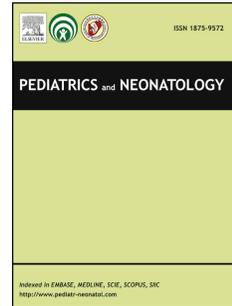


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Vitamin D Status Correlates With The Markers of Cystic Fibrosis-Related Pulmonary Disease

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Title Page**Title: Vitamin D Status Correlates With The Markers of Cystic Fibrosis-Related Pulmonary Disease**

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Short title: Vitamin D status and pulmonary disease in cystic fibrosis

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Keywords: cystic fibrosis; pulmonary exacerbation; vitamin D deficiency; bacterial colonization; lung function

Abbreviations:

CF-cystic fibrosis

CFTR-cystic fibrosis transmembrane regulator

25-OHD-25-hydroxy vitamin D

IQR-interquartile range

CI-confidence interval

FEV-forced expiratory volume

Manuscript

Abstract

Background: The prevalence of Vitamin D deficiency remains high in cystic fibrosis despite daily supplementation. Vitamin D as an immunomodulator has been related to lower respiratory tract infections in children.

Objective: The present study was undertaken to examine the association between vitamin D status and markers of cystic fibrosis-related pulmonary disease including exacerbations, bacterial colonization and pulmonary function.

Methods: The study includes review of records of 51 cystic fibrosis patients. Baseline patient variables and serum vitamin D levels were recorded. Based on vitamin D levels study patients were divided into three groups: vitamin-D sufficient (≥ 20 ng/mL), vitamin-D insufficient (12 to 20 ng/mL), and vitamin D-deficient (≤ 12 ng/ml).

Results: The proportion of children with deficient, insufficient and sufficient vitamin D levels were 47.1%, 15.7%, and 37.2%, respectively. Female sex, bacterial colonization and a greater number of exacerbations were associated with highest odds of developing vitamin D deficiency in patients with CF with 1.77(0.22–14.61)($p = 0.002$), 2.9(0.57–14.82)($p = 0.011$), and 5.12(1.28–20.50)($p = 0.021$) respectively. The comparison of vitamin-D levels taken during exacerbations, colonization and during routine follow-up were significant [16.04(7.42–27.91), 24.3(15.5–32.4) and 48.54(18.37–78.7)ng/ml, $p < 0.001$]. The FEV1 was determined in 24 patients; the comparison was significant between vitamin D-deficient and -sufficient groups [0.75(0.717–0.777) vs. 0.82(0.74–0.92) $p < 0.05$].

Conclusion: We concluded that vitamin D deficiency was highly prevalent in children with CF, despite daily supplementation of the vitamin in diet. Further, vitamin D deficiency was associated with a higher rate of pulmonary exacerbations and higher incidence of pulmonary bacterial colonization. In addition, in younger patients, low vitamin D levels were associated with reduced pulmonary function.

Introduction

Vitamin D deficiency is common in patients with cystic fibrosis (CF) and prevalence ranges from 40 to 90%, depending on age and definition of deficiency.¹⁻⁴ The etiology of vitamin D deficiency in CF is multifactorial, including reduced intake, pancreatic insufficiency, poor body fat stores, reduced sunlight exposure, reduced absorption and decreased vitamin D-binding protein^{4,5}. The prevalence of Vitamin D deficiency remains high in CF despite daily supplementation of the vitamin in the diet.⁵ Insufficient vitamin D in the body leads to decreased intestinal absorption of calcium, which results in secondary hyperparathyroidism. The elevated parathyroid hormone causes calcium resorption from bone, resulting in bone weakness, skeletal losses, and early osteoporosis.⁶ Related to the high rates of vitamin D deficiency, individuals with CF have also been shown to have low bone mineral density and a relatively higher fracture prevalence.⁷ In addition to effects on bone health, vitamin D deficiency is associated with an increased risk for the development of autoimmune diseases, cancer, infections, and cardiovascular disease among individuals without CF.^{8,9} There is also evidence to suggest that vitamin D plays a role in modulating the innate immune system, and vitamin D receptors have been found on Th1 cells and macrophages.¹⁰ Vitamin D deficiency has been related to a higher predisposition to more severe respiratory infections in healthy infants and children.¹¹⁻¹³ In CF, vitamin D concentrations have been associated with lung growth and preserving lung function.^{12,14-16} Further, in vitro studies on CF bronchial epithelial cells have shown that vitamin D up-regulates cathelicidin, an antimicrobial with activity against *Pseudomonas aeruginosa*.¹⁷ *Pseudomonas aeruginosa* infection and colonization are important in CF, as they can significantly decrease lung function and increase morbidity and mortality in this population.¹⁸

