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Serum 25 *Hydroxy Vitamin D Levels In Adult Asthmatic Patients*

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Purpose: Patients with chronic lung disease as asthma appear to be at increased risk for vitamin D deficiency for reasons that are not clear.

Methods: A cross sectional study including 75 asthmatic adults aged older than 18 years and 75 adults healthy control aged older than 18 years (35 males and 40 females for both groups) assessing the relationship between serum 25 hydroxy vitamin D levels and lung function.

Result: In our study only (12،31%) of our asthmatic adults had sufficient vitamin D levels (≥ 30 ng/ml) , wherase (85%) of healthy control subjects expressed sufficient levels. Vitamin D deficiency (<20 ng/ml) was observed in 59 (78.66%) asthmatic patients (17.28 ±2.4 ng/ml). Deficiency was not observed in controls (33.67±6.3). In asthmatic patients Serum 25(OH) vitamin D was positively correlated with forced expiratory volume in 1 second % (FEV1 %) predicted &forced expiratory/forced vital capacity ratio (FEV1//FVC)(P=<0.05 for all). There was no significant association between serum vitamin D level and eosinophil count.

Conclusion: Reduced vitamin D levels are highly prevalent in adult asthmatic patients and are associated with impaired pulmonary functions.

INTRODUCTION

A connection between vitamin D status & asthma has been reported.. Vitamin D deficiency has been blamed as one cause of increased asthma prevalence in the last decades **(Litonjua and Weiss,2007)** A recent clinical investigation showed that high vitamin D levels associated a better lung function, less airway higher responsiveness & improved glucocorticoid response **(Sutherland *et al*.,2011).**

Vitamin D is a nutrient and hormone that can be obtained from a few natural foods (e.g. fatty fish and fish liver oils) and for fortified foods (e.g. Milk and cereal), and it can be generated endogenously from sunlight exposure via photosynthetic mechanism in the skin **(Holick,2007).** Increased maternal vitamin D intake during pregnancy has been linked to a decreased incidence of wheezing during childhood. A decreased risk of doctor-diagnosed asthma or recurrent wheezing episodes at three years of age was noted in those whose mothers had higher vitamin D intake during prenatal period **(Camargo*et al*.,2007).** The underlying mechanisms ,that vitamin D modulates the pathogenesis of asthma are not clear. Vitamin D may protect from developing respiratory infections that could serve as trigger for a deterioration of asthma **(Urashima *et al*.,2010).**Interestingly, application of vitamin D is potentially capable to overcome the poor glucocorticoid responsiveness in severe asthmatics by up regulation of IL-10 production from $CD4^+$ T cell **(Xystrakis *et al*.,2006).**

Studies further demonstrate that asthma may be linked to vitamin D on a molecular genetic basis. **Raby *et al*., 2004 and Poon *et al*.,2004)**,identified polymorphism of the vitamin D receptor (VDR) that influence asthma and allergy susceptibility. **Raby *et al*.,2003**, has suggested a link, located on chromosomes 12q, between asthma, airway responsiveness and pulmonary function indexes. In a different study, **Raby *et al* ., 2004** was able to demonstrate a genetic link between asthma and VDR located in close proximity on that same chromosome 12q. **Poon *etal* ., 2004**) discovered an association between VDR variants and the presence of asthma and atopy in a Quebec Cohort .

The present study examined the relationship of serum 25 hydroxy vitamin D levels with adult asthmatic patients and pulmonary function measurements.

SUBJECTS AND METHODS

Diagnosis of asthma

This cross-sectional study was carried out on 150 adults, 75 adult with asthma and 75 adult healthy control (70 women and 80 men for both groups) aged older than 18 years who had been referred to the Internal Medicine Department of Dr. Abdul Rahman Al-Mishari Hospital, KSA during the period from May 2011 to July 2011. All cases had current asthma symptoms including wheezing, cough, shortness of breath, waking up at night. Participants who had a history of consumption of any supplements of vitamin D or drugs that modulate serum vitamin D levels, such as anti-inflammatory medications were excluded. Asthma diagnosis was made based on the patients symptoms plus objective evidence from pulmonary function tests according to the criteria defined by the American thoracic Society in 1987.

Lung function test

Pulmonary function tests using the Master Screen system **(**Jaeger Co., Hochberg, Germany) were performed in all asthmatic subjects in the chest clinic laboratory of Abdul Rahman Al Mishari Hospital. The following parameters were documented:Forced expiratory volume in 1 second (FEV1),Forced vital capacity (FVC,) and peak expiratory flow rate and flow- volume loop. The best FEV1, FVC and FEV1/FVC values were selected for analysis.

Peripheral blood eosinophil count

A peripheral blood eosinophil count was measured using Coulter counter (LH 750 Model) technique.

