SERUM 25-OH VITAMIN D IN CHILDREN WITH BRONCHIAL ASTHMA

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ABSTRACT

Vitamin D comprises a group of fat-soluble sterol compounds that can be ingested orally or synthesized endogenously in the skin after exposure to Ultraviolet (UV) B light. Several hypotheses have been proposed that vitamin D plays role in development of allergy and asthma. We determined 25-OH vitamin D in 36 cases of bronchial asthma versus 16 controls. Vitamin D level in asthmatic cases ranged from 88.64-252.6 nmol/l , while in control from 80-99nmol/l . Both levels still in normal range (80-250nml/l) according to current literatures. When we make stratification of vitamin D level in both cases and controls ,we found only 2 cases 5.6 % have level in range of control 80-100 nmol/l " control ranges , Remaining patient 94.4 **%** remained above control range ,. Also, we found significant statistical higher vitamin D level in asthmatic cases in relation to control (178.03±36..44 versus, 88.52±4.91nmol/l P was 0.000) , No significant difference in vitamin D in asthmatic cases in relation to age ,sex ,weight or respiratory distress .Also , no relation found to laboratory parameters including Hb level ,Htc. value .,,WBCs and ESR . These results highlight the necessity to updates and revise the reference range of vitamin D in relation to respiratory and immunological function . Also highlight importance of revision of multivitamin and vitamin D supplements for infants

INTRODUCTION

Vitamin D comprises a group of fat-soluble sterol compounds that can be ingested orally or synthesized endogenously in the skin after exposure to Ultraviolet (UV) B light at a wavelength of 290–315nm **(1)** Vitamin D does not, however, meet this classical description, as some of its characteristics are more like those of a hormone than of a vitamin. Such characteristics include, its metabolism at sites distant from its origin, its principle mode of action, which is mediated through stimulation of specific receptor proteins and the production of the active form, 1,25-dihydroxyvitamin D which is strongly feedback regulated at the physiological level **(2, 3).**

It has been known for many years that 1, 25(OH)2D3 is involved in bone mineralization by regulating calcium and phosphate metabolism [4]. However, in recent years other function has been suggested through detection vitamin D receptors (VDRs) in cells, which play a critical role in the human immune system, most notably monocytes, tissue macrophages and activated T- and B-lymphocytes **[5,6]**

Several hypotheses have been proposed to suggest that vitamin D status may play a role in autoimmune disorders rheumatoid arthritis (RA), multiple sclerosis (MS), type I diabetes mellitus (DM) and psoriasis **[7,8]**

Several studies have reported that lower maternal vitamin D status during pregnancy or during early childhood increases the risk of asthma and wheezing in the offspring and later childhood, respectively **[9,10].** Vitamin D has both in-utero and post-natal effects on lung development and immune system development and function. In the in utero period, vitamin D appears to play a role in fetal lung development, and recent findings support this. {**11]** Additionally, adequate vitamin D status in the postnatal period likely continues to affect lung development and immune system functioning**.{12}**

Asthma is a chronic inflammatory condition of the lung airways resulting in episodic airflow obstruction .Genetic, environmental factors cause disturbed immunological response in asthma with predominance of Th2 lymphocytes over Th1 phenotype **{13}** Significant interest exists in the immune-modulatory properties of the steroid hormone vitamin D. The vitamin D receptor is widely expressed throughout the immune system with possible effect on their balance (Th1&Th2) IL8 ,production of antigen presenting cell (APCS) ,inflammatory cytokines **(14&15)**

Some epidemiological data found association between vitamin D deficiency and bronchial asthma Vitamin D deficiency is more common among African American (AA) individuals**,(16)** especially those from urban environments**(17)** or with obesity**(18)** Similar epidemiologic patterns exist among youth with asthma. Robert J. et al 2010 founded associations between low vitamin D levels and asthma . in urban African American youth with persistent asthma with some consideration should be given to routine vitamin D testing in urban AA youth, particularly those with asthma. The same association was found in asthmatic children living in Mediterranean area (Italy) .Lower level more in cases with poor control **(19)**

PATIENTS & METHODS

36 children aged ≥ 2 years diagnosed as bronchial asthma was selected for this study from Banha Teaching Hospital . Detailed pediatric history including recurrent attacks ,

smoking exposure , medications , family history of asthma was taken , Detailed physical examination was done including systemic and local. Respiratory function was assessed (FEV) in aged 6 ys and older Diagnosis of asthma based on history of acute attack of cough ,dyspnea and wheezes in child more than 2 years in response to provocation which is associated with impaired lung function " in 6ys or older " and improved with bronchodilator β agonist in addition to family history and eosinophilia Those with congenital lung lesion , gastro-esophageal reflux disease (GEORD) , possible cardiac or mediastinal lesion were excluded from the study .Also those with history of vitamin D intake . were excluded

