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Relationship between serum 25-hydroxyvitamin D and inflammatory cytokines in Nigerian children with asthma

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ABSTRACT

Background: Childhood asthma involves a network of mutually interacting inflammatory mediators and cytokines. The influence of serum vitamin D (an immunomodulatory agent) on these cytokines is poorly understood. This study sets out to determine the relationship between serum T-helper cells type 1 cytokines (Interleukins ILs-1 β , 2, 6, 8 and 10), type 2 cytokines (ILs 3, 4, 5, 9, 13), ILs-12, 17 and 25-hydroxyvitamin D (25-OHD) in Nigerian children with asthma.

Methods: Children with physician-diagnosed asthma and non-asthmatics aged 2-15 years were consecutively recruited at a tertiary center in Nigeria. Serum 25-OHD and cytokines were assayed using chromatography method and association between these parameters determined.

Results: A total of 180 children (90 for each group; mean age 7.5 ± 3.9 years; M:F 1.9:1) were recruited. Fifty-six (76.7%) asthmatics had mild intermittent form and symptoms were sub-optimally controlled in 16 (21.9%). The mean (SD) serum Vitamin D was lower in children with asthma (38.8 ± 17.0 ng/ml vs. 45.5 ± 16.6 ng/ml vs. $p = 0.023$). Also more proportion of children with asthma had suboptimal serum vitamin D level (26.0% vs. 8.8%; $p = 0.022$). Deficient vitamin D state was associated with higher serum Th₁ and Th₂ cytokines compared to insufficient state, but the highest cytokine levels were observed in normal vitamin D state. There was significant positive correlation between serum vitamin D and Th₁ cytokines IL-2 and IL-8 as well as Th₂ cytokines (ILs-3, 4, 5 and 9), but negative correlation with IL-13

Conclusions: Serum Vitamin D and cytokines were lower in a sample of Nigerian children with asthma than controls. Vitamin D status was not related to asthma severity and control. Serum 25-OHD seems to have a non-linear relationship with Th₁ and Th₂ cytokines in Nigerian asthmatics.

Abbreviations: ILs: interleukins; Th: T helper cells; 25-OHD: 25-hydroxyvitamin D; LMICs: low and middle income countries; VDR: vitamin D receptors

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Introduction

Asthma is chronic inflammatory disease of the small airways in which cells and cellular elements play significant roles in its pathogenesis (1). It manifests with recurrent episodic cough, breathlessness, chest tightness and wheezing which resolves spontaneously or with medications (1). The burden of asthma is enormous as it is estimated to be responsible for about 15 million disability adjusted life years per annum (2). Childhood asthma is a common cause of hospitalization and emergency hospital visits, school absenteeism and impaired quality of life of affected children and their parents/caregivers (3,4). Unfortunately, the burden of childhood asthma is on the increase particularly in Low and Middle Income Countries (LMICs)

where hitherto low prevalence had been reported (5,6). This has been linked to increasing indoor and outdoor pollution, use of breast milk substitutes, westernized diet, sedentary lifestyle and childhood obesity among others (1,5,6).

The immunopathologic basis of asthma involves a number of networks of mutually interacting inflammatory cells and mediators (7). These mediators are influenced by vitamin D - a fat soluble vitamin with immunomodulatory properties (8,9). Vitamin D receptors (VDRs) are found on the surface of immune cells including lymphocytes, macrophages and dendritic cells (10,11). These receptors modify inflammation of respiratory epithelium by regulating the transcription of various genes implicated in inflammation (10,11).

Vitamin D often assayed as the more stable 25-hydroxyvitamin D (25-OHD) with longer half-life of three weeks (12) has also been reported to regulate the functions of T-helper cells (Th) (13–15). Mixed effects (both stimulatory and inhibitory) of vitamin D have been reported on T-helper cells type 2 (Th₂) cytokines in human and animal studies, while inhibitory effects of vitamin D on Th₁-associated cytokines including the pro-inflammatory IL-17 had been consistently reported (13–15). Vitamin D enhances the production of anti-infective peptides such as cathelicidin and β -defensin-2 thereby reducing the prevalence of respiratory tract infections which are common triggers of asthma exacerbation in children (15). Hence increasing prevalence of vitamin D deficiency had been hypothesized as one of the possible explanations for the increasing prevalence of childhood asthma and other allergic diseases (16).