Measurements of serum 25 (OH) vitamin D levels

Blood samples (serum or plasma) were collected and frozen to -20 C° until analyzed for a maximum three months. Serum concentrations of vitamin D 25(OH) were analyzed using fully automated machine (ABBOTT, Architect i1000, Germany) according to the manufacture's recommendations. The limit of detection of vitamin D was (8.0 – 160.0 ng/ml) & the normal range of vitamin D concentration was (30-40 ng/ml).Vitamin D level values were used as a continuous variable and were categorized in descriptive analyses as desirable (or sufficient) when scores were at least 30 to 40 ng/mL, insufficient between 20 and 30 ng/mL and deficient when <20

ng/mL.We examined the relationship between vitamin D levels and the following outcomes: eosinophil count, base line forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), FEV1/FVC ratio.

Principle of the Procedures

The Architect 25-OH vitamin D assay is a delayed one-step immunoassay including a sample pre-treatment for the quantitative determination of vitamin D in human serum or plasma using chemiluminescent microparticles immunoassay (CMIA) technology with flexible assay protocols, referred to as chemiflex. Sample and pre-treatment reagent are combined. An aliquot of the pre-treatment sample is combined with assay diluent and paramagnetic anti-vitamin D coated microparticles to create a reaction mixture.

A questionnaire completed through an interview included questions regarding age, sex, body mass index (BMI) and some outcome measures related to asthma and severity. The body mass index was calculated as Weight kg/Height (m^2).

Statistical Analysis

All data were statistically analyzed using Student's *t*-test, and chi-square(linear by linear correlation) tests, as applicable (with a preset probability of $P<0.05$). Experimental results are presented as arithmetic mean ± SD. Statistical tests were conducted using SAS version 9.1 (SAS institute, Inc., Cary, N.C.,USA). Additionally, using simple, multiple, and logistic regression analysis, the simultaneous effects of confounding variables such as, age, sex, vitamin D levels and body mass index (BMI) on the asthmatic state were measured

RESULTS

A total of 75 adult participants with persistent asthma and 75 adult healthy controls were examined. Characteristic of both asthmatic and control subjects are shown in table 1. The mean level of vitamin D 25 in the control and asthmatic subjects were 33.67±6.3 and 17.28±2.4 respectively. This difference was statistically significant (table1; P=0.001).

Table 1. Characteristics of subjects

Parameters	GROUPS		***P Value***
	Control Subjects	Asthmatic Subjects	
Age(yrs):	41.0± 1.5	39.5±1.2	0.009
Sex(female/male)	35/40	35/40	1[1]
BMI(Kg/m2)	20.2±0.3	24.42±2.5	0.866
Vitamin D (ng/ml)	33.67±6.3	17.28±2.4	0.001

•*Two Sample t-test; chi-square test.*

BMI, body mass index

Table 2 shows classification of asthmatic subjects according to vitamin D quartiles. As shown in table 2, there were statistically significant differences between quartiles in term of Sex, BMI, and Predicted FEV1(P<0.05). No other statistically significant difference among the quartiles (P>0.05).

Table2. Classification of asthmatic subjects according to Vitamin D quartiles.

Characteristics	**Asthmatic Patients**	**Quartiles of vitamin D Level**			**p value**
	17.28±2.4	**1st quartile**	**2nd quartile**	**3rd quartile**	**4th quartile**
No of patients	75	19	19	19	18
Age ***(yrs)***	39.51±1.2	41.3±3.3	39.3±2.3	35.9±1.5	40.5±2.4
Sex ***(female/Male)***	35/40	10/12	9/10	8/9	8/9
BMI***(Kg/m²)***	24.42±2.5		22.56±2.4	*0.002*ǂ	
Eosinophil Count ($x10^9$/L)	1.82±0.03	0.59±0.03	0.38±0.03	25.13±1.2	23.26±3.1
Eosinophil %	6.6±0.5	7.1±0.5	6.3±0.5	*0.006**	
FEV1, L ***(absolute)***	2.0±0.05	2.1±0.05	2.0±0.05	0.44±0.03	0.41±0.03
FEV1,% ***(predicted)***	67.95±2.9	63.1±2.9	62.8±2.9	*0.850**	
FEV1/FVC ratio	62.75±1.1	62.9±1.1	60.5±1.1	6.8±0.5	6.2±0.5
				1.91±0.05	*0.17**
				*0.643**	2.0±0.05
				65.1±2.9	
				*0.028**	70.8±2.9
				60.7±1.1	
				66.9±1.1	
				*0.090**	

*** linear by linear correlation (chi-square).**

BMI, body mass index; FEV1, forced expiratory volume in 1 sec.; FVC; forced vital capacity.

There was a significant association between higher serum vitamin D concentration and better lung function as evident by the absolute FEV1 % predicted and FEV1/FVC ratio (P<0.05 for all). (table2). Linear association between vitamin D levels and peripheral blood eosinophil count shows no significant association (P>0.05).

