

Serum Vitamin D Levels Amongst Asthmatic and Non-Asthmatic Children in Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria: A Comparative Cross-Sectional Study

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ABSTRACT

Background: Asthma is a common chronic inflammatory disease of the airways. Recent studies have found an additional important role of Vitamin D (VD) in extra-skeletal health, particularly in the aetio-pathogenesis of childhood asthma due its effects in immune modulation and inflammation systems.

Aim and objective: This study was aimed to determine and compare the serum VD amongst asthmatic and non-asthmatic children, and also to determine the relationship between serum VD level and lung function test (LFT) in asthmatic children in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto.

Materials and methods: This was a comparative cross-sectional study. A total of 120 children were enrolled (60 asthmatic and 60 non-asthmatic) over a period of 4 months. Serum VD was assayed with ELISA kit, portable spirometer was used to assess the LFT of the participants. Data was analysed using SPSS version 21.0.

Results: The median serum VD levels among asthmatic children was 16.25ng/mL and that of non-asthmatic children was 16.80ng/mL with no significant difference in their median VD levels ($p = 0.550$). Significant difference in the median FEV₁/FVC among asthmatic and non-asthmatic was found ($p = 0.001$). No relationship was found between serum VD and LFT (FEV₁: $r = 0.034$, $p = 0.080$, FEV₁/FVC: $r = 0.067$, $p = 0.623$, PEFr: $r = 0.045$, $p = 0.152$).

Conclusion: Vitamin D deficiency was highly prevalent (76%) among study participants. No significant difference was found between the median serum VD levels of the asthmatic and non-asthmatic children and no relationship was found between serum VD and LFT of asthmatic children. However, there was difference in the LFT between asthmatic and non-asthmatic subjects. Emphasis should be made in routine serum VD screening and improve serum VD status among study population through health education, dietary supplementation and fortification.

Key words: Asthma, Vitamin D, Lung function test, Vitamin D deficiency, children

BACKGROUND:

Asthma is an important respiratory disease with increasing public health problem affecting children worldwide.¹ The aetiology of asthma is multifactorial; this includes genetics and environmental factors like urban lifestyles or dietary habits among which deficiency in micro nutrient – vitamin D (VD)- deficiency is a known factor.²⁻⁴

Vitamin D (VD) has long been identified to be important in bone and calcium metabolism.⁵ Recent studies have found an additional important role of VD in extra-skeletal health, particularly childhood asthma (allergic conditions).^{6,7} Vitamin D is a fat-soluble vitamin that is made mainly in the epithelial cells of the skin, which occurs due to UV rays and the conversion of 7-dehydrocholesterol to VD. It has basic regulatory roles in immune function of almost all cells especially on the airways.^{6,8} by affecting Th1 and Th2 cytokines which contribute to the development of atopy (including asthma).⁹⁻¹¹

Furthermore, VD has effects on epithelial T and B lymphocytes, antigen presenting cell functions¹² and induction of regulatory T (T reg) cells to produce interleukin (IL) – 10 which modulates inflammatory processes and could help to control asthma severity.¹³ Also VD modulates foetal lung maturation and smooth muscle cell proliferation thereby promotes lung development.¹⁴

Reduced VD levels in adult and pediatric patients with asthma are associated with impaired lung function, increased airway hyperreactivity, and reduced corticosteroid response.¹⁵ Nevertheless, there are uncertainties in defining the

appropriate vitamin D cutoffs for respiratory health and lung disorders.¹⁶ Spirometry provides an objective criterion for assessment of lung function test (LFT) in asthma diagnosis, follow up, monitoring and objective assessment of response to interventions.¹⁷ In addition, it helps in screening of apparently healthy children as part of medical checkup. Spirometry confirms airflow limitation with a reduced forced expiratory volume in the first second (FEV₁), FEV₁/FVC ratio (forced expiratory volume/forced vital capacity). In general, a FEV₁/FVC ratio of <0.80 in adults, and <0.90 in children indicates a significant airflow obstruction.¹⁷ In a typical case of bronchial asthma, the FVC is normal, while FEV₁ is reduced. Reversibility is detected by a >12% and 200ml increase in FEV₁ 15minutes after inhaled short acting beta-2 agonist or 2-4 weeks trial of oral corticosteroids.¹⁷

Aim and objective: This study aimed at determination of serum VD among asthmatic and non-asthmatic children, and also determined the relationship between serum VD and lung function test among asthmatic children in UDUTH, Sokoto.