With reported increasing prevalence of childhood asthma in LMICs, where there is double burden of infectious and non-communicable diseases like asthma, and in the light of paucity of reports on the interaction between serum inflammatory cytokines and vitamin D, this study therefore set out to characterize the socio-demographic and phenotypic characteristics of children with asthma and their age and sex-matched controls and to determine the relationship between serum Th₁ and Th₂ inflammatory cytokines and 25-OHD in children with asthma attending a Pediatric chest clinic of a Nigerian tertiary health facility. This may assist in better understanding of immunomodulatory properties of vitamin D as related to serum inflammatory cytokines in childhood asthma and may hold prospects for its therapeutic use in the management and symptoms control in children with asthma.

Methods

Study design and location

This was a hospital-based cross-sectional study carried out at the pediatric chest clinic of the Wesley Guild Hospital (WGH), Ilesa, south west Nigeria over a 10-month period (October, 2018 to July 2019). The clinic attends to children with respiratory conditions predominant of which is bronchial asthma. The control group were apparently healthy children without asthma or allergic diseases who presented for routine school health related medical checkup at the Child Welfare Clinic of the hospital. The WGH is a tertiary arm of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife which provides

general and specialized care for children in the south west part of Nigeria.

Ilesa is a semi-urban city with typical tropical climate (7°35'N, 4°51'E). It is in the rain forest zone of the country with average ambient temperature of 25.6° C, relative humidity 17-40%, and 1317 mm (variation 208 mm) annual rainfall (17).

Sample size determination

The minimum sample size for this study was estimated using open Epi^(R) sample size software (18). Using 5% significance (alpha) level, 80% study power and 95% confidence interval. With a reported mean difference in Serum 25-OH vitamin D between children with Asthma and controls as 1.6 ng/ml and standard deviations from the mean for the two groups as 8.5 and 19.5 ng/ml respectively (19), and ratio of cases to control being 1. The calculated sample size was approximately 180 i.e 90 each for asthmatics and controls.

Ethical consideration

Ethical approval for this study was obtained from the Ethics and Research Committee of the OAUTHC, Ile-Ife. Informed consent and assent were obtained from the caregivers and children > 6 years respectively.

Study procedure

The study participants were children (aged two to 15 years) with physician diagnosed asthma defined thus: history of recurrent cough, wheezing, chest tightness, and shortness of breath which either resolves spontaneously or with the use of bronchodilators (1). Additionally for those ≥ 6 years, demonstration of expiratory airflow obstruction reversibility i.e. $\geq 12\%$ increase in forced expiratory volume in one second (FEV₁) post bronchodilator was ensured before recruitment (1). Recruitment was consecutive and asthma severity (at first clinic visit) and level of symptoms control (over the last four weeks) were assigned using the National Asthma Education Prevention Program Expert Panel Report (NAEPP) and the Global Initiative on Asthma (GINA) criteria respectively (20,21). Well controlled symptoms was defined as few ($\leq$ twice per week) or absence of daytimes asthma symptoms, no limitation of activities and no use of reliever medications for more than twice a week (21). Use of inhaled steroids and other medications were also noted.

Socio-demographic characteristics, history about housing environment, household pets, family size and parental socio-economic class (SES) defined using ranked assessment of parental occupation and highest educational qualifications using a validated SES tool (22) were obtained.

Anthropometric parameters of the study participants including weight in kilograms (kg), height in meters (m) and Body Mass Index (BMI) in kg/m² were taken using standard methods. These were compared to age-appropriate standards using the WHO growth reference charts (23,24) to assign nutritional status to the study participants.

Serum cytokines and 25-OHD estimation

Serum of study participants were obtained by centrifuging five mls of their blood at 3000 revs/min for 15 min. This was then separated in aliquots into acid washed (10% (v/v) HNO₃) plain screw cap specimen bottles to remove contaminants and subsequently stored frozen at -20 °C till analyzed at the central laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria. The Interleukins and 25OHD were analyzed using automated Waters 616/626S transducer pump High-Performance Liquid Chromatography (HPLC) (Waters Incorporate, California, U.S.A) at a wavelength of 198 nm

Quality control

Accuracy was ensured by assaying control samples obtained from Waters Instrument Incorporate, California, USA along with samples of participants. Results gotten were within the kit manufacturers stipulated control reference interval for each analyte and levels of standards before accepting the results. Precision (reproducibility) of method used was determined using control samples with an intra-assay coefficient of variation (CVs') below 5% for all the assayed interleukins and 25OHD. For 25-OHD estimation, the solvent, internal standard, and reference standard were methanol and acetonitrile, laurophene and 25-OD2 and 25OHD3, respectively. The lower limit of detection in serum was 5 ng/ml, and 25-OHD was measured within a range of 5-100ng/ml. The CVs for intra-day accuracy was also less than 5%.

