Associations between Vitamin D, Pulmonary Function, Asthma Control and Asthma Outcome: The Role of Vitamin D Receptor and Its Binding Protein.

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Abstract:
Background*: The mechanisms linking vitamin D and asthma are unknown. Distinct roles played by vitamin D binding protein (*VDBP*) and its receptor (*VDR*) may provide an explanation.*
Objectives*: To study the link among the level of Vitamin D, VDR, and VDBP with the pulmonary function, control of asthma and outcome in asthmatic Saudi females.*
Methods*: randomized case control study included 161 Saudi pre-menopausal women aged 25 to 45 years, 81 were asthmatics and 80 were age and weight matched controls normal. Data were collected using a structured questionnaire and baseline spirometry. Assessment of asthma control test (ACT) were done according to GINA guidelines. The 25-hydroxy vitamin D (25(OH)D), VDBP, and VDR were analyzed using ELISA.*
Results: *asthmatic women especially at risk of exacerbation, had significantly low levels of 25(OH)D. Uncontrolled asthmatics had significantly lower 25(OH)D and higher VDR than those with better control. The 25(OH)D correlated negatively with VDR but not with VDBP. The 25(OH)D predicted asthmatics (OR 0.924, 95% CI: 0.900-0.949), (OR=3.86, 95% CI: 1.27-11.76) and ACT (B=0.192, 95% CI: 0.173-0.211). VDR predicted ACT (B= -0.011 (95% CI:-0.014- -0.007).*
Conclusions*: Vitamin D deficiency are frequent in asthmatic women and are associated with poor control and outcome. Serum levels of VDR are linked to 25(OH) and behave in its opposite direction. The role of VDBP in clinical asthma parameters may be independent to the level of 25(OH)D and needs further studies.*
Key Words: <u>Asthma Control Test</u>, 25-hydroxy Vitamin D, Vitamin D Receptor, Vitamin D Binding Protein

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I. Background

Currently, the approach that accurately predicts response to treatment and risk of future exacerbation in asthma patients is still undefined (1, 2). A growing evidence from previous observational studies has offered a strong link between 25 hydroxy vitamin D and bronchial asthma (3,4) based on findings of inverse relation among level of vitamin D (25(OH)D) and increased hospital admission, emergency room visits, along with lung function affection, and airway hyper-responsiveness in patients with bronchial asthma (4,5). Moreover, a link with asthma exacerbation and treatment resistance has also been suggested (5-8). Unfortunately, clinical trials using vitamin D supplementation in asthma found conflicting results (9-10) with promising protective effects in some studies (6-8).

Vitamin D exerts its effects by binding to its cognate receptor protein (VDR) which has been described as a ligand-activated transcription factor that control gene expression (11)) and has traditionally been associated with calcemic activities, namely, calcium and phosphorus homeostasis and maintenance of bone content beside its importance as a modulator of cell growth and differentiation
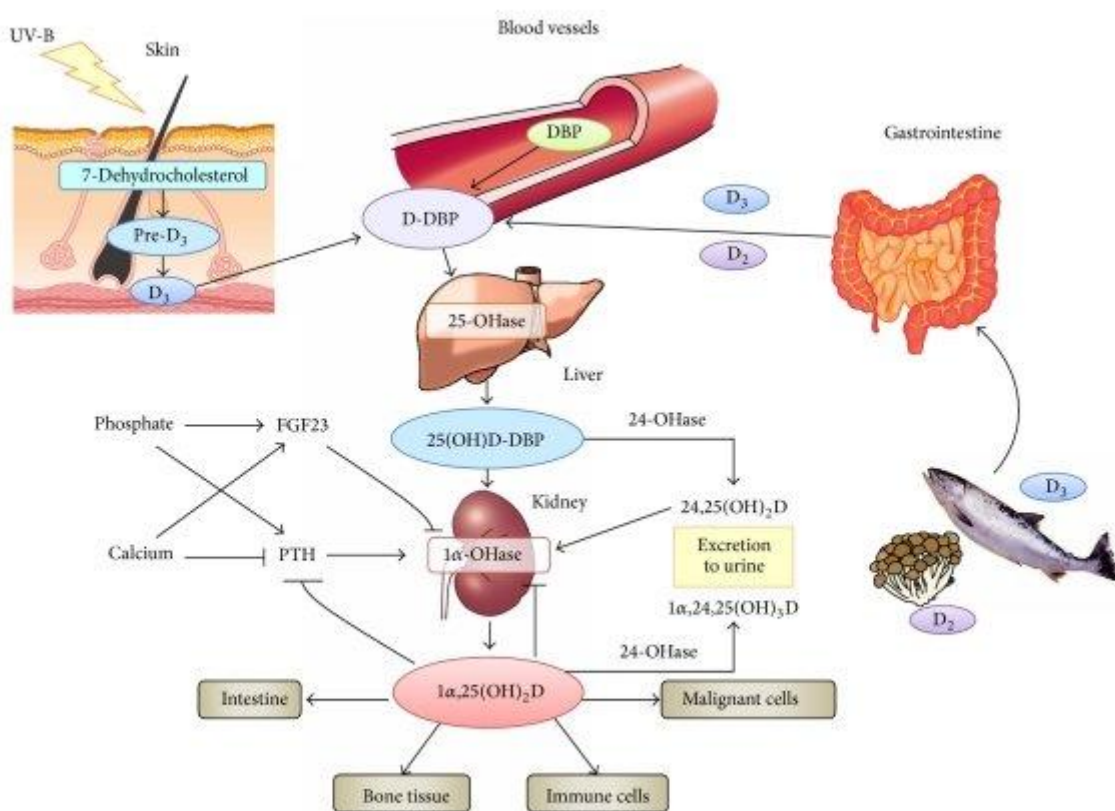