Materials and methods:

This was a hospital based comparative cross-sectional study conducted in UDUTH, Sokoto from October 2019 - February 2020.

The minimum sample size (n) per group based on the study design was calculated as follows:

$$n = \frac{(Z_{1-\alpha/2} + Z_{\beta})^2 \sigma^2}{D^2} \quad 18$$

$Z_{1-\alpha/2}$ = percentage point of the normal distribution corresponding to the

required (two-sided) significance level (α) of 0.05 = 1.96.

Z_β = the value of the standard normal distribution cutting off probability β , which is 0.84 for 80% power.

σ = standard deviation of variable under study.

D = margin of error

Sample size adjustment was done for a finite population of less than 10,000.

A total of 120 (60 asthmatic and 60 non-asthmatic children) age-matched children who were from five to 15 years (15 years is the upper limit of age of children seen in the Department of Paediatrics, UDUTH) were enrolled into the study by a systematic random sampling technique. The age range of 5-15 years was chosen in this study due to fact that children with childhood asthma below the age of five years may be unreliable and fraught with inconsistency¹⁷. Participants were enrolled from the Paediatric Pulmonology and Allergy clinic; and Emergency Paediatric Unit of department of Paediatric, UDUTH, Sokoto with diagnosis made according to GINA guideline¹⁷.

About 12-14 children with age less than 15 years (cut-off age for paediatrics in UDUTH) was the average population of asthmatic children seen in Pulmonology clinic weekly (runs on Fridays and has a consultant pulmonologist and two residents who oversee the clinic); while 10-12 of these asthmatic children were in the age bracket of 5-15 years. Balloting technique was used to enroll the 1st subject and subsequently participants were enrolled at every 3rd subject.

In addition, there were age-matched children who attended Paediatric Out-

Patient Department of UDUTH Sokoto which runs every weekday (Monday-Friday) with average population of about 25 patients per clinic. Out of this population, 20 non-asthmatic children who were of the age bracket 5-15 years were recruited as controls for the study. Participants were enrolled once a week and such clinic was chosen randomly by balloting. Balloting technique was used to enroll the 1st subject and subsequently participants were enrolled at every 5th subject till the required sample size was enrolled.

Patients with chronic respiratory or cardiac diseases like pulmonary tuberculosis, congenital heart diseases and on VD supplement intake were excluded. History, examination, anthropometric measurements (weight was measured using seca digital weighing machine to the nearest 0.1kg, height measured using seca stadiometer to the nearest 0.1cm, body mass index BMI = weight(kg)/height(m²) were recorded in a study questionnaire, and BMI percentile calculated and plotted on the growth chart accordingly).¹⁹ Socio - economic class was also classified based on Oyediji social class scheme (Higher, Middle and lower class).

Detailed spirometry was performed by the trained researcher on all the children enrolled in the study to assess their LFT according to American Thoracic Society guideline²⁰ using BTL- 08 Spiro Pro portable spirometer under aseptic procedure. The spirometer was calibrated daily and procedure done under ambient temperature, pressure and humidity. The procedure was explained and demonstrated to all the study participants. An incentive spirometer was

used to encourage the participants while the subjects' seat on a chair and instructions were given. The subjects were allowed 2-3 practice trial blow and three test blows for four - six seconds and the personal best FVC, FEV₁, FEV₁/ FVC, peak expiratory flow rate (PEFR) were recorded.

Two mls of blood was collected by a trained medical personnel from all the study subjects in an aseptic procedure and analysed by a chemical laboratory scientist for serum VD levels. The blood was centrifuged, serum was stored at - 20°C until time (2 weeks) for assay. Serum levels of VD was quantified by ELISA kit immunodiagnostic systems.

