Effect of Vitamin D3 on Lung Function and Exercise Tolerance in D3 Deficient COPD Patients

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PROJECT SUMMARY

Background: Chronic Obstructive Pulmonary Disease (COPD) is a major cause of morbidity and mortality throughout the world which is a preventable as well as treatable disease. It has some important extra pulmonary effects which may contribute to the magnitude of the severity of this disease. Standard therapeutic treatment alone does not optimize its remedy. Vitamin D_3 has been found to improve the physical efficiency of patients with various morbid disorders, including respiratory ailments. **Objectives**: To evaluate the effects of Vitamin D_3 on lung functions and exercise tolerance in patients with stable moderate COPD. **Methods**: For this, a prospective interventional randomized double blinded study will be carried out on 46 (eighty) vitamin D_3 deficient (serum 25 dihidroxycholecalceferol less than 30 ng/ml), male, stable (diagnosed patient, who has not experienced any acute exacerbation , hospitalizations , urgent care visits, or changes in routine medication within 4 weeks prior to study), moderate (post bronchodilator $FEV_1/FVC<0.70$ of predicted value and $FEV_1=50$ to 79% of predicted value) COPD patients (age ≥40 years), who will be selected from the Out Patient Department (OPD) of the National Institute of Diseases of Chest and Hospital (NIDCH) and will be grouped as A (control) and B (study) groups, respectively. All the patients will be again designated as A0, A90 (with placebo) and B0, B90 (with D_3) for before and after 90 days of follow up. All the participants will be matched in terms of duration of COPD, history of smoking, occupation and socioeconomic status. Along with the standard pharmacological treatment of COPD, the patients of the 'Study group' will be prescribed for 80000 IU of oral vitamin D_3 per week for consecutive 3 months. Along with this, all patients both the groups will be advised to continue ad lib (according to their own choice) diet. At the very 1st day of the study, the lung functions will be assessed by measuring Forced vital capacity (FVC), Forced expiratory volume in one second (FEV_1), Forced expiratory ratio ($FEV_1/FVC\%$), Peak expiratory flow rate (PEFR) and Forced mid expiratory flow of FVC($FEF_{25-75\%}$), 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 (SpO_2) by Pulse Oximeter and degree of dyspnoea by Modified Borg Scale (MBS) will also be measured both before and after 6MWT to evaluate their change in both the groups. All these variables will be measured again after 90 days standard pharmacological treatment of COPD with D_3 intervention (B group) and also without D_3 intervention (A group). For statistical analysis, Chi-square test, independent sample 't' test between two groups, paired

Student's 't' test within two specific measurements of different durations of each group ,will be done. In the interpretation of results, ≤0.05 level of probability (p) will be accepted as significant.

Expected outcome:

Supplementation of vitamin D_3 may cause statistically significant improvement in lung function test along with exercise tolerance in moderate stable COPD patient.

Keywords:

COPD, VitaminD_3, FVC, FEV1, FEV1/FVC%, FEF25-75%, PEFR, SpO2, 6 Minute Walk Distance, Modified BORG scale

1.Introduction:

Chronic Obstructive Pulmonary Disease (COPD) is one of the major causes of chronic morbidity and mortality throughout the world (GOLD 2015). Many people suffer from the disease for years and die too early from its complications. COPD is the fourth leading cause of death in adults of United States and also projected to be the third by 2020 (Sabit et al 2007; Eickhoff et al 2008; Finkelstein and Scharf 2009). Though it is a preventable and treatable disease, once developed the disease along with its comorbidities cannot be cured. But its progression and morbidity can be reduced.

In European countries, on the basis of different variables, the prevalence of COPD was ranged from 2.1% to 26.1% (Atsou et al 2011). Additionally it was 8.9% in India (from 6.2% to 13.5%) (Afonso 2011), 17.4% in Copenhagen (aged≥ 35 years) (Fabricious et al 2011) and 3.7% in Abu Dhabi (age 40 to 80 years) (Al Zaabi et al 2011). In Bangladesh among >40 years population it is 21.24%, which suggests an overall prevalence of 4.32% in the total population of all age group (Habib et al 2010). Furthermore 90% of COPD deaths occur in low and middle income countries (Murray and Lopez 1997).

