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Vitamin D levels in asthmatic patients with and without allergic bronchopulmonary aspergillosis

[Running title: Vitamin D levels in ABPA]

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ABSTRACT

Vitamin D deficiency is believed to a pathogenetic factor in patients with allergic bronchopulmonary aspergillosis (ABPA) and cystic fibrosis. Whether vitamin D deficiency is also prevalent in ABPA complicating asthma, remains unknown. Herein, we evaluate vitamin D levels in asthmatic patients with and without ABPA.

In a prospective study, plasma vitamin D (25[OH]D) levels were measured in consecutive subjects with asthma (n=75), ABPA (n=158) and healthy volunteers (n=50). Vitamin D levels <20 ng/mL were considered as vitamin D deficiency.

There was no difference in mean (95% CI) vitamin D levels between healthy controls (15.3 [12.7-17.9]), asthmatics (19.2 [16.3-22.1]) and subjects with ABPA (18.9 [16.9-20.8]) (p=0.22). Vitamin D deficiency was encountered in 70%, 64% and 65% of the healthy controls, asthmatics and ABPA subjects, respectively, and was not different between the groups (p=0.79). There was no difference in the asthma control, pulmonary function, immunological findings and the severity of bronchiectasis, in patients with ABPA, with and without vitamin D deficiency.

Vitamin D deficiency is equally prevalent in asthmatic patients with or without ABPA in the Indian subcontinent, and does not appear to play a major role in the pathogenesis of ABPA complicating asthma.

Key-words: allergic bronchopulmonary aspergillosis; aspergillus; abpm; allergic bronchopulmonary mycosis; asthma; allergy

INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is an immunological pulmonary disorder caused by hypersensitivity reactions mounted against *Aspergillus fumigatus* colonizing the tracheobronchial tree of patients with asthma and cystic fibrosis (CF).[1] The condition clinically presents with poorly controlled asthma, recurrent pulmonary opacities and bronchiectasis. The disorder has a worldwide presence, and the global burden of ABPA complicating asthma has been estimated to be about 5 million cases, with about 1.4 million cases in India alone.[2, 3] The prevalence of ABPA is as high as 13% in the special asthma or chest clinics.[4] However, despite six decades of research, the pathogenesis of ABPA remains poorly understood.

The normal response of human host against *A.fumigatus* is a Th1 response, however ABPA is characterized by a heightened Th2 response against *A.fumigatus*.[5] This Th2 response is unable to clear the fungi, however the resultant exuberant inflammation leads to the characteristic immunological and pathological changes associated with this condition.[6] Recently, it has been shown that peripheral CD11c+, thymic stromal lymphopoietin-activated dendritic cells from patients with ABPA and CF induce robust Th2 cytokine responses from CD4+ T-cells; and, in vitro treatment with vitamin D blunted these responses.[7, 8] Moreover, vitamin D levels have been shown to be reduced in patients with ABPA complicating CF.[7, 9] Thus, it is possible that vitamin D deficiency may be one of the pathogenetic factors in the causation of ABPA complicating CF. Patients with CF have malabsorption that may predispose to vitamin D deficiency, which is not seen in asthmatic patients. Whether vitamin D deficiency is also prevalent in ABPA complicating asthma remains unknown. In this study, we evaluated vitamin D (25[OH]D) levels in asthmatic patients with and without ABPA.

MATERIAL & METHODS

This was a prospective study conducted between 1st January 2015 and 31st December 2016 in the chest clinic of this Institute. Our Institute is a 1500-bedded tertiary care referral center with a huge catchment area of about 75 million cases (www.pgimer.edu.in). The outpatient attendance is about 4600 patients every day, and the chest clinic caters to about 800 patients every week. The study protocol was approved by the Institute Ethics Committee and a written informed consent was obtained from all the study subjects.