Vitamin D deficiency (serum 25OHD level < 20 ng/ml) and insufficiency (< 30 ng/ml) were classified as suboptimal serum vitamin D, while levels ≥ 30 ng/ml were optimal (25).

Data analysis

This was done using Statistical Program for Social Sciences (SPSS) Software version 17.0 (SPSS Inc., Chicago, IL, USA, 2008). Age range, sex, socioeconomic classes and other categorical were summarized using proportions and percentages, while all continuous variables were tested for normality using Kolmogorov-Smirnov test and were summarized using means and standard deviations (SDs) for Gaussian variables or median and interquartile ranges for non-Gaussian ones. Differences between continuous variables were analyzed using Student's *t*-test or Mann Whitney U test and analysis of Variance (ANOVA) or Kruskal-Wallis test as appropriate, while categorical variables were analyzed using Pearson's Chi-square test and Fisher's exact test as appropriate. Pearson's or Spearman Rho coefficient was used to determine the relationship between serum 25-OHD and inflammatory cytokines. The level of significance at 95% confidence interval (CI) was taken at $p < 0.05$.

Results

Characteristics of the study participants

One hundred and eighty children (90 each for asthmatics and non-asthmatics) participated in the study. The mean (SD) age was 7.5 (3.9) years, range 2–15 years and male: female ratio was 1.7:1. The sample population was enriched with well-nourished participants (76.1%) from upper and middle social class (86.1%). No significant difference in the socio-demographic characteristics, home environment and nutritional status of children with asthma and controls (Table 1).

Study participants with asthma

The majority (75.8%) of the children with asthma had intermittent form, two-third were steroid naïve, and 16 (17.7%) had suboptimal symptoms control at the point of enrollment (Table 2).

Serum 25-hydroxyvitamin D (25-OHD)

This ranged from 4.3–77.1 ng/ml with a mean (SD) of 37.4 (13.6) ng/ml. Children with asthma had significantly lower serum 25-OHD than controls (Table 3), and more proportion (37.8% vs. 21.1%) of children with asthma had suboptimal serum Vitamin D than controls (Table 1).

Table 1. Socio-demographic, general information and vitamin D categories of the study participants.

Variables	Children with Asthma <i>n</i> = 90 (%)	Controls <i>n</i> = 90 (%)	Total <i>n</i> = 180	χ^2	<i>p</i> -value
Age range (years)					
≤ 6	32 (35.6)	28 (31.1)	60	0.400	0.819
>6–12	45 (50.0)	48 (53.3)	93		
>12	13 (14.4)	14 (15.6)	27		
Sex					
Male	56 (62.2)	58 (64.4)	114	0.096	0.757
Female	34 (37.8)	32 (35.6)	66		
SES					
Upper	52 (57.8)	32 (35.6)	84	8.931	0.011
Middle	28 (31.1)	43 (47.8)	71		
Low	10 (11.1)	15 (16.7)	25		
Household pets					
Yes	17 (18.9)	26 (28.9)	43	2.102	0.147
No	83 (81.1)	64 (71.1)	137		
Indoor air pollution					
Yes	43 (47.8)	54 (60.0)	97	1.997	0.158
No	47 (42.2)	36 (40.0)	83		
Crowded homes					
Yes	25 (27.8)	22 (24.4)	47	0.418	0.518
No	65 (72.2)	68 (75.6)	133		
Nutritional status					
Normal	67 (74.5)	70 (77.8)	137	2.858	0.582
Overweight/obese	11 (12.2)	12 (13.3)	23		
Underweight	12 (13.3)	8 (8.9)	20		
Serum Vitamin D					
Normal	56 (62.2)	71 (78.9)	127	10.029	0.005*
Insufficient	19 (21.1)	16 (17.8)	35		
Deficient	15 (16.7)	3 (3.3)	18		

Figures in parentheses are percentages of the total along each column; SES socio-economic status.

*Fischer's exact test applied.

Table 2. Asthma severity and symptoms control as related to serum vitamin D and cytokines.

Asthma characteristics	<i>n</i> (%)	Serum 25-OHD Mean (SD) ng/ml	Test statistics	<i>p</i> -value
Asthma severity				
Intermittent	68 (75.8)	32.9 (13.6)	0.712	0.547*
Mild persistent	13 (14.4)	38.5 (14.5)		
Moderate persistent	7 (7.8)	37.4 (22.3)		
Severe persistent	2 (2.2)	31.1 (11.6)		
Use of ICS				
Steroid naïve	61 (67.7)	33.7 (13.7)	0.292	0.771#
Steroid use	29 (32.2)	34.7 (15.8)		
Level of asthma symptoms control				
Well controlled	74 (82.3)	33.5 (13.9)	0.255	0.776*
Partly controlled	12 (13.3)	36.2 (16.1)		
Uncontrolled	4 (4.4)	36.5 (18.9)		

*Analysis of variance (ANOVA) applied.