Figure (1) Shows Vitamin D metabolism

However, the observation that VDR is also present in cells other than those of the intestine, bone, kidney, and parathyroid gland led to the recognition of noncalcemic actions of VDR ligands. As a result, VDR is also known to be involved in cell proliferation, differentiation, and immunomodulation (12).The link between VDR gene polymorphisms and willingness to adult and childhood asthma was analyzed in 2 recent meta-analysis (13) with inconsistent findings. Vitamin D-binding protein (VDBP), a glycosylated-globulin, is the main holder protein of 25(OH)D3. It plays a key role for vitamin D axis in the lung as a part of the circulating actin scavenger system (14, 15). Many studies have found a link between plasma VDBP and different asthmatic parameters (16, 17).

To clearly understand the effect of the vitamin D pathway on the pathogenesis of asthma, a systematic study of the different roles of its various components is required. Therefore, this work aimed to study the link among the level of Vitamin D 25(OH)D, VDR, and VDBP with the pulmonary function, control of asthma and outcome in asthmatic Saudi females.

II. Subjects and Methods

This randomized case controlled study was done at the out-patient clinic in King Fahd Hospital, Madinah, KSA to collect cases and at the Taibah University female medical unit to collect controls. The size of sample of 202 was determined using "openEpi Sample Size Calculation for case-control sectional study" (Version 3.04.04) with two-sided significance level (α) of 0.05, Power (1-β, % chance of detecting) of 90%, proportion with disease in non-exposed (comparison) group of 0.4 and 0.5 in exposed group. This study was carried out in compliance with the Helsinki declaration and in accordance with Taibah University Ethics Committee regulations (No. 6230/35). Informed written consent were obtained from all the attended adult Saudi females before their inclusion into the study.

Pre-menopausal Saudi females, aged 25 to 45 years old, previously diagnosed with bronchial asthma were selected from the out-patient clinic of King Fahd Hospital with any degree of severity, and who were receiving any form of treatment. Age matched healthy Saudi females working or studying at Taibah University were recruited from medical unit's attendants for different reasons. Exclusion criteria were patients currently on oral steroid, cigarette smoking and who had history of repeated periods of immobility during hospitalizations, endocrine disorders, diabetes, or patients on drugs that could have effect on bone (antiepileptic, corticosteroids, antidepressants, vitamin D, and calcium). All of the followings were done to all patients' clinical history taking,

complete examination, chest X-ray and pulmonary function test. After implementation of the study, data were completed only for 161 women, eighty –one patients had intermittent asthma and 80 were healthy control women.

A well-structured questionnaire and demographic data of the patients (marital status, age, lactation, special habit and family history of atopy and bronchial asthma), were performed. Bronchial asthma history was taken in details (duration, provocations, frequency, and severity) with especial emphasis on current medications for bronchial asthma (oral and inhaled corticosteroids doses, frequency, and response).
Pulmonary function test; FVC and FEV1; was done according to ATS criteria (18) using the spirometer (Master Screen IOS; Jaeger, Höchberg, Germany) the use of short-acting bronchodilators was stopped for at least 6 hours before performing the test.

Asthma control test (ACT), asthmatic subjects were classified according to asthma control test (ACT) into controlled, partly controlled and uncontrolled according to Global Initiative for Asthma 2009. Available from: www.ginasthma.com. Asthma exacerbation (Poor asthma outcome), assessment of risk factors for asthma future exacerbations as an indicator for poor asthma outcomes was performed using the Global Strategy for Asthma Management and Prevention.