COPD is a multicomponent disease, comprising emphysema in the lung parenchyma, large central airway inflammation and mucociliary dysfunction, bronchiolitis and small airway structural changes (Agusti 2005). Together, these separate factors contribute to the chronic airflow limitation that characterizes the condition (Agusti 2005; Lapperre et al 2004). In addition, there is evidence that systemic inflammation and extra pulmonary effects are also common in COPD, although the association between systemic inflammation and systemic manifestations of COPD is still not entirely clear.

The airflow obstruction in this disease is generally persistent as well as progressive (Lapperre et al 2004). It has two clinical phases (stable phase and exacerbation phase), both of which are associated with inflammation (Barbu et al 2011). Independent risk factors for COPD are male gender, advanced age, low socioeconomic status, occupational exposure and cigarette smoking

(both active and passive) (Caballero 2008), reactivity of airways, occupational factors and air pollution (Reilly et al 2004).

Vitamin D is a steroid important in bone mineralization and calcium homeostasis. The prevalence of vitamin D deficiency has been increasing in the general population in recent decades. The majority of circulating 25-hydroxyvitamin D [25(OH)D] is derived from sun exposure with a limited dietary contribution. The increased prevalence of vitamin D deficiency is attributed to sun avoidance, indoor lifestyle, use of sunscreen, and decreased intake of vitamin D-containing foods (Reilly et al 2004). Because vitamin D is sequestered in adipose tissue, the increasing prevalence of obesity also increases the prevalence of vitamin D deficiency (Holick 2007).

Recently, research has found that vitamin D may play a role in multiple chronic diseases such as cancer, autoimmune diseases, infections, and cardiovascular disorders (Holick 2007; Holick and Chen 2008). Vitamin D may also have a role in several diseases involving the respiratory system. Recently Heideri et al (2015) reported that a significant proportion of young COPD patients may have insufficient (20 to 29 ng/ml) serum 25-OHD (Heideri et al 2015). They also found a statistically significant positive relationship between serum 25-OHD and FEV1 in this group of patients. Furthermore, it has been suggested that, lower vitamin D status in COPD may be due to diminished production of pre-vitamin D_3 associated with skin aging caused by smoking and limited Ultra Violet B radiation exposure (Holick 2007; Janssens et al 2009). Moreover, higher serum vitamin D concentrations, assessed by [25(OH)D], have been associated with better lung function as measured by FEV_1 in a large cross-sectional study of the U.S. population (Black and Scragg 2005). 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 (Mahon et al 2003; Xystrakis et al 2006; Searing and Leung 2010), inducing antimicrobial peptides (De Smet and Contreas 2005), and/or its action on muscles (Hopkinson et al 2008; Forli et al 2009).

It has also been suggested that, patients with COPD have a high prevalence of vitamin D deficiency, ranging from approximately 30% in mild COPD to over 75% in severe COPD (Black and Scragg 2005; Janssens et al 2010; Ferrari et al 2010; Kunisaki et al 2011). Particularly for COPD, vitamin D deficiency may enhance chronic airway and systemic inflammation, reduce

bacterial clearance, and increase the risk for infectious exacerbations at the same time (Kunisaki et al 2011). In addition, Ferrari et al (2010) also demonstrated that the maximal exercise capacity and carbon monoxide transfer in the single breath method were both positively correlated with serum [25(OH)D] (Ferrari et al 2010). It has also been suggested that the impairment of exercise capacity in COPD may be related to a reduction in muscle strength (Forli et al 2009).

To provide a quick, acceptable as well as repeatable and reproducible lung function data spirometry is a safe and practical procedure (Johns et al. 2014). The ventilatory functions of the lung such as, forced vital capacity (FVC), forced expiratory volume in 1st second (FEV_1), FEV_1/FVC ratio (FEV_1/FVC %), forced mid expiratory flow rate between 25% and 75% of FVC ($FEF_{25-75\%}$) and peak expiratory flow rate (PEFR), can be assessed by spirometry (Wildhaber et al. 2007; Mehrparvar, Mirmohammadi and Sohrabi 2010). The indices derived from this forced exhaled maneuver have become the most accurate and reliable way of supporting a diagnosis of COPD (Bangladesh Lung Health Manual 2010). This test should be undertaken in all patients who may have COPD. It is needed to make a confident diagnosis as well as to exclude other diagnoses that may present with similar symptoms. Although spirometry does not fully capture the impact of COPD on a patient's health, it remains the gold standard for diagnosing the disease and monitoring its progression. It is the best standardized most reproducible and most objective measurement of airflow limitation available (GOLD 2015).