25-OH vitamin D was determined by Enzyme- Linked –Assay (ELIA) provided from Immune-Diagnostics company .The samples were collected ,centrifuged and separated. Serum were stored at -20|°C Sample were precipitated with precipitation reagent to extract OH- vitamin which is bound to Vitamin D Binding Protein (VDBP). VDBP- antibodies was added in solid phase It binds vitamin bound protein Washing were done to remove excess unbound antibodies to bounded OH vitamin D bound protein .After washing quantification of VDBP is achieved by incubation with host specific peroxidase libeled Abs using TMB (Tetra-methybenzidine) as enzyme substrate .An acidic solution then added to stop the reaction The color converts to yellow .The intensity of yellow color is proportional to concentration of OH vitamin D in the sample (20)

Written consent have been taken from parents of all children in this study . The study also was approved from Research Ethics Committee in General Organization For teaching Hospital and Institutes in Cairo

Statistical Analysis

Data were entered and analyzed by using SPSS 20.Data were expressed as mean ±SD for categorized variables .Chi-square, student-T test and correlation when appropriate P< 0.05 was statistical significant

RESULTS

Table (1) Demographic Data

		Control n=16	Cases n=36	P
Age (ys)	Ẍ ±SD	5.44±1.99	4.59±1.54	0.104
	Range	2-9	2-7	
Sex	♀	6 37.5 %	13 36.1%	0.346
	♂	10 62.5 %	20 55.56%	
Wt.(kg)	Ẍ ±SD	18.25±3.97	16.55±3.5	0.128
	Range	11-26	10-22.5	

Table (2) Frequency of Symptoms

Symptoms		Number	percentage
Dyspnea	moderate	27	75%
	Severe	9	25%
Family History. Of Asthma	-ve	22	61.1%
	+ve	14	38.9%
Color	Cyanosis	10	27.8%
	Pallor	3	8.3%
	normal	23	63.9%
Family H. of Smoking	-ve	22	61.1%
	+ve	14	38.9%
Previous attacks	-ve	7	19.4%
	+ve	29	80.6%

Table (3) Cases Versus Control Vital Signs

	Control n=16	Cases n=36	P
Pulse	102.5±11.4	106.25±18.45	0.457
	80-120	90-170	
Respiratory Rate.	30.5± 6.14	52.527± 14.7	0. 000
	22-40	40-95	
Temperature	36.82±0.42	36.78±0.47	0.829
	36-37.3	36-37.6	

Table (4) Cases Versus Control Regarding Laboratory Data

		Cntrol=16	Cases=36	P
Hb	Ẍ ±SD	13.33±1.2	11.5±0.95	0.000
	Range	11-16.20	9.2-12.5	
Htc	Ẍ ±SD	36.37±1.86	33.1±4.15	0.001
	Range	33.00-40.00	26.00-40.00	
RBCs	Ẍ ±SD	4.33±0.461	40.6±0.45	0.052
	Range	3.50-5.30	3.20-4.900	
WBCs	Ẍ ±SD	6050.625±1499.224	6962.777±696.865	0.032
	Range	4000.00-8500.00	5500.-8700.00	
CRP	Ẍ ±SD	00	00	00
	Range	00.00-00.00-	00.00-00.00	
ESR1STH	Ẍ ±SD	11.87±3.95	15.16±5	0.011
	Range	10.00-20	7.00-25.00	
ESR2NDH	Ẍ ±SD	23.75±7.18	30.36±9.3	0.016
	Range	20-40	15.00-45.00	
Platelets	Ẍ ±SD	381.25±94.44	325.27±73.23	
	Range	250.00-550.000	140.00-450.00	0.024
VITD LEVEL	Ẍ ±SD	88.52±4.91	178.03±36..44	0.000
	Range	80-99	88.64-252.6	

Table (5) Vitamen D Stratification In Cases , Control

Vitamin D level	Contro=16	Cases =36	Percentage
80-100 nmol/l	16	2	5.6 %
101-150nmol/l	0	4	11.1 %
151-200nmol/l	0	21	58.3 %
201-250nmol/l	0	8	22.2 %
>250 nmol/l	0	1	2.8 %

