

Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital



UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Imraan Kola

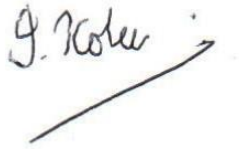
Student number 362488

A research report submitted to the Faculty of Health Sciences, University of the
Witwatersrand, in partial fulfilment of the requirements for the degree of
Master of Medicine

Johannesburg, 2023

DECLARATION

I, Imraan Kola, declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Medicine in the branch of Internal Medicine at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



27th day of July 2023 in Johannesburg

Supervisors:

Dr SA van Blydenstein

MBBCh, DCH, FCP(SA), MMed(IntMed), Cert Pulm(SA)

**Specialist Physician, Division of Pulmonology, Department of Internal Medicine, Chris
Hani Baragwanath Academic Hospital
University of the Witwatersrand**

Prof S Omar

MBChB, FCpath(SA)CHEM, DA(SA), Critical Care (SA)

ICU Department

Chris Hani Baragwanath Academic Hospital

University of the Witwatersrand

DEDICATION

I dedicate this work to my mother, Zareena Kola and father, Mohamed Kola for unwavering moral and financial support, encouragement and prayers.

PRESENTATIONS ARISING FROM THE RESEARCH PROJECT

Nil presentations.

PUBLICATIONS ARISING FROM THE RESEARCH PROJECT

Submitted for publication to the African Journal of Thoracic and Critical Care Medicine – awaiting editorial review.

ABSTRACT

Background: There has been a growing interest in nutritional/ lifestyle factors, including vitamin D, that may impact Chronic Obstructive Pulmonary Disease (COPD). Most data are in Caucasian populations and temperate climates with minimal African (ethnicity and geographical) data.

Objectives: The primary objective was to determine amongst COPD patients the prevalence of vitamin D deficiency ($25[\text{OH}]\text{D} \leq 20 \text{ ng/ml}$) and insufficiency ($25[\text{OH}]\text{D} = 21\text{-}29 \text{ ng/ml}$). Secondary objectives were to investigate the association between vitamin D and demographic/lifestyle factors; lung function parameters; markers of COPD severity and corticosteroid usage.

Methods: A prospective, cross-sectional study of 76 COPD patients was conducted at a tertiary Johannesburg hospital. Patients were interviewed regarding demographic/lifestyle factors, COPD severity markers and corticosteroid therapy. The most recent spirometry was recorded. Blood samples were taken for calcium, alkaline phosphatase and vitamin D. Patients were stratified according to vitamin D status (deficiency and non-deficiency) and statistical analysis was performed to assess for associations.

Results: The sample included 72% males and 63% black/ African patients. The prevalence of vitamin D deficiency and insufficiency were 48% (95% confidence interval [CI] 42 -54) and 35% (CI 30-41), respectively. The Modified Medical Research Council (MMRC) dyspnoea scale of ≥ 2 was associated with a relative risk of 1.34 (CI 1.05-1.7) for vitamin D deficiency in univariate analysis. In multivariate regression only sunlight exposure ($<1 \text{ hour/day}$) was an independent predictor of vitamin D deficiency (Odds ratio 2.4, CI 1.3 -4.5)

Conclusion: A high prevalence of suboptimal vitamin D levels exists within this sample COPD population. A higher MMRC score was associated with an increased risk of vitamin D deficiency while low sunlight exposure was the only independent predictor of vitamin D deficiency.

ACKNOWLEDGEMENTS

The author would like to thank Professor M. Wong, Head of Pulmonology at Chris Hani Baragwanath Academic Hospital (CHBAH) for allowing the study to happen and assisting at the Respiratory Outpatient Department (OPD). Special thanks are also extended to my supervisors, Dr S.A. van Blydenstein and Prof S. Omar, for their constant guidance. A special thanks and acknowledgement to all the patients that elected to join the study, without which this research would not be possible. A special thanks to Mohau La-Donna Kapa, Amore Visagie and Annie Maphthu, respiratory technicians that assisted with spirometry and data collection. Thanks are extended to Jean Johnstone for assisting with formatting.

