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CHRONIC ASTHMA IS ASSOCIATED WITH LOW LEVELS OF VITAMIN D

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ABSTRACT

Introduction: asthmatic patients may be at increased risk for vitamin D deficiency, but risk factors for deficiency among these patients have not been extensively reported. **Methods:** Serum 25(OH)D was estimated by enhanced chemiluminescent assay in subjects aged 20-60 years from Burdwan Medical College, Burdwan, West Bengal, India, including 552 asthmatic patients and 521 controls. Levels 20 ng/ml defined deficiency. Season, sex, age, diets, body complexion, body mass index (BMI), smoking, comorbidities (Charlson score), were examined for associations with 25(OH)D in both linear and logistic regression models. **Results:** Asthmatic patients had an increased risk for vitamin D deficiency compared to controls after adjustment for age, smoking and BMI, season, diet, skin complexion. Variables associated with lower 25(OH)D levels in adsthmatic patients were obesity (-6.65), current smoking (-4.09), Vegetarian (-3.94), dark skin complexion (-1.58) and depression (-3.26). Summertime decreased the risk of vitamin D deficiency (OR = 0.24). Conclusion: Asthma was associated with an increased risk of vitamin D deficiency even after adjustment for confounding factors that are responsible for lowering vitamin D.

Key Word: Asthma, 25 (OH) D, Confounding factors.

1. INTRODUCTION

Asthma represents one of the most common chronic diseases and is a major public health problem worldwide. [1] In the majority of patients control of asthma as defined by guidelines can be achieved with long-term maintenance medications. [1] However, a substantial proportion of patients do not achieve optimal asthma control despite even high dose

treatment. In particular inadequately controlled patients with severe persistent asthma are at high risk of severe exacerbations and asthma-related mortality. These patients represent the greatest unmet medical need among the asthmatic population today.

Several recent studies in different populations have associated a deficiency in vitamin D with increased risk of asthma.[2-6] However, these findings cannot be considered conclusive since the association may be confounded by important variables, such as smoking and sunlight exposure, which were not accounted for in the analysis. So, present study was conducted to compare levels of vitamin D in asthmatic patients and age and sex-matched controls and to investigate the association between important confounding factors with plasma circulation level of vitamin D in asthma.

As 25 – hydroxyvitamin D (25-OHD) is the principal circulating vitamin D metabolite and recognised as the best short-term biomarker of total exposure to vitamin D, [7] 25-OHP was measured in asthmatic patients to detect vitamin D status.

2. MATERIAL AND METHODS

2.1 Study area

The present study was conducted in the department of Biochemistry with the collaboration of department of Medicine of Burdwan Medical College, Burdwan, West Bengal, India.

2.2 Selection of subjects

A total of 622 from 1846 patients aged 18-60 years who had been diagnosed with asthma who attended Burdwan Medical College of Burdwan district were selected as case and 617 subjects having no asthma as age and sex-matched control by simple random sampling after informed consent had been received between February 2011 and October 2013. The subjects were 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 [8]. They were divided into Mild intermittent, Mild persistent, Moderate persistent and severe persistent using same guideline. Based on clinical history taking and clinical records, patients with human immunodeficiency virus (HIV) infection, hepatic disease, renal failure, malignancy, diabetes mellitus, pregnancy, sarcoidosis, hyperparathyroidism or those taking any corticosteroids, immunosuppressive agents, thiazide

diuretics or drugs known to interfere with vitamin D levels (phenytoin, phenobarbital, carbamazepine, theophylline) were excluded from the study. Asthma diagnosis was made based on the patients symptoms plus objective evidence from pulmonary function tests according to the criteria defined by the American thoracic Society in 1987.

2.4 Pulmonary function tests

Pulmonary function was measured both pre-and post-inhalation of 0.4 mg salbutamol, on a spirometer (HELIOS 401) by trained study staff. As the FEV_1 is the most reproducible lung function test parameter therefore is best adopted to assessment of large group of people. Spirometric values were post-bronchodilator measurements, and absolute values were expressed as percentage predicted of reference values.[9]

2.5Anthropometric measurements

Weight and height measurements were obtained, using standardized technique.[10] BMI was calculated as the weight in kilograms divided by the square of height in meters. Body mass index (BMI) was calculated as the weight (kg) divided by the square of height (m^2), and was categorized as underweight (BMI < 18.5), normal (BMI 18.5–24.99), overweight (BMI 25.0–29.99), and obese (BMI 30.0 or more) according to the current World Health Organization (WHO) classification.