A 25-OH VD ELISA assay kit (VID31-K1) was used, which is packaged and produced by Eagle Biosciences. This was a complete kit for quantitative determination of serum levels of VD with sensitivity of 1.6ng/mL and uses newly monoclonal antibody which is specific for VD2 and VD3 at 100% specificity.

Values obtained and recorded for serum VD levels was categorized into four as follows:²¹

- The VD levels < 20ng/mL were considered deficient;
- The VD level between 20-30ng/mL was considered insufficient;
- The VD levels above 30-150ng/mL were considered sufficient, and values greater than 150 ng/mL were considered as hyper-vitaminosis D

Data analysis was done using Statistical Package for social sciences version 20.0 (Chicago Illinois). Non- parametric tests were used after testing for normality with

Shapiro-Wilk test. Descriptive statistical analysis such as median and interquartile range were done for quantitative variables, Wilcoxon rank sum test was used to compare if differences existed in serum VD between asthmatic and non-asthmatic subjects. Chi square was used to determine if any relationship existed between categorized serum VD levels amongst asthmatic and non- asthmatic children.

Level of significance was set at $P < 0.05$ and p- values were generated. The study was approved by the Research Ethics committee of UDUTH (UDUTH/HREC/2020/995/V1). All patients' parents/caregivers were required to sign a written informed consent form; parents/caregivers who cannot sign had thumb print on the consent form. Assent was obtained from children aged 7 years and above.

RESULTS

Subjects enrolled

A total of 120 subjects aged 5- 15 years were studied for serum vitamin D levels among which 60 were asthmatic children and 60 were non – asthmatic children.

Socio – demographic characteristic of the study participants

One hundred and twelve (93%) of the study participants came from the urban area. Males accounted for 62 (51.7%) while females accounted for 58 (48.3%) of the study participants with a ratio of 1.07: 1.0. Almost 50% of the study participants belong to low socio- economic class (26 asthmatics and 31 non- asthmatic children as presented in table I below.

Table III. Comparison of Median serum vitamin D level amongst asthmatic and non-asthmatic children

Subjects	N	VD levels(ng/mL)		z - test	p- value
		Median	IQR		
Asthmatic Children	(60)	16.25	13.38	-0.598	0.550
Non-Asthmatic Children	(60)	16.80	8.89		
Age group 5 <10 years					
- Asthmatic Subjects	33 (55%)	16.60	10.75	-0.525	0.599
- Non- Asthmatic subjects	29 (48.3%)	17.90	8.18		
Age group 10 -15 years					
- Asthmatic subjects	27 (45%)	15.90	15.50	-0.446	0.656
- Non- asthmatic subjects	31 (51.7%)	15.80	9.60		
Male study Participants	62 (51.7%)	17.90	11.60	-0.670	0.430
Female study Participants	58 (48.3%)	16.05	8.27		

N number of subjects, ng/mL nanogram per milliliter

Categorized serum Vitamin D levels among study participants

Eighty (67%) of the study participants were VD deficient. There was no relationship between categorised serum vitamin D levels amongst male and female participants nor was there relationship

with aged group 5-<10 years and 10-15 years ($r = 0.067$, $p = 0.324$, $r = 0.023$, $p = 0.421$) respectively. There was no relationship between categorised Vitamin D levels amongst asthmatic and non-asthmatic children ($\chi^2 = 6.063$, $r = 0.042$, $p = 0.194$) as shown below in table IV:

Table IV. Categorized serum vitamin D levels among study participants

Subjects	Asthmatic	Non- Asthmatic	Total	p
Serum vitamin D levels				
Vitamin D deficient	38 (63.3%)	42 (70%)	80 (67%)	0.194
Vitamin D insufficient	17 (28.3%)	16 (26.7%)	33 (27.5%)	
Vitamin D sufficient	5 (8.3%)	2 (3.3%)	7 (5.8%)	
Hypervitaminosis D	0	0	0	
Total	60 (100%)	60 (100%)	120 (100%)	

