

RESEARCH PROTOCOL

TITLE

**Effects of vitamin D₃ supplementation on lung functions and
exercise tolerance in D₃ deficient Asthma COPD Overlap
(ACO) patients**

Date:06/09/2018

Project Summary

Background: Asthma COPD Overlap (ACO) has been described as a distinct disease now-a-days. Several studies have shown positive relationship between serum concentration of 25 hydroxyvitamin D, lung function and exercise tolerance in different group of patients. However, vitamin D supplementation showed significant improvement in lung function status and exercise tolerance in both asthma and COPD patients but no study was found in ACO patients. **Objectives:** To evaluate the effects of vitamin D₃ supplementation on lung function status and exercise tolerance in D₃ deficient, stable patients with ACO. **Methods:** This double blinded placebo controlled trial will be conducted by Department of Physiology Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbag, Dhaka from March 2018 to February 2019. For this study a total number 70 vitamin D₃ deficient (serum 25 hydroxycholecalceferol <30 ng/ml) male, stable (diagnosed patient, who was not experienced any acute exacerbation, hospitalization, urgent care visits or changes in routine medication within 4 weeks prior to study) patients with ACO of age 40-70 years will be selected. They will be selected from the Out Patient Department (OPD) of the National Institute of Diseases of Chest and Hospital (NIDCH) and will be randomly grouped as A (control) and B (study). All the participants will be matched in terms of age, duration of ACO, duration of smoking, body mass index (BMI), mid upper arm circumference (MUAC), waist hip ratio, occupational status, socioeconomic status, serum

25hydroxycholecalciferol [25(OH)D], serum parathormone (PTH) , serum calcium (Ca^{2+}), serum phosphate (PO_4^{3-}) ,serum alkaline phosphatase (ALP), serum glutamate pyruvate transaminase (SGPT), serum creatinine, fasting blood sugar (FBS), serum glycated haemoglobin ($\text{HbA}_{1\text{C}}$), serum cholesterol, serum high density lipoprotein (HDL), serum low density lipoprotein (LDL), serum triglyceride (TG). Any patient with history of any acute exacerbation of any pulmonary, liver, endocrine or renal disease, or with any malignancy or with any drug known to affect vitamin D metabolism within 1 month prior to study, will be excluded from the study. All the baseline data at day 0 will be recorded as A0 (Control) and B0 (Study). Along with the standard pharmacological treatment of ACO (according to GOLD criteria), the patients of the ‘Study group’ will be prescribed for 80,000 IU of oral vitamin D_3 per week and ‘Control group’ for placebo for consecutive 13 weeks. Subsequently, all the patients will be cordially requested to again visit the Department of physiology, BSMMU, on 13th week of their follow up to reexamine all the spirometric variables along with serum 25(OH)D and Ca^{2+} (to check the toxicity). These data will be recorded as values of ‘13th week’ (B_1). Then according to serum level of 25(OH)D and Ca^{2+} (American Vita min D council 2017), 40,000 IU / 1 to 6 week oral vitamin D_3 will be prescribed, for further 13 weeks. At 26th week of follow up, all the study variables will be again reexamined, and the data will be recorded as values of ‘26th week’ (B_2). Similarly, all the patients of ‘control group’ will be prescribed for placebo (along with standard pharmacological treatment of ACO, prescribed by pulmonologist) for consecutive 26 weeks and their respective data will be recorded as A_1 and A_2 . With this, all patients of both the groups were

advised to continue ad lib (according to their own choice) diet. At the very 1st day of the study, the spirometric lung functions will be assessed by measuring forced vital capacity (FVC), forced expiratory volume in 1st second (FEV₁), forced expiratory ratio (FEV₁/FVC%), peak expiratory flow rate (PEFR) and forced mid expiratory flow of FVC (FEF_{25-75%}), maximum expiratory flow₇₅ (MEF₇₅), maximum expiratory flow₅₀ (MEF₅₀), maximum expiratory flow₂₅ (MEF₂₅) with a portable digital spirometer with a portable digital spirometer. In addition, exercise tolerance will be assessed by change in 6 minute walk distance (6MWD) in 6 minute walk test (6MWT). Changes in peripheral capillary oxygen saturation (SpO₂) by Pulse Oximeter and degree of dyspnea and fatigue by Modified Borg Scale (MBS) will also be measured both before and after 6MWT, to evaluate their changes in both the groups. All these variables will be again measured on their follow-up in 13 weeks and 26 weeks with standard pharmacological treatment of ACO with D₃ intervention (B group) and also without D₃ intervention (A group). The data will be statistically analyzed by Graphpad prism (Version 7) using independent sample 't' test (between two groups), paired Student's 't' test (between paired groups before and after intervention). Group comparisons will be done using one way ANOVA. In the interpretation of results, ≤0.05 level of probability (p) will be accepted as significant.

Expected outcome of the study:

- Impaired lung functions and exercise tolerance in vitamin D₃ deficient ACO patients will be detected and the good impact of vitamin D₃ supplementation for six months on restoration of the lung function status and exercise tolerance in ACO patients will be proved.

Key words: Asthma COPD Overlap, Vitamin D₃, FVC, FEV₁, FEV₁/FVC%, FEF_{25-75%}, PEF_R, MEF_{75%}, MEF_{50%}, MEF_{25%}, SpO₂, 6 Minute Walk Distance, Modified BORG scale.