2.6 Categorization of Comorbidities

Comorbidities were categorized using the Charlson Comorbidity Index (CCI).[11]

2.7 Measurement of depression symptomatology

Depressive symptomatology was measured using the Centre for Epidemiologic Studies Depression Scale (CES-D), where a positive score of 16 or more defined depression.[12]

2.8 Collection of samples

Peripheral venous blood was drawn and allowed to coagulate at room temperature for 30–45 min, followed by centrifugation at 2500Xg for 15 min. All serum samples were stored at -70^0C and kept under these conditions until chemical analysis was performed.

2.9 Parameters assay

Serum 25(OH)D was estimated by enhanced chemiluminescent assay (ECI) using instrument VITROS *eci* (Johnson &Johnson) & dedicated reagent.[13]

2.10 Statistical analysis

The data for biochemical analysis was subjected to standard statistical analysis using the Statistical Package for Social Science (SPSS) 11.5 software for windows. Serum concentrations of 25(OH)D were normally distributed. Differences in mean 25(OH)D by bivariate predictors were examined with parametrical tests. We analysed the relation between study category and levels of 25(OH)D both by linear and logistic regression after adjusting for possible confounders: age, sex, diet, skin complexion, season, BMI, smoking, and comorbidities. A cut off level for vitamin D deficiency were defined as 20 ng/ml for the logistic model. When building the regression models for the levels of vitamin D among tuberculosis patients only, a backward stepwise method was used for both the linear and logistic models. The following variables were included from the start: Age, sex, BMI, comorbidity. Variables remained in the model if their significance level were less than 0.10. After the first run, all excluded variables were reintroduced one at a time and retained in the final model if their significance level were less than 0.10.

3. RESULT

3.1 The characteristics of the study population are shown in Table 1.

Personal profile and clinical details of the asthmatic patients and age, sex-matched control population are shown in Table 1.Unpaired t-test was performed to compare the mean of individual demographic profile.

Table 1 Personal profile and clinical details of healthy persons and patients suffering from asthma

Demographic profiles	Controls (n = 521)	Patients with asthma (n = 552)	p
Age (Years)	62.68±5.33	62.4±5.64	NS
Sex Male (%) Female (%)	 297 (57) 225 (43)	 319 (58) 233 (42)	NS
BMI (Kg/m^2) < 18.5 18.5 – 24.9 25-30 > 30	26.40±3.45 5(1) 245(47) 193(37) 78(15)	22.62±3.51 22(4) 315(57) 160(29) 55(10)	S
Smoking habits Never Ex-smoker Current Smoker	 115(22) 177(34) 229(44)	 0(0) 259(47) 293(53)	S

Pack-years ŧ Quit-years ŧ	39(30-52) 1(0-8)	47(33-63) 2(0-8)	
Comorbidity, CCI score			S
1	67	0	
2	17	53	
3	9	21	
4	7	26	
Depression, CES-D score ≥16			S
Yes	5	19	
Season[#]			
Winter	55	52	NS
Spring	21	23	
Summer	16	15	
Autumn	8	10	
Pulmonary function tests			
FEV1*	72.73±3.19	50.92 ±11.56	
Dietary habits			S
Vegetarian	286(54.89)	291(52.72)	NS
Non-vegetarian	235(45.11)	261(47.28)	
Skin complexion			
Clear	77	85	NS
Fair	181	198	
Black	263	269	

Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values ± SD for continuous variables. When variables were not normally distributed, median values (Q1-Q3 IQR in parentheses) are given instead (indicated by ŧ); IQR means Interquartile range.

Pack-years were expressed as the numbers of packs of cigarette smoked per day X the number of years the person has smoked.

Ex-smokers are those who have stopped smoking at least 1 year.

Quit-years were the number of years since a patient stopped smoking.

[#]Seasonal variation was defined as winter (December-March), spring (April-May), summer (June-September), and autumn (October–November).

*FEV1: Forced expiratory volume in 1 sec that is expressed in % of VC.