The risk of future exacerbation is increased if the patient had history of intubation or ICU admission, $\geq$ 1 severe exacerbation in last 12 months. Also, the risk of exacerbation is increased if the patient had one or more of the following risk factors:

- Uncontrolled asthma symptoms
- High short acting beta agonist use
- Inadequate inhaled corticosteroids
- Low EFV1
- Major psychological or socioeconomic problems
- Exposures (smoking and allergens)
- Comorbidities
- Sputum or blood eosinophilia
- Pregnancy

Blood samples collections: Blood samples were taken by venipuncture in the morning between 8:00 and 10:00 in winter season in Madina from December 2015 to February 2016. After an overnight fasting using complete aseptic condition by puncture of an ante-cubital vein. At room temperature the sample were left in a plain tube for 30-60 minutes for spontaneous clotting then serum separation was done by centrifugation at 3000 rpm for 10 minutes. The obtained serum was frozen immediately at −70 °C for future analysis. Total serum levels of vitamin D 25(OH)D, VDR and VDBP in samples were carried out by ELISA technique, using available commercial kit (CUSABIO and R&D SYSTEMS) using horseradish peroxidase detection in accordance with the manufacturer's instructions (50, 100 and 50 μL of sample respectively were used). Samples were assayed as duplicates with a minimal detection level of 5 μg/L for 25(OH)D, 1.65 pg/ml for VDR, and 0.65 ng/ml for VDBP. Deficiency of vitamin was defined as if 25(OH)D level of less than 50 nmol/L(20ng per milliliter), insufficient Vitamin D if 25(OH)D levels were between 50 and 75 nmol/L (20-30 ng per milliliter); and normal if levels of 25(OH)D levels were 75 nmol/L(30 ng per milliliter) to 100 nmol/L. Serum levels of 25(OH)D greater than 150 nmol/L. was defined as vitamin D intoxication (19).

III. Statistical Analysis

Statistics for baseline characteristics were calculated with the median (range). Comparison between different groups were done using non parametric tests. The associations were studied using Spearman's correlation and scatter plot graphs. Linear and binary logistic regression were used to estimate the Coefficient B, odds ratio (OR) and 95% confidence interval (CI) of predictors of asthma, asthma control test and asthma future exacerbation. All statistical tests were done using SPSS 23 software (SPSS Inc, Chicago, USA) and a p-value < 0.05 was recognized as statistically significance in all calculations.

IV. Results

The study included 161 premenopausal women 25 to 45 years old, 81 of them were asthmatic women and 80 were healthy age and BMI matched women. No statistically significant difference was found in age, BMI value, as well as concentration of circulating VDR, and VDBP on comparing patients with control group. The plasma 25(OH)D in asthmatic cases were significantly lower in comparison with the controls (p-value = 0.001). For vitamin D status, prevalence of insufficiency was 55 (67.9%) in the asthma group in comparison to 27 (33.8%) in the control group (p=0.000) (Table 1).

Table 1: The comparison between the asthmatic patients and the control subjects.

Characteristics	Asthmatic group N=81 Median (Range)	Control group N=80 Median (Range)	P-value
Age: years	44 (29)	43 (20)	0.745
BMI	30.11 (22.22)	30.11 (20.42)	0.201
Vitamin D status Insufficient: Sufficient: Normal:	27 (33.8%) 41 (51.2%) 13 (15%)	55 (67.9%) 25 (32.1%) 0 (0%)	0.000
25(OH)D: nmol/L	31.18 (59.2)	58.72 (45.5)	0.000
VDR: pg/ml	141.50 (530.50)	171.00 (227.00)	0.247
VDBP: ng/ml	135.2 (203.5)	113.50 (171.50)	0.320

Abbreviations: BMI, body mass index; 25(OH) D, 25-hydroxyvitamin D; VDBP, vitamin D binding protein; VDR: vitamin D receptor

In this study, asthmatic women were characterized by significant reduction of plasma 25(OH)D level than healthy controls with 67.9% of them has insufficient level compared to 33.8% in the control group. Low 25(OH)D levels was an independent predictor for all aspects of asthma: asthma development (OR 0.924), poor control (B 0.192) and poor outcome (OR 3.86). The VDR behaved in opposite direction to 25(OH)D in most associations, where it was significantly higher in asthmatics with poor outcome compared to those with better outcome (p=0.003). Moreover, VDR was found to be significant predictor for poor asthma control (OR -0.011). While we did not detect a direct connection between 25(OH)D or VDR with respiratory function, only VDBP showed association with poor respiratory function (OR -0.003 for FVC & OR -0.003 for FEV1). Interestingly, the correlations for all parameters of pulmonary function (FVC and FEV1) were in the same direction. Moreover VDBP was associated with poor asthma outcome (OR= 0.95). Uncontrolled asthmatics had significant lower levels of 25(OH)D and higher VDR than both partially controlled or controlled women (p=0.000 for all). There is no significant difference in the level of VDR between partially controlled or controlled asthmatics (p=0.133). Comparison between asthma patients with and without risk of future exacerbations (Table 3) showed that asthmatic at risk of future exacerbation had significantly lower levels of 25(OH)D (p=0.001) and higher levels of VDR (p=0.003) despite having significantly lower BMI. The VDBP levels did not show any significant differences in any comparisons, between patients and control groups (Table 1), between different groups of asthma control (table 2), between patients with or without high risk for future exacerbation (Table 3).