Introduction

Chronic obstructive pulmonary disease (COPD) and bronchial asthma both are common airway diseases contributing to mortality and morbidity worldwide. There is growing consensus that typical asthma and COPD characteristics can both exist simultaneously in one patient. Approximately one in four patients with COPD has asthmatic features and patients with asthma may present with fixed airway obstruction over time. A joint project of Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD) has recommended the term “asthma COPD overlap (ACO)” for these patients. ACO is defined as persistent airflow limitation with several features associated with asthma and several features associated with COPD. ACO is associated with increased disease severity and worse health status and poorest health related quality of life. These patients experience greater exacerbation than those with COPD alone, have high hospital burden, and require a large amount of medical resources compared to patients with asthma or COPD.

Though all of airway diseases (ACO/ COPD/ Asthma) are preventable and treatable, once developed the disease along with its comorbidities cannot be cured. But their progression and exacerbation of morbidity can be reduced. Medicine treatment alone cannot do this. Now-a-days the

interest of role of different non-pharmacological supplements on different lung diseases are outgoing.

Vitamin D is now known to be of physiological importance outside of bone health and calcium homeostasis. There is a large amount of evidence supporting the influence of vitamin D on immune function and inflammatory diseases. In addition it plays beneficial role in the prevention of cancer, autoimmune disease, cardiovascular disorders, hypertension and influenza.

In respiratory health, higher serum vitamin D concentration has been associated with better lung function as measured by FEV1 in a large cross-sectional study of the US population. Recently a group of researcher found a statistically significant positive relationship between FEV1 and serum 25(OH)D in young COPD patients. On the other hand, statistically significant association between vitamin D deficiency and asthma has been reported and this vitamin's deficiency is associated with severe asthma exacerbation and need for emergency department evaluation or hospitalization. High vitamin D levels are associated with better lung function, less airway hyperresponsiveness and improved glucocorticoid response in adult asthma. Although the precise connection between vitamin D status and lung function is not clear at this point, it is postulated that vitamin D may improve lung function through its action on regulating inflammation inducing antimicrobial peptides, and/or its action on muscles.

Plasma vitamin D concentration has been positively associated with exercise capacity in COPD patients and it was observed that exercise capacity was decreased in vitamin D deficient COPD patients.

In patients with bronchial asthma significant improvement in FEV1 was shown after 50,000 IU /day of vitamin D for consecutive 3 months. In addition, in D₃ deficient COPD patients 50,000 IU/week vitamin D for 8 weeks followed by 800 IU /day for 1 year did significant improvement in exercise tolerance with decrement of frequency of COPD exacerbations. In a similar group of patients, only 50,000 IU / week vitamin D for 3 months showed significant improvement in exercise tolerance.

However, as far as it has been reviewed, association of vitamin D status with ACO has only been observed by Odler et al. (2015). In that study 60% of ACO patients had vitamin D deficiency and a positive correlation was found between serum 25(OH)D and spirometric lung function status. Strikingly no study have been conducted to observe the effects of this fat soluble vitamin on lung function and exercise capacity in vitamin D₃ deficient, stable patients with ACO.

Therefore, on the basis of this background the present study has been designed to evaluate the effects of Vitamin D₃ on the spirometric lung function status, exercise tolerance in D₃ deficient, stable patients with ACO. This study may draw attention of the physicians about the beneficial effects of the vitamin D₃ on both pulmonary and extrapulmonary manifestation in ACO patients.

Rationale

In recent years, ACO has gained much attention and have extensively reviewed worldwide. There is a scarcity of population-based data on ACO in Bangladesh except Habib et al. As patients with ACO seem to be at risk for a poor outcome, high risk of exacerbations, it is of utmost importance to perform more research on this significant disease.

However, as far as it has been searched association of vitamin D status with ACO has only seen by Odler et al. (2015) who reported significant decrement of serum 25(OH)D in elderly ACO patients. Strikingly no study have been conducted to observe the effects of this fat soluble vitamin on lung function and exercise capacity in vitamin D₃ deficient, stable patients with ACO.

To the best of our knowledge, as far as we reviewed with a loading dose followed by upto 50,000 IU/week of oral D₃ for 12 weeks, could not improve the spirometric variables in stable COPD patients (Said and Elnaeem 2014; Hornikx et al. 2015; Moosavi and Shoushtari 2015; Rezk et al. 2015). Whereas, Nazarian et al. (2011) found significant improvement of insulin sensitivity in patients with Type II Diabetes meliitus after 4 weeks oral supplementation of 10,000IU/day vitamin D₃ (Nazarian et al. 2011). On the basis of this observation, we have selected 80,000 IU/ week of oral vitamin D₃ , for consecutive 13 weeks followed by 40,000 IU / 1 to 6 week for further consecutive 13 weeks, as the dosage schedule for our D₃ deficient patients of stable ACO. In addition,

as toxicity dose of vitamin D₃ is very high (50,000IU/day for several months) (Veith 1999), this dose schedule will be safe for our patients.

Therefore, on the basis of this background the present study has been designed to evaluate the effects of Vitamin D₃ on the spirometric lung function status, exercise tolerance in D₃ deficient, stable patients with ACO. This study may draw attention of the physicians about the beneficial effects of the vitamin D₃ on both pulmonary and extrapulmonary manifestation in ACO patients.

Research question

Is there any improvement in lung function status and exercise tolerance in vitamin D₃ deficient ACO patients after having vitamin D₃ supplementation?