NS=p value is not significant; S=p value is statistically significant ($p < 0.05$)

3.2 Distribution of concentration of serum 25(OH)D among study population

Among all the asthmatic patients studied, the median serum 25-hydroxyvitamin D level was 26 ng/ml. Based on changes in parathyroid hormone levels and intestinal calcium transport

that have been noted that values of 20-30 ng/ml are considered vitamin D relative insufficient,[14] deficient serum levels are < 20ng/ml,[13] desirable circulating vitamin D level (OH)D) is 30 to 40 ng/ml.[14-16] But there are suggestions levels higher than 40 ng/ml may be necessary for optimal immune functioning and overall health. [17-19] In present study almost 53% of the tubercular patients studied had vitamin D levels in the insufficient range, whereas near-about 9% was vitamin D deficient as shown in the Figure 1.

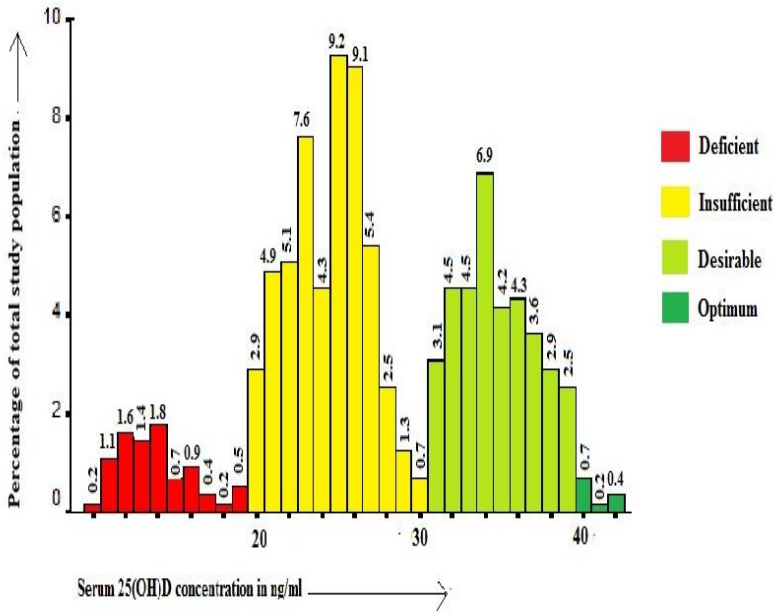

Figure 1 Frequency distribution of serum 25(OH)D concentration in study population suffering from asthma.

3.3 Factors associated with 25(OH)D in asthmatic patients and controls - bivariate analysis

Concentrations of 25(OH)D among asthmatic patients and controls for different explanatory variables are shown in Table 2. During unadjusted state, mean 25-OHD levels was differed significantly between patients with asthma and healthy controls (27.36±6.84ng/ml in cases vs 35.25±3.5ng/ml in controls; $p < 0.001$). Among the cases and controls, obesity and underweight, FEV1, seasonal variation, or depression all factors were associated with significantly lower levels of serum 25(OH)D.

Table 2. Serum concentration of 25(OH)D in ng/ml, for different potential explanatory variables in patients suffering from asthma and controls

Baseline characteristics of the study sample	Control		Asthma	
	Mean±SD [25(OH)D in ng/ml]	p value	Mean±SD [25(OH)D in ng/ml]	p value
	35.25±3.5		27.36±6.84	
Sex				
Male	35±3.12	0.43	27 ±6.89	0.35
Female	35.60±3.97		27.85±6.76	
BMI(Kg/m^2)				
< 18.5	33.56±2.67		20.7±9.8	
18.5-24.9	40.86±5.54	0.245	31.87±7.93	0.001
25-30	35.46±8.94		33.93±6.31	
>30	30.67±5.93		26.21±8.75	
Smoking habits				
Never	41.56±9.28	0.014		0.012
Current smoker	28.84±7.58		25.23±9.56	
Ex-smoker	33.13±10.48		28.73±10.26	
FEV1	34.84 ± 5.52		27.28±9.91*	
Depression				
Yes			25.97±9.96	0.019
No			29.32±7.32	
Season				
Winter	33.61±9.25		23.45±6.29	
Spring	37.36±5.37	0.017	20.28±8.16	0.001
Summer	39.37±6.26		27.95±9.74	
Autumn	34.62±8.47		21.54±8.35	
Comorbidity				
< 2			28.85±11.34	0.04
≥ 2			26.59±7.36	
Food habits				
Vegetarian	27.95±3.23	0.002	27.23±3.98	0.009
Non-vegetarian	32.78±4.78		30.27±3.84	
Complexion				
Clear	33±7.21	0.037	31.89±6.23	0.043
Fair	31.56±6.34		29.45±4.35	
Black	27.97±3.72		27.02±3.76	