The present study was undertaken to examine the association between vitamin D status and markers of CF-related pulmonary disease including exacerbations, bacterial colonization and pulmonary function. We further studied the impact of various patient-related risk factors on development of vitamin D deficiency in CF.

Material and methods

Study subjects

This retrospective study was performed in the department of pediatrics, SKIMS hospital, Srinagar. Study includes a review of records of 62 cystic fibrosis patients under follow-up of pediatric pulmonology clinic, with age less than 15 years. Our unit uses a protocolized approach for patients with CF; after initial diagnosis is made, patients are put on a close four-weekly follow-up. Microbiological cultures of respiratory secretions are done every three months. Pulmonary secretions for cultures are retrieved either by active coughing or oropharyngeal swab in case of non-expectorating younger patients. Bronchoalveolar lavage is performed if patients fail to respond to treatment directed at pathogens cultured using the above mentioned methods. Pulmonary function tests are done yearly in patients above 6 years of age or earlier if patients are cooperative enough for spirometry. All the patients with CF are routinely started on daily vitamin D supplementation (400–800 IU per day). Further, patients with intestinal disease are started on pancreatic enzyme replacement therapy (PERT).

In our study, patients were included if they were diagnosed with CF either by a positive sweat chloride test, defined as a chloride concentration ≥ 60 mmol/l using pilocarpine iontophoresis, or

with two known CFTR mutations positive in presence of a chloride concentration of 30-59mmol/l. CFTR mutations analyzed were $\Delta F508$, 3849+10kbc->T, R560T and R117H.

The study was approved by the hospital ethics committee. Informed consent was obtained from the parents/guardians of the study patients.

Data collection and data elements

The recorded vitamin D levels were measured between January and December 2016. Data abstracted from the records include: age, sex, weight, height, age at diagnosis, sweat chloride at diagnosis, mutation analysis, pancreatic sufficiency, number of exacerbations in the preceding year (prior to vitamin D measurement), sputum/oropharyngeal swab/broncho-alveolar lavage culture and dose of pancreatic enzyme supplementation. Pulmonary exacerbations enumerated in the study were defined as acute or sub-acute worsening of respiratory symptoms severe enough to warrant oral or intravenous treatment with antibiotics. Pulmonary colonization was defined as positive respiratory cultures in the absence of increase in baseline signs and symptoms. We excluded the patients with normal pancreatic function, as such patients do not have impaired vitamin D absorption, and also patients with evidence of liver dysfunction. Serum vitamin D levels as 25-hydroxyvitamin D(25-OHD) were measured using liquid chromatography. Based on vitamin D levels, study patients were divided into three groups: vitamin D-sufficient (≥ 20 ng/mL), vitamin D-insufficient (12 to 20 ng/mL), and vitamin D-deficient (≤ 12 ng/ml). These definitions were based on the 2016 Global Consensus recommendations.¹⁹

Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) version 20. Demographic and clinical parameters were compared among the vitamin D groups using Chi-

square (or analysis of variance) and Student t tests (table.1). To determine risk factors for vitamin D deficiency, univariate comparisons were performed between groups with and without deficiency. The variables with significant univariate comparison ($p < .05$) were then included in a multivariate logistic regression model. Further, Pearson's correlation was performed to determine the relationship between different variables. Results are presented as percent (%), median, interquartile range (IQR), and 95TH percent confidence interval (95th CI).