Hypothesis

Null: Vitamin D₃ supplementation does not improve lung functions and exercise tolerance in vitamin D deficient ACO patient.

Alternate: Vitamin D₃ supplementation improves lung functions and exercise tolerance in vitamin D deficient ACO patient.

Objectives:

General objectives:

To evaluate the effects of vitamin D₃ supplementation on lung function status and exercise tolerance in D₃ deficient, stable patients with ACO.

Specific objectives

- To measure the FVC, FEV₁, FEV₆, FEV₁/FVC%, PEFR and FEF_{25-75%} of vitamin D₃ deficient, stable patients with ACO, in order to assess their lung function status at baseline.
- To measure the 6MWD of all the patients, in order to assess their functional exercise capacity at baseline.
- To measure the SpO₂ at rest of all the patients, in order to assess the basal oxygenation status at baseline.
- To measure the level of dyspnea and fatigue at rest of all the patients in order to assess the basal breathlessness level at baseline.
- To measure the both the SpO₂ and level of dyspnea and fatigue again after 6MWT, for the assessment of their exercise tolerance at baseline.
- To measure the all these variables in study group at 13th weeks and at 26th weeks of their follow up.
- To measure the all these variables in control group at 13th weeks and at 26th weeks of their follow up.
- To compare all these variables in study group at baseline and endline.
- To compare all these variables in control group at baseline and endline
- To compare all these variables in both group at baseline and endline.

Methods

Type of study	: Double blinded placebo controlled trial
Place of study	: Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka
Period of study	: March 2018 to February 2019
Study population	: Stable patients of ACO with vitamin D ₃ deficiency
Sample size	: 60
Sampling	: Consecutive sampling
Grouping	:
Group A (Control)	: 30 (thirty) D ₃ deficient ACO patients with placebo administration
Group B (Study)	: 30 (thirty) D ₃ deficient ACO patients with vitamin D ₃ administration

For the assessment of all the variables of all the patients on day 0, at 13th week and as well as at 26th week, they will be designated as A₀, A₁ and A₂ (control) and B₀, B₁ and B₂ (study). In addition, for the mean of SpO₂, level of dyspnea and level of fatigue at resting condition (before 6MWT), all the groups will be further designated as A_{0before}, A_{1before}, A_{2before}, B_{0before}, B_{1before} and B_{2before} respectively. Similarly after 6MWT all these six subgroups will be again designated as A_{0after}, A_{1after}, A_{2after} and B_{0after}, B_{1after}, B_{2after}, for measurement of these three variables.

Sample size calculation

Using the following statistical formula

$$n = [(Z\alpha + Z\beta)^2 \times (\sigma_1^2 + \sigma_2^2)] / (\mu_1 - \mu_2)^2$$

(Kirkwood and Sterne 2003)

$$\text{Mean of control, } \mu_1 = 364.1$$

$$\text{Mean of study group, } \mu_2 = 357.3$$

$$\text{SD of control, } \sigma_1 = 7.21$$

$$\text{SD of study group, } \sigma_2 = 12.25$$

(Hassan 2018)

$$\text{Type I Error, } Z\alpha = 1.96$$

$$\text{Type II Error, } Z\beta = 0.84$$

(Kirkwood and Sterne 2003)

Therefore,

$$\begin{aligned} n &= [(Z\alpha + Z\beta)^2 \times (\sigma_1^2 + \sigma_2^2)] / (\mu_1 - \mu_2)^2 \\ &= [(1.96 + 0.84)^2 \times (7.21^2 + 12.25^2)] / (364.1 - 357.3)^2 \\ &= [7.84 \times (51.9841 + 150.0625)] / (6.8)^2 \\ &= 1584.04534 / 46.24 \\ &= 34.2570359 \\ &= \text{approximately 35 for each group.} \end{aligned}$$

Subject selection:

Inclusion criteria

- Pulmonologist diagnosed stable patient of ACO
(GINA and GOLD 2015)
- Duration of ACO: > 4 year
- Vitamin D₃ deficient : Serum 25(OH)D <30 ng/ml
(Vitamin D Council 2017)
- Age: 40 -70 years
- Sex: Male
- Socioeconomic status: middle class
- Smoker
- Anthropometric status:
 - BMI=18.6-24.9 kg/m²
(WHO 2006)
 - mid upper arm circumference>25.1 cm
(Sultana et al. 2015)
 - waist-hip ratio<0.89
(Sultana et al. 2015)
- Serum parathormone: 10-65 pg/ml
- Serum Ca²⁺: 8.5-10.5 mg/dl
- Serum PO₄³⁻: 2.3-4.7 mg/dl
- Serum alkaline phosphatase: 30-120 U/L
- SGPT: <50 U/L
- Serum creatinine: 0.7-1.3 mg/dl
- FBS: 3.5-6.1 mmol/L
- Serum HbA1c: 4.5-6.3 %

- Serum cholesterol: <200 mg/dl
- Serum HDL: >40 mg/dl,
- Serum LDL: <130 mg/dl
- Serum TG: <150 mg/dl

Exclusion criteria

With history of:

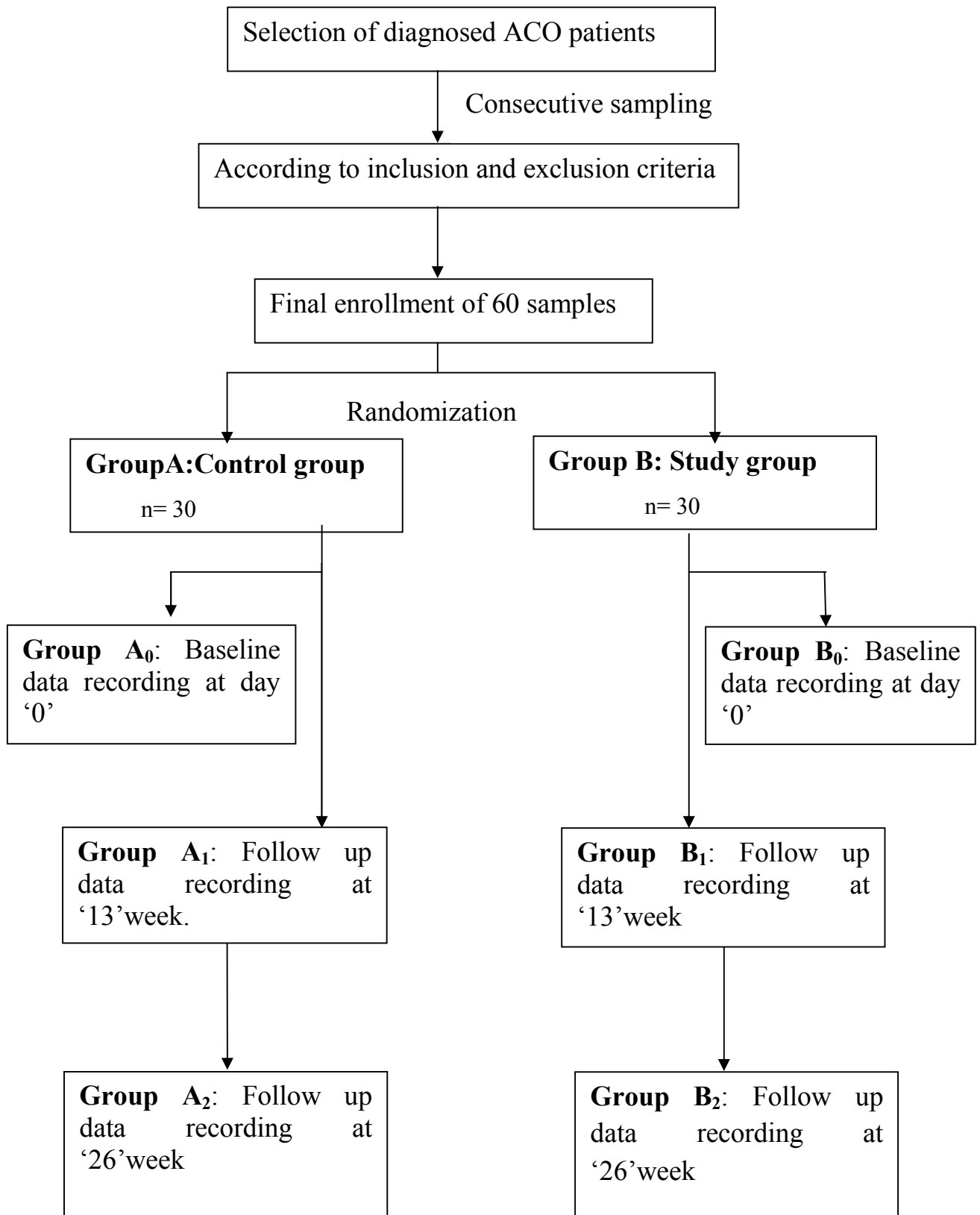
- any other pulmonary diseases, as,
 - ✓ chronic obstructive pulmonary disease
 - ✓ bronchial asthma
 - ✓ respiratory tract infection
 - ✓ bronchiectasis
 - ✓ pleural effusion
 - ✓ tuberculosis
 - ✓ interstitial lung disease
 - ✓ pneumonectomy or pulmonary lobectomy
- any cardiac disease, like –
 - ✓ unstable angina pectoris
 - ✓ congestive heart failure
 - ✓ myocardial infarction
 - ✓ cardiac arrhythmia
- systemic hypertension
- any liver disease
- any malignancy

- use of any drugs known to affect vitamin D metabolism within 1 month prior to study, as,
 - ✓ antiepileptics (Phenytoin, Carbamazepine)
 - ✓ antibiotics (Clotrimazole, Rifampicin)
 - ✓ antihypertensives (Nifedipine, Spironolactone)
 - ✓ antiretroviral drugs (Ritononavir, Saquinavir)
 - ✓ endocrine drugs (Cyproterone acetate)
 - ✓ glucocorticoids
 - ✓ bisphosphonate
 - ✓ calcium supplement

- With biochemical evidence of
 - ✓ diabetes mellitus
 - ✓ renal insufficiency

All the criteria mentioned above will be scrutinized by taking history or clinical examination or laboratory investigations

Study outline:



Site of sample collection

All the patients will be collected from the Out Patient Department (OPD) of National institute of the diseases of chest and hospital (NIDCH), Mohakhali, Dhaka..

Study procedure

On the first day of enrollment, the objectives, nature, purpose and potential risk of all the procedures used for the study will be explained in detail to each ACO patients (diagnosed by pulmonologist), with a cordial attitude giving emphasis on the benefits he might obtain from this study. He will be encouraged for voluntary participation and will be allowed to withdraw himself from the study even after participation, whenever he felt uneasy. If he agreed to be enrolled in the study, an informed written consent will be taken in a prescribed form. Detailed family history, medical history and thorough physical examination of each patient will be done and all the information will be recorded in a standard data sheet. Then all the patients will be requested to attend the Department of Physiology at 7:30 am (after overnight fasting) on the examination day.