Patients

The study included consecutive subjects with ABPA and asthma along with healthy volunteers. The diagnosis of asthma was made if the subjects met two of the following clinical criteria: (a) history of recurrent attacks of chest tightness, breathlessness and cough; (b) evidence of wheeze on auscultation of the chest; and, (c) obstructive defect on spirometry with presence of bronchodilator reversibility documented at any point in their illness. *A.fumigatus*-sensitized asthma was diagnosed if the subject met all the following criteria: (a) asthma; (b) *A.fumigatus* specific IgE level >0.35 kUA/L; and (c) total IgE level <1000 IU/mL.[10]

ABPA was diagnosed by a multidisciplinary team (pulmonary physicians, radiologist, microbiologist) according to the ISHAM working group consensus criteria.[1] The subject should meet all the following criteria: (a) asthma; (b) serum *A.fumigatus*-specific IgE >0.35 kilounit of antibody [kUA]/L) or immediate cutaneous hyperreactivity to *A.fumigatus* antigen; (c) serum total IgE levels >1000 IU/mL; and, two of the following criteria: (a) presence of precipitating antibodies (by double diffusion method) against *A.fumigatus* in serum; (b) total eosinophil count >500 cells/µL; and, (c) radiographic pulmonary opacities consistent with ABPA. Subjects were excluded if they met any of the following criteria: (a) treatment with oral vitamin D within the last twelve weeks; (b) intake of systemic glucocorticoids within the last four weeks; (c) immunosuppressive conditions

including uncontrolled diabetes mellitus, chronic renal failure, chronic liver failure, cytotoxic therapy and others; (e) pregnancy; and, (e) failure to provide informed consent.

The controls were otherwise asymptomatic healthy volunteers with normal physical examination. The controls were recruited from the attendants accompanying the patients in the outpatient department of our Institute.

Methods

IgE (total and *A.fumigatus*-specific): The total IgE and *A.fumigatus*-specific IgE concentrations in serum were measured using the commercial ImmunoCAP system (Phadia 100, Phadia, Uppsala, Sweden) that utilizes the automated fluorescent enzyme immunoassay technology.[11]

Aspergillus skin test: was performed intradermally by injecting 0.2 mL of 100 protein nitrogen units (PNU)/mL of the *Aspergillus* antigen (1 PNU = 0.00001 mg/mL) in the forearm, as previously described.[12]

Aspergillus fumigatus precipitins: were detected using the Ouchterlony gel double-diffusion technique.[13]

Total eosinophil count: The total leucocyte count was determined using an auto-analyzer (LH-750, Beckman Coulter). The differential white cell count was measured by counting and classifying 100 white blood cells on a peripheral blood smear. The total eosinophil count was obtained by multiplying the percentage with the total leucocyte count.

Pulmonary function test: was performed on a dry rolling seal spirometer to determine the lung function measurements and bronchodilator reversibility, as previously described.[14]

High-resolution computed tomography (HRCT) of the chest: was performed using a multidetector CT scanner with a matrix size of 512x512.[15, 16] The diagnosis of bronchiectasis and high-attenuation mucus was made on HRCT of the chest using previously described criteria.[17] Subjects meeting all

the criteria for ABPA but without the presence of bronchiectasis were labeled as serologic ABPA (ABPA-S).

Asthma control questionnaire (ACQ) 7: Asthma control was assessed using the ACQ7, which scores the patient's symptoms (nocturnal awakening, symptoms on waking, limitation of activity, breathlessness and wheeze) and bronchodilator use (rescue short-acting β2-agonist use) in the previous week on a seven-point scale (0-6, no impairment to maximum impairment). The forced expiratory volume in the first second (FEV1, percentage predicted pre-bronchodilator) is also scored on a seven-point scale. The ACQ7 score is the mean of the seven items, and ranges between 0 (fully controlled) and 6 (severely uncontrolled).[18]

Vitamin D levels: Total plasma 25-hydroxyvitamin D [25(OH)D] levels were estimated by a competitive electrochemiluminescence immunoassay on a fully automated analyzer (E 601, Roche Diagnostics GmbH, Mannheim, Germany) using kits, calibrators, and controls from the same manufacturer. The minimum detection limit of the assay used is 3.0 ng/mL with inter-assay and intra-assay coefficient of variation being 13.1% and 6.8%, respectively. Vitamin D levels ≥30 ng/mL, 21-29 ng/mL and <20 ng/mL were considered as normal status, vitamin D insufficiency and vitamin D deficiency, respectively.[19]