Table 2: Comparison between levels of asthma control (GINA guidelines) in asthmatic women.

	Uncontrolled asthmatics Median (range)	Partially controlled asthmatics Median (range)	Controlled asthmatics Median (range)	P-value
Age years	43.50 (9)	44 (19)	43 (20)	0.438
FVC	2.79 (1.24)	2.34 (1.04)	2.66 (1.12)	0.005
FEV1	1.84 (2.22)	1.54 (1.08)	1.58 (1.49)	0.032
BMI	28.90 (14.86)	30.20 (9.92)	30.92 (22.22)	0.171
25(OH)D	6.80 (6.16)	21.55 (14.71)	55.53 (22.46)	0.000
VDR	232.25 (448.5)	114.25 (89.5)	116.50 (261)	0.000
VDBP	140.80 (203.5)	143.50 (78.90)	113.70 (106.1)	0.190

Abbreviations: FVC, forced vital capacity; FEV1 forced expiration first second; BMI, body mass index; 25(OH) D, 25-hydroxyvitamin D; VDBP, vitamin D binding protein; VDR: vitamin D receptor

Table 3: Comparison between asthma patients with and without risk of future exacerbation (asthma outcome).

	Asthma patients with risk of future Exacerbation (n=17) Median (Range)	Asthma patients without risk of future Exacerbation (N= 64) Median (Range)	p-value
Age	44 (9)	44 (29)	0.859
FVC	2.92 (1.03)	2.43 (1.51)	0.000
FEV1	2.04 (1.73)	1.58 (1.72)	0.000
BMI	28 (89)	30.30 (22.22)	0.047
25(OH)D	6.62 (5.51)	46.47 (59.20)	0.00
VDR	167 (448.5)	121 (262.5)	0.003
VDBP	146.4 (168.2)	129.6 (160.8)	0.251

Abbreviations: FVC, forced vital capacity; FEV1 forced expiration first second; BMI, body mass index; 25(OH) D, 25-hydroxyvitamin D; VDBP, vitamin D binding protein; VDR: vitamin D receptor

Figure 2 shows the correlations between 25(OH)D, VDR, VDBP and ACT. ACT correlated positively with 25(OH)D (r=0.921, p=0.001) and negatively with VDR (R=-0.448, p=0.001). Figure 3 shows the correlations between VDBP and respiratory function tests. VDBP correlated negatively with FVC (r= -0.228, p=0.041) and FEV1 (r=-0.307, p=0.005). Figure 3 shows that a significant negative correlation exists between 25(OH)D and VDR (r=-0.316, p=0.004) but not with VDBP (r=0.117, p=0.298).

Predictors of respiratory function are seen in Table 4. Linear logistic regression analysis showed that age, BMI, 25(OH)D, VDR, VDBP could explain 21% of variability of FVC (R^2= 0.208, F= 3.949, p=0.003). Age (B=-0.016, 95% CI:-.030- -0.001)), BMI (B=-0.020, 95% CI: -0.037- -0.004), and VDBP (B=-0.002, 95% CI:-.004-0.000) were significant negative predictors of FVC. Age, BMI, 25(OH)D, VDR, VDBP could explain 29% of variability of FEV1 (R^2= 0.285, F= 5.99, p=0.000). Age (B=-0.029, 95% CI:-0.045- -0.012), and VDBP (B=-0.003, 95% CI:-0.005-0.000) were significant negative predictors of FEV1.

Predictors of asthma, the risk of future exacerbation (outcome) and the degree of asthma control are seen in Table 5. By binary logistic regression analysis, age, BMI, 25(OH)D, VDR, VDBP could explain 45% of asthma variance (R^2= 0.45, p=0.00). BMI was the significant positive predictor (OR 1.078, 95% CI: 1.001-1.161, p=0.047) and 25(OH)D was the negative predictor (OR 0.924, 95% CI: 0.900-0.949, p=0.000) of asthma development. Binary regression showed also that same predictors explained 86% of the variance of future exacerbation (p=0.000, R2 =0.86). The 25(OH)D (p=0.017) was negative predictor (OR=3.86, 95% CI: 1.27-11.76), p=0.017 and VDBP was positive predictor (OR= 0.95, 95% CI: 0.91-0.99), p=0.031 of exacerbations.