#Students' *t*-test applied.

Serum 25-OHD and asthma severity and control

No significant association was found between serum 25-OHD and asthma severity, use of inhaled corticosteroids and level of symptoms control ($p > 0.05$) Table 2.

Serum inflammatory cytokines in the study participants

The T-helper cells type 1 and 2 inflammatory cytokine profile of the study participants are highlighted in Table 3. Serum Inflammatory cytokines are generally lower in the children with asthma compared to the controls.

Relationship between serum inflammatory cytokines and 25-OHD in children with asthma

Vitamin D and Th1 cytokines

Children with asthma that were vitamin D deficient had significantly higher serum levels of IL-2, 6, 8 and 10 than those with insufficient vitamin D levels. However, these cytokines were significantly higher in those with sufficient vitamin D levels than those with insufficient levels (Table 4).

Vitamin D and Th2 cytokines

Serum IL-3, 5 and 9 as well as IL-12 were significantly lower in children with suboptimal (both deficient and insufficient) serum 25-OHD compared to those with

Table 3. Serum inflammatory cytokines and vitamin D in children with asthma and apparently healthy controls.

Serum cytokines	Range	Total <i>n</i> = 180 Mean (SD)	Children with asthma Mean (SD)	Controls Mean (SD)	<i>p</i> -value
Th1 (pg/dl)					
IL-1 β	0.4–10.0	3.7 (2.0)	3.5 (1.8)	3.9 (2.1)	0.223
IL-2	0.1–13.4	6.1 (3.2)	5.0 (3.0)	7.1 (3.0)	<0.001
IL-6 Median (IQR)	0.2–15.1	0.8 (0.5–2.1)	0.8 (0.4–2.0)	0.9 (0.5–2.2)	0.055*
IL-8	0.1–23.0	6.1 (3.3)	5.6 (3.0)	6.6 (3.5)	0.047
IL-10	0.2–11.3	3.4 (1.9)	3.4 (2.1)	3.4 (1.7)	0.949
Th2 (pg/dl)					
IL-3	0.1–19.5	7.9 (5.2)	7.0 (4.7)	8.9 (5.5)	0.013
IL-4	0.01–15.6	5.1 (2.7)	4.6 (2.8)	5.7 (2.5)	0.007
IL-5	1.4–8.2	4.3 (1.7)	3.9 (1.7)	4.7 (1.7)	0.001
IL-9	0.1–13.8	5.9 (3.40)	4.8 (3.3)	7.0 (3.2)	<0.001
IL-13 Median (IQR)	0.01–10.6	0.3 (0.1–2.4)	0.5 (0.1–2.2)	0.1 (0.06–3.0)	0.585*
Others					
IL-12	0.1–10.6	3.4 (3.0)	3.4 (2.8)	3.4 (3.2)	0.883
IL-17	0.01–10.1	1.9 (1.7)	1.8 (1.6)	1.9 (1.7)	0.777
Serum 25-OHD (ng/ml)	4.3–77.1	37.4 (13.6)	34.0 (14.3)	40.8 (12.0)	0.001

*Mann Whitney U applied; 25-OHD 25-hydroxyvitamin D.

Table 4. Serum inflammatory cytokines as related to vitamin d categories in the children with asthma.

Serum cytokines	Vitamin D categories			<i>p</i> -value
	Deficient <i>n</i> = 15	Insufficient <i>n</i> = 19	Normal <i>n</i> = 56	
Th1 (pg/dl)	Mean (SD)	Mean (SD)	Mean (SD)	
IL-1 β	3.3 (1.5)	3.2 (1.8)	3.6 (1.9)	0.642
IL-2	3.7 (1.9)	3.1 (1.6)	6.1 (3.2)	<0.001
IL-6 Median (IQR)	1.9 (1.0–2.1)	0.3 (0.2–0.4)	0.8 (0.5–2.3)	<0.001*
IL-8	4.4 (2.4)	3.8 (1.4)	6.3 (3.2)	0.004
IL-10	3.5 (2.8)	2.4 (1.0)	3.3 (1.6)	0.002
Th2 (pg/dl)				
IL-3	4.5 (1.7)	5.8 (2.6)	8.0 (5.5)	0.016
IL-4	5.5 (2.4)	2.8 (3.3)	4.9 (2.6)	0.008
IL-5	2.3 (0.7)	3.1 (0.9)	4.5 (1.7)	<0.001
IL-9	2.1 (1.4)	2.8 (1.4)	6.2 (3.3)	<0.001
IL-13 Median (IQR)	1.1 (0.5–2.3)	1.5 (0.3–1.7)	0.2 (0.1–2.7)	0.486*
Others				
IL-12	1.4 (1.1)	3.6 (2.2)	3.8 (3.0)	0.010
IL-17	1.8 (1.0)	1.5 (0.9)	1.9 (1.6)	0.660

Analysis of Variance (ANOVA) applied unless otherwise stated.