Statistical analysis: Statistical analysis was performed using the statistical package SPSS for MS Windows (version 22; IBM SPSS Inc; Chicago, IL). Data are presented in a descriptive fashion as mean with 95% confidence intervals (CI) or number (percentage). The differences between categorical variables were analyzed using chi-square test (or Fisher's exact test) while the differences between continuous variables were analyzed using the Mann-Whitney U test or Kruskal-Wallis test, as applicable. The relationship between vitamin D levels and immunological tests (total IgE, *A.fumigatus* specific IgE and eosinophil counts) was analyzed using Spearman's correlation. Statistical significance was assumed at a p-value of <0.05.

RESULTS

During the study period, 188 and 108 subjects with ABPA and asthma were screened for inclusion in the study. Finally, 158 subjects with ABPA, 75 asthmatics and 50 healthy controls were enrolled. The baseline characteristics of the study population are shown in Table 1. The mean age of the study population was 37.7 (95% CI, 36.1-39.4) years. Subjects with ABPA had significantly lower body mass index compared to those with asthma. The other demographic characteristics and lung function were similar in the two groups. The serum total and *A.fumigatus*-specific IgE were significantly higher in ABPA subjects compared to asthma. Bronchiectasis and high-attenuation mucus were observed in 89% and 33% of the study population, respectively. HRCT chest revealed no bronchiectasis in 18 subjects, and these subjects were labeled as ABPA-S. *A.fumigatus* sensitized asthma was diagnosed in 13 of the 75 subjects with asthma.

There was no difference in vitamin D (25[OH]D) levels between healthy controls, asthmatics (including *A.fumigatus* sensitized asthma subjects) and subjects with ABPA (Figure 1 and Table 2). Vitamin D deficiency was encountered in 70%, 64% and 65% of the healthy controls, asthmatics and ABPA subjects, respectively and was not different between the three groups (p=0.79). There was no difference in the asthma control (ACQ7 scores), pulmonary function, immunological findings and findings on HRCT chest, in patients with ABPA, with and without vitamin D deficiency (Table 3). There was no significant correlation between vitamin D levels and total IgE (r=-0.093, p=0.25), *A.fumigatus* specific IgE (r=-0.071, p=0.37) and total eosinophil counts (r=0.102, p=0.22) in subjects with ABPA.

DISCUSSION

The results of the study suggest that vitamin D (25[OH]D) deficiency is equally prevalent in asthmatic patients with or without ABPA. We found no relationship between vitamin D deficiency and asthma control, lung function, immunological severity and extent of bronchiectasis in patients with ABPA.

Vitamin D has multitude of effects on the immune system, and vitamin D receptor has been shown to be present on peripheral blood mononuclear cells, dendritic cells and activated T lymphocytes.[20] Vitamin D also has multiple cytokine modulating effects, and it helps in blunting the Th2 response, thereby linking vitamin D deficiency with allergy.[20] Findings from several previous studies support a relationship between vitamin D status and asthma prevalence.[21] A recent Cochrane review also found that vitamin D supplementation could reduce the risk of asthma exacerbations.[22]

Only a few studies have investigated the association between vitamin D and ABPA. In one study, vitamin D levels were found to be low in patients with ABPA-complicating CF.[7] The same study also found that lower serum vitamin D levels correlated with enhanced Th2 reactivity in subjects with ABPA.[7] Interestingly, in vitro addition of calcitriol substantially reduced the expression of OX40L on dendritic cells while increasing the expression of TGF-β. Further, it also resulted in increased expression of TGF-β on regulatory T cells and reduced Th2 responses by CD4+ T cells.[7] Another unpublished study (conference abstract) found that not only the vitamin D levels were lower in patients with ABPA and CF but also there was a significant negative correlation between *A.fumigatus*-specific IgE and vitamin D levels.[23] In a recent study, supplementation with vitamin D (4000 IU daily for 6 months) blunted *Aspergillus*-induced IL-13 responses from peripheral CD4+ T cells and reduced *Aspergillus*-specific IgE levels, in patients with ABPA complicating CF.[9]

Only one unpublished study (conference abstract) has reported the association between vitamin D levels and ABPA complicating asthma.[24] The study found vitamin D deficiency in 44% of

the total cohort of asthma and ABPA, with no significant difference in vitamin D levels in asthmatic patients with and without ABPA.[24] The present study population had a higher prevalence of vitamin D deficiency (65%), similar to previous studies.[25] However, no difference was found in vitamin D levels between asthmatic patients with and without ABPA. Chishimba et al. previously reported FEV1 to be significantly lower in patients with vitamin D deficiency,[24] which was not observed in the current study. Further, our study found no relationship between vitamin D levels and immunological or radiological severity.

