**ORIGINAL ARTICLE**

**Association of vitamin D status in the pathogenesis of chronic obstructive pulmonary disease**

Adel H. Ghoneim a, Mahmood A. Al-Azzawi b,c, Samir A. Elmasry b, Mohamed Y. Nasr b, Mohamed M.N. AboZaid a,*

a Department of Chest Diseases, Zagazig Faculty of Medicine, Zagazig University, Egypt
b Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, Sadat City University, Egypt
c Al-Mustansiriyah University, Baghdad, Iraq

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**Abstract**  
Background: The most important pathogenic mechanisms involved in the development of chronic obstructive pulmonary disease (COPD) are protease–antiprotease imbalance, inflammation, lung remodeling and oxidative stress. Interestingly, lower vitamin D levels have been related to regulation of each of these processes, that is, higher expression of proteases, modulation of inflammation, modulation of extracellular matrix turnover, and increased oxidative stress. Thus, one can speculate that lower vitamin D levels may be linked to increased risk of developing COPD. In some clinical studies, lower levels of vitamin D, measured as plasma 25-hydroxyvitamin D (25(OH)D), have been associated with lower lung function and faster lung function decline, but the results are conflicting.

**Aim of the study:** The aim of this study is to evaluate the evidence of the effect of vitamin D on COPD.

**Subjects and methods:** The present study, included 120 subjects, 80 patients diagnosed as COPD and 40 healthy volunteers (20 smokers and 20 nonsmokers). Plasma 25-OHD was measured by Enzyme Linked Immunosorbent Assay (DiaSorin, Stillwater, Minnesota, USA) in all study participants.

**Results:** The mean concentration of 25-OH-D was significantly higher in the reference groups (smokers and nonsmokers) compared with severe COPD group \((p = 0.001, 0.001)\) respectively.

The mean concentration of 25-OH-D was significantly higher in the reference groups (smokers and nonsmokers) compared with moderate COPD group \((p = 0.029, 0.049)\) respectively.

The mean concentration of 25-OH-D was not significantly higher in the reference groups (smokers and nonsmokers) compared with the mild COPD group.
Introduction

Vitamin D refers to a group of fat-soluble secosteroids responsible for enhancing intestinal absorption of calcium, iron, magnesium, phosphate and zinc. In humans, the most important compounds in this group are vitamin D3 (also known as cholecalciferol) and vitamin D2 (ergocalciferol). Cholecalciferol and ergocalciferol can be ingested from the diet and from supplements. The body can also synthesize vitamin D (specifically cholecalciferol) in the skin, from cholesterol, when sun exposure is adequate (hence its nickname, the “sunshine vitamin”). Synthesis from exposure to sunlight and intake from the diet generally contributes to the maintenance of adequate serum concentrations [1].

Vitamin D controls calcium absorption in the small intestine and works with parathyroid hormone to mediate skeletal mineralization and maintain calcium homeostasis in the blood stream. In addition, recent epidemiologic studies have observed relationships between low vitamin D levels and multiple disease states, probably caused by its anti-inflammatory and immune-modulating properties and possible effects on cytokine levels [2].

Vitamin D and COPD

Chronic obstructive pulmonary disease (COPD) has several systemic symptoms and consequences. Malnutrition, muscle weakness and osteoporosis are common comorbidities [3] in COPD that can be linked to vitamin D deficiency. Treatment with calcium and vitamin D supplementation reduces not only the risk of osteoporotic fractures [4], but interestingly also increases muscle strength and prevents falls [5].

This effect is mainly thought to be mediated by vitamin D. Vitamin D status is most commonly evaluated by measuring 25-hydroxyvitamin D (25-OH-D) instead of the active 1,25-dihydroxy vitamin D. The mechanism by which vitamin D affects the pathogenesis of COPD is unclear. Comorbidities of COPD such as reduced bone mineral density and skeletal muscle weakness have been associated with low vitamin D serum concentrations [3]. However, studies show that vitamin D can modulate the activity of various immune cells which inhibit inflammatory responses and regulate airway smooth muscles [6]. A review of molecular and animal experiments showed that vitamin D regulates airway contraction, inflammation, and remodeling in airway smooth muscles characteristic of COPD [6]. A cross-sectional study found that higher plasma levels of vitamin D are associated with increased bone mineral density and exercise capacity in people with COPD [7].

Evidence also showed that a high dose of vitamin D supplementation improved respiratory muscle strength and exercise capacity in people with COPD [7]. A study among 414 smokers with COPD showed that vitamin D deficiency is highly prevalent in this population, and correlates with disease severity. The study also found that genetic determinants for low vitamin D levels were associated with an increased risk of COPD [8].

