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Corresponding author:
Maksuda Bintey Mahmud, Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Dhaka. email: maksuda.lima999@gmail.com

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Does Vitamin D_3 supplementation affect antioxidant enzymes of D_3 deficient patients with asthma COPD overlap (ACO)? - A randomized controlled trial

Maksuda Bintey Mahmud[1], Taskina Ali[1], Kazi Saifuddin Bennoor[2], Md. Ali Hossain[2], Mustafizur Rahman[2], Mohammad Abdus Shakur Khan[2], Md. Saiful Islam[1], Samia Hassan[3], Salsa Bil Nahar[4], Sharkia Khanam Rosy[5], Naznin Sultana[1], Salma Anjum[1]

1. Department of Physiology, Bangabandhu Sheikh Mujib Medical University, Dhaka.
2. Department of Respiratory Medicine, National Asthma Center, National Institute of the Diseases of Chest and Hospital, Dhaka.
3. Department of Physiology, Anwer Khan Modern Medical College, Dhaka
4. Department of Physiology, Abdul Malek Ukil Medical College, Noakhali.
5. Department of Physiology, Shaheed Suhrawardy Medical College, Dhaka

Abstract

Background: Asthma COPD overlap (ACO) is a new disease entity where respiratory tract is continuously exposed to oxidants. Supplementation of vitamin D_3 have shown evidence of increasing antioxidant enzymes level which neutralize these oxidants. **Objectives**: To evaluate the effects of vitamin D_3 supplementation on two antioxidant enzyme levels in D_3 deficient ACO patients. **Methods:** A double blinded placebo controlled randomized clinical trial (RCT) was carried out on 60 (sixty) D_3 deficient [serum 25-hydroxycholecalciferol, 25(OH) D<30 ng/ml], male, smoker, stable ACO patients of age 40 to 80 years. This RCT was registered at www.clinicaltrials.gov identifier NCT03931889. After the final selection, all the patients were randomly allocated to vitamin D_3 supplemented 'Study' group (n=30) or placebo treated 'Control' group (n=30). Two antioxidant enzyme levels [plasma superoxide dismutase (SOD) and catalase (CAT)] were measured at their baseline. Subsequently, along with standard pharmacological treatment of ACO, 'Study' patients received 80,000 IU (2 oral capsules) of vitamin D_3 per

week for first 13 weeks. Thereafter, according to their serum 25(OH)D or calcium concentration, they received 40,000 IU (1 oral capsule) of D_3 per 1 week or per 2 weeks or per 6 weeks or no further supplementation, for another 13 weeks. Whereas, all the 'Control' patients received two oral capsules of placebo weekly, for consecutive 26 weeks. All patients of both groups were also advised to have sunlight exposure (within 11 am to 4 pm) only for 20 minutes daily. After 26 weeks of follow up, both enzyme levels of all patients were again measured. Both enzymes were measured by ELISA method using spectrophotometry. Data were analyzed by Student's paired and unpaired 't' test, where $p \leq 0.05$ was accepted as significant. **Results:** Initially a total 60 patients were enrolled and randomized, but ultimately 40 of them completed the trial. The baseline antioxidant enzymes levels of two groups were not significantly different. However, the mean level of both enzymes increased in both groups after 26 weeks of follow up but it was statistically significant ($p<0.001$) only in 'Study' patients. In addition, SOD ($p<0.05$) and CAT ($p<0.01$) were significantly higher in the vitamin D_3 supplemented patients than those of placebo treated patients after 26 weeks of follow up. **Conclusion:** The present study reveals that vitamin D_3 supplementation increases the antioxidant enzyme level in vitamin D_3 deficient stable ACO patients.

Keywords: Asthma COPD overlap (ACO), Vitamin D_3, Superoxide dismutase (SOD), Catalase (CAT)

Introduction

The obstructive lung disases (OLDs) including asthma and COPD are very common and are associated with substantial morbidity. Asthma is characterized by chronic airway inflammation with wheeze, shortness of breath, chest tightness and cough, but its airflow obstruction is fully reversible after treatment with bronchodilator[1]. In contrast, COPD is a progressive inflammatory disorder affecting the airways and characterized by chronic airway inflammation, increased airway resistance, fibrosis, mucus hypersecretion, parenchymal lesions with reduced elastic recoil and loss of alveolar attachments, leading to airflow limitation, which is not fully reversible with bronchodilator[2].