Table (6) Vitamin D Regarding Clinical Data In Patients Group

		No.	Ẍ ±SD	P
Sex	♀	13	170.32±35.2	0.347
	♂	23	182.5±37.18	
Wt.	Normal	24	192.25±31.827	0.28 1 was overweight not included
	Under wt.	11	167.54±46.2	
Dyspnea	Moderate	27	183.25±28.098	0.291
	Severe	9	162.36±53.82	
ecurrent attacks	Recurrent	29	177.906±35.907	0.967
	1st attack	7	178.55±41.59	
mily H.of asthma	+ve	16	166.85±47.34	0.1
	-ve	20	186.97±22.33	
Family smoking	+ve	14	190.887±23.7	0.092
	-ve	22	169.85±41.05	
Color	Normal	23	185.92±33.18	0.203
	Cyanosis	10	168.96±36.58	

Table (7) Vitamin D Correlation With Clinical Data

	R	P
Age	-0.139	0.418
Wt.	0.034	0.843
Pulse	-0.167	0.330
Resp. Rate	-.032	0.854

Table(8) Vitamin D Regarding Laboratory Data

	R	**P**
Hb	**-0.175**	**0.175**
Htc	**-0.281**	**0.097**
RBCs	**0.065**	**0.706**
WBCs	**0.107**	**0.534**
ESR	**0.136**	**0.429**

We selected 36 asthmatic patient from Banha teaching hospital 13 were male 23 were female .Their mean age was 4.59±1.54 years ,their mean weight was 16.55±3.5 kg .No significant statistical difference regarding sex, age and weight between our patients and control (P was 0.104, 0.346 and 0.128 respectively) "**Table 1"**

14 patients had family history of asthma while 22 had no similar history 9 had previous attacks of asthma while 7 presented in 1st attack. 14 had history of passive smoking while 22 did not have. All our patients presented with dyspnea 27 had moderate distress while 9 had severe distress. Also 10 cases presented with cyanosis , 3 presented with pallor ,while 23 had normal colored skin "**Table 2"**

No significant differences between cases and control regarding pulse rate and temperature (P was 0.457&0.829) However cases showed significant increase in respiratory rate compared to control (P was 0.000) **"Table 3"**

Asthmatic patients showed significant lower Hb , Htc ,platelet compared to control (P was 0.000 , 0.001 , 0.024 respectively) .However these cases showed higher WBCS ,ESR compared to control (P was 0.032 ,0.011 respectively) .RBCS was non-significantly lower in cases compared to control . (P was 0.052) "**Table 4 "**

Regarding vitamin D asthmatic cases showed significant higher level compared to control but still in normal ranges for both (P was0.000)" **Table 4**" With stratification , no deficient (< 50 nmol/l) or insufficient (< 75nmol/l) **(29)** individuals among either cases or controls. All control ranged between 80-99nmol/l ,only 2 cases 5.6 % have level in range of control 80-100 nmol/l " control ranges ' , Remaining patient remained above control range: "**Table 4**" 4 cases "11.1 %" ranged from 101-150 nmol/l ,21 cases "58.3 %" ranged from 151-200 nmol/l ,8 cases "22.2 %" ranged from 201-250nmol/l,1 case "2.8 %" > 250 nmol/l "**Table 5**" . .Moreover, no significant relation regarding regarding , sex, wt. and dyspnea (P was 0.347 , 0.28 , 0.291) found . Also no significant relation found regarding other clinical data (previous attacks , family history of asthma or passive smoking} P was 0.967 0.1 and 0.092 respectively "**Table 6**" No significant correlation found between vitamin D and age ,Wt., pulse and respiratory rate (P was 0.418 ,0.843 ,0.330 and 0.854 respectively) "**Table 7**" Vitamin D showed no relation no relation to Hb , Htc, RBCS , WBCS ,ESR, (P was 0.175 ,0.097 ,0.706 , 0.534 and 0.429 respectively) "**Table 8**"

DISCUSSION

In this study we determine vitamin D in 36 cases with asthma .we compared these case with 16 age , sex and weight matched controls (P was 0.104 , 0.346 ,0.128 respectively)

We measured 25-OH vitamin D rather than 1,25 vitamin D because it is reliable measure for vitamin D .It reflect the store of vitamin D It is bounded to vitamin binding protein (VDBP) .It constitute the major circulating form . The active form 1,25 OH vitamin D occur locally in renal and extra renal tissues . The local production of 1,25 (OH)2D3 by cells and the ability for the hormone to act directly on surrounding tissues, emphasizes the possibility that circulating plasma levels of 1,25(OH)2D3 may not truly reflect what is going on in specific tissues **(21)**