*p<0.05(Statistically significant)

Current smoking and co-morbidity were significantly associated with lower levels of serum 25(OH)D in both groups. High BMI and under-weight both were significantly negative

correlated with serum vitamin D concentration in both cases and controls as shown in the Table 3.

Table 3 Bivarient correlation between continuous baseline predictors and baseline serum concentration of 25(OH)D in asthma and control

Variables of 25(OH)D	Control		Asthma	
	Pearson's correlation	p	Pearson's correlation	p
Body complexion	0.765	0.032	0.671	0.011
Dietary habits	0.964	0.018	0.817	0.007
FEV1	0.064	0.276	0.966	0.016
BMI				
< 18.5	-0.375	0.034	-0.805	0.038
18.5-24.9	0.071	0.086	0.14	0.092
25-30	0.052	0.181	0.10	0.121
>30	-0.25	0.011	-0.842	0.041
Co-morbidies			0.456	0.017
Smoking	-0.957	0.013	-0.924	0.024
Depression (CES-D score ≥ 16			-0.948	0.018

p<0.05(Statistically significant)

3.3 Difference in Estimated Risk for Vitamin D Deficiency: Asthma vs. Controls

To difference in estimated risk for Vitamin D deficiency of asthma vs. controls, it was found that astham was associated with lower levels of 25(OH)D (Coefficient = 4.34, p< 0.001) and a more than doubled risk of being categorised as deficient (OR =2.32, p= 0.001), after adjustment for sex, age, BMI, smoking, comorbidities and season (Table 4).

Table 4 Regression coefficients for the relationship between subject status and serum levels of 25(OH)D, adjusted for sex, age, BMI, smoking, comorbidities and season.

	25(OH)D †			**25(OH)D* (<20 ng/ml)**		
Study category	Coefficient	CI	p	OR	CI	p
Controls	0			1		
Asthma	-4.34	-6.31, -2.37)	<0.001	2.23	1.43, 2.75	0.001

† Linear regression model

*Logistic regression model

3.4 Factors Associated with 25(OH)D in asthma patients - Multivariate Analysis

To demonstrate the role of lung function, obesity, smoking, and season in reduction of serum vitamin D, multiple regression analysis was performed and it was found that mean levels of 25(OH)D remain reduced in serum even after adjustment as shown in the table 5.

Table 5. Multiple linear regression and logistic regression analysis showing the relationship between baseline predictors and serum levels of 25(OH)D in asthmatic patients.

Explanatory variables	25(OH)D			25(OH)D <20 ng/ml		
	Coefficient	95% CI	p value	OR	95% CI	p value
BMI(Kg/m^2)	-0.38	-0.57 to -0.18	<0.001	1.03	1.04 to 1.18	0.007
<18.5	-2.78	-6.56 to 1.09	0.12	1.36	0.57 to 3.89	0.42
18.5-24.9	0			1		
25-30	-1.56	-3.75 to 0.68	0.15	1.04	0.56 to 1.99	0.7
>30	-6.65	-9.65 to -3.65	<0.001	4.18	1.94 to 9.03	<0.001
Season						
Winter		0		1		
Spring	3.23	-0.05 to 6.12	0.051	0.74	0.35 to 1.53	0.41
Summer	7.03	4.32 to 9.45	<0.001	0.24	0.12 to 0.45	<0.001
Autumn	2.74	-1.75 to 7.12	0.22	0.58	0.21 to 1.65	0.28
Depression (CES-D score ≥ 16						
Yes	-3.26	-5.79 to -0.81	0.006	1.72	0.89 to 3.27	0.078
No	0			1		
Smoking						
Ex	0			1		
Current	-4.09	-6.15 to -1.93	<0.001	3.18	1.78 to 5.37	<0.001
Comorbidity						
<2	0			1		
≥2	0.148	0.139 to 0.157	<0.001	5.32	1.67 to 9.75	<0.001
Dietary habits						
Non-vegetarian	0			1		
Vegetarian	-3.94	-4.89 to -3.12	0.002	2.98	1.12 to 3.47	<0.001