Results

Patient characteristics and prevalence of vitamin D deficiency

Retrospective data were analyzed for 62 patients aged less than 15 years. Nine patients with pancreatic sufficiency and two patients with liver dysfunction were excluded. Final data were collected for 51 patients. The median (IQR) age of the patients was 60(36–72) months, and 47% patients were females (Table 1). CFTR mutation analysis was positive in 36(70.6%) patients, with $\Delta 508$ mutation in 30(58.8%) and 3849 in 6(11.8) patients. Sweat chloride was positive (≥ 60 mmol/l) in 39(76.5%) patients. In our study patients, the median (IQR) vitamin D level was 16.04(9.29–26.69) ng/ml; levels across different seasons were: 13.56(7.92–24.57) in spring in 18 patients, 20.54(15.37–45.7) in summer in 9 patients, 17.63(9.39–27.09) in autumn in 14 patients, and 5.88(2.9–10.99) in winter in 10 patients. The intergroup analysis of vitamin D between different seasons showed that the only significant comparison was between the levels taken in summer and those taken in winter($p < 0.05$) with highest recorded levels during summer months of the year. The proportions of children with deficient, insufficient and sufficient vitamin D levels were 47.1%, 15.7%, and 37.2% respectively(Table 1)(Figure 1). At the time of vitamin D

level measurement 6(11.76%) patients were in exacerbation of CF and 9(17.65%) patients had bacterial colonization. Organisms isolated during exacerbation were: *Pseudomonas aeruginosa* 3(50%), *Staphylococcus aureus* 2(33.3%), *Klebsiella pneumonia* 2(33.3%) and *Streptococcus pneumonia* 1(16.7%). Organisms isolated during colonization were: *Pseudomonas aeruginosa* 5(55.5%), *Klebsiella pneumonia* 2(22.2%) and *Enterococcus* 2(22.2%).

Vitamin D and markers of CF-related pulmonary disease

There was a significant difference in mean number of exacerbations (in preceding 1 year) between the vitamin D-sufficient, -insufficient, and -deficient groups 1(1–3.25), 1(1–1.5), and 0(0–1), respectively; $P < 0.001$) (Table 1). However, the greatest difference was between the vitamin D-sufficient and -deficient groups. Further, the comparison of vitamin-D levels taken during exacerbations, colonization and during routine follow-up were significant [16.04(7.42–27.91), 24.3(15.5–32.4) and 48.54(18.37–78.7)ng/ml, $p < 0.001$]; the inter-group comparison was significant between vitamin D during exacerbations and routine follow-up ($p < 0.001$), and colonization and routine follow-up ($p < 0.05$)(Figure 2). The FEV1 was determined in 24 patients; the comparison was significant between vitamin D-deficient and -sufficient groups [0.75(0.717–0.777)% versus 0.82(0.74–0.92)% $p < 0.05$]. However, there was no difference in FEV1 between vitamin D-insufficient and -sufficient groups [0.79(0.77–0.79)% versus 0.82(0.74–0.92)% $p > 0.05$].

Risk factors for vitamin D deficiency in CF

To determine the possible impact of different patient parameters on vitamin D level in patients with CF, Pearson's correlation was performed. We observed that vitamin D deficiency was positively correlated to female sex (0.483, $p = 0.001$), number of exacerbations (0.507, $p =$

0.001), age at diagnosis (0.335, 0.016), bacterial colonization (0.500, $p = 0.035$), and pancreatic enzyme supplementation (0.063, $p=0.60$), and that it was negatively correlated to age (-0.584, $p = 0.001$). In multivariate regression analysis, we observed that female sex, bacterial colonization and greater number of exacerbations were associated with the highest odds of developing vitamin D deficiency in patients with CF (Table 2).