Among our cases 14 (38.9%) patients had family history of asthma while 22 (61.1%) had no similar history , Also 14 (38.9%) had history of passive smoking while 22 (61.1%) did not have such history .The similar percentage between family history of asthma and positive passive smoking in our report is accidental finding .This relative small percentage of family history of asthma and passive smoking indicate that environmental cause other than smoking appear to have role in pathogenesis of asthma. We found in previous report that copper is increased in wheezy chest compared to control **(22)** Among environmental risk factor of asthma ,inhalant, food allergen sensitization , bronchiolitis requiring hospitalization, pneumonia severe lower respiratory tract infection **(13)** Recent reports described new environmental air pollutants as a risk factor of asthma. Among this pollutants, benzene **(23)** 4-nonylphenol **,(24)** , ambient air zinc **(25)**

Among asthmatic patients ,we found significant decrease in Hb ,Ht. Platelet , non-significant decrease in RBCs(P was 0.000 , 0.001 , 0.024, 0.052 respectively) .Lower Hb. Level in asthmatic children may be due chronic inflammation associated with asthma and recurrent exacerbation which is precipitated with infection which is possible cause of anemia **(26)** .Another possible explanation is anemia may be a risk factor of asthma **(27)**

Also, asthmatic patients showed significant higher WBCs than control P was (0.032) this higher values due to infection , inflammatory reaction associated asthmatic attacks ,and due to medication " β agonist, steroids " **(28)**

Regarding vitamin D level , asthmatic cases showed higher level compared to control (P was 0.00) without relation to clinical data . In patient it ranged from 88.64-252.6 nmol/l, while in control 80-99 nmol/l Both values still in normal range (80-250nml/l) Most studies consider vitamin D deficiency < 50nmol/L ,insufficiency 50-80 nmol/L ,sufficiency 80-250 ,excess > 250 nmol/L **"29"** Others consider deficiency < 30 nmol/l , insufficiency 30-75 ,sufficiency >75nmol/l **(30)**

According to our study , no evidence of vitamin D deficiency or excess in both cases asthmatic cases and control according to current data in literatures .When we did stratification of vitamin D level ,only 2 cases 5.6% was in ranges of control range. Remaining 94.4 % of cases above upper limit of control >100 nmol/l. Still significant higher level in cases compared to control .The previous reference range for vitamin D based for studies not related to pulmonary function or allergies. This results need further investigation for revision of upper limit of vitamin D in relation to other newly detected functions related to allergies, respiratory function and cardiovascular diseases . The relation between vitamin D (25- OH vitamin D) and bronchial asthma in children still shows some controversy. **Menon J. et al 2012(31**) did not find any case with vitamin D deficiency in asthmatic children compared to control (.Also he did not found significant difference regarding vitamin D between asthmatic cases and control no relation found to asthma severity The decrease level of vitamin D in asthmatic cases(28.64 +/- 10.09ng/l) compared to our results(71.21+/-15.18ng/l) may be related to difference in age group. Our age group ranged from 2-9 ys , but their age groups ranged from 2-19ys. . There is some evidence that vitamin D effect on asthma is more evident in children rather than adult population (32) Still his control cases show lower values to our reference control results (71.05 +/- 26.175 vs 88.52±4.91 nmol/l) **.** Higher values in our cases and control relative to his values may explained on basis of possible medication abuse in our population including multivitamins and sunny atmosphere.

In our study we did not measure accurate indoor and outdoor stays .We did not assess housing in our patient sample. .Sunlight exposure showed some racial differences among different populations **(33&34)** .No available reports about effect of sun exposure and vitamin D level and sun exposure in our relative sunny near rural area compared to most reports done in less sunny northern atmosphere .This environmental factor in relation to vitamin D biology may need further study in our area .

On other hand , **Goleva E . et al 2012** have shown that vitamin D deficiency (<50 nmol/L) is more common in non-asthmatics (57%) than asthmatics (48%) **(32)** . No deficient or insufficient individual among our cases or control in our study "Table 4" This report supports our finding that no vitamin D deficiency or insufficiency in asthmatic cases Higher values of vitamin D level in our study in both cases and controls may be explained on environmental and cultural differences as mentioned before

We found significant higher level of vitamin D in asthmatic cases compared to control, while both in normal range. Some reports supports that excess vitamin D can be risk factor for development of asthma **HYPPÖNEN E. et al 2004 (35)** found an association between large-dose vitamin D supplementation in infancy(> 2000 i.u./day) and an increased risk of atopy, allergic rhinitis, and asthma later in life . A recent report by **Maija e t al 2013 (36)** described higher 25(OH)D_3 concentrations are associated with increased risk of wheezing and flexural dermatitis in children . Also, **Gale CR. 2008 (37)** found children whose mother concentration of 25(OH) vitamin D in excess of 75 nmol/l at late pregnancy had an increase risk of eczema at 9 month and asthma at 9 years compared to children whose mother with concentration < 30 nmol/l . A woman's serum concentration of 25(OH)-vitamin D during pregnancy is strongly predictive of her child's 25(OH)-vitamin D concentration at birth **(38) Wjst M. et al 2009 (39)** hypothesized positive association between oral supplementation with vitamin D and allergy pandemics