*Kruskal-Wallis test applied.

25-OHD, 25-hydroxyvitamin D; IQR, interquartile range.

sufficient levels. However vitamin D deficiency was associated with elevated serum IL-4 and 13 compared to insufficient state, while sufficient state was associated with the highest Th₂ cytokine levels (Table 4).

Correlation between vitamin D and inflammatory cytokines

There was significant positive correlation between serum 25-OHD and IL-2, IL-6 and 8 as well as the Th₂ cytokines (IL-3, 5 and 9), while IL-4 and IL-13 had negative correlation with serum vitamin D. These and other correlations are highlighted in Table 5 and Figures 1–7.

Discussion

This study demonstrated low serum vitamin D in a population of Nigerian children with predominantly intermittent, steroid naïve and well controlled asthma compared to age and sex matched children without

Table 5. Relationship between serum inflammatory cytokines and serum 25-hydroxyvitamin D.

Serum cytokines	Correlation with serum 25-OHD
Th1 (pg/dl)	
IL-1 β	$r = 0.079$; $p = 0.460$
IL-2	$r = 0.548$; $p < 0.001$
IL-6	$r = 0.226$; $p = 0.055^*$
IL-8	$r = 0.247$; $p = 0.019$
IL-10	$r = -0.113$; $p = 0.287$
Th2 (pg/dl)	
IL-3	$r = 0.368$; $p < 0.001$
IL-4	$r = 0.251$; $p = 0.017$
IL-5	$r = 0.612$; $p < 0.001$
IL-9	$r = 0.621$; $p < 0.001$
IL-13	$r = -0.252$; $p = 0.031^*$
Others	
IL-12	$r = 0.137$; $p = 0.199$
IL-17	$r = -0.008$; $p = 0.942$

*Spearman Rho applied.

asthma and other allergic diseases. Similarly this study also highlighted reduced serum Th₁ and Th₂ inflammatory cytokines in children with asthma compared to controls and demonstrated the relationship between

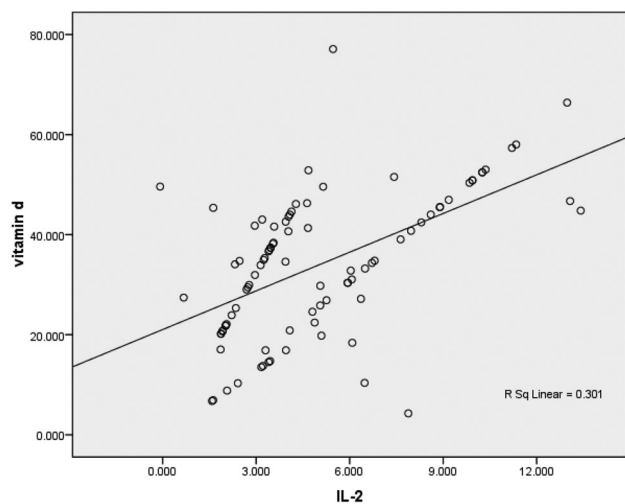


Figure 1. Scattered plot of vitamin D vs. interleukin-2.

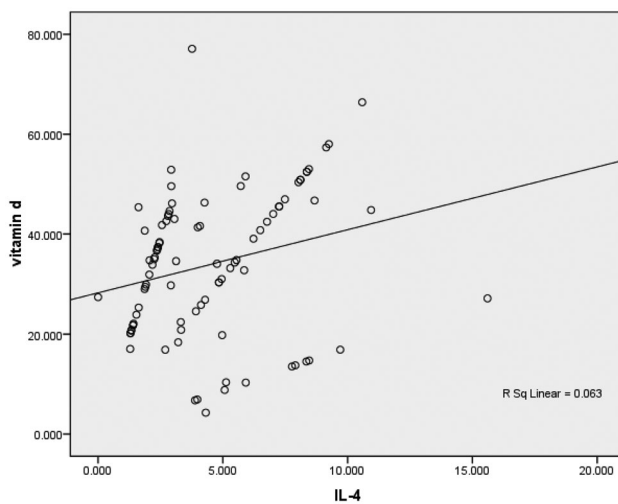


Figure 4. Scattered plot of vitamin D and interleukin-4.

