM</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>25</td>
<td>21.54</td>
<td>11.05</td>
<td>9.50</td>
<td>48.00</td>
<td>&lt;0.001</td>
<td>[significant]</td>
</tr>
<tr>
<td>Moderate</td>
<td>25</td>
<td>15.43</td>
<td>4.60</td>
<td>9.50</td>
<td>25.50</td>
<td></td>
<td>ANOVA F=10.64</td>
</tr>
<tr>
<td>Severe</td>
<td>25</td>
<td>10.60</td>
<td>5.57</td>
<td>4.10</td>
<td>21.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>15.57</td>
<td>8.52</td>
<td>4.10</td>
<td>48.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 5: Serum Vitamin D level with % of predicted FEV1

<table>
<thead>
<tr>
<th>% Predicted FEV1</th>
<th>MEAN S. VITAMIN D3 (ng/ml)</th>
<th>±</th>
<th>SD</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;60</td>
<td>11.30</td>
<td>4.748</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>60-79</td>
<td>15.07</td>
<td>5.934</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>&gt;80</td>
<td>19.79</td>
<td>9.704</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>15.33</td>
<td>7.750</td>
<td>75</td>
<td></td>
</tr>
</tbody>
</table>
V. Discussion

Bronchial asthma is a major health problem especially among children. It has dramatically increased worldwide over the last few decades, in both developed and developing countries. Vitamin D deficiency may predispose to allergic phenotype of asthma and epidemiological evidence suggests that lack of vitamin D has been linked to increased incidence of asthma and increased severity of asthma in children. Vitamin D is a potent modulator of the immune system[11].

Being a tropical country, Vitamin D deficiency is supposed to be uncommon in India[31]. However from the data available in published literature vitamin D deficiency is very common in India in all the age groups and both the sexes across the country. This had been shown by Harinarayan and Marwaha[20,23,24] in Bangalore,India which show vitamin D deficiency is associated with an increased incidence of asthma and other allergy symptoms. In a cross sectional study carried out in 170 children of age range from 2-14 years by Uysalol et al, it show that 90.6% (n=77 out of 85) of asthmatic children show raised serum alkaline phosphatase (ALP) as compared to 37.5% (n=14 out of 40) of control have normal ALP; p value is <0.001 which is highly significant[11]. In the our study we observe the elevated serum levels of alkaline phosphatase. It was highest in severe asthmatics group 248.38±97.138mg/dl and was lowest in mild asthmatics group180.7±74.982mg/dl. This difference is statistically significant [p=0.006]. This could be explained by vitamin D deficiency which causes secondary hyperparathyroidism leading to increase turnover of osteoid tissue and elevated serum levels of alkaline phosphatase.

Several studies in the past have concluded that vitamin D deficiency is associated with an increased incidence of asthma and other allergy symptoms. In a cross sectional study carried out in 170 children of age range from 2-14 years by Uysalol et al, it show that 90.6% (n=77 out of 85) of asthmatic children show raised serum alkaline phosphatase (ALP) as compared to 37.5% (n=14 out of 40) of control have normal ALP; p value is <0.001 which is highly significant[11]. In the our study we observe the elevated serum levels of alkaline phosphatase. It was highest in severe asthmatics group 248.38±97.138mg/dl and was lowest in mild asthmatics group180.7±74.982mg/dl. This difference is statistically significant [p=0.006]. This could be explained by vitamin D deficiency which causes secondary hyperparathyroidism leading to increase turnover of osteoid tissue and elevated serum levels of alkaline phosphatase.

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and 16% had insufficiency. Hence our study finding is in accordance with the previous studies and vitamin D deficiency is common in asthmatic patient.