On other hand contrary to our results , some reports some reports described low level of vitamin D in bronchial asthma in children **Freishtat RJ ,et al 2010** found most of cases urban African American with asthma are either insufficient 86% ,< 30 ngm /l or deficient 54 % ,< 20 ngm/l **(40).**This report was done in northern latitude with less sun exposure, also it was done in population known already within increased risk of vitamin D deficiency due darker skin **(41**) and a diet inadequate to compensate for deficiencies (**42).**,also with lower socioeconomic standard ,urban inner city population with different age group 6-20 ys with mean age 11.1±0.4 y . In equatorial area , with sun repletion evidence of vitamin D deficiency and insufficiency was found only in 28 % of asthmatic ,while its serum

level negatively correlate with marker of allergy and asthma severity .In this study no control was taken **(43)** .The average daily intake in this area was185 iu /**day (44) ,** below daily recommended intake 200 i.u.. /l at normal sun exposure and 800-1000 i.u. when sun exposure is not adequate **(45)** We have no available reports about daily intake of vitamin D in area of our report. In mediterranean area ,no significant differences between mean 25-OH vitamin D between control (23.30 ±4.57 ng/mL) and .patients (20.89 ±7.51 ng/mL;) P was 0.12. However, when patients and controls were divided according to vitamin D classes, significant differences were observed Sufficient vitamin D levels observed only in six asthmatics (15.38%). Insufficient, deficient levels were found in 16,17 (41%,43.59%) patients respectively .Insufficient level was found only in 20 % of healthy control with no deficient level .(**46)** The detail of this study is not available

Association between high vitamin D and bronchial asthma in children has different explanations .One explanation what is called gut microbiome/vitamin D hypothesis .This hypothesis drawn links between the potential for vitamin D to regulate the gut microbiome and the emergence of asthma and autoimmune disease . Vitamin D is an important modulator of the signaling traffic between gut bacteria surface antigens via its effects on dendritic cells and the T regulatory cells .and has direct effect on gut bacterial flora to increase or decrease the number of specific species of bacteria. Composition of gut flora plays important role in development of allergy and gut immunity early in life **[47}**

Another explanation ,that 1,25(OH)2D increases allergen-induced T cell proliferation, IL-4 and IL-13 cytokine levels, and serum IgE production. induce a shift in the balance between Th1- and Th2-type cytokines toward Th2 domination **(48).**At higher values of 25(OH)D, some sort of vitamin D resistance may occur**.(49)**

Conflicting results regarding serum vitamin D in asthma in literatures can be explained on basis variable genetically determined vitamin D sensitivity. Genetic sensitivity to high or low vitamin D intake through low or high sensitive form of genes may be acause of this contradiction (50). Positive association of several vitamin D receptor (VDR) variants with asthma has been shown by previous U.S. [51] as well as one Canadian study **[52].**

It seems that widespread "historical" rickets in industrial countries was also a genetic disease. A formal twin analysis yielded a 91% concordance rate in monozygotic twins compared to 23% in dizygotic twins **[53)** This last hypothesis needs further investigation

Another explanation , it is possible that vitamin D is protected against asthma at recommended daily intake (45) and normal serum level (possible in range of our control or little higher) .At lower intake (<200- 400 i.u./ & 800-1000) **(45)** which is possibly associated with lower serum level (deficiency or insufficiency) or higher intake (> 2000 i.u./day)(35) which is associated with higher serum level (possible in range of our cases) , is associated with increased risk of asthma .This means vitamin D deficiency or excess is associated with risk of asthma ,while in normal range is protective regarding pulmonary function because it function as hormone rather than simple vitamin . Further studies are required to confirm this suggestion and define upper safe level regarding pulmonary and anti-allergic function

Conclusion:We determined 25-OH vitamin D in 36 asthmatic cases versus 10 controls .We found significant higher vitamin D level in asthmatic cases versus control but still in normal range according to current literatures Most cases(94.4 %) remained above control range . These results needs to revise the reference range of vitamin D in serum regarding respiratory function .Also needs to revise vitamin D and multivitamin supplements for infants

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