The relatively long half-life of 25-OH-D makes it a better marker for both dietary intake and skin synthesis from sun exposure. The National Health and Nutrition Examination Survey (NHANES) III study showed a positive correlation between serum 25-OH-D levels and spirometry performance [9]. A subsequent study conducted in the UK on a small group of patients with COPD did not reach the same conclusion. However, it was found that a higher dietary intake of vitamin D was associated with better lung function and a lower prevalence of COPD [10]. Another study from Belgium showed that COPD patients often have vitamin D deficiency and a low serum level of vitamin D correlates with the severity of the disease [8].

The latest consensus suggests levels below 25 nmol/L indicate deficiency, between 25 mol/L and 50 mol/L indicate insufficiency, between 50 nmol/L and 75 nmol/L indicate satisfactory levels and above 75 nmol/L indicate optimal levels [11].

Materials and methods

Patients and controls

The COPD group consisted of 80 adults, who were diagnosed as COPD at the Chest Department, Zagazig University, Faculty of Medicine, Egypt. The diagnosis was based on the signs and symptoms, as well as on the results of pulmonary function tests including: forced expiratory volume by the end of the first second (FEV1) percentage of predicted value (FEV1/predicted FEV1%) using portable vitalograph copd-6 model 4000 (Vitalograph Ltd, Gort Road business Park, Ennis, Co. Clare, Ireland) (Fig. 1).

Classification of the disease severity (mild, moderate or severe) was done according to [12] as follows depending upon the obtained value of FEV1/predicted FEV1%:

- Mild: 80% or above (symptoms should be present to diagnose COPD in people with mild airflow obstruction).
- Moderate: 50–79%.
- Severe: 30–49%.
- Very severe: below 30% (less than 50% but with respiratory failure). Very severe cases were excluded from this study.

Classification of the severity of cigarette smokers was done according to the number of pack-years (P-Y); number of cigarettes smoked per day multiplied by the duration in years (Smoking Index) and divided by 20 as follows [13]:

- Mild smokers: less than 20 P-Y.
- Moderate smokers: 20–49 P-Y.
- Heavy smokers: more than 49 P-Y.

Note: (1 pack has 20 cigarettes).

Healthy controls (smokers and nonsmokers) collected from non-chesty patients referred from Outpatient Clinics and inpatients of different Departments of Zagazig University Hospitals to the Chest Outpatient Clinic for clinical and functional assessment e.g. before abdominal, eye operations etc. and also from voluntary people who work in this hospital.

All control cases had no historical, clinical or radiological data suggestive of chest problems and they had normal spirometric data and even smokers and their pulmonary function tests showed an FEV1/FVC ratio > 70%.

**Determination of vitamin D**

Plasma 25-OHD was measured by Enzyme Linked Immunosorbent Assay (DiaSorin, Stillwater, Minnesota, USA) in all study participants, as previously described.

**Method description**

The IDS 25-Hydroxy vitamin D EIA kit is an enzyme immunoassay for the quantitation of 25-OH D and other hydroxylated metabolites in serum or plasma. Calibrators, controls and samples are diluted with biotin labeled 25-OH D. The diluted samples are incubated in microtitre wells which are coated with a highly specific sheep 25-OH D antibody for 2 h at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labeled avidin, is added and binds selectively to complexed biotin and, following a further wash step, color is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtitre plate reader, color intensity developed being inversely proportional to the concentration of 25-OH D [14].

**Specimen collection and storage**

The assay should be performed using serum or plasma (EDTA or heparin) specimens. Specimens should be separated as soon as possible after collection. For long term storage, it is stored at −20 °C. Avoided repeated freeze/thaw of samples.

**Quality control**

The regular use of control samples at several analyzed levels is advised to ensure day-to-day validity of results. Two kit controls are provided. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.

**Expected values**

The following range has been determined using the IDS 25-Hydroxy vitamin D EIA kit and is provided for guidance only. Each laboratory should determine ranges for their local population.

- Normal adults: 47.7–144 nmol/L (n = 36).

**Statistical analyses**

All statistical analyses were carried out using the SPSS (statistical package for the social science software) statistical package version 20.0 (SPSS Inc., Chicago, IL, USA) for Windows. Quantitative data were expressed as mean and standard deviation (X ± SD) and analyzed by applying student’s t-test for comparison of two groups of normally distributed variables. The results of the “t”-value are then checked on student’s “t”-table to find out the significance level (p-value) according to the degree of freedom. All these tests were used as tests of significance at P < 0.05. [15].