Figure 2: The association between VDBP and respiratory function tests.
(r= -0.228, p=0.041 for FVC and r=-0.307, p=0.005 for FEV1).

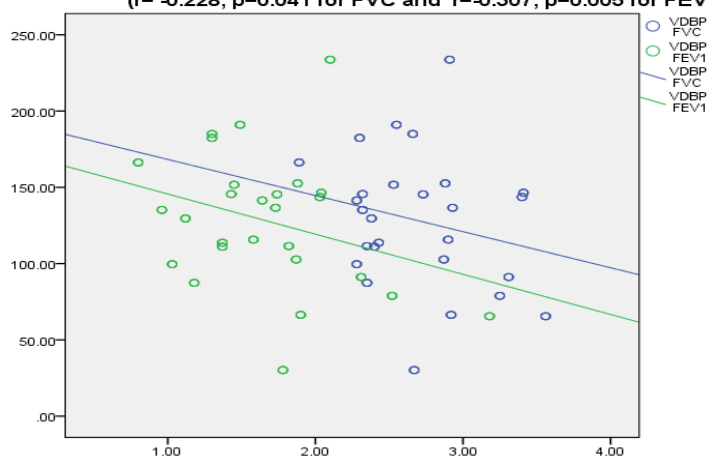Figure 3: The associations between 25(OH), VDR and VDBP.
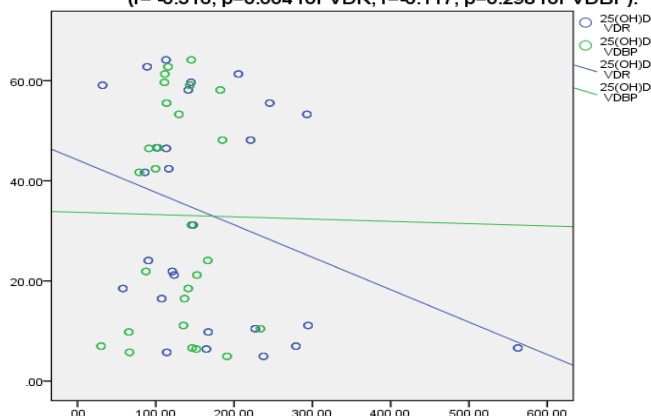(r= -0.316, p=0.004 for VDR, r=-0.117, p=0.298 for VDBP).

Table 4: Predictors of respiratory function

	Predictors of FVC		Predictors of FEV1	
	B (95% C.I.)	P	B (95% C.I.)	P
Age	-0.016 (-.030- -0.001)	0.034	-0.029 (-0.045- -0.012)	0.001
BMI	-0.020 (-0.037- -0.004)	0.014	-0.016 (-0.035-0.002)	0.088
25(OH)D	-0.001 -0.005-0.004)	0.735	-0.004 (-0.009-0.001)	0.122
VDR	0.001 (0.000-0.002)	0.084	0.000 (-0.001-0.001)	0.742
VDBP	-0.002 (-0.004-0.000)	0.044	-0.003 (-0.005-0.000)	0.026

Abbreviations: FVC; Forced vital capacity; FEV1, forced expiratory volume first second; BMI, body mass index; 25(OH) D, 25-hydroxyvitamin D; VDBP, vitamin D binding protein;VDR: vitamin D receptor

Table 5: Predictors of asthma, asthma control test and the risk of future exacerbation.

	Predictors of asthma development		Predictors of the risk of future exacerbation (outcome)		Predictors of asthma control test	
	OR (95% C.I.)	P	OR (95% C.I.)	P	B (95% C.I.)	p
Age	0.959 (0.895-1.027)	0.232	2.960 (0.97-9.05)	0.057	-0.079 (-0.142- -0.017)	0.014
BMI	1.078 (1.001-1.161)	0.047	0.609 (0.27-1.37)	0.232	0.138 (0.068- 0.208)	0.000
25(OH)D	0.924 (0.900-0.949)	0.000	3.86 (1.27-11.76)	0.017	0.192 (0.173- 0.211)	0.000
VDR	0.996 (0.991-1.001)	0.134	1.004 (0.99-1.021	0.659	-0.011 (-0.014- -.007)	0.000
VDBP	1.000 (0.991-1.010)	0.995	0.95 (CI: 0.91-0.99)	0.031	0.003 (-0.006- 0.012)	0.555

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; OR, odds ratio; VDBP, vitamin D binding protein, VDR: vitamin D receptor.