Finally, our study has a few limitations. This is a single center study with a small sample size. We also did not investigate the cytokine responses to *A.fumigatus* in patients with ABPA with and without vitamin D deficiency. This is probably a more sensitive measure than lung function and other immunological parameters such as IgE (total and *A.fumigatus* specific) and eosinophil counts in assessing the functional effects of vitamin D deficiency in ABPA. Also, given the high prevalence of vitamin D deficiency in the general population, a large cohort (3700 subjects) would be required, to have a 90% chance of detecting as significant at the 5% level, a 5% difference in prevalence of vitamin D deficiency in asthmatic patients with and without ABPA. Thus, it would be better to examine whether supplementation of vitamin D improves the control of asthma with or without ABPA, to conclude the role of vitamin D deficiency in the pathogenesis of ABPA. In fact, a randomized trial evaluating the role of vitamin D in ABPA, has completed recruitment (clinical trials.gov: NCT03133299). There is very little evidence about effects of vitamin D in non-Caucasian population;[26] the association of vitamin D deficiency with ABPA-CF in the United States,[7] but not with ABPA-asthma in India, might be due to the difference in racial backgrounds. Finally, vitamin D levels would vary depending on the race, ethnicity and the geographic locale (sun exposure and other factors). Hence, the results cannot be generalized beyond India.

In conclusion, the current study found no difference in the prevalence of vitamin D deficiency in patients with ABPA compared to asthma. There was also no association between

vitamin D deficiency and clinical, spirometric, radiological and immunological severity in patients with ABPA. Thus, it appears that vitamin D deficiency does not play a major role in the pathogenesis of ABPA complicating asthma. However, given the fact that vitamin D levels could vary depending on the geographic locale, the results cannot be generalized.

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Table 1: Baseline characteristics of the study population

	ABPA (n=158)	Asthma (n=75)	Healthy controls (n=50)	P value
Demographic characteristics				
Age in years	35.9 (33.9-37.9)	39.4 (35.7-43.1)	40.8 (36.7-44.9)	0.09
Sex (M: F)	78: 80	39: 36	29: 21	0.57
BMI (kg/m^2)	21.7 (20.9-22.4)	24.6 (23.6-25.7)	22.6 (21.9-23.2)	0.0001
Clinicoradiographic features				
History of hemoptysis, n (%)	44 (27.8)	-	-	
History of expectoration of mucus plugs, n (%)	24 (15.2)	-	-	
History of prescription of anti-tuberculosis therapy, No (%)	41 (25.9)	-	-	
Fleeting opacities on chest radiograph, n (%)	68 (46.3)	-	-	
Spirometry				
FEV1 (L)	1.9 (1.8-2.0)	2.1 (1.9-2.3)	-	0.12
FVC (L)	2.7 (2.5-2.8)	2.8 (2.6-3.0)	-	0.29
FEV1/VC ratio	68.2 (66.2-70.2)	70.6 (67.9-73.4)	-	0.19
Immunological findings				
Aspergillus skin test positivity	148 (93.7)	-	-	
Total eosinophil count, (cells/μL)	1343 (1156-1529)	-	-	
Aspergillus precipitins, n (%)	39 (27.3)	-	-	
IgE levels (total), IU/mL	8841 (7710-9972)	984 (541-1427)	-	0.0001
A.fumigatus specific IgE levels, kUA/L	38.5 (27.3-49.7)	0.19 (0.13-0.25)	-	0.0001
HRCT chest findings				
Bronchiectasis, n (%)	140 (88.6)	-	-	
High-attenuation mucus, n (%)	53 (33.5)	-	-	
No. of segments involved by bronchiectasis	8.6 (7.6-9.5)	-	-	