In addition, oxygen saturation is an indicator of the percentage of hemoglobin saturated with oxygen at the time of the measurement. Peripheral capillary oxygen saturation values obtained from pulse oximetry (SpO_2) is one part of a complete assessment of the patient's oxygenation status. Normal oxygen saturation values are 97% to 99% in the healthy individual and of 95% is clinically accepted in a patient with a normal hemoglobin level (Schutz 2001). This value may vary with the amount of oxygen utilization by the tissues. For example, in some patients, there is a difference in SpO_2 values at rest compared with those during activity, such as ambulation or positioning. However it does not reflect the patient's ability to ventilate (Schutz 2001).

Moreover, dyspnea is one of the most significant symptoms occurring during the progression of COPD and results from pulmonary hyperinflation, weakness of inspiratory muscles, increased ventilation, voluntary hyperventilation, increased respiratory work load and impaired function of

the inspiratory muscles (Schutz 2001). The evaluation of dyspnea is very important in any chronic respiratory ailment. The effort dyspnea determined at the end of exercise is accepted as the best indicator of dyspnea (Kendrick et al 2010). There are several scales are available to evaluate dyspnea. Though the interpretation of dyspnea scales depends solely on the statements of the patients, but Modified Borg Scale (MBS) is known to be simple and partially objective, have usually been used to evaluate effort dyspnea in clinical practice (Kendrick et al 2010).

Respiratory disease often presents with limited activity level and exercise capacity and reduced exercise tolerance is a hallmark of patients with COPD (Balke 1963). In 1963, Balke developed a simple test to evaluate the functional capacity by measuring the distance walked during a period of time. Walk test are typically administered as a means of evaluating functional status, monitoring treatment effectiveness and establishing prognosis. The 6 minutes walk test (6 MWT) is a practical simple test that requires a 30 meter hallway but no exercise equipment or advance training for the observer. The test measures the distance that a person can quickly walk on a flat, hard surface in a period of 6 minutes. The self paced 6 MWT assesses the submaximal level of functional capacity. However, because most activities of daily living are performed at submaximal levels of exertion, the 6 minutes walk distance (6 MWD) may better reflect the functional exercise level for daily physical activities (ATS statement 2002).

2. Rationale:

Standard therapeutic treatment schedule has a limited role in improving the physical capacity in COPD patients (Singh et al 2003). Various supplementations and extra-therapeutic measures have been tried to improve the functional capacity of the COPD patients (Bool2012; Biswas et al 2013; Ismail et al 2015). Vitamin D_3 supplementation is one of them. The principal goals of adding vitamin D_3 in the treatment schedule of these patients are to reduce symptoms and exacerbations, to improve quality of life and to increase physical and emotional participation in everyday activities which may not be adequately addressed by standard pharmacological regime alone for COPD (GOLD 2015). Recently, a number of studies have shown an association between vitamin D deficiency and severity of COPD (Janssens et al 2010; Ferrari et al 2010). In addition in a prospective study, FEV_1 was measured in patients with severe and very severe COPD both before and after vitamin D_3 supplementation and significant improvement was found (Zendedel et al 2015). On the other hand, one recent study reported no significant improvement in FVC, FEV_1 and FEV_1/FVC% in Vitamin D_3 deficient COPD patients after vitamin D_3 administration as compared to that of the control group (Jabbari et al 2013).

However the volume of information regarding the effect of vitamin D_3 administration in COPD patients is not enough for reaching any final conclusion. Moreover, with the best of our knowledge no study have been conducted to observe the effects of this fat soluble vitamin on the spirometric lung function status, oxygen saturation and exercise tolerance in vitamin D_3 insufficient, stable patients with moderate COPD.