However, it is evident that typical asthma and COPD characteristics can both exist simultaneously in one patient. Approximately one in four patients with COPD have asthmatic features consist of wheeze, airway hyper-responsiveness or atopy[3]. On the other hand, patients with asthma may present with fixed airway obstruction over time[4]. In 2015, a joint project GINA and global standard of obstructed lung disease(GOLD) described ACO as, persistent airflow limitation with several features usually associated with asthma and several features usually associated with COPD[5].

The patients with OLD are continuously exposed to oxidants generated from endogenous or exogenous source such as exposure to cigarette smoking, dust particles and air pollutants such as, allergens, bacterial or viral spores[6]. This increment of oxidants is the main component of

oxidative stress (imbalance between reactive oxygen species production and antioxidant defenses)[7].

For protection from oxidative stress, human body possesses antioxidants, derived from both endogenous and exogenous sources. They may delay or prevent direct oxidation of substrates or scavenge oxidative free radicals neutralizing the oxidants[8]. The major antioxidants enzymes include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and glutathione reductase[9].

Recent research evidence showed role of vitamin supplement on lung diseases[10]. Vitamin D_3 is the active form, synthesized from 7-dehydro-cholesterol in the skin through the action of ultraviolet B (UVB) radiation[11]. This vitamin is now recognized for its classical role in bone mineralization and calcium homeostasis[12]. Deficiency of this vitamin D might cause rickets, osteomalacia, low bone mass, osteoporosis as well as fractures[13]. In addition, in respiratory health, vitamin D deficiency has also been shown to increase the risk of upper respiratory tract infections[14] and active tuberculosis[15]. This vitamin deficiency frequently occurs in COPD patients and correlates with severity of COPD[16]. Moreover, association between vitamin D deficiency and asthma has also been reported[14]. Very recently it has been found that vitamin D_3 supplementation can improve exercise tolerance with D_3 deficient stable COPD [17] as well as ACO[18] patients.

Previous studies found that plasma antioxidant enzyme was positively correlated with serum vitamin D_3 and erythrocyte SOD and CAT activity[19]. Furthermore, both SOD and CAT were found increased in diabetic mice[20], hemodialysis patient[21] and preeclamptic women[22] after vitamin D_3 supplementation.

Nevertheless, the volume of data on effect of vitamin D_3 supplementation on plasma antioxidant enzymes is not enough for conclusive remarks.

On the basis of this background, this study was designed to observe the effect of vitamin D_3 supplementation on plasma SOD and CAT level in vitamin D_3 deficient stable ACO patients.

Methods

Subjects. This double blinded placebo controlled RCT was carried out in the Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU) and National Institute of the Diseases of Chest and Hospital (NIDCH), Dhaka from March, 2018 to August, 2019. This clinical trial was registered at www.clinicaltrials.gov, ID: NCT03931889 and approved by Institutional Review Board of BSMMU. For this purpose, 60 (sixty) vitamin D_3 deficient (serum 25-hydroxycholecalciferol <30 ng/ml)[23], male, smoker, stable[5] patients with asthma COPD overlap (ACO) of age 40 to 80 years were selected by pulmonologists through clinical, physical and radiological signs of chronic airway disease, usually with spirometric evidence of chronic airflow limitation (post bronchodilator FEV1/FVC<0.7)[5], but its absence did not absolutely exclude ACO. The ultimate diagnosis was confirmed by 'tick box' approach advocated by joint committee of GINA and GOLD (2019)[5]. The inclusion criteria were duration of ACO (1 to 5 years), duration of smoking (>10 pack years)[24], body mass index (18.6 to 24.9 kg/m^2)[25], mid upper arm circumference (>25.1 cm)[26], serum total calcium (8.5 to 10.5 mg/dl)[27], serum inorganic phosphate (2.3 to 4.7 mg/dl)[27] and serum parathormone (10 to 65 pg/ml)[27]. In addition, patients with uncontrolled systemic hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg, with anti-hypertensive medication)[28], uncontrolled diabetes mellitus (fasting blood sugar ≥7 mmol/l and/or HbA1c ≥6.5%)[29], dyslipidemia (total cholesterol ≥240 mg/dl and/or HDL <40 mg/dl and/or LDL ≥160 mg/dl and/or triglyceride ≥200 mg/dl and/or with use of any lipid lowering drug)[30], renal insufficiency (serum creatinine >1.36 mg/dl)[31] as well as H/O any

pulmonary, liver, endocrine or cardiac disease, malignancy or with consumption of any drug known to affect vitamin D metabolism (phenytoin, carbamazepine, clotrimazole, rifampicin, nifedipine, spironolactone) within 1 month prior to study, were excluded.