Linear logistic regression was used for testing the association between ACT and predictors including age, BMI, 25(OH)D, VDR, VDBP. Predictors could explain 90% of asthma control test scores ($R2= 0.90$, F= 136.67, p=0.000). All were significant except the VDBP. BMI and vitamin D were the significant positive predictors for ACT (OR= 0.138, 95% CI: 0.068-0.208, p=0.000 for BMI, and OR=0.192 (95% CI: 0.173-0.211), p=0.000 for 25(OH)D3). Age and VDR were the significant negative predictors for ACT (OR= -0.079, 95% CI:-0.142- -0.017, p=0.014 for age and OR= -0.011, 95% CI:-0.014- -0.007, p=0.000 for VDR).

V. Discussion
Vitamin D and its metabolites are steroid hormones and hormone precursors. About 80% derive from ultraviolet B (UVB) induced photoconversion in the skin of 7-dehydrocholesterol to previtamin D_3, and the remainder from the diet and from food supplements. Whether derived from skin, food, or supplements, previtamins D_2 and D_3 are biologically inactive and, in the liver and in the kidney, undergo two stages of

hydroxylation to the biologically active form of vitamin D, $1,25(OH)_2D$. Vitamin D and its metabolites are transported in the circulation by vitamin D binding protein (VDBP), and having reached their target cells, they dissociate from the VDBP and enter the cells.

In this study, we investigated Vitamin D and its related metabolites with incidence and severity of asthma in women. We found that asthmatic women were characterized by significant reduction of plasma 25(OH)D level than healthy controls and was an independent predictor for all aspects of asthma. The VDR behaved in opposite direction to 25(OH)D in most associations, where it was significantly higher in asthmatics with poor outcome compared to those with better outcome. Moreover, VDR was found to be significant predictor for poor asthma control. While we did not detect a direct connection between 25(OH)D or VDR with respiratory function, only VDBP showed association with poor respiratory function and was associated with poor asthma outcome. Interestingly, the correlations for all parameters of pulmonary function (FVC and FEV1) were in the same direction.

The exact consequence of these complex associations remains largely unexplained. The primary problem seems to be the low level of the 25(OH)D as both VDR and VDBP levels did not show any significant difference in asthmatics compared with control group. Because of the significant negative correlation between 25(OH)D and the VDR (Figure 3), we can postulate that low 25(OH)D causes compensatory elevation of serum VDR levels in uncontrolled asthmatic and those with high risk of exacerbation .

VDBP usually circulates at a much higher concentration than 25(OH)D (20). Therefore, deficiency of vitamin D may not have a strong influence on elevating the level of VDBP to a significant level as seen in our results. Besides, the high levels of VDBP may further insulate more 25(OH)D leading to less free and bioavailable 25(OH)D. Therefore, VDBP may play a unique role in different clinical parameters of asthma independent to the level of 25(OH)D. VDBP is responsible for transport of vitamin D metabolites and it can modulate the effect of vitamin D status on asthma severity because of its immune-modulatory actions. Neutrophil-expressed VDBP may assign directly to the chronic inflammatory response seen in asthma (21-24).

In this study, other independent predictors were found: age for poor respiratory function, BMI for asthma development, and age and BMI for poor asthma control. However, poor outcome is a function of vitamin D and its binding protein independent to age and BMI.

Our findings are in agreement with others' who demonstrated that insufficient vitamin D status is affiliated with an increase in the risk of asthma exacerbation indicating poor outcome (4, 25, 26). In our study, the absence of link between vitamin D and respiratory function is not in agreement with the finding from the Third National Health and Nutrition Examination Survey that included 14000 subjects (27). Although deficiency of D was found to be inconsistently prevalent in asthma (4,5, 28-30), it was found to decrease lung function in severe asthma (31) and predict asthma outcomes (32,33). One study (16) showed that low vitamin D is associated with steroid resistant asthma, decreased lung function, increased corticosteroid use, and asthma exacerbation independent of cathelicidin deficiency and other risk factors for asthma severity. There is a growing evidence that vitamin D has an influence on innate immunity through production of antimicrobial (34) and anti-inflammatory peptides especially against viruses (35). Hence decreased level of vit D exposes asthmatics to respiratory viral infections and exacerbation of attacks of asthma. The active 1.25(OH)D3 hormone itself is known to exert an inhibitory effect on airway smooth muscles and increases their glucocorticoid bioavailability (36,37).

While we did not find any association between serum VDBP and asthma control, Jiang's study supposed that serum VDBP may act as a significant biomarker for predicting steroid resistance in asthma patients. (38). This difference could be explained by genetic variation as the construction and function of VDBP may be affected by the presence of more than 120 single nucleotide polymorphisms in its gene (39).