Therefore, on the basis of this background the present study has been designed to evaluate the effects of Vitamin D_3 on the spirometric lung function status, peripheral capillary oxygen saturation and exercise tolerance in D_3 deficient, male patients with stable moderate COPD. This study will draw attention of the physicians about the beneficial effects of the vitamin D_3 on both pulmonary and extrapulmonary complications in COPD patients.

Research Question:

Does vitamin D_3 has any effect on lung function and exercise tolerance in Vitamin D_3 deficient stable COPD patients?

Hypothesis:

Null: Vitamin D_3 administration does not improve lung function and exercise tolerance in vitamin D_3 deficient stable COPD patients.

Alternate: Vitamin D_3 administration improves lung function and exercise tolerance in vitamin D_3 deficient stable COPD patients.

Objectives:

General Objective

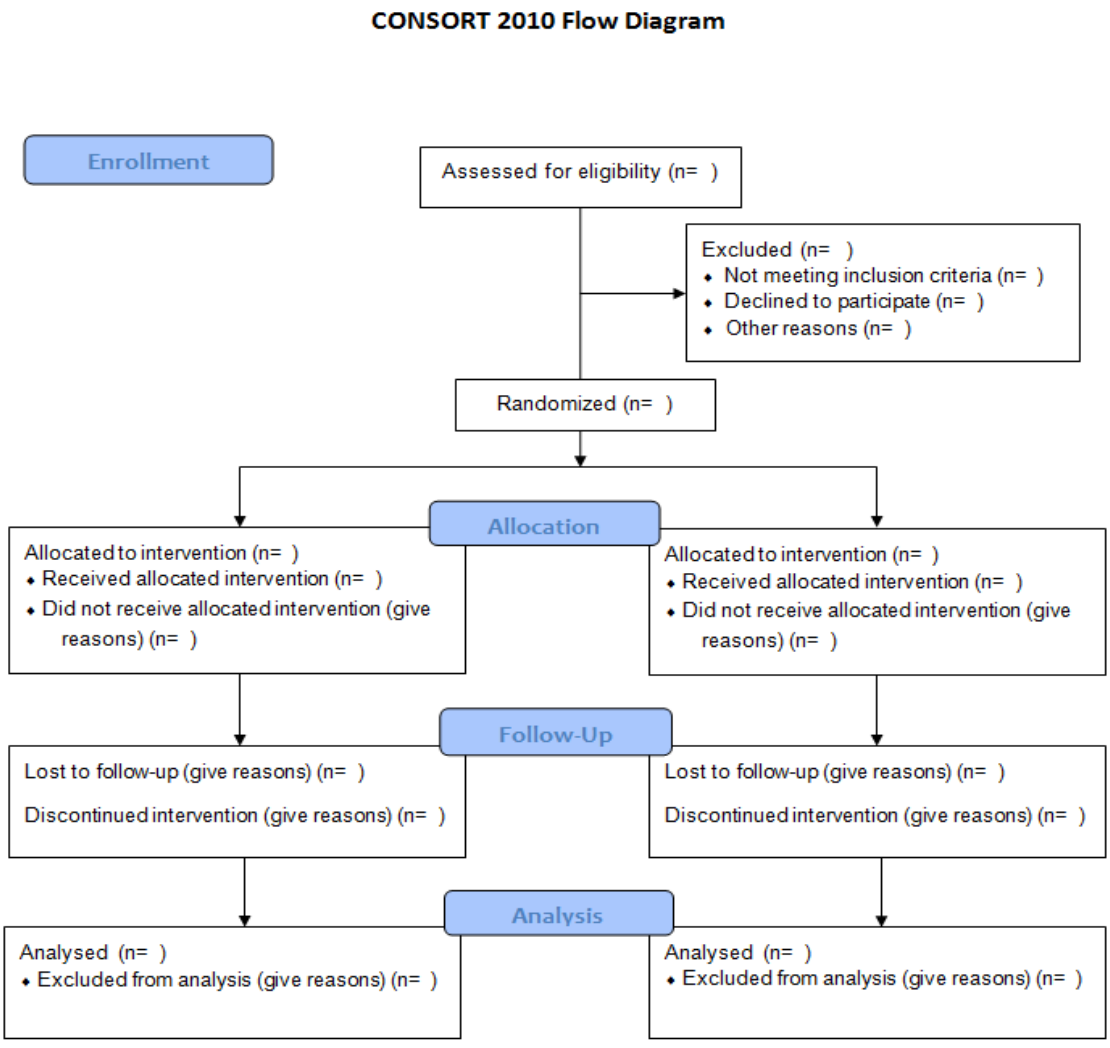
To evaluate the effects of vitamin D_3 administration on lung functions and exercise tolerance in vitamin D_3 deficient male patients with stable COPD.