Discussion

Taking a cut-off of $<12\text{ng/ml}$ for vitamin D deficiency as per the consensus recommendation for pediatric age group,¹⁹ we reported a prevalence of 47.1% in our study patients. Although few studies from large CF centers observed that $>90\%$ of patients had vitamin D levels $<30\text{ ng/mL}$, recent data suggested a trend toward higher levels.^{1,5} This recent trend has been attributed mainly to greater awareness about vitamin D in CF resulting in additional supplementation.¹ We supplemented our patients with 400 IU of vitamin D daily as per the CF foundation evidence-based guidelines for children.²⁰ Further, the clinical practice guidelines for preschoolers from the Cystic Fibrosis Foundation were consistent with CF foundation guidelines in terms of daily dosage.²¹ However, the reported presence of low vitamin D in our study emphasizes the continued deficiency despite recommended daily supplementation. The Cystic Fibrosis Foundation evidence-based guidelines recommend that vitamin D status should be checked annually in all patients with CF, ideally at the end of winter as the vitamin D status fluctuates by season in the CF population.²⁰ It further recommended 12-weekly assessment of vitamin D after a change in vitamin D dosage, to keep levels above 30ng/ml .²⁰ However, targeted and frequent vitamin D assessment-guided treatment has its downside as the financial issues and treatment burden are highly relevant to individuals with CF, especially in patients from resource-poor

countries. Despite pancreatic enzyme replacement therapy and routine oral vitamin D supplementation, 25-OHD levels $<30\text{ng/ml}$ were present in 82.4% of our study patients. Although studies have shown that vitamin D deficiency increases with age, we observed a higher prevalence in lower age groups [OR 0.937(0.901–0.975)]. We attributed this trend to higher mean patient age at the time of diagnosis, due to lack of widespread neonatal screening for CF.

Studies have shown that vitamin D has pleiotropic effects on the immune system and its daily supplementation modulates immune activation in CF in a complex manner.²² These studies have conflicting results, with only one study reporting a significant positive relationship between vitamin D level and pulmonary function.⁴ Further, some studies reported vitamin D deficiency was related to other markers of CF lung disease, such as pulmonary exacerbations and bacterial colonization with *P. aeruginosa*.^{4,18} We observed a significant difference in the number of pulmonary exacerbations between those with vitamin D levels $<20\text{ng/ml}$ and those with vitamin D levels $>20\text{ng/ml}$. The greatest difference was between the vitamin D-sufficient and -deficient groups. McCauley and co-workers observed that the rate of exacerbations for the deficient vitamin D group, aged 15 to 18 years, was 13.1 per 10 patient-years, which was significantly higher than 4.3 per 10 patient-years for the insufficient and sufficient vitamin D groups ($P < 0.05$).⁴ They concluded that higher vitamin D levels in children with CF were associated with lower rates of pulmonary exacerbations.⁴ The mechanism by which vitamin D may prevent pulmonary exacerbations has been related to protection from viral infections which account for significant cause of pulmonary exacerbations in children with CF.^{11,12,16} This is important in the management of CF, wherein pulmonary exacerbations at various ages have been shown to negatively impact lung function, increase mortality, and decrease quality of life.²³

We reported significantly lower vitamin D levels in patients with exacerbation and bacterial colonization, as compared to vitamin D levels taken during routine follow-up. This observation suggests that vitamin D deficiency in CF may be a predisposing state for bacterial colonization and exacerbation. To the best of our knowledge, this is the first study that reports a relationship between vitamin D and markers of CF-related pulmonary disease in patients receiving daily vitamin D supplementation. Simoneau et al observed that *Pseudomonas aeruginosa* was a more common pathogen in the patients who were vitamin D-insufficient/deficient as compared to patients with sufficient vitamin D status (18 of 63 vs. 11 of 85, $P = 0.018$).⁹ The possible explanation for this association is the observation that patients with low serum 25 (OH)D levels have higher levels of airway vitamin D-binding protein, which is expressed by inflammatory cells which play an important role in CF-related inflammation.^{9,24} Further, vitamin D has been shown to decrease B-cell proliferation, plasma-cell differentiation and immunoglobulin secretion, which has a moderating influence on inflammatory response in the airways that otherwise plays a central role in the progression of lung damage in CF.²⁵