Sampling and Data collection. An informed written consent was taken from each preliminarily selected patient and his serum 25(OH)D was estimated. When serum 25(OH)D was <30 ng/ml (D_3 deficiency) but >10 ng/ml[23], then he was finally selected and randomly assigned as 'Study' (n=30) or 'Control' (n=30) patient (Figure 1). The baseline plasma level of SOD and CAT of all patients were measured. Then along with the standard pharmacological treatment of ACO (according to GOLD criteria)[32], 'Study' patients received 80,000 IU (2 oral capsules) of vitamin D_3 per week for first 13 weeks. Detail of the dose schedule for D_3 supplementation for last 13 weeks[23] are shown in Table I. Whereas, all the 'Control' patients received two oral capsules of placebo weekly for consecutive 26 weeks. Additionally, all the patients of both groups were also advised to have sunlight exposure (within 11 am to 4 pm) only for 20 minutes daily[33]. On the other hand, if serum 25(OH)D was <10 ng/ml (severely deficiency)[23] of any patient, then he was dropped out (Figure 1) from the study (for ethical purpose). After 26th week of follow up, both the enzyme levels were again measured.

Preparation of vitamin D_3 and placebo capsules. All capsules were prepared and supplied by Beximco Pharmaceuticals Limited, Bangladesh. Ingredients of vitamin D_3 capsules were cholecalciferol (40,000 IU), microcrystalline cellulose (58.1 gm), butylated hydroxy toluene (0.2 mg), magnesium stearate (3 mg), gelatin capsule shell (1 mg). Ingredients of placebo were same as vitamin D_3 except Cholecalciferol.

Assessment of serum vitamin D_3 and antioxidant enzymes. Serum 25(OH)D was assessed by Chemiluminescent microparticle immunoassay (CMIA) method (Abbot Laboratory, Ireland). Plasma SOD and CAT enzymes level were

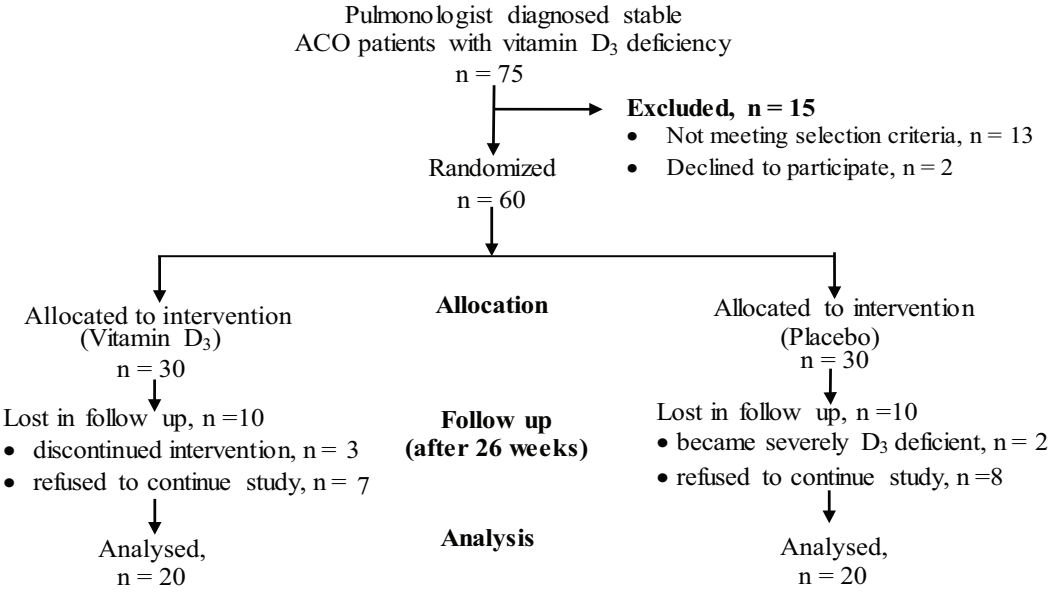

Figure 1: CONSORT (Consolidated Standards of Reporting Trials) diagram; ACO: Asthma COPD Overlap

assessed by colorimetric method using SOD assay kit (Elabscience, USA) and CAT assay kit (Elabscience, USA).

Statistical Analysis. The results were expressed as mean±SD and the data were analyzed by SPSS (Version 16), and for statistical analysis, independent sample 't' test as well as paired Student's 't' test was used. In the interpretation of results, ≤0.05 level of probability (p) was accepted as significant.