Specific Objectives

- To measure the FVC, FEV_1, FEV_1/FVC%, PEFR and $FEF_{25-75\%}$ of vitamin D_3 insufficient male patients with stable moderate COPD, in order to assess their lung function status.
- To measure the SpO_2 at rest, in order to assess the basal oxygenation status in this group of patients.
- To determine level of dyspnea at rest, in order to assess the basal breathlessness level in the same group of patients
- To measure the 6MWD of all the patients, in order to assess their functional exercise capacity.
- To measure both the SpO_2 and level of dyspnea again after 6MWT, for the assessment of their exercise tolerance.
- To measure all these variables after 90 days standard pharmacological treatment with vitamin D_3 and also without vitamin D_3 in stable COPD patients.
- To compare the results of baseline and endline.

Methodology:

Consolidated Standard of Reporting Trials: (Moher et al 2010)



TYPE OF STUDY	:	Prospective interventional randomized double blinded study
PLACE OF STUDY	:	Department of Physiology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Shahbagh, Dhaka
STUDY PERIOD	:	March 2017 to February 2018
STUDY POPULATION	:	Stable, moderate COPD patients with vitamin D_3 deficiency.
SAMPLE SIZE	:	46
SAMPLING	:	Simple random sampling .
GROUPING OF THE SUBJECTS	:	
Group A (Control group)	:	23 D_3 deficient COPD patients without D_3 administration
A0	:	On day 0
A90	:	On day 90
Group B (Study group)	:	23 D_3 deficient COPD patients with D_3 administration
B0	:	On day 0
B90	:	On day 90

Sample size calculation:

Using the following statistical formula

$n = [(Z\alpha+Z\beta)^2 \text{ X } (\sigma1^2+\sigma2^2)] / (\mu1-\mu2)^2$

(Kirkwood and Sterne 2003)

	Mean of control,	$\mu1$	=	47.1
Therefore,	Mean of study group,	$\mu2$	=	67.4
$n = [(Z\alpha+Z\beta)^2 \text{ X } (\sigma1^2+\sigma2^2)] / (\mu1-\mu2)^2$	SD of control,	$\sigma1$	=	26.9
	SD of study group,	$\sigma2$	=	27.5

(Martineau et al. 2015)

	Type I Error,	$Z\alpha$	=	1.96
$= [(1.96+1.64)^2 \text{ X } (26.9^2+27.5^2) / (47.1-67.4)^2$	Type II Error,	$Z\beta$	=	1.64

(Kirkwood and Sterne 2003)

$= [12.96 \text{ X } (756.25+723.61)] / (20.3)^2$

$= 19178.98 / 412.09$

$= 46.54$

$= 47$

Subject Selection:

Inclusion Criteria

- Age: >40 years (Heideri 2015)
- Socioeconomic condition: Middle class (Martineau et al 2015)
- Smoking status: (Janssens et al 2010)

  Current smoker = with history of active smoking

  Ex-smoker = with history of 0 – 8 quit year

  Duration = for >30 pack years

- BMI: 25 – 29.9 kg/meter-sq (Janssens et al 2010)
- COPD patients at moderate stage according to GOLD criteria with > 4 years duration (Rees et al 2013)
- Stable COPD patients (Mehrparver et al 2010)
- Vitamin D_3 deficient: 30 ng/ml (Vitamin D Council 2017)

Exclusion Criteria (Said and Abd-Einaeem 2015)

With history of –

- Chronic pulmonary diseases, like –

  bronchial asthma

  respiratory tract infection

  bronchiectasis

  pneumothorax

  pleural effusion

tuberculosis

pulmonary fibrosis

pneumonectomy or pulmonary lobectomy

- Chronic liver disease
- Malignancy
- Systemic hypertension
- Thyrotoxicosis
- Chronic cardiac diseases, like –

  angina

  congestive heart failure

  myocardial infarction

  cardiac arrhythmia

  cardiomegaly

- Use of drugs known to affect vitamin D metabolism within 1 month prior to study, as,

  Anticonvulsants

  Glucocorticoids

  Calcium

With biochemical evidence of –

- Diabetes mellitus
- Renal insufficiency

All the criteria mentioned above will be scrutinized by taking history and clinical examination, except vitamin D_3 deficiency, diabetes mellitus and renal insufficiency, which will be diagnosed biochemically.