The strength of our study is the investigating the complex connection between the serum level of a three metabolite of Vitamin-D relate them to both clinical and functional asthma measures. However, many limitations of our study should be consigned. First, the relatively small sample size that could not represent the general population and would reduce the statistical power. Second: selecting only women to be included in the study may make it difficult to generalize results on asthmatic men; however this helps to avoid the gender influence on certain parameters as VDBP. Third, multiple other confounding factors are not considered in the analysis including sun exposure, dietary habits, and physical activity. Finally: all samples were collected and analyzed at one point and this would not represent the total status of vitamin D, VDR and VDBP.

VI. Conclusion,

Asthmatic women were characterized by low vitamin D which was inversely linked to low VDR and was significant predictors for all clinical and functional aspects of asthma. VDR significantly predicted poor asthma control but its role may be dependent on low vitamin D. VDBP showed association with poor respiratory function and poor asthma outcome independently to low levels of vitamin D. These results might

give us new measures in treating bronchial asthma but further studies are required on large sample size and on male patients.

References

[1]   Asthma fast stats. Centers for disease control and prevention website. [Accessed January 05, 2017 (internet)]. Available from; http://www.cdc.gov/nchs/fastats/asthma.htm.
[2]   Sears MR. Trends in the prevalence of asthma. Chest Journal. 2014;142(2):219–225. [PubMed].
[3]   Baeke F, Takiishi T, Korf H, et al. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010;10:482–496. [PubMed]
[4]   Chinellato I, Piazza M, Sandri M, et al. Vitamin D serum levels and markers of asthma control in Italian children.. J Pediatr. 2011;158:437–441. [PubMed
[5]   Devereux G, Wilson A, Avenell A, et al. A case-control study of vitamin D status and asthma in adults. Allergy. 2014;65:666–667. [PubMed]
[6]   Urashima M, Segawa T, Okazaki M, et al. Randomized trial of vitamin D supplementation to prevent seasonal influenza A in schoolchildren. Am J Clin Nutr. 2010;91:1255–1260. [PubMed]
[7]   Majak P, Olszowiec-Chlebna Mg, Smejda K, et al. J Vitamin D supplementation in children may prevent asthma exacerbation triggered by acute respiratory infection. Allergy Clin Immunol. 2011;127:1294–1296. [PubMed]
[8]   Yadav M, Mittal K. Effect of vitamin D supplementation on moderate to severe bronchial asthma. Indian J Pediatr. 2014;81:650–654. [PubMed]
[9]   Hall SC, Agrawal DK. Vitamin D and Bronchial Asthma: An Overview of Data From the Past 5 Years. Clin Ther. 2017 May;39(5):917-929
[10]   Jensen ME, Mailhot G, Alos N, et al. Vitamin D intervention in preschoolers with viral-induced asthma (DIVA): a pilot randomised controlled trial. Trials. 2016;17:353–361
[11]   Pike JW and Meyer, MB. The Vitamin D Receptor: New Paradigms for the Regulation of Gene Expression by 1,25-Dihydroxyvitamin D3. Endocrinol Metab Clin North Am. 2010 Jun; 39(2): 255–269.)
[12]   Sunil Nagpal, Songqing Na, and Radhakrishnan Rathnachalam Noncalcemic Actions of Vitamin D Receptor Ligands Endocrine Reviews 26(5):662– 687 doi: 10.1210/er.2004-0002
[13]   Zhao DD, Yu DD, Ren QQ, Dong B, Zhao F, Sun YH. Association of vitamin D receptor gene polymorphisms with susceptibility to childhood asthma: A meta-analysis. Pediatr Pulmonol. 2017;52:423-429.
[14]   Chishimba, L., Thickett, D. R., Stockley, R. A. & Wood, A. M. The vitamin D axis in the lung: a key role for vitamin D-binding protein. Thorax 65, 456–462 (2010).
[15]   Zhang, J. & Kew, R. R. Identification of a region in the vitamin D-binding protein that mediates its C5a chemotactic cofactor function. J Biol Chem 279, 53282–53287 (2004).
[16]   Arikoglu T, Kuyucu S, Karaismailoglu E, Batmaz SB, Balci S. The association of vitamin D, cathelicidin, and vitamin D binding protein with acute asthma attacks in children. Allergy Asthma Proc. 2015 Jul-Aug;36(4):51-8.