On the examination day, 10 ml of venous blood will be collected from antecubital vein of patient in different vacutainer tubes and will be taken to the laboratory of Department of Biochemistry and Molecular Biology . as soon as possible for the estimation of serum creatinine and serum 25(OH)D. and rest of the blood will be preserved at -4°C.

If the patient with D₃ deficiency [Serum 25(OH)D <30 ng/ml] and not with renal insufficiency (Serum creatinine >1.3 mg/dl), then the Fasting blood sugar, serum HbA_{1c}, serum parathormone (PTH), serum calcium (Ca²⁺), serum phosphate (PO₄³⁻), serum alkaline phosphatase (ALP), serum SGPT, serum cholesterol, serum High Density Lipoprotein (HDL), serum low density lipoprotein (LDL), serum triglyceride (TG), will be assessed from the preserved blood.

After that their height and weight will be measured and the spirometric lung function test will be done by using a portable spirometer (PONY FX, cosmed, Italy]. After getting all the biochemical and spirometric reports the final selection will be done, according to the inclusion and exclusion criteria. All the eligible patients will be randomly assigned to either 'Control' or 'Study' groups and will be thoroughly informed about the objectives and detailed study procedure, once again.

Subsequently a standard therapeutic treatment (according to GOLD guideline) will be prescribed to all the selected stable ACO patients of both groups. Proper education will be given about drug, method of taking medication and medication plan.

Along with the standard pharmacological treatment of ACO, the patients of the 'Study group' will be prescribed for 80,000 IU/ week of oral vitamin D₃, for consecutive 13 weeks. Subsequently, all the patients will be cordially requested to again visit the Department of physiology, BSMMU, on 13th week of their follow up to reexamine all the study

variables along with serum 25(OH)D and Ca^{2+} (to check the toxicity). These data will be recorded as values of '13th week' (B13).

Then according to serum level of 25(OH)D and Ca^{2+} (American Vitamin D council 2017), 40,000 IU / 1 to 6 week oral vitamin D₃ will be prescribed, for further 13 weeks. At 26th week of follow up, they will be requested again to visit the Department of Physiology, BSMMU, to reexamine all the study variables, and the data will be recorded as values of '26th week' (B26).

Similarly, all the patients of 'control group' will be prescribed for placebo (along with standard pharmacological treatment of ACO, prescribed by pulmonologist) for consecutive 26 weeks and their respective data will be recorded as A13 and A26.

With these, all patients of both the groups will be advised to continue ad lib (according to their own choice) diet as well as to have sunlight exposure (within 10 am to 2 pm) only for 20 minutes daily (Manjeeta 2016).

In the mean time, a good rapport will be built up to take time to time follow-up over telephone and visiting patient's place with scheduled appointment to maintain a proper follow up at 1st (day 1) 2nd (at 13th week) and 3rd (at 26th week) visit of the study , in addition to the hematological values.

Any patient, who will be failed to follow the study procedure exactly during study period, will be dropped and a new one will be included to fulfill the desired total sample number.

Test procedure for spirometry

(Hassan 2018)

- At first, the subject will be asked to take rest sitting on a chair and remain calm and quiet for 5 minutes.
- The detailed procedure will be explained to him to ensure his maximum cooperation.
- The switch of spirometer will be on and windows will be open.
- Patients' information will be filled up and saved.
- The patient will be asked to hold a disposable paper mouthpiece (connected to spirometer) in his hand horizontally and to place it in between his two lips.
- To make good seal, he will also be asked to put the lips tightly around the outside of the mouthpiece.
- Then he will be asked to inhale first as deeply and as rapidly, as possible, which was followed by a forceful expiration for the possible longest period into the mouthpiece.
- The readings of different variables will be recorded from the spirometer monitor.
- Three (3) consecutive readings at 5 minutes interval for each parameter will be taken and the best value will be noted.

Test procedure for pulse oximetry

(Hassan 2018)

- The patient will be asked to take rest and remain calm and quiet for 10 minutes (MacAllister et al. 2007).
- The detailed procedure will be explained to him to ensure his maximum cooperation.
- Then his right index finger will be washed with alcohol and pulse oximeter sensor will be applied.
- The switch button will be on.
- The reading will be recorded from the display screen.
- Three (3) consecutive readings at 5 minutes interval will be taken and the best value will be noted.

Procedure for six minute walk test (6MWT)

(Hassan 2018)

The 6MWT will be performed at indoors, along a long, flat, straight, enclosed corridor with a hard surface that was seldom traveled. The walking course will be 30 meter in length which was marked every 3 meter. The turnaround points will be marked with a cone. A starting line, which marked the beginning and end of each 6 meter lap, will be marked on the floor using brightly colored tape.

The subject will be asked to walk at his own maximal pace along the corridor from the end, covering as much ground as he can, during the allotted time, without running. He will be also advised to take rest if he

was too exhausted to continue the test. Standard phrases at regular intervals (every 60 seconds) will be used, like: “you are doing well,” “keep up the good work”. At the same time he will be also informed about the past time and how much left before the test completed.