Few authors have studied the cross-sectional and longitudinal relationship between vitamin D status and lung function in CF.⁴ We observed a significant difference in FEV1 between vitamin-deficient and -sufficient groups [0.75(0.717–0.777) versus 0.82(0.74–0.92) $p < 0.05$]. Although most studies observed no association between vitamin D deficiency and pulmonary function, two studies reported a significant positive linear relationship between vitamin D and FEV1 in adolescent age groups.^{4,26} However, the median age in our study patients was 60(36–72) months and only two patients were above 10 years of age. There is a possibility that this observation may be skewed as vitamin D deficiency in our study was most prevalent in younger age groups, as opposed to most studies reporting higher deficiency rates with increasing age.⁴

Our study has some limitations. First, this was a retrospective study from a single center with a small sample size. Second, owing to the smaller patient sample size adjustment for various variables like sex, vitamin D therapy, anthropometry, seasonal variations in vitamin D levels and other similar confounders could not be determined. Further, we excluded patients with sufficient pancreatic function, so our results cannot be generalized to such patients. However, our study has strengths as well. This is the first study that examines the relationship between vitamin D and various markers of CF pulmonary disease in patients receiving routine vitamin D supplements.

We concluded that vitamin D deficiency was highly prevalent in children with CF, even after routine daily supplementation in diet. Further, vitamin D deficiency is associated with a higher rate of pulmonary exacerbations and higher incidence of pulmonary bacterial colonization than those with sufficient vitamin D levels. In addition, in younger patients with CF, lower vitamin D levels were associated with lower FEV1. This study proposes a possible immunomodulatory role of vitamin D in mitigating progression of lung disease in CF. Therefore, extends the possibility that treatment with vitamin D in doses higher than routine daily supplementation may preserve lung function and improve quality of life in children with CF.

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Figures:

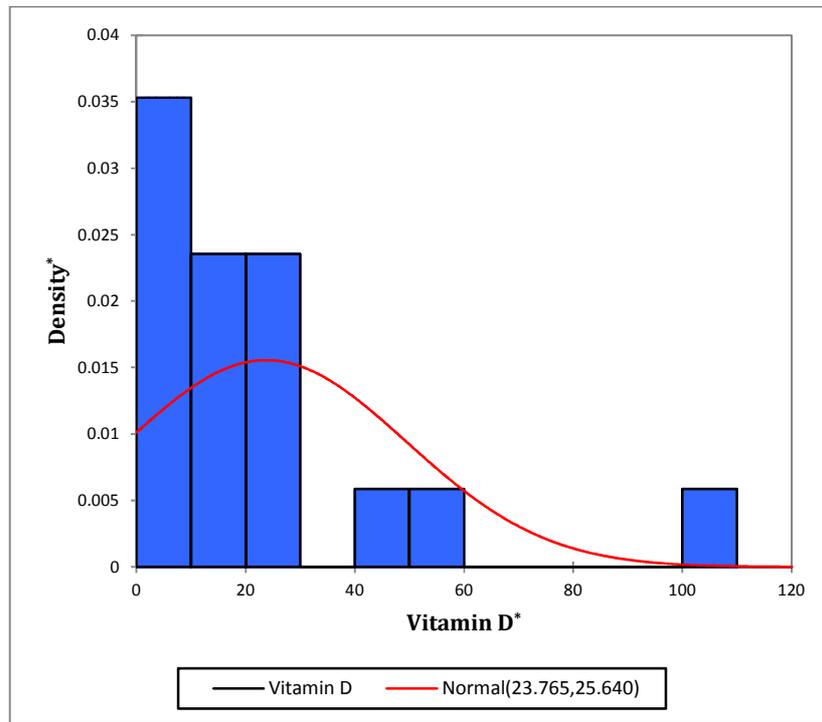


Figure 1 Histogram showing distribution of patients based on serum vitamin D levels with a positively skewed distribution curve.