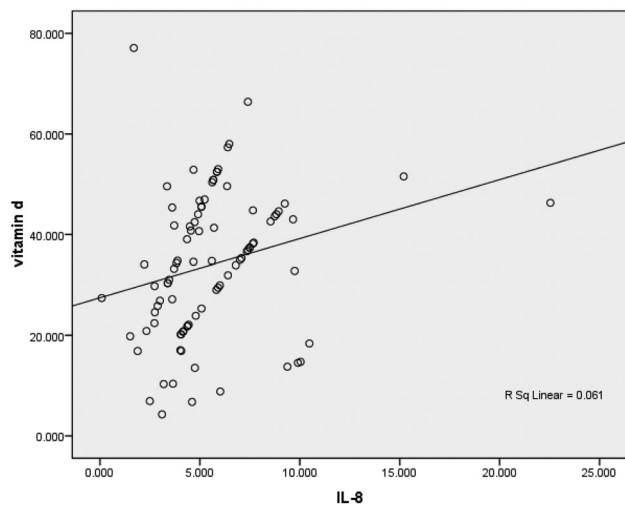


Figure 2. Scattered plot of vitamin D vs. interleukin-8.

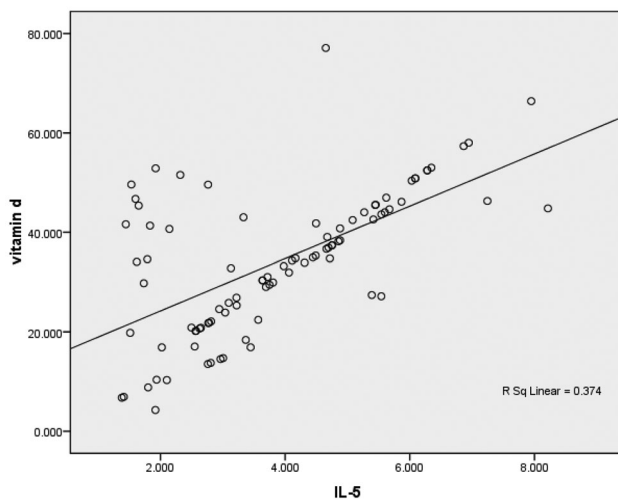


Figure 5. Scattered plot of vitamin D vs. interleukin-5.

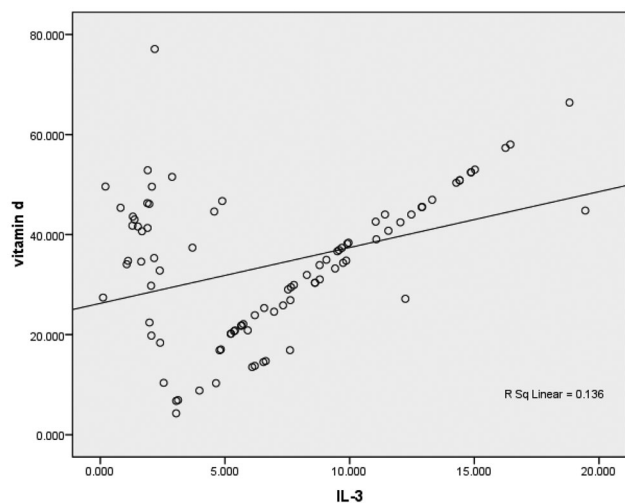


Figure 3. Scattered plot of vitamin D vs. interleukin-3.

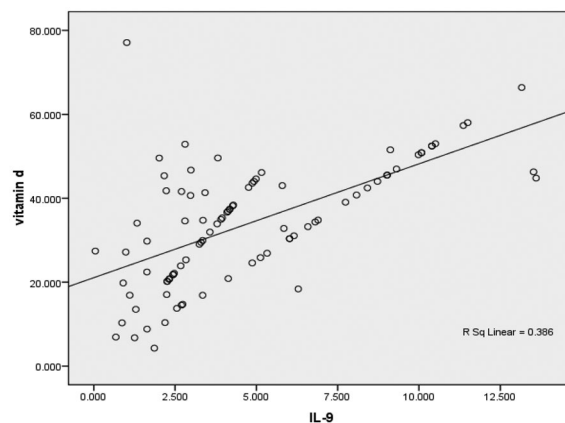


Figure 6. Scattered Plot of vitamin D vs. interleukin-9.