If any of the subjects develop chest pain, intolerable dyspnea, leg cramps, staggering, diaphoresis or pale appearance the test will be immediately stopped and the subject will be dropped. Again, when any subject will complete the total test procedure (according to the exact protocol), he will be congratulated for good effort.

Vitamin and placebo administration

	Vitamin D₃	Placebo
Collection	Beximco Pharmaceuticals Limited, Bangladesh	Beximco Pharmaceuticals Limited, Bangladesh
Dose	80,000 IU/wk	80,000 IU/wk
Route	Oral	Oral
Ingredient	<ul style="list-style-type: none">• Cholecalciferol (40,000IU)• Microcrystalline Cellulose (58.1 gm)• Butylated Hydroxy Toluene (0.2mg)• Magnesium Stearate (3mg)• Gelatin Capsule Shell (1mg)	<ul style="list-style-type: none">• Microcrystalline Cellulose (303.8gm)• Butylated Hydroxy Toluene (0.2mg)• Magnesium Stearate (3mg)• Gelatin Capsule Shell (1mg)

STATISTICAL ANALYSIS

TITLE

**Effects of vitamin D₃ supplementation on lung functions and
exercise tolerance in D₃ deficient Asthma COPD Overlap
(ACO) patients**

Date: 06/09/2018

The results will be expressed as mean with standard error (mean \pm SE). The data will be statistically analyzed by Graphpad prism (Version 7) using independent sample 't' test (between two groups), paired Student's 't' test (between paired groups before and after intervention). Group comparisons will be done using one way ANOVA. Correlations between variables will be tested with Pearson correlation coefficient.

In the interpretation of results, ≤ 0.05 level of probability (p) will be accepted as significant.

INFORMED CONSENT STATEMENT

TITLE

**Effects of vitamin D₃ supplementation on lung functions and
exercise tolerance in D₃ deficient Asthma COPD Overlap
(ACO) patients**

Date :06/09/2018

Title of the study: Effects of Vitamin D₃ supplementation on lung functions and exercise tolerance in D₃ deficient Asthma COPD Overlap (ACO) patients.

Researcher:

Dr. Naznin Sultana

MD Resident

Department of Physiology

Bangabandhu Sheikh Mujib Medical University (BSMMU)

Shahbag, Dhaka, Bangladesh.

Supervisor:

Dr. Taskina Ali

Associate Professor

Department of Physiology

Bangabandhu Sheikh Mujib Medical University (BSMMU),

Shahbag, Dhaka, Bangladesh.

You are being asked to take part in a research study. Your participation is voluntary. Before agreeing to participate in this study, it is important that you read the following explanation of the study, if you do not understand what you are reading, do not sign it. Please ask the researcher to explain anything, you do not understand. If you decide to participate, you will be asked to sign this form and a copy will be given to you.

Purpose of the study: The present study will be conducted to assess lung function status and exercise tolerance in D₃ deficient ACO patients before and after taking vitamin D therapy for a period of 6 months. This study may find out the benefit of vitamin D therapy on lung function and exercise tolerance in ACO patients.

Explanation of procedures: The researcher will take an interview and will do general examination of the body. Then the spirometric variable will be measured and exercise tolerance, oxygen saturation, the level of dyspnea and fatigue (Borg

scale) will be measured before and after six minute walk test . Then 5 ml venous blood will be collected under all aseptic precaution. The same procedure will be done on his follow up.

Risks and discomfort: This study involves almost no physical risk. However, while drawing the blood sample the subject may experience slight discomfort but it will be negligible. Adequate precautions will be taken to avoid any error.

Withdrawal without prejudice: Each participant is free to withdraw the consent and discontinue participation in this study at any time.

Confidentiality: Your identity in this study will be treated as confidential. Your name or identity will not be linked in any way to the research study.

Costs and payments to subjects for participation in research: There is no financial support (compensation) for your participation in this research.

Available medical treatment for research related injuries: If you are injured as a direct result of taking part in this research study, emergency medical care will be provided by transporting to your personal doctor or medical centre.

Questions: Any question concerning the research, study participants can call Dr. Naznin Sultana, Mob no-01798598493.


Authorization: I have read and understood this consent form and I volunteer to participate in this research study, but I understand that my consent does not take away any legal rights in the case of negligence or other legal fault of anyone who is involved in this study.

Participant's Signature:.....

Date:.....

Appendix-I

Clearance from Institutional Review Board (IRB) of BSMMU

 **বঙ্গবন্ধু শেখ মুজিব মেডিক্যাল বিশ্ববিদ্যালয়**
Bangabandhu Sheikh Mujib Medical University

রেজিস্ট্রার অফিস Office of the Registrar

No.BSMMU/2018/ 10786 Date:06-9-2018


Dr. Naznin Sultana
MD(Phase-B)
Department of Physiology
Bangabandhu Sheikh Mujib Medical University
Shahbag, Dhaka- 1000.

Sub: **Institutional Review Board (I.R.B) Clearance.**

With reference to your application on the above mentioned subject, this is to inform you that your Research Proposal entitled “Effects of vitamin D₃ Supplementation on lung functions and exercise tolerance in D₃ deficient Asthma COPD Overlap (ACO) patients” has been reviewed and approved by the Institutional Review Board (IRB) of Bangabandhu Sheikh Mujib Medical University in its 163rd meeting held on 07 July 2018.

You are requested to follow the Institutional Review Board (I.R.B) guidelines.

Expected Examination date January' 2019.