*patient number density versus serum vitamin-D levels

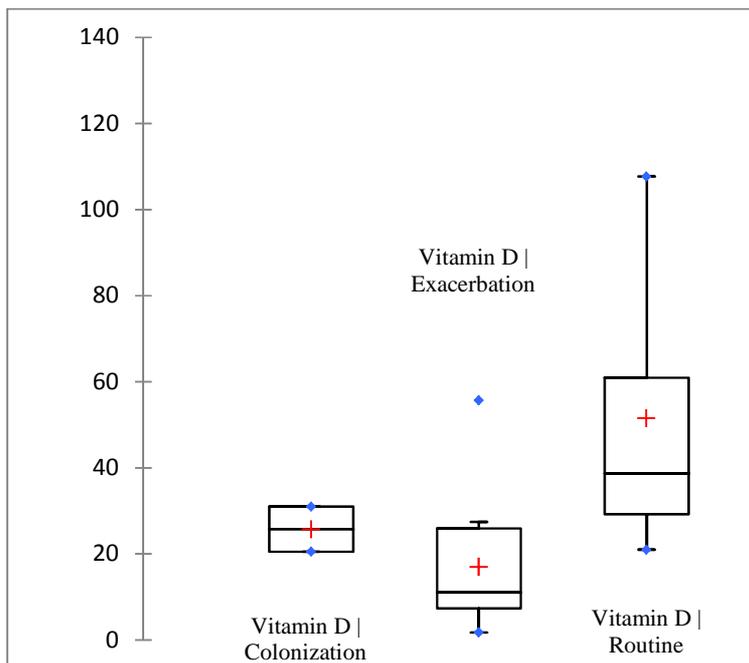


Figure 2 Box-plot comparison of vitamin D levels during exacerbations, colonization and during routine follow-up.

Tables:**Table 1** Patient characteristics compared by serum vitamin D levels.

	All subjects	Vitamin D deficient	Vitamin D insufficient	Vitamin D sufficient	P value
Number(%)	51(100%)	24(47)	8(15.7)	19(37.3)	-
Age, months, median(IQR)	60(36–72)	72(54–96)	42(24–46.5)	60(36–72)	0.17
Male sex, n(%)	27(52.9)	14(58.3)	3(37.5)	10(52.6)	0.84
Weight, kg, median(IQR)	15(12–17)	15(13.75–20)	12.6(10.2–14.5)	16(11–17)	0.38
Height,cm, median(IQR)	97.5(87.4–102.2)	98.2(93.2–104.4)	89.5(80.2–92.5)	101(88.2–104.5)	0.22
Age at diagnosis, months, median(IQR)	36(12–48)	48(30.75–63)	36(10–39)	24(12–24)	0.009
Sweat chloride, mmol/l, median(IQR)	80(63–100)	79(53.5–103.3)	66(65.25–97)	85(75–109)	0.64
Exacerbations ^a , n, median(IQR)	1(0–2)	1(1–3.25)	1(1–1.5)	0(0–1)	<0.001
Pancreatic enzyme replacement, IU/kg, median(IQR)	2000(1810–4000)	3000(1538–4000)	3300(2000–3475)	2000(1810–5000)	0.90

a-number of exacerbations in preceding one year

IQR-interquartile range

Table 2 Multivariate odds ratio of risk factors for vitamin D deficiency.

Variable	OR ^a	95% CI	P value
Age	0.937	0.901–0.975	0.001
Female sex	1.77	0.22–14.61	0.002
Age at diagnosis	1.035	1.005–1.066	0.022
Number of exacerbations	5.12	1.28–20.50	0.021
Bacterial colonization	2.9	0.57–14.82	0.011

OR-odds ratio; CI-confidence interval

a-Model fit was assessed using Hosmer-Lemeshow test, where the null hypothesis that the model fit the data was accepted for $p > 0.05$.