ABPA- allergic bronchopulmonary aspergillosis; FEV1- forced expiratory volume in the first second; FVC- forced vital capacity; HRCT- high resolution computed tomography

All values are expressed as mean (95% confidence intervals) unless otherwise stated

Table 2: Vitamin D characteristics in the study population

	ABPA (n=158)	*A.fumigatus* sensitized asthma (n=13)	Asthma without *A.fumigatus* sensitization (n=62)	Healthy controls (n=50)	P value
Vitamin D levels, ng/mL	18.9 (16.9-20.8)	17.7 (9.7-25.7)	19.5 (16.3-22.7)	15.3 (12.7-17.9)	0.22
Vitamin D status, n (%)					0.79
Normal	25 (15.8)	3 (23.1)	8 (12.9)	4 (8)	
Vitamin D insufficiency	30 (19)	2 (15.4)	14 (22.6)	11 (22)	
Vitamin D deficiency	103 (65.2)	8 (61.5)	40 (64.5)	35 (70)	

All values are expressed as mean (95% confidence intervals) unless otherwise stated

Vitamin D (25[OH] D) levels ≥30 ng/mL, 21-29 ng/mL and <20 ng/mL were considered as normal status, vitamin D insufficiency and vitamin D deficiency, respectively

Table 3: Clinical and immunological characteristics of subjects with ABPA with and without vitamin D deficiency (<20 ng/mL)

	Vitamin D deficiency present (n=103)	Vitamin D deficiency absent (n=55)	P value
Body mass index (kg/m^2)	21.1 (20.2-22.0)	22.7 (21.3-24.0)	0.08
Asthma control questionnaire	3.7 (3.4-4.1)	3.3 (2.8-3.8)	0.17
Spirometry			
FEV1 (L), mean (SD)	1.9 (1.7-2.0)	1.9 (1.8-2.2)	0.21
FVC (L), mean (SD)	2.6 (2.4-2.8)	2.8 (2.5-3.0)	0.07
FEV1/FVC ratio	67.8 (64.9-70.2)	69.4 (66.2-72.6)	0.35
Immunological findings			
Aspergillus skin test positivity, n (%)	96 (93.2)	52 (94.5)	0.93
Total eosinophil count, (cells/μL)	1260 (1038-1483)	1501 (1156-1529)	0.24
Aspergillus precipitins, n (%)	27 (26.2)	12 (21.8)	0.87
IgE levels (total), IU/mL	9595 (8086-11104)	7455 (5843-9067)	0.13
IgE levels (*Af*), kUA/L	44.5 (27.7-61.3)	27.0 (21.9-32.2)	0.09
HRCT chest findings			
High-attenuation mucus, n (%)	36 (34.9)	17 (30.9)	0.96
No. of segments involved by bronchiectasis	8.9 (7.8-10.1)	7.8 (6.3-9.4)	0.28

FEV1- forced expiratory volume in the first second; FVC- forced vital capacity; HRCT- high resolution computed tomography

All values are expressed as mean (95% confidence intervals) unless otherwise stated

LEGEND TO FIGURES

Figure 1: Box and whisker plots showing vitamin D levels in healthy controls and subjects with asthma, *A.fumigatus*-sensitized asthma and allergic bronchopulmonary aspergillosis. Box plots represent the 25th and 75th percentiles, with the internal horizontal lines showing the median and T bars showing the 10th and 90th percentiles. The vitamin D levels were not different between the four groups (p=0.22)

REFERENCES

1 Agarwal R, Chakrabarti A, Shah A, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013;43: 850-73.

2 Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmonary aspergillosis with asthma and its complication chronic pulmonary aspergillosis in adults. *Med Mycol* 2013;51: 361-70.

3 Agarwal R, Denning DW, Chakrabarti A. Estimation of the burden of chronic and allergic pulmonary aspergillosis in India. *PLoS One* 2014;9: e114745.

4 Agarwal R, Aggarwal AN, Gupta D, Jindal SK. *Aspergillus* hypersensitivity and allergic bronchopulmonary aspergillosis in patients with bronchial asthma: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2009;13: 936-44.