[17]   Gupta A, Dimeloe S, Richards DF, Bush A, Saglani S, Hawrylowicz CM. Vitamin D binding protein and asthma severity in children. J Allergy Clin Immunol. 2012;129:1669–1671.
[18]   American Thoracic Society. Standardization of spirometry: 1994 update. Am J Respir Crit Care Med. 1995; 152: 1107–1136.
[19]   Heaney RP, Holick MF. Why the IOM recommendations for vitamin D are deficient. J Bone Miner Res. 2011;26:455–457
[20]   Cooke NE, Haddad JG. Vitamin D binding protein (Gc-globulin). Endocr Rev 1989;10:294–307
[21]   Yamamoto N, Homma S. Vitamin D3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. Proc Natl Acad Sci U S A. 1991;88(19):8539–8543. [PMC free article] [PubMed]
[22]   Piquette CA, Robinson-Hill R, Webster RO. Human monocyte chemotaxis to complement-derived chemotaxins is enhanced by Gc-globulin. J Leukoc Biol. 1994;55(3):349–354. [PubMed]
[23]   Kew RR, Sibug MA, Liuzzo JP, Webster RO. Localization and quantitation of the vitamin D binding protein (Gc-globulin) in human neutrophils. Blood. 1993;82(1):274–283. [PubMed]
[24]   Binder R, Kress A, Kan G, Herrmann K, Kirschfink M. Neutrophil priming by cytokines and vitamin D binding protein (Gc-globulin): impact on C5a-mediated chemotaxis, degranulation and respiratory burst. Mol Immunol. 1999;36(13–14):885–892. [PubMed]
[25]   Brehm, J.M., Schuemann, B., Fuhlbrigge, A.L., Hollis, B.W., Strunk, R.C., Zeiger, R.S. et al. Childhood Asthma Management Program Research Group. Serum vitamin D levels and severe asthma exacerbations in the Childhood Asthma Management Program study. J Allergy Clin Immunol. 2010; 126: 52–58
[26]   Font-Ribera L, Villanueva CM, Nieuwenhuijsen MJ, Zock JP, Kogevinas M, Henderson J. Swimming Pool Attendance, Asthma, Allergies, and Lung Function in the Avon Longitudinal Study of Parents and Children Cohort Am J Resp Crit Care Med. 2011;183(5):582–588.
[27]   Black, P.N. and Scragg, R. Relationship between serum 25-hydroxyvitamin D and pulmonary function in the Third National Health and Nutrition Examination Survey. Chest. 2005; 128: 3792–3798)
[28]   Shahin MYA, El-lawah AA, Amin A, et al. Study of serum vitamin D level in adult patients with bronchial asthma. Egypt J Chest Dis Tuberc. 2017;66:5–9.
[29]   Thuesen BH, Skaaby T, Husemoen LLN, et al. The association of serum 25OH vitamin D with atopy, asthma, and lung function in a prospective study of Danish adults. Clin Exp Allergy. 2015;45:265–272.[PubMed]
[30]   Camargo CA, Ingham T, Wickens K, et al Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma.. Pediatrics. 2011;127:180–187. [PubMed]
[31]   Gupta A, Dimeloe S, Richards DF, et al. Defective IL-10 expression and in vitro steroid-induced IL-17A in paediatric severe therapy-resistant asthma. Thorax. 2014;69:508–515. [PubMed]
[32]   Freishtat RJ, Iqbal SF, Pillai DK, et al. High prevalence of vitamin D deficiency among inner-city African American youth with asthma in Washington, DC. J Pediatr. 2010;156:948–952. [PMC free article][PubMed]
[33]   Montero-Arias F, Sed-Meja G, Ramos-Esquivel A. Vitamin D insufficiency and asthma severity in adults from Costa Rica. Allergy Asthma Immunol Res. 2013;5:283–288.
[34]   Adams, J.S. and Hewison, M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. Nat Clin Pract Endocrinol Metab. 2008; 4: 80–90

[35]   Hansdottir, S., Monick, M.M., Lovan, N., Powers, L., Gerke, A., and Hunninghake, G.W. Vitamin D decreases respiratory syncytial virus induction of NF-kappaB-linked chemokines and cytokines in airway epithelium while maintaining the antiviral state. J Immunol. 2010; 184: 965–97.

[36]   Song, Y., Qi, H., and Wu, C. Effect of 1,25-(OH)2D3 (a vitamin D analogue) on passively sensitized human airway smooth muscle cells. Respirology. 2007; 12: 486–494

[37]   Bosse, Y., Maghni, K., and Hudson, T.J. 1a,25-dihydroxy-vitamin D3 stimulation of bronchial smooth muscle cells induces autocrine, contractility, and remodeling processes. Physiol Genomics. 2007; 29: 161–168.

[38]   Jiang H, Chi X, Zhang X, Wang J. Increased serum VDBP as a risk predictor for steroid resistance in asthma patients. Respir Med. 2016 May;114:111-6.

[39]   Speeckaert, M., Huang, G., Delanghe, J. R. & Taes, Y. E. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta. 2006; 372, 33–42.