Principles Of Bangladesh Good Clinical Practice:

(Directoriate 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.

Ethical Implications:

Before conducting the study the researcher will take approval from International Review Board of BSMMU. In addition ,the institutional permission will also be taken for data collection.
While conducting this study the researcher will focus special attention to protect the life, health, dignity, integrity, privacy and confidentiality of the personal information of the study subjects.

After selection of subject the researcher will sit for an interview with the subject. The researcher will at first introduce himself mentioning his institutional affiliation, conflict of interest and will supply address, contact telephone number and information about sponsorship. After that the subject will be thoroughly informed about the objectives and outcome of the study. Brief explanation of the procedure will be given to them. The subject will also be assured if any problem arises during the test; it will be taken care of. The result of the test will be sent to him free of charge and if any abnormality is detected, then appropriate management will be given to him, if possible or will be referred to specific treatment facility as appropriate. He will be encouraged for voluntary participation in a cordial and friendly attitude and will be allowed freedom to withdraw from the study whenever he likes even after participation. They will be informed about the method of confidentiality of their identification. Their identity will be recorded only in questionnaire which will be kept in safe custody with the researcher. It will not enter in the computer and will not go in any publication. No personal identification will be used in data analysis, report writing or publication. They will also be assured that interview time will minimum for maintaining their comfort, some questions will be asked about the personal, family and medical history without any private enquiry. The conflict of interest will be told to him if there is any. After he has understood these entire procedure, if he agrees to participate, then a willingly given informed written consent will be taken from him.

There is no possibility of any physical, social or mental risk of the respondent. A pretested questionnaire will be filled up by the research assistant.

All gathered information will be kept secret and only will be used for medical research and analysis.

SITE OF SAMPLE COLLECTION:

All the patients will be collected from the Out Patient Department (OPD) of National Institute of Diseases of Chest and Hospital (Annexure - I).

STUDY PROCEDURE:

Investigator has applied for formal Clinical trials through www.Clinicaltrials.gov and was granted permission to go on with the trial (Annexure II) .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 subject, 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 (Annexure - IV). 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 (Annexure - V). Then all the patients will be requested to attend the Department of Physiology at 9 am (about 1 and 1/2 hours after his breakfast) on the day of biochemical and spirometric examination.

On the examination day, 5 ml of venous blood will be collected and taken to the Hematology laboratory as soon as possible for the estimation of serum 25-hydroxycholecalceferol, serum glucose 2 hours after breakfast and serum creatinine. After that his height and weight will be measured and the spirometric lung function test will be done by using a portable spirometer. After getting all the biochemical and spirometric reports the final selection will be done, according to the inclusion and exclusion criteria.

Subsequently, 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. Then the patients will be examined for the baseline value of all the study variables in Day 1.

Again after 90 days the study variables will be collected from same 46 patients.

CONFOUNDING VARIABLES:

- Age: >40 years (Heideri 2015)
- Socioeconomic condition: Middle class (Martineau et al 2015)
- Smoking status: (Janssens et al 2010)

  Current smoker = with history of active smoking

  Ex-smoker = with history of 0 – 8 quit year

  Duration = for >30 pack years

- BMI: 25 – 29.9 kg/meter-sq (Janssens et al 2010)

  COPD patients at moderate stage according to GOLD criteria with > 4 years duration (Rees et al 2013)
- Stable COPD patients (Mehrparver et al 2010)
- Vitamin D_3 deficient: 30 ng/ml (Vitamin D council 2017)

All the above mentioned variables will be similar ,statistically non significant in both the groups to ensure the minimization of their effects.