5 Tracy M, Okorie C, Foley E, Moss R. Allergic Bronchopulmonary Aspergillosis. *Journal of Fungi* 2016;2: 17.

6 Moss RB. Pathophysiology and immunology of allergic bronchopulmonary aspergillosis. *Med Mycol* 2005;43 Suppl 1: S203-6.

7 Kreindler JL, Steele C, Nguyen N, et al. Vitamin D3 attenuates Th2 responses to Aspergillus fumigatus mounted by CD4+ T cells from cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Clin Invest* 2010;120: 3242-54.

8 Nguyen NL, Chen K, McAleer J, Kolls JK. Vitamin D regulation of OX40 ligand in immune responses to Aspergillus fumigatus. *Infect Immun* 2013;81: 1510-9.

9 Nguyen NL, Pilewski JM, Celedon JC, et al. Vitamin D supplementation decreases Aspergillus fumigatus specific Th2 responses in CF patients with aspergillus sensitization: a phase one open-label study. *Asthma Res Pract* 2015;1.

10 Agarwal R. Severe asthma with fungal sensitization. *Curr Allergy Asthma Rep* 2011;11: 403-13.

11 Agarwal R, Aggarwal AN, Sehgal IS, Dhooria S, Behera D, Chakrabarti A. Utility of IgE (total and Aspergillus fumigatus specific) in monitoring for response and exacerbations in allergic bronchopulmonary aspergillosis. *Mycoses* 2016;59: 1-6.

12 Agarwal R, Noel V, Aggarwal AN, Gupta D, Chakrabarti A. Clinical significance of Aspergillus sensitisation in bronchial asthma. *Mycoses* 2011;54: e531-8.

13 Agarwal R, Dua D, Choudhary H, et al. Role of Aspergillus fumigatus-specific IgG in diagnosis and monitoring treatment response in allergic bronchopulmonary aspergillosis. *Mycoses* 2017;60: 33-9.

14 Aggarwal AN, Gupta D, Jindal SK. Development of a simple computer program for spirometry interpretation. *J Assoc Physicians India* 2002;50: 567-70.

15 Agarwal R, Gupta D, Aggarwal AN, Behera D, Jindal SK. Allergic bronchopulmonary aspergillosis: lessons from 126 patients attending a chest clinic in north India. *Chest* 2006;130: 442-8.

16 Agarwal R, Gupta D, Aggarwal AN, Saxena AK, Chakrabarti A, Jindal SK. Clinical significance of hyperattenuating mucoid impaction in allergic bronchopulmonary aspergillosis: an analysis of 155 patients. *Chest* 2007;132: 1183-90.

17 Agarwal R, Khan A, Garg M, Aggarwal AN, Gupta D. Pictorial essay: Allergic bronchopulmonary aspergillosis. *Indian J Radiol Imaging* 2011;21: 242-52.

18 Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. *Respir Med* 2006;100: 616-21.

19 Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96: 1911-30.

20 Searing DA, Leung DY. Vitamin D in atopic dermatitis, asthma and allergic diseases. *Immunol Allergy Clin North Am* 2010;30: 397-409.

21 Hall SC, Agrawal DK. Vitamin D and Bronchial Asthma: An Overview of Data From the Past 5 Years. *Clin Ther* 2017;39: 917-29.

22 Martineau AR, Cates CJ, Urashima M, et al. Vitamin D for the management of asthma. *Cochrane Database Syst Rev* 2016;9: Cd011511.

23 Green C, Lim AYH. Vitamin D level in cystic fibrosis patients with allergic bronchopulmonary aspergillosis and aspergillus sensitization. *Eur Respir J* 2013;42: P1170.

24 Chishimba L, Zhang W, Andrew Mochridge AM, Niven RM, Al-Shar K, Denning D. Vitamin D deficiency in asthma patients with allergic bronchopulmonary aspergillosis (ABPA). *Eur Respir J* 2013;42: P4178.

25 Gupta R, Gupta A. Vitamin D Deficiency in India: Prevalence, Causalities and Interventions. *Nutrients* 2014;6: 729-75.

26 Powe CE, Evans MK, Wenger J, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med* 2013;369: 1991-2000.