Relationship between serum Vitamin D levels and Lung Function Test amongst study participants

There is no relationship between serum VD level and LFT (FEV₁/FVC) among asthmatic subjects ($r = 0.624$, $p = 0.625$),

and no relationship was found between serum VD levels and LFT among non - asthmatic children ($r = 0.576$, $p = 0.781$) as shown in Table V below:

Table I. Socio – demographic characteristics of study participants

Groups		Asthmatic children	Non – Asthmatic children	p
5-<10years		33 (55%)	31(51.7%)	0.170
10-15 years		27 (45%)	29(48.3%)	0.145
Gender	Males	35 (58.3%)	27 (45.0%)	0.189
	Females	25 (41.7%)	33 (55.0%)	0.230
Domicile	Urban	55 (91.7%)	57 (95.0%)	0.119
	Rural	5 (8.3%)	3 (5.0%)	0.167
Social				
Class	Low class	26 (43.3%)	31 (51.7%)	0.062
	Middle class	28 (46.7%)	25 (41.7%)	0.085
	High class	6 (10.0%)	4 (6.6%)	0.156

Age and Anthropometric Characteristics of study population

The median age of the study population was 9.5 years, interquartile range = 5.75 years. There was significant difference in the BMI between asthmatic and non –

asthmatic children ($p = 0.001$) with almost 50% of the study participants underweight for age (20 asthmatics and 36 non- asthmatic children) as depicted in table II. below:

Table II. Age and anthropometric characteristics of study population

Character	Asthmatic children (N = 60)		Non-asthmatic children (N = 60)		z- test	p- value
	Median	IQR	Median	IQR		
Age (years)	9.47	3.98	9.81	7.45	-0.852	0.796
Weight (kg)	24.25	14.25	22.00	13.00	-1.767	0.077
Height (cm)	131.00	26.05	130.000	17.75	-0.914	0.361
BMI (kg/m ²)	14.68	2.60	13.45	3.35	-3.453	0.001
BMI	Underweight	22(36.7%)		36 (60%)		
percentile	Normal weight	34(56.6%)		23(38.3%)		
	Overweight	1 (1.7%)		0		
	Obesity	3 (5%)		1 (1.7%)		

Kg kilogram, cm centimetre, m² metre square, z-test Wilcoxon Rank sum test, N number of subjects, IQR interquartile range

Comparison of Median serum vitamin D level amongst study participants

The median VD level amongst asthmatic children was 16.25ng/mL while that of the non- asthmatic children was

16.80ng/mL. There was no significant difference in the median VD levels among asthmatic and non – asthmatic children ($p = 0.550$) as shown below (table III).

Table V: Lung function status in relation to median vitamin D level among study participants

Characteristics Subjects	Median LFT	IQR	Median VD level (ng/mL)	IQR	p
FEV ₁ /FVC					
- Asthmatic subjects	0.856	0.150	19.84 ±16.85		0.625
- Non- Asthmatic subjects	0.972	0.090	18.27 ± 6.83		0.781
FEV ₁					
- Asthmatic subjects	68.00	14.50	19.84±16.85		0.604
- Non – asthmatic subjects	72.00	14.25	18.27± 6.83		0.367
PEFR					
- Asthmatic subjects	67.50	16.96	19.84±16.85		0.347
- Non – asthmatic subjects	73.00	16.75	18.27±6.83		0.612

Spekman correlation values differ significantly at $p < 0.05$, ng/mL nanogram per milliliter, LFT lung function test, FEV₁ forced expiratory volume at 1st second, FVC functional vital capacity, PEFR peak expiratory flow rate.

DISCUSSION

The median serum VD level amongst asthmatic children was 16.25ng/mL and that of non- asthmatic children was 16.8ng/dL. This is similar to the findings by Freishtal *et al*²² and Chinellato *et al*²³. The prevalence of VD deficiency among asthmatic children in this study was 63.3% while that of non- asthmatic children was 70%. Metin *et al*²⁴ made a similar observation with a prevalence of 67% of VD deficiency among asthmatic children. These findings were also reported by these previous studies^{16,25,26}. This was also in consistent with other studies²⁷⁻²⁹.