Table I: D_3 Supplementation schedule for D_3 deficient ACO patients[23]

If, **serum 25 (OH)D and/or serum Ca^{2+} =**	**Then,** **for next 13 weeks, dose was -**	**So,** **for next 13 weeks, dose schedule was -**
At 1st visit / at day 1: Vitamin D_3 80,000 IU (2 capsules of 40,000 IU) / week, for consecutive 13 weeks		
At 2nd visit / after 13 weeks:		
30-40 ng/ml and/or 8.5-10.5 mg/ml	4600 IU/day	4600 IU X 7 = 32,200 IU / 7 days = 1 cap (40,000 IU) / week
40-50 ng/ml and/or 8.5-10.5 mg/ml	3000 IU/day	3000 X 15 = 45,000 IU / 15 days = 1 cap (40,000 IU) / 2 weeks
60-80 ng/ml and/or 8.5-10.5 mg/ml		1 cap (40,000 IU) / 6 weeks
> 80 ng/ml and/or 8.5-10.5 mg/ml	stop taking drug & symptom analysis	no dose for further 13 weeks
>150 ng/ml and/or >10.5 mg/ml	close monitoring and ask for – • feeling sick or being sick • poor appetite or loss of appetite • feeling very thirsty • passing urine often • constipation or diarrhea • abdominal pain • muscle weakness and pain • bone pain • feeling confused • feeling tired	no dose for further 13 weeks
At 3rd visit / after 26 weeks: Patients were referred to pulmonologist and suggested to follow the above-mentioned schedule		

Results

A total of 60 patients were initially randomized and 40 of them ultimately completed the study (20 in each group was dropped out). There was no significant difference in the baseline characteristics including antioxidant enzymes (SOD and CAT) between vitamin D_3 supplemented and placebo groups (Table II). However, the plasma level of both enzymes increased after 26 weeks of their follow up in the 'Study' as well as in 'Control' groups in comparison to their corresponding baseline values, but these differences were statistically significant ($p<0.001$) only is 'Study' patients (Figure 2). In addition, the mean plasma levels of SOD ($p<0.05$) and CAT ($p<0.01$) were significantly higher in the vitamin D_3 supplemented patients than those of placebo after 26 weeks of follow up (Figure 2).

Table II: Baseline characteristics of ACO patients in both groups (N=60)

Parameters	Vitamin D_3 group (n=30)	Placebo group (n=30)
Age (years)	60.13±9.75	57.66±10.90
Duration of ACO (years)	3.63±0.80	3.53±0.81
Duration of smoking (pack years)	14.62±4.46	16.28±5.72
Body Mass Index (kg/m2)	22.51±3.34	21.75±3.01
Mid Upper Arm	26.73±2.88	26.93±3.43
Circumference (cm)	116±7.23	116±7.23
Systolic blood pressure (mm of Hg)	75.33±7.76	75.33±7.76
Diastolic blood pressure (mm of Hg)	86.80±17.93	83.02±16.97
FEV1/FVC (%) FEV1 (% of PV)	62.88±21.11	58.22±21.93
25-hydroxcholecalciferol (ng/ml) serum	19.20±4.44	19.58±3.79
parathormone (pg/ml)	53.29±9.28	49.93±10.92
Total calcium (mg/dl)	9.15±0.33	9.39±0.4
Inorganic Phosphate (mg/dl)	3.15±0.63	3.27±0.39
Fasting blood sugar (mmol/l)	5.09±0.79	5.14±0.77
Glycosylated hemoglobin (%)	6.14±0.44	6.12±0.49
Plasma Superoxide dismutase (U/ml)	14.13±5.34	12.29±5.55
Plasma Catalase (U/ml)	11.90±6.81	17.16±7.30

Data were expressed as mean±SD; Statistical analysis was done by independent sample t test; n: number of subjects; Pack year: (number of cigarette smoked per day ÷ 20) X no. of year smoked; ACO: Asthma COPD Overlap; FEV1: Forced expiratory volume in 1st second; FVC: Forced Vital Capacity; PV: Predicted Value.