Body complexion						
Clear	0			1		
Fair	2.95	-0.45 to 5.56	0.28	0.43	0.22 to 0.69	0.12
Black	-0.158	-0.96 to -0.194	<0.001	4.92	2.87 to 6.23	<0.001

CI = Confidence interval; p value is statistically significant (p <0.05)

4. DISCUSSION

Evidence is increasing that suggests an expanded role for vitamin D in health outcomes apart from its classic actions on the gut and bone.[20] These include modulation of a variety of processes and regulatory systems including host defence, immunity, inflammation, and repair.[21] Several lung diseases, all inflammatory in nature, may be related to activities of vitamin D including asthma. [22,23] As vitamin D status measured by circulating 25-(OH)D reflects the dynamic equilibrium between vitamin D synthesis in the skin by sun exposure, vitamin D intake via food or dietary supplements and vitamin D degradation by catabolising enzymes, [24] this study demonstrated that prevalence of vitamin D deficiency as assessed by 25(OH)D level in serum, was high in patients suffering from asthma as shown in previous study, [25] even after adjustment for potential confounder such as season, age, smoking, comorbidities, and BMI.

Cigarette smoking is a potential explanatory variable of vitamin D and is the most important single causal factor for hypovitaminosis D. In this study, it was found that serum vitamin D concentration was reduced significantly in both cases and controls, as smoking induces increased skin ageing [24] and leads to reduced capacity of aging skin for vitamin D synthesis.[21]

Season was a strong explanatory variable of 25(OH)D levels, and relatively more patients than controls were examined during spring in our study sample as in recent study.[26] The decreased vitamin D levels in the spring are thought to follow reduced sun exposure during winter months as the circulating form of vitamin D, 25-hydroxyvitamin D has an average half-life of 2–8 weeks.[27-30]

Dietary factors also appear to influence vitamin D status and susceptibility to asthma. The fear of allergic reaction against diet enriched with vitamin D might be reason in this area.
Increasing the melanin in human skin increases the length of exposure to UV light that is needed to maximize synthesis of previtamin D3.[31] Deeply melanised skins become

nonadaptive under conditions where the concentration of melanin is too high to permit sufficient amounts of vitamin D3 precursor to be synthesized in the skin under conditions of available UV radiation.[32-34], If the duration of UV exposure is not sufficient to catalyze previtamin D3 synthesis, individuals are at much higher risk of vitamin D deficiency and its manifestations as has been demonstrated by one previous study.[35]

Adiposity has in previous studies been a significant predictor of low levels of vitamin D in both asthmatic patients and subjects without asthma. [36] Obesity has been demonstrated to increase asthma risk, [37] and one of the most significant effects of obesity in asthma relates to its association with an impaired response to glucocorticosteroids. [38,39] Higher vitamin D levels in adults with asthma are not only correlated with improved lung function and reduced bronchial hyperresponsiveness, but also with an improved in vitro response to glucocorticosteroids. [40] The present findings suggest that reduced 25(OH)D levels in overweight and obese asthma patients may contribute to the reduced glucocorticosteroid response in this population.

Several studies have found an increased prevalence of vitamin D deficiency in subjects with depression [41,42] or depressive symptoms.[43] Patients with depression typically spend less time outdoors and exhibit less physical activity. These factors are also associated with asthma, and could confound a relationship between vitamin D deficiency and asthma.

Epidemiological and mechanistic evidence in humans indicates that vitamin D deficiency is associated with many chronic diseases, such as cardiovascular disease, autoimmune disease, cancer and chronic infections as in several previous studies.[44,14] However, evidence also indicates that vitamin D may causally contribute to different chronic disorders.[45,46]

After adjustment of confounding factors of Vitamin D, it was clearly demonstrated that all patients of asthma had low level of vitamin D in blood.

5. CONCLUSION

In conclusion, asthmatic patients had an increased risk for having vitamin D deficiency even after adjustment for a large number of known and potential confounders of blood level of vitamin D.

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