DISCUSSION

Low serum vitamin D has been recognized as a possible risk factor for several chronic lung diseases, including asthma and other respiratory disorders **(Glind *et al*., 2009,a&b).** In addition, reduced serum vitamin D levels are associated with increased expression of TNF-α suggesting that enhanced expression of this pro inflammatory cytokine is one potential pathway by which decreased vitamin D levels could exert a pro inflammatory effect in asthma. **(Berry *et al*., 2006 and Mora *et al*., 2008),** observational studies suggest that vitamin D deficiency increases the risk of respiratory infection which may contribute to the incidence of wheezing illnesses in adults and children and cause asthma exacerbations **(Ginde *et al*.,2009).**

In our study, vitamin D levels were compared between adult asthmatic patients and healthy control subjects. Although we find higher prevalence of vitamin D deficiency in asthmatic adults, this difference was not statistically

significant. In this study, linear association between serum vitamin D levels and measurement of lung function showed statistically significance as evident by FEV1 and FEV1/FVC ratio- (P<0.05 for all). **Black and Scraggy2005**, found that higher serum 25-OH vitamin D concentrations were associated with lung functions inducing FEV1 and FVC in united states and was positively associated in adults general population.

Our findings are also consistent with findings from a recent study of 54 US adult asthmatics, where vitamin D levels were also positively associated with lung function **(Sutherland *et al*.,2010).** A recent study reported by **Shaheen *et.al*.,2011**,.in an older adult UK population did not show a positive relationship between serum vitamin D levels and lung function in Spiro metrically defined COPD patient **(Shaheen *et al*.,2011).** Recently, **Zosky *et al*.,2011** reported that vitamin D deficiency decreased lung function **(Zosky *et al*.,2011).** Similary **Li *et al*.,2011**, reported that vitamin D deficiency was highly prevalent in chineese asthma patients, and vitamin D status was correlated with lung function

Hypponen *et al*.,2009, found significantly non-linear association between serum concentration of vitamin D levels and eosinophil count This study is an agreement with our study which shows non-significant association between vitamin D levels and eosinophil count. **Brehm *et al*.2009**, found an inverse relationship between serum vitamin D levels and markers of allergy such as total IgE and eosinophil count **(Brehm *et al*.,2009).**

Several studies suggested that vitamin D deficiency could lead to immune malfunctioning **(Zosky *et al*.,2011).** Although the exact mechanisms of lower vitamin D levels in chronic inflammatory states are not yet elucidated in asthma, this deficit probably interferes with immunoregulatory functions of vitamin D.Immune cells (T and B lymphocytes, macrophages and dendritic cells) express vitamin D receptors (VDR) and are affected by vitamin D deficiency during their maturation process.

In summary, in our study we found that, there is a direct relationship between vitamin D deficiency and impaired lung function, and there is no significant association between vitamin D deficiency and markers of allergy such as eosinophil count. These findings should be confirmed prospectively through the generation of an efficient multivaiate model allowing further research about the use of vitamin D supplementation in patients with asthma.

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مستويات المصل 25 هيدروكسي فيتامين(د) ومرضى الربو فى البالغين

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يعانى المصابين بامراض الرئة المزمنة مثل الربو من خطر متزايد نتيجة لنقص فيتامين(د) ولكن بدون سبب واضح. من هنا أجريت الدراسة الحالية على عينة من 75 مريض بالربو للبالغين للفئة العمرية أكثر من 18 عاما و مقارنتها مع 75 شخص من الأصحاء فى نفس المرحلة السنية فى محاولة للتعرف على علاقة مستوى فيتامين د بالدم بوظائف الرئة .

وتم أخذ العينات من المرضى فى قسم الباطنة العامة فى مستشفى الدكتور عبد الرحمن المشارى بالمملكة العربية السعودية فى الفترة من مايو 2011 وحتى يونيو 2011 وتم قياس مستوى فيتامين (د) فى الدم ووظائف التنفس فى معمل عيادة الصدر. وتم استبعاد الحالات التي تتناول جرعات من فيتامين (د) أو الأدوية التي تعدل مستويات فيتامين (د) ، مثل الأدوية المضادة للالتهابات.

وقد وجد فى 59 من عدد 75 مريض ربو (78.66%) نقص فى مستوى فيتامين(د)أقل من 20ng/ml ، كما كان لمستوى فيتامين(د) علاقة واضحة بوظائف الرئة متمثلة فى(FEV1 و FEV1/FVC) .

الا انه لم يثبت وجود رابط قوى بين مستوى فيتامين (د) ودلائل الحساسية المتمثلة فى خلايا الدم (Eosinophil cells).ومن هنا استنتجنا ان معظم حالات الربو فى البالغين تعانى من نقص فيتامين (د) بالدم مما ادى الى خلل فى وظائف الرئة .

لذلك فانه يوصى بالمزيد من الأبحاث حول استخدام مكملات فيتامين (د) في المرضى الذين يعانون من الربو لتأكيد هذه النتائج مستقبلا.