Several studies have examined the relationship between vitamin D deficiency and asthma exacerbations and markers of more severe disease. Many studies have shown that low vitamin D levels were associated with increase in severity of bronchial asthma \cite{7,8,11,12,16,28,30,42}. El-naggar et al observe that serum vitamin D level in intermittent asthma is 41.1±11.8ng/ml, mild persistent asthma is 35.1±6.4ng/ml, moderate persistent asthma is 24.7±3.8ng/ml and severe persistent asthma is 20.7±3.8ng/ml with p value of <0.001 which is statistically significant. It conclude that there is an inverse correlation between vitamin D level and severity of asthma \cite{39}. In another study conducted by Elnady et al in Egyptian children show that there is highly significant negative correlation between the vitamin D level and the grade of severity (r = -0.903, \( P < 0.000 \)) and the degree of control of bronchial asthma (\( r = -0.923, \ P < 0.000 \)). The accuracy of vitamin D as a predictor of asthma was found to be 88% by automatic linear modeling \cite{41}. Avinash Kumar et al observed serum vitamin D level of <10ng/ml in 7.69% of intermittent asthma, 3.5% of mild persistent asthma, 35% of moderate persistent asthma and 100% of severe persistent asthma; \( p \) value is < 0.001 which is highly significant and conclude that vitamin D deficiency is associated with severity of childhood asthma \cite{48}. In our study, the mean levels of serum vitamin D in asthmatic patients are: mild persistent: 21.54±11.05ng/dl, moderate persistent: 15.43 ± 4.60ng/dl and severe persistent: 10.60±5.57ng/dl; \( p \) value of < 0.001 which is highly significant. 64% (n=16 out of 25) of severe asthmatic patients have serum vitamin D level of < 10ng/ml while none of the mild asthmatic patient have levels < 10ng/ml; \( p \) value is 0.021 which is also statistically significant. This means that the higher the level of serum vitamin D, the lesser the degree of bronchial asthma severity. This finding is consistent with the above mention studies. Our finding is also in accordance with the work conducted by Litonjua et al. \cite{16}, who proved that low vitamin D levels were associated with worse bronchial asthma symptoms and poorer lung function measures. Thus our study demonstrated a highly significant negative correlation between serum vitamin D levels and the severity of asthma.

Few studies have shown that children with insufficient vitamin D levels were found to have a slightly lower mean FEV1 with lower vitamin D3 levels \cite{19,26}. A.R Somashekar et al observe that predicted FEV1 for serum vitamin D level of <15ng/ml is 68±4.1% and 81.5±3.7% for level of >15ng/ml - <20ng/ml; \( p \) value of <0.001 which is statistically significant and show a direct correlation between vitamin D levels and predicted FEV1 \cite{42}. Significant positive correlations were found between the serum vitamin D level and \% of predicted FEV1 (\( r = 0.871, \ P < 0.001 \)) in a study conducted by Elnady in Egypt \cite{41}. In our study, we observe that the predicted FEV1 for the mean value of vitamin D level of 11.50 ±4.74ng/ml, 15.07 ± 5.9ng/ml, and 19.79 ± 9.70ng/ml is <60, 60-79 and >80% respectively; \( p < 0.001 \), which is significant. This is also supported by study conducted by Brehm which conclude that insufficient vitamin D status predicts a lower FEV1 \cite{19}. The same is observe by Alyasin and suggest that lower predicted FEV1 were significant predictors of lower 25(OH) D levels \cite{19}.

Irreversible loss of lung function can be caused by airway adaptation occurring early on in asthma. Airway adaptation or remodelling involves structural changes in the walls of the airways caused by continual injury and repair processes. Vitamin-D may modulate airway remodelling and lung function by affecting the growth and contractility of airway smooth muscle. Vitamin-D inhibits transforming growth factor- \( \beta \) (TGF-\( \beta \)), matrix metalloproteinases and fibroblast proliferation \cite{9}.

Our study has some limitations: The sample size was relatively small (\( n = 75 \)). A larger sample size would have increased our statistical power to detect associations. As with most other studies investigating the role of vitamin-D in asthma, our design was cross-sectional, thus limiting our ability to establish a causal link between vitamin-D and asthma morbidity. A healthy control was not taken but at the same time age match patients were compared in each group of patient like control group. In our study we tried but could not match the sex of the patients because boys were out numbered as compared to girls and secondly total numbers of patient studied were limited i.e 25 in each group of severity of asthma. Even though there is a strong relation of lung function and 25(OH) D level, we could not establish a direct mechanistic link between vitamin D deficiency and asthma in children Furthermore, many confounding factors can affect vitamin-D levels or asthma severity. Long-term follow-up studies focusing on changes in vitamin-D status and asthma parameters will be needed to elucidate the effect of vitamin-D status on asthma.

### VI. Conclusion

The present study suggests that vitamin D deficiency is relatively frequent in asthmatic patients (97.3\%) who were attending to pediatric department of S.P.M.C.H.I, S.M.S Medical college, Jaipur.

There was a significant inverse relationship between vitamin D levels and severity of asthma symptoms. Measuring serum levels of vitamin D may be considered in patients with bronchial asthma especially
un-controlled or severe asthmatic patients. Further studies are needed to determine the role of vitamin D in the treatment of asthma in terms of improvement of lung function test and severity of asthma.

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