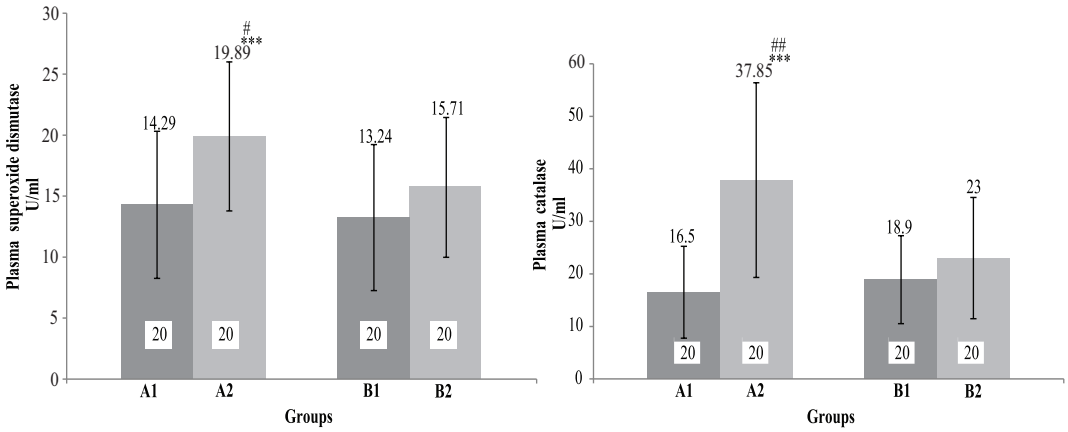

Figure 2: Antioxidant enzymes level on pre and post intervention in both groups. Each bar symbolizes mean±SD of 20 patients; A1: Patients with vitamin D_3 on day 1; A2: Patients with vitamin D_3 after 26th week; B1: Patients with placebo on day 1; B2: Patients with placebo after 26th week; ***: $p<0.001$ in A1 vs A2; #: $p<0.05$ in A2 vs B2; ##: $p<0.01$ in A2 vs B2

Discussion

The present study observed the effects of vitamin D_3 supplementation on antioxidant enzymes in vitamin D_3 deficient Asthma COPD overlap (ACO) patients. In the present study, both the antioxidant enzyme levels at their baseline status were almost similar to other studies[19,21].

In this study, SOD was significantly higher in vitamin D_3 supplemented patients compared to placebo treated patients after 26th week of follow up. Similar higher SOD in erythrocytes of atopic dermatitis patients on 60th day[19], plasma of neonates with hypoxic-ischemic encephalopathy on 5th day[34] and serum of preeclamptic pregnant women on 8th week of vitamin D_3 supplementation[22] were reported. In addition, this antioxidant enzyme was found higher in cardiac tissue and liver tissue of vitamin D_3 supplemented rats on 5th week[35] and 28th day[36] of follow up, respectively.

In addition, CAT was also significantly increased in ACO patients with vitamin D_3 supplementation in comparison to that of patients with placebo after 26th week of follow up. Similar result was reported, where CAT was significantly higher in erythrocytes of patients with atopic dermatitis after 60th day[19] and serum of pre-eclamptic pregnant women after 8th week of vitamin D_3 supplementation[22]. In addition, this antioxidant enzyme was found higher in hippocampus of vitamin D_3 supplemented rats with multiple sclerosis on 21st day[37] and in liver tissue of diabetic rats on 28th day[36] of follow up, respectively.

In the present study, all of our ACO patients were long standing smoker (≥ 10 pack year). It is well known that cigarette contains reactive oxygen species (ROS)[38] which can damage epithelial cells lining the airways by inducing peroxidation of lipids and other cell membrane constituents and/or by activating oxidant-sensitive cellular pathways and/or by inducing DNA damage[39]. These ROS also activate epithelial cell intracellular signaling cascades leading to gene activation for inflammatory cytokines production[40]. These mediators promote chronic immune cell (neutrophils, macrophages, T cell) recruitment to produce more ROS or oxidants[41] and ultimately increase the consumption of antioxidant enzymes[42] followed by decrement of

their plasma concentration[43]. In addition, cigarette smoke could also reduce the antioxidant defenses of the lung by irreversibly modifying glutathione (GSH) and various redox-sensitive signaling proteins. Smoking also could compromises transcription factors, such as the nuclear factor-erythroid 2-related factor 2 (Nrf2), which could activate an antioxidant response element (ARE) that regulates antioxidant[8]. However, the activities of these enzymes could also be increased[44] to combat the over burden of oxidants. Nevertheless, it has been proposed that vitamin D_3, structurally having a steroid nucleus[11] might induce the mRNA gene expression of the antioxidant enzymes, SOD and CAT[45] by its genomic action to regulate one or more of the mitogen activated protein kinase (MAPK) signaling pathways[46]. This vitamin might also increase Sirtuin1 (SIRT1) expression through phosphorylation of the extracellular signal-regulated kinases (REK1/2)[47], which is induce the expression of the antioxidant enzymes (SOD, CAT).

Conclusion

The present study reveals that vitamin D_3 supplementation increases the antioxidant enzymes in D_3 deficient ACO patients. However, further experimental study is needed to elucidate the exact component and mechanism responsible for these effects. In addition, vitamin D_3 supplementation may be recommended to ACO patients with D_3 deficiency to raise their antioxidant status to protect them from oxidative stress.

Conflict of interest: None

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