**Results**

A total of 120 subjects, including 80 COPD patients, 20 healthy smokers and 20 nonsmokers as control subjects were studied. The mean age of the COPD patients was (54.9 ± 9), male/female ratio was (27/7), number of pack-years was

<table>
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<tr>
<th>Table 1</th>
<th>Clinical characteristics of the COPD patients.</th>
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<tr>
<td>Character</td>
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<tr>
<td>Age</td>
<td>54.9 ± 9</td>
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<tr>
<td>Sex (M/F)</td>
<td>27/7</td>
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<tr>
<td>Cigarette (pack-years)</td>
<td>46.4 ± 1.7</td>
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<td>FEV1 (%)</td>
<td>59 ± 3.7</td>
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<td>Age</td>
<td>52.2 ± 2.1</td>
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<tr>
<td>Sex (M/F)</td>
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<tr>
<td>Cigarette (pack-years)</td>
<td>33.7 ± 1.2</td>
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<th>Table 3</th>
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<td>Character</td>
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<tr>
<td>Age</td>
<td>55.7 ± 2.3</td>
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<tr>
<td>Sex (M/F)</td>
<td>11/9</td>
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</table>
The mean concentration of 25-OH-D was significantly higher in the reference groups (smokers and nonsmokers) (53.4 ± 13.3), (52.5 ± 19.5) ng/mL, respectively compared with (30.7 ± 9.2) ng/mL in the severe COPD group (p = 0.001, 0.001), as shown in Table 4, Fig. 2.

The mean concentration of 25-OH-D was significantly higher in the reference groups (smokers and nonsmokers) (53.4 ± 13.3), (52.5 ± 19.5) ng/mL, respectively compared with (44.7 ± 11.1) ng/mL in the moderate COPD group (P = 0.029, 0.049) respectively, as shown in Table 5, Fig. 3.

The mean concentration of 25-OH-D was not significantly higher in the reference groups (smokers and nonsmokers) (53.4 ± 13.3), (52.5 ± 19.5) ng/mL, respectively compared with (48.9 ± 15.4) ng/mL in the mild COPD group, as shown in Table 6, Fig. 4.
Discussion

Vitamin D is a seco-steroid hormone important in bone mineralization and calcium homeostasis. Recently, research has found that vitamin D may play a role in multiple chronic diseases such as cancer, autoimmune diseases, infections, and cardiovascular disorders (see Fig. 5).

Vitamin D may also have a role in several diseases involving the respiratory system. Higher vitamin D concentrations, assessed by 25-hydroxyvitamin D [25(OH)D], have been associated with better lung function as measured by forced expiratory volume in 1s (FEV1) in a large cross-sectional study of the U.S. Population in the NHANES III. Although the precise connection between vitamin D status and lung function is unclear at this point, the mechanism by which vitamin D improves lung function may be through its action on regulating inflammation, inducing antimicrobial peptides, and/or its action on muscle. There have been numerous studies looking at vitamin D status in association with various lung diseases focusing on asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), and respiratory infections. These studies have demonstrated a high prevalence of vitamin D deficiency in their participants [16].

Our study indicates that the mean concentration of 25-OH-D was significantly higher in the reference groups (smokers and nonsmokers) (53.4 ± 3.3), (52.5 ± 19.5) ng/mL, respectively compared with (30.7 ± 9.2) ng/mL in the severe COPD group (p = 0.001).

Also the mean concentration of 25-OH-D was significantly higher in the reference groups (smokers and nonsmokers) (53.4 ± 13.3), (52.5 ± 19.5) ng/mL, respectively compared with (44.7 ± 11.1) ng/mL in the moderate COPD group (p = 0.029, 0.049 respectively).

But the mean concentration of 25-OH-D was not significantly higher in the reference groups (smokers and nonsmokers) (53.4 ± 3.3), (52.5 ± 19.5) ng/mL, respectively compared with (48.9 ± 15.4) ng/mL in the mild COPD group.

We found that there is a correlation between low levels of vitamin and the severity of the disease; this is consistent with [8]. A number of studies have shown an association between vitamin D deficiency and severity of COPD [8,17]. Lower vitamin D status in COPD may be due to diminished production of pre-vitamin D3 associated with skin aging caused by smoking and limited UVB exposure [18,19].

Studies have shown that the degree of vitamin D deficiency correlates with the severity of the disease as measured by the reduction of FEV1 [9,8,17].