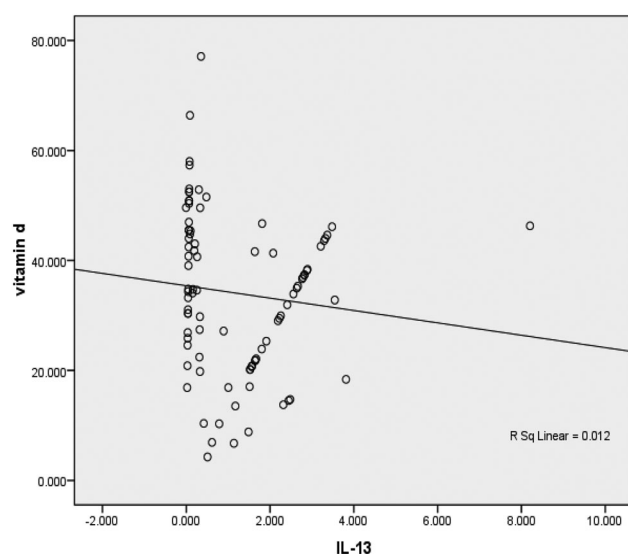


Figure 7. Scattered Plot of vitamin D vs. interleukin-13.

selected T helper cells type 1 and 2 inflammatory cytokines with serum vitamin D (25-OHD) in Nigerian children with asthma.

The relative hypovitaminosis D observed in the children with asthma was also reported by other workers in tropical (9,26,27) and non-tropical regions (28,29). Similarly as observed by many workers (27–29), more proportion of children with asthma had vitamin D deficiency and insufficiency compared to controls. Hypovitaminosis D observed in children with asthma may be related to increased need for immune-regulatory functions of vitamin D as asthma is associated with chronic inflammation, free radical generation and increased oxidative stress (7–9). Also environmental and dietary factors have also been proposed to explain the relative hypovitaminosis in asthmatics (30). Children with asthma may have exercise intolerance which often limit their exposure to sunlight and food allergy which may limit their intake of vitamin D rich dairy food (30,31). It is believed that the association between vitamin D and asthma is multifactorial and may be a cause-and-effect relationship (14). Children with Vitamin D deficiency/insufficiency may be at increased risk of asthma or asthma may predispose children to being vitamin D deficient (14,15).

Some researchers however reported that the effect of vitamin D deficiency on increased incidence of childhood asthma may be pronounced only in preschool children and this wean off during the school age and adolescent years (32,33). and some workers even linked vitamin D supplementation in infancy with higher prevalence of allergic diseases and asthma in adulthood (34,35). There is therefore more to be unraveled pertaining to vitamin D and childhood asthma.

Concerning asthma severity and level of symptoms control as related to serum Vitamin D levels, we observed no significantly relationship between serum 25-OHD in children with various categories of asthma severity and control. Similar findings were reported by Menon et al (36), Krobtrakulchai et al (37) and Neagu et al (38). Asthma severity and symptoms control are affected by other factors including socio-economic, presence of co-morbidities, exposure to triggers of exacerbations and even emotional and psychological factors (39,40). These cofounders may mask the effect of vitamin D in asthma severity and levels of symptoms control. Nevertheless, some workers reported positive association between asthma control and vitamin D (9,28,41), while Elnady et al (42) even reported inverse relationship between serum 25-OHD and asthma severity in Egyptian children. The effects of vitamin D supplementation on asthma severity and level of asthma symptoms control also yielded conflicting results (43–45). These call for more carefully designed studies on the relationship between serum vitamin D and childhood asthma severity and control.

Vitamin D had been reported to affect inflammatory cytokines produced by immune regulatory cells in culture and animal models (13–15). Unexpectedly in this study, both Th₁ and Th₂ serum inflammatory cytokines were generally reduced in children with asthma compared to controls. This may be related to the fact children with asthma often use corticosteroids either persistently as daily controller medications or intermittently to treat exacerbations and steroids have been reported to suppress the activities of immune cells and reduce the secretion of pro-inflammatory cytokines as one of the many anti-inflammatory properties (46).