VITAMIN ADMINISTRATION :

Collection:

Vitamin D3

Ingredient

• Cholecalciferol (40,000IU)

• Microcrystalline Cellulose (58.1 gm)

• Butylated Hydroxy Toluene (0.2mg)

• Magnesium Stearate (3mg)

- Gelatin Capsule Shell (1mg)

Dose : 80,000 IU/wk

Route : Oral

Placebo : Courtesy of Beximco Pharmaceuticals LimitedBangladesh

Ingredient : • Microcrystalline Cellulose (303.8gm) • Butylated Hydroxy Toluene (0.2mg)

- Magnesium Stearate (3mg)
- Gelatin Capsule Shell (1mg)

STUDY VARIABLES:

(1) Spirometric variables

- FVC (L) : Forced Vital Capacity
- FEV_1 (L) : Forced Expiratory Volume in 1^{st} second
- FEV_1/FVC Ratio (%) : Forced Expiratory Ratio
- PEFR (L/min) : Peak Expiratory Flow Rate
- FEF_{25-75} (L/S) : Forced Expiratory Flow in the middle of FVC

(2) Oxygenation variables

- Resting SpO_2 (%) : Resting Peripheral Capillary Oxygen saturation

(3) Exercise tolerance variables

- 6MWD (meter) : Six Minute Walk Distance
- Level of Dyspnea : Modified Borg Scale

(4) Hematological variables

- 25(OH)D (ng/ml) : 25-hydroxycholecalceferol
- PTH (pmol/l) : Parathyroid hormone
- Ca^{2+} (mg/ml) : Ionic calcium
- PO_4^{3-} (mg/ml) : Ionic phosphate
- AP (U/L) : Alkaline Phosphatase

Subsequently a standard therapeutic treatment (according to GOLD guideline) (Annexure - VI) will be prescribed to all the selected stable moderate COPD patients. In addition, the patients of

the ‘Study group’ will be prescribed for 80000 IU of oral vitamin D_3 per week for consecutive 90 days . Along with this, all the patients of both the groups will be advised to continue ad lib (according to their own choice) diet.

Proper education will be given about drug , method of taking medication and medication plan.Afterwards,a good rapport will be built up to take time to time follow up over telephone and visiting patient’s place .Schedule appointment ,hotline and follow up will be maintained properly. They will be requested to attend the Department of Physiology again on the 90th day, to have the assessment of all the above-mentioned study variables

Any patient, who 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.Thats why extended sample should be taken to fulfill the total sample number.

Collection of blood sample (Afroza 2015)**:**

With all aseptic precaution, 5 ml of venous blood will be drawn from the antecubital vein by a disposable plastic syringe and was transferred immediately into a dry, clean test tube with a gentle push after removing needle, to avoid hemolysis. The test tube will be kept in slanting position till formation of clot. After centrifuging the clotted blood (at 3000 rpm for 10 minutes), the serum will be separated and taken into an Eppendorf tube and will be preserved in the refrigerator at -4˚C in the laboratory. The serum level of all the intended variables will be done by an auto analyzer in the hematology lab of Department of Physiology, BSMMU.

Test procedure for spirometry (Afroza 2015)**:**

- 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 will be 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 (Afroza 2015)**:**

- 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) (Ahmed, Begum and Ali 2014)**:**

The 6MWT will be performed at indoors, along a long, flat, straight, enclosed corridor with a hard surface that is seldom traveled. The walking course will be 30 meter in length which will be marked every 3 meter. The turnaround points will be marked with a cone. A starting line, which marked

the beginning and end of each 60 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 will be 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 has left before the test was completed.

If any of the subjects developed chest pain, intolerable dyspnoea, 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 study procedure (according to the exact study design), he will be congratulated for good effort.

STATISTICAL ANALYSIS:

The results will be expressed as mean with standard deviation (mean±SD).

The data will be statistically analyzed by a computer with SPSS (Version 16.0) using Chi-square test,(to know the qualitative difference between the observed and expected result).Spirometric variables, oxygenation variables, exercise tolerance variables and hematological variables will be found between two groups of patients. If the variables are normally distributed , statistical analysis will be done by parametic statistical test. But if it is not normally distributed then it will be transformed into natural logarithm and again into anti logarithm, to make it normally distributed, then parametric statistical test will be done. Independent sample 't' test between two groups, paired Student's 't' test within two specific measurements of different durations of each group will be done.