The connection between Vit.D status and COPD has attracted attention in the recent years. This is based on data from observational studies that determined levels of Vit.D in COPD patients. Black and colleagues examined data from the NHANES III data set (cross-sectional survey of 14091 adults in the US). After adjustment for potential confounders, a strong relationship between serum levels of Vit.D and lung function (FEV1 and FVC) was found [9].

A number of studies have reported on 25-(OH)D3 levels in COPD patients. [20] found Vit.D deficiency (in this study...
defined as below 20 ng/ml) in more than 50% of a cohort waiting for lung transplantation [20].

A recent study showed that Vit.D deficiency is highly prevalent in COPD and correlates with variants in the Vit.D binding gene [8]. There are several factors that could account for Vit.D deficiency in COPD patients: poor diet, a reduced capacity of aging skin for Vit.D synthesis, reduced outdoor activity and therefore sun exposure, an increased catabolism by glucocorticoids, impaired activation because of renal dysfunction, and a lower storage capacity in muscles or fat due to wasting [21]. Many steps of the Vit.D pathway (intake, synthesis, storage, and metabolism) can potentially be disturbed in COPD patients. A single nucleotide polymorphism (SNP) of the DBP was shown to be associated with a decreased risk of COPD by a mechanism that is unclear [22]. Similar SNPs in the gene coding for DBP may influence levels of circulating 25-(OH)D₃ and 1,25-(OH)₂D₃ [23,24].

Therefore it has been hypothesized that their protective role might be mediated by the bioavailability of 1,25-(OH)₂D₃ [19]. The mechanisms that link Vit.D biology with the development of COPD are largely speculative:

(1) The association of Vit.D deficiency and reduced lung function could depend on the calcemic effects of Vit.D. The vital capacity and total lung capacity were found to decline with an increasing number of thoracic vertebral fractures as a direct consequence of Vit.D deficiency. [25,26] 3030 ambulatory COPD patients were observed and a strong association between COPD severity and fractures was found [26]. Kyphosis related to osteoporosis caused limitation in rib mobility and inspiratory muscle function and correlated with a reduction in FEV₁ and FVC [27]. The altered properties of the thoracic skeleton could result in failure of the respiratory muscles contributing to the pathophysiology of COPD.

(2) Vit.D deficiency could result in altered host defense of the lung with subsequent growth of an abnormal flora that triggers inflammation. Acute exacerbations of COPD are an important cause of hospitalization and lead to a faster decline in FEV₁ [28]. Exacerbations are triggered by viruses, bacteria, atypical strains, or a combination of these [29–31]. Potential bacterial pathogens are detected in about 50% of exacerbations. A therapeutic consequence would be the up-regulation of the innate immune defense system. Wang and colleagues demonstrated that genes coding for the antimicrobial peptide cathelicidin (LL-37/hCAP-18) are regulated by VDRE-containing promoters [32]. In cultured monocytes, a local increase of the 1,25D3-VDR complex stimulates the production of LL-37, resulting in an improved intracellular eradication of Mycobacterium tuberculosis [33].

Figure 4 25-OH-D levels, in mild COPD patients and control groups (smokers and nonsmokers), where (1 = mild COPD, 2 = Smoker, 3 = nonsmoker).

Figure 5 25-OH-D levels, in Severe, Moderate, mild COPD patients and control groups (smokers and nonsmokers) [mean (±SD)].
The data demonstrated that the activation of TLRs on human monocytes triggers a microbicidal pathway that is dependent on both the endogenous production and action of 1,25-(OH)2D3 through the VDR.

(3) The effect of Vit.D on extracellular matrix homeostasis not only in bone tissue, but also within the lung may have a role in COPD development. Boyan et al. found Vit.D to be an autocrine regulator of extracellular matrix turnover and growth factor release via matrix metalloproteinases [34]. Matrix metalloproteinasis-9 (MMP-9) has been shown to be elevated in induced sputum of COPD patients and a causative role has been suggested in the development of COPD [35]. Vit.D also to attenuates TNF alpha induced up regulation of MMP-9 in keratinocytes [36].

Vit.D deficiency may lead to a reduced attenuation of MMP-9 activity resulting in enhanced degradation of lung parenchyma. Recently, it has been recognized that COPD is a systemic disease with several closely related comorbidities [37]. Interestingly, Vit.D deficiency is associated with an equivalent spectrum of diseases including coronary heart disease, cancer; inflammatory disease and infection [18]. Comorbidities of COPD such as reduced bone mineral density and skeletal muscle weakness [5] have been associated with low Vit.D serum concentrations.

Conflict of interest

No conflict of interest.

References