Effects of vitamin D on inflammatory cytokines in children with asthma have been reported to involve complex and diverse pathways with conflicting results (13–15). In this study, we observed that children with low serum 25-OHD at deficient levels (< 20 ng/ml) had significantly higher levels of Th₁ cytokines (IL-2, 6, 8 and 10) and to a lesser extent IL-17 compared to those with insufficient (20 - <30 ng/ml) levels. This supports the findings that vitamin D deficiency is associated with more inflammation as vitamin D had been reported to inhibit Th₁ function in animal and human in-vitro culture model (8–9) and cause a shift in Th₁/Th₂ cytokine balance in favor of Th₂ (8,9,13–15). However, the findings that children with asthma at serum vitamin D sufficiency level had relatively higher Th₁ cytokines may be related to the fact

that at sufficient vitamin D level (> 30 ng/dl), its inhibitory functions on Th₁ cytokines may not be as pronounced as in deficient state. In addition, IL-12 (a Th₁ stimulating cytokine) was significantly lower in children with suboptimal vitamin D in this study which further supports the inhibitory effects of vitamin D on Th₁ cytokines.

Serum IL-10, (a known anti-inflammatory and immunosuppressive cytokine with ability to inhibit immune cells and cytokine production) (47) was found in this study to be significantly higher in children with sufficient vitamin D compared to the insufficient group. This finding was supported by the work of Heine et al (48) who reported that IL-10 secretion is increased in B-cells that are in-vitro stimulated by vitamin D. This may however be dose related (48). The poor stimulation of production of this important anti-inflammatory cytokine in vitamin D deficient state may explain the net increase of the Th₁ pro-inflammatory cytokines in deficient state compared to insufficient state as observed in this study and others (47–48).

In this study serum IL-4 was higher in children with vitamin D deficiency than the insufficient and sufficient groups which reflect an inhibitory effect of Vitamin D on serum IL-4 which is an important Th₂ cytokine in asthma pathogenesis. This may be a dose-dependent effect as the IL-4 level increases significantly in sufficient group compared to the insufficient group. This finding was supported by findings of Thiem et al (49) in which $1\alpha, 25$ -dihydroxyvitamin D₃ ($1\alpha, 25$ [OH] 2D₃) inhibits IL-4 production in-vitro by activated human peripheral polymorphonuclear cells. This inhibitory effect of vitamin D on IL-4 may be pronounced only at insufficient serum vitamin D level.

Pertaining to the relationship between the other Th₂ cytokines (IL-3, 5 and 9) and vitamin D, our study shows increasing serum levels of these cytokines with increasing serum vitamin D level. There are mixed reports on the effects of vitamin D on Th₂ cytokines in children with asthma as both inhibitory and stimulatory effects were reported in complex and diverse immunopathologic pathways in different animal models and T-cell culture models (13–15). It has been suggested that the effects of vitamin D on Th₂ inflammatory cytokines may also be a non-linear dose-dependent relationship in that serum vitamin D reached a level that eventually stimulate rather than inhibit Th₂ cytokine response (14–15). Also Hypponen et al (35) reported elevated immunoglobulin E (IgE) which is Th₂ cytokine driven in cohort of

asthmatics with very low or high vitamin D levels in a non-linear relationship. These support the finding in this study that asthmatics with sufficient vitamin D had significantly increased serum cytokines compared to those with suboptimal levels. Serum 25-OHD in this study also shows direct correlation with Th₂ cytokines except IL-13. This may be due to the fact that the baseline vitamin D levels of the children are relatively high hence there is no significant effect on inflammatory cytokines.

Vitamin D has been observed to affect immune responses in a number of other immunopathologic pathways which directly and indirectly affects the inflammatory cytokines. These include effects on regulatory T cells (T-regs), lymphocyte Adenosine triphosphate (ATP) metabolism and antigen presenting cells (8,9). Others include induction of antimicrobial peptides like cathelicidin and b-defensin-2 (8,9). All these actions coupled with the heterogeneous nature of asthma may influence the overall impact of vitamin D on inflammatory cytokines and may explain the non-linear and mixed relationship between serum vitamin D and inflammatory Th₁ and Th₂ cytokines in children with asthma (13–15).

This study has attempted to determine the relationship between serum 25-OHD and inflammatory cytokines in relatively poorly studied population of children with and without asthma in a tropical region. We assayed the serum 25-OHD and cytokines using quality assured standard HPLC methods in adequate sample of the population. We did not assay for other chemokines and inflammatory mediators, also bronchoalveolar lavage samples which could have been more representative of the local actions of these cytokines were not assayed. Nevertheless, this study highlights hypovitaminosis D in a population of children with asthma in a tropical region with abundant sunshine with immune regulatory implications. Dietary intake of food rich in vitamin D, exposure to abundantly available sunlight may improve serum vitamin D levels of these children and by extension inflammatory mediators balance and this is thereby recommended.

Declaration of interest

The authors have no conflicts to declare concerning the conduct and the reporting of the study.

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