This study showed generally high prevalence of VD deficiency (67.0%) among study participants, with no difference in the median serum VD amongst asthmatic and non-asthmatic children. Similar observations were made by Dogru *et al*³⁰ and Menon *et al*³¹, with no difference in the median VD levels amongst the study group. Surupa *et al*²⁷ found out that VD deficiency was highly prevalent among Indian children

generally. Vitamin D deficiency was also found to be highly prevalent among study population in other studies.^{28-9,32} However, previous studies^{33,34} have reported different finding with disparity in the serum VD levels among study population where asthmatic children had lower VD levels compare to the non – asthmatic children. The reasons for these contrasted observations could be attributed to the presence of risk factors for VD deficiency in the index study population viz dark skin pigmentation, which contain melanin, thus inhibit absorption of VD from the sunlight.³⁵⁻⁶ The concealing clothing style³⁵⁻⁶ (being that the majority of the study population were from Muslim family who are known to cover their body), thus preventing cutaneous absorption of VD, seasonal variation³⁵(this study was conducted during harmattan, with no or little sunshine and children tend to stay indoors to protect themselves against the hazardous dusty and cold weather), low socio – economic status (which make up 47.5% of the study population) who may

not be able to buy VD high rich diets and urbanization³⁶ (more than 90% of the participants were from the urban area) were children tend to spend more time indoors thereby preventing them from getting exposed to sunlight. In addition, the high prevalence of under nutrition among study population (48.3%) which will present as VD micro deficiency is also a major culprit for VD deficiency among study participants. Thus, causing decrease in the VD binding protein in blood, which reduces the ability of the body to conserve and store VD.³⁷⁻⁸. Thus, these risk factors for VD deficiency are the environmental and social factors (climate, nutritional, religious, cultural and socio – economic status, urbanization {lifestyle changes}, dark skin pigmentation and seasonality) which affected both study groups equally, hence the general low serum VD and lack of difference in the median VD amongst asthmatic and non-asthmatic children. Furthermore, this finding could be a reflection of the global hypovitaminosis epidemic in children.^{39,40}

This study reported no relationship between serum VD levels and LFT amongst asthmatic children. This was also reported by Sebnem *et al*⁴¹. Similar observations were made by these studies.^{42,43} However, Sakr *et al*⁴⁴ and Alyasin *et al*⁴⁵ reported a significant positive correlation between VD deficiency and LFT such as FEV₁, FVC, and FEV₁/FVC among Egyptian and Iranian children respectively. The contrasted reports could be explained by a dose-dependent association effect between serum VD and LFT. This was demonstrated by El- Gamal *et al*⁴⁶ among Egyptian children who found no relationship between serum VD and LFT

but, however, reported a critical value of serum VD (7.5ng/mL) to be associated with poor LFT. This was also supported by Saber *et al*⁴⁷ findings, which showed no significant difference in the baseline characteristics and of those spirometry functions (FVC, FEV₁, and FEV₁/FVC) between the healthy individuals with normal and deficient VD levels. Thus, the relationship between serum VD and LFT has not been clearly specified yet. Hence the need for longitudinal studies using the goal standard (Liquid chromatography-tandem mass spectrometry (LC-MS)) of serum VD assay to determine the relationship between serum VD and LFT, also determine the threshold effect of serum VD levels on lungs especially in populations with generally high prevalence of VD deficiency.

Conclusion and recommendations:

Majority of the study participants were VD deficient with no difference in their median VD. There was no relationship between serum VD and LFT amongst asthmatic children. There is need to improve serum VD status among the children population through health education and food fortification. With the emerging association between low VD levels and asthma, serum VD assay using LC-MS in children is recommended so as to ascertain this relationship for better management outcome, especially in asthmatic children.

Limitation of the study

1. Cross- sectional study was used in this study which is not ideal to establish causality between serum VD level and asthma

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