06.9.2018

(Dr. Ferdous Alam)
Member Secretary
Institutional Review Board
BSMMU, Shahbag, Dhaka.

Appendix-II

DATA SCHEDULE

Title : Effects of vitamin D₃ supplementation on lung functions and exercise tolerance in D₃ deficient ACO patients .

SL No. :

ID No. :

Date :

Subject information:

Name :

Age :

NID No :

Contact address
:

Permanent
address :

Contact No : Patient:
Attendant:

Monthly income
(taka) :

No. of family
member :

Residence : Urban/Rural

Occupation : Homemaker-1, Student-2, Retired-3,
Business-4, Service-5, Day labourer-6

Education : Illiterate-0, Primary-1, High school-2, SSC-3,
HSC-4, Graduate-5, Masters-6

Marital status : Married-1, Unmarried-2, Widower-3,
Separated-4, Divorced-5, Livetogether-6

Biomass fuel : Yes/No
exposure

Smoking habit : Current/Past smoker
: Duration of smocking:
: Stopped for (in years, if past smoker):
: Packs-year: (sticks per day/20 X
year)

Past medical history:

H/O Hypertension	: Yes/No	If yes, duration	<input type="text"/> months
H/O Diabetes mellitus	: Yes/No	If yes, duration	<input type="text"/> hs
H/O Cardiac disease	: Yes/No	If yes, duration	<input type="text"/> hs
H/O Renal disorder	: Yes/No	If yes, duration	<input type="text"/> hs
H/O Endocrine disorder	: Yes/No	If yes, duration	<input type="text"/> hs
H/O Psychosis	: Yes/No	If yes, duration	<input type="text"/> hs
H/O Rheumatoid arthritis	: Yes/No	If yes, duration	<input type="text"/> hs
H/O Other disease	: Yes/No	If yes, duration	<input type="text"/> hs
Drug history	:		

History of ACO:

Place of diagnosis :

Duration of illness :

Features	Favors Asthma	Favors COPD
Age of onset	<input type="checkbox"/> Before age 20 years	<input type="checkbox"/> After age 40years
Pattern of respiratory symptoms	<input type="checkbox"/> Variation in symptoms over minutes, hours or days <input type="checkbox"/> Symptoms worse during night or early morning <input type="checkbox"/> Symptoms triggered by exercise, emotions including laughter, dust or allergen	<input type="checkbox"/> Persistence of symptoms despite treatment <input type="checkbox"/> Good or bad days but always daily symptoms & exertional dyspnea <input type="checkbox"/> Chronic cough & sputum preceded dyspnea, unrelated to triggers
Lung function	<input type="checkbox"/> Record variable airflow limitation (spirometry, peakflow)	<input type="checkbox"/> Record persistent airflow limitation (post BD <0.7)
Lung function between symptoms	<input type="checkbox"/> Normal	<input type="checkbox"/> Abnormal
Past history or family history	<input type="checkbox"/> Previous doctor diagnosis of asthma <input type="checkbox"/> Family history of asthma and other allergic condition	<input type="checkbox"/> Previous doctor diagnosis of COPD, chronic bronchitis or Emphysema <input type="checkbox"/> Heavy exposure to tobacco smoke, biomass fuel
Time course	<input type="checkbox"/> Symptoms vary seasonally, or from year to year <input type="checkbox"/> May improve spontaneously or have an immediate response to BD or to ICS over weeks	<input type="checkbox"/> Symptoms slowly worsening over time (progressive) <input type="checkbox"/> Rapid acting bronchodilator provides only limited relief.
Chest X-ray	<input type="checkbox"/> Normal	<input type="checkbox"/> Severe hyperinflation

History of sun exposure:

Time : /day or week or month

Duration : /min or hour

Anthropometric data:

Height : cm Weight: kg BMI: kg/m²

Waist: cm Hip: cm Waist-hip ratio:

MUAC: cm

General examination: (present = +, absent = -)

Anaemia - / + / ++ / +++

Jaundice - / + / ++ / +++

Cyanosis - / + / ++ / +++

Clubbing - / + / ++ / +++

Edema - / + / ++ / +++

Ascites - / +

Pulse rate :

(Beats/min)

BP : Systolic:

(mmHg) Diastolic:

Systemic

examination:

Respiratory system:

Shape of the chest : Normal-1;abnormal-2

Movement of chest : Abdominothoracic-1;
thoracoabdominal-2

Tracheal position : Central-1;shifted to right-2;
shifted-3

Use of accessory respiratory muscle : Yes-1;no-2

Percussion note : Dull-1;resonant-2

Breath sound : Vesicular-1;bronchial-2

Added sound : No-1;ronchi-2;creps-3

Other system:

Heart	:	Not palpable-1;palpable-2	<input type="text"/>
Kidney	:	Not palpable-1;palpable-2	<input type="text"/>
Spleen	:	Not palpable-1;palpable-2	<input type="text"/>
Liver	:	Not palpable-1;palpable-2	<input type="text"/>

Lung function test:

Variables	MV	% of PV	MV	%	of	MV	% of PV
	Day 1	Day 1	Day 90	PV		Day 90	Day 180
	(post		(post	Day		(post	
	BD)		BD)	90		BD)	

FVC(L)

FEV1(L)

FEV1/FVC(L)

PEFR(L/S)