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

PUBLICATION POLICY

- Scientific article to be published in a scientific journal.
- Presentation in scientific seminar or conference orally or through poster presentation.
- Website dissemination through internet version of JBSP.
- The supervisor of the study will take initiative for publication.

INFORMED CONSENT STATEMENT

Title of the study: Effect of vitamin D3 on lung function and exercise tolerance in D3 deficient COPD patients.

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 in male patients with stable COPD by spirometric lung function variables before and after supplementation therapy for a period of 3 months.

This study may find out the benefit of vitamin D_3 on lung function in COPD 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. The researcher will be cautious in using the measurement tools so that the procedure does not harm you physically. 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. Samia Hassan at 0191174400.

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:.....

DATA SCHEDULE

Title: Effects of Vitamin D_3 on lung functions & exercise tolerance in male COPD patients

ID No:_____ SL No:_____ Date:…….

Subject information

Name:_____ Age (years):_____

Contact address: _____

_____

Permanent Address:_____

_____

_____

_____

E-mail ID :_____

Contact No: Tel:_____ Cell:_____

Occupation: Dependent-1; Student-2; Unemployed-3; Business-4;, Service-5; Day labour-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; Seperated-4; Divorced-5; Live together-6;

Socioeconomic status:

Monthly income (Taka):

Number of family members:

Residence: Urban/Rural

Personal History

1. Smoking habit: Current smoker/Past smoker

Packs- year:

Duration of smoking:

Stopped for (in years, in case of past smoker):

2. Marital status: Married/Unmarried.

3. Past medical history:

History of hypertension: Yes/No

History of Cardiac disease: Yes/No

History of Liver disease: Yes/No

History of Psychic and neurological disorder: Yes/No.

History of diabetes mellitus: Yes/No

Others: Yes/No

(If yes)

Duration of disease:

4. Drug History:

5. Anthropometrical data:

Height: cm. Weight: Kg.

BMI: kg/m^2

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

Anemia: - / + / ++ / +++

Jaundice: - /+ / ++ / +++

Pulse rate: .....beat/min.

Cyanosis: -/+ / ++ / +++

Clubbing: - /+

Edema : - /+

Ascites : -/+

BP: Systolic : ..... mm of Hg.

Diastolic: ..... mm of Hg

7. Systemic examination:

P/A examination:

Kidney- Palpable/Not

Spleen- Palpable/Not

Liver- Palpable/Not

Heart- Normal/Abnormal.

Respiratory System:

Shape of the chest - Normal/ Abnormal

Movement of chest- Abdominothoracic/ Thoracoabdominal

Tracheal position - Central/Shifted to right /Shifted to left

Use of accessory respiratory muscles - Yes/No

Percussion note - Dull/Resonance

Auscultation -

Clear/Creaps/Ronchi

Breath sound - Vesicular/Bronchial

Added sound- Yes/No.

Study variables:

Lung Function variables	PV	MV (day 0)	% of PV (day 0)	MV (day 90)	% of PV (day 90)
FVC:	(L)				
FEV_1:	(L)				
FEV_1/FVC	(%)				
PEFR:	(L/S)				
FEF_{25-75}	(L/S)				
MEF_{75}	(L/S)				
MEF_{50}	(L/S)				
MEF_{25}	(L/S)				

(PV=Predicted Value, MV=Measured Value)

Variables	Before (day 0)	After (day 0)	Before (day 90)	After (day 90)
Oxygen Saturation % (SpO_2)				
Level of dyspnea (Borg Score)				

Level of fatigue (Borg Score)

Total Distance (in meter) Walked In 6 Minute :

The 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

Hematological variables:

Serum 25(OH)D	ng/ml
Serum PTH	pg/ml
Serum Ca^{2+}	mg/dl

Serum Alkaline phosphatase	IU/L
Serum SGPT	IU/L
Serum Creatinine	mg/dl
Fasting blood glucose	mg/dl
Serum HbA1c	%
Serum Cholesterol	(mg/dl)

Serum (mg/dl)

TG

Serum (mg/dl)

HDL

Serum (mg/dl)

LDL

Serum mg/dl)

PO_4^{3-}