FEF 25-75%

(PV =Predicted Value , MV=Measured Value) \

Exercise tolerance variables

(a) 6MWD	On day	On day	On day
(meter)	0	90	180

No. of laps.....(X 60 min)+ final
partial lap.....meter

Stopped or paused before 6min (yes/no)

Reason

Symptoms at the end of exercise:

angina/dizziness/hip,leg or cuff pain

	On day 0		On day 90		On day 180	
	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT
(b)(SpO2)(%)						

(c) Level of
dyspnea(score)

(d) Level of
fatigue(score)

Haematological variables:

Variables	On day 1	On day 90	On day 180
Serum 25(OH)D (mg/ml)			
Serum Ca ² (mg/dl)			
Serum PO ₄ ³⁻ (mg/ml)			
Serum PTH (pg/ml)			
Serum creatinine(mg/dl)			
Serum SGPT (U/L)			
Serum cholesterol (mg/dl)			
Serum TG (mg/dl)			
Serum HDL (mg/dl)			
Serum LDL(mg/dl)			
Serum FBS (mmol/L)			
Serum HbA1c (%)			

Modified borg scale:

SCALE	SEVERITY
0	No Breathlessness At All
0.5	Very Very Slight (Just Noticeable)
1	Very Slight
2	Slight Breathlessness
3	Moderate
4	Somewhat severe
5	Severe Breathlessness
6	
7	Very Severe Breathlessness
8	
9	Very Very Severe (Almost Maximum)
10	Maximum

AppendixIII

Standard pharmacotherapy for stable patient of ACO:

(GOLD 2015)

- **Inhaled corticosteroid** (in low or moderate dose, depending on level of symptoms)
- **Inhaled long acting bronchial agonist (LABA), and /or**
- **long acting muscarinic agonist (LAMA)**
- **Advice-**
 - on smoking cessation.
 - Appropriate self management strategies.
 - Regular follow up.

Appendix IV

Cholecalciferol supplementation schedule for D₃ deficient [serum 25(OH)D<30 ng/ml] ACO pts

Our aim is

- to avail (within 6 months) and maintain serum 25(OH)D @ 60-80 ng/ml + serum Ca²⁺ @ 8.5-10.5 mg/ml,
- for having extra-calcaemic effect of vitamin D₃,
- on lungs and other tissues

At 1st visit / at day 0:

Cholecalciferol 80,000 IU (2 capsules of 40,000 IU) / wk, for 12 weeks (3 months)

At 2nd visit / at day 90 / after 3 months:

<i>If,</i> serum 25 (OH)D + serum Ca ²⁺ =	<i>Then,</i> for next 3 months, prescribed <u>dose</u> will be -	<i>So,</i> for next 3 months, <u>dose schedule</u> will be -
30-40 ng/ml + 8.5-10.5 mg/ml	4600 IU/day	4600 IU X 7 = 32,200 IU / 7 days = 1 cap (40,000 IU) / wk
40-50 ng/ml + 8.5-10.5 mg/ml	3000 IU/day	3000 X 15 = 45,000 IU / 15 days = 1 cap (40,000 IU) / 2 wks
60-80 ng/ml + 8.5-10.5 mg/ml	not suggested in guideline, but, to get extra-calcaemic effect of D₃	1 cap (40,000 IU) / 6 wks
> 80 ng/ml + 8.5-10.5 mg/ml	stop taking drug & symptom analysis	no dose for 3 months
>150 ng/ml + >10.5 mg/ml	close monitoring and ask for – <ul style="list-style-type: none"> • feeling sick or being sick • poor appetite or loss of appetite • feeling very thirsty • passing urine often 	no dose for 3 months

	<ul style="list-style-type: none">• constipation or diarrhea• abdominal pain• muscle weakness• pain• bone pain• feeling confused• feeling tired	
--	---	--

At 3rd visit / at day 180 / after 6 months:

Pt will be referred to physician and will be suggested to follow the above-mentioned schedule

Ref: Vitamin D Council. I tested my vitamin D level. What do my results mean? 2017 [Internet]. [Cited 2018 March 25]. Available from: <https://www.vitamindcouncil.org/i-tested-my-vitamin-d-level-what-do-my-results-mean/>

Appendix-V

Principles of Bangladesh Good Clinical Practice

(Directorate general of drug administration 2011)

Before conducting the study the researcher will follow all the principals of Bangladesh GCP, written below:

1. Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirement(s).
2. Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial participant and society. A trial should be initiated and continued only if the anticipated benefits justify the risks.
3. The rights, safety, and well-being of the trial participants are the most important considerations and should prevail over interests of science and society.
4. The available nonclinical and clinical information on an investigational product should be adequate to support the proposed clinical trial.
5. Clinical trials should be scientifically sound, and described in a clear, detailed protocol.
6. A trial should be conducted in compliance with the protocol that has received prior institutional review board (IRB)/ independent ethics committee (IEC) approval/ favorable opinion.
7. The medical care given to, and medical decisions made on behalf of, participants should always be the responsibility of a qualified physician or, when appropriate, of a qualified dentist.
8. Each individual involved in conducting a trial should be qualified by education, training, and experience to perform his or her respective task(s).
9. Freely given informed consent should be obtained from every participant prior to clinical trial participation.
10. All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification.

11. The confidentiality of records that could identify participants should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirement(s).

12. Investigational products should be manufactured, handled, and stored in accordance with applicable good manufacturing practice (GMP). They should be used in accordance with the approved protocol.

13. Systems with procedures that assure the quality of every aspect of the trial should be implemented .