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NOMENCLATURE

1,25 [OH] ₂ D	- 1,25–dihydroxy vitamin D
25[OH]D	- 25 hydroxy-vitamin D
ATS	- American Thoracic Society
BMI	- Body Mass Index
BOLD	- Burden of Obstructive Lung Disease
CAT	- COPD Assessment Test
CHBAH	- Chris Hani Baragwanath Academic Hospital
CI	- 95 % confidence interval
COPD	- Chronic Obstructive Pulmonary Disease
COVID-19	- Coronavirus Disease 2019
ERS	- European Respiratory Society
FEV ₁	- Forced Expiratory Volume in the first second
FEV ₁ %	- Forced Expiratory Volume % predicted
FVC	- Forced Vital Capacity
FVC%	- Forced Vital Capacity % predicted
GMCSF	- Granulocyte Monocyte Colony Stimulating Factor
GOLD	- Global Initiative for Obstructive Lung Disease
HIV	- Human Immunodeficiency Virus
HOD	- Head of Department
IFN γ	- Interferon γ
IL	- Interleukin
IQR	- Interquartile range
IV	- Intravenous
LTOT	- Long term oxygen therapy
MAC	- Medical Advisory Committee
MMRC	- Modified Medical Research Council
n	- Number of variable
OPD	- Outpatient department
QOL	- Quality of Life
RCT	- Randomised Control Trial
SD	- Standard deviation
SGRQ	- St. George's Respiratory Questionnaire
SPIROMICS	- Subpopulations and Intermediate Outcome Measures in COPD Study

T reg	- Regulatory T cell
Th1	- Type 1 T helper
Th2	- Type 2 T helper
UK	- United Kingdom
US	- United States
USA	- United States of America
UVB	- Ultraviolet B
UVS	- Ultraviolet score
VDR	- Vitamin D Receptor

CHAPTER 1

INTRODUCTION AND EXTENDED LITERATURE REVIEW

1.1 Epidemiology

Globally more than 170 million people are affected by Chronic Obstructive Pulmonary Disease (COPD) and COPD accounted for approximately 3.2 million deaths in 2015 (1). Low and middle-income countries bear a significant burden of COPD mortality. Limited data on the epidemiology of COPD in Africa is available. There is no robust South African (national) COPD prevalence data. The BOLD study (2) found a prevalence exceeding 20% in Cape Town. It should be noted that the demographics of Cape Town differs significantly from the remainder of South Africa and it would be inaccurate to infer that these estimates represent a national prevalence.

There is an increasing interest in lifestyle and dietary factors that may impact disease severity in the COPD population. There have been a large number of studies investigating the role of Vitamin D deficiency in COPD and its association with markers of disease severity. From the review of the available literature, there is a paucity of data available from South Africa or Africa in general concerning this subject. George *et al.* (3) in a healthy cohort in Johannesburg found a prevalence of vitamin D < 30 nmol/l (12 ng/ml) ranging between 5.1% (in black/African patients) to 28.6 % (in Asian/ Indian patients) emphasising the impact of race/ethnicity on vitamin D level. This finding may seem counterintuitive, owing to higher skin melanin content (and reduced vitamin D activation) in black/ African participants. The authors attribute the high vitamin D deficiency prevalence in Asians/ Indians to conservative cultural dressing practices limiting direct skin sunlight exposure.

1.2 Vitamin D in calcium and phosphate homeostasis

Vitamin D₃ is predominantly synthesised in the skin. Ultraviolet (UVB) radiation converts previtamin D₃ to vitamin D₃ (cholecalciferol). This step is influenced by melanin content in the skin and sunlight exposure. The other less significant source of vitamin D is dietary intake. Vitamin D₃ is hydroxylated in the liver to 25 hydroxy-vitamin D (25[OH]D). 25 [OH] D is the major storage form and is used to ascertain vitamin D status in populations (4,5). Vitamin D is predominantly hydroxylated in the kidney to 1,25–dihydroxy vitamin D (1,25

[OH]₂D). This represents the active form of vitamin D which enhances gastrointestinal absorption of calcium and phosphate and impacts positively bone turnover and bone mineral density (6).

1.3 Non-calcaemic vitamin D effects and role in COPD

Vitamin D exerts pleiotropic effects unrelated to its role in calcium and phosphate homeostasis. It is now evident that vitamin D plays a significant immunomodulatory effect. Antigen Presenting Cells can be inhibited by vitamin D. Vitamin D inhibits the innate and adaptive immune response through Vitamin D Receptors (VDRs) on inflammatory cells. Vitamin D deficiency fails to restrain macrophage and dendritic cell maturation through major histocompatibility class II restriction. Vitamin D also plays an important function in T cell differentiation. Vitamin D inhibits the Type 1 T helper (Th1) response and its associated cytokines i.e. interleukin (IL)-2, Granulocyte Monocyte Colony Stimulating Factor (GM-CSF) and interferon γ (IFN γ) in favour of Type 2 T helper (Th2) and regulatory T cell (T_{reg}) responses. Thus, vitamin D deficiency is postulated to result in dysregulation of the immune and inflammatory response resulting in chronic lung inflammation and structural lung changes promoting the onset and progression of COPD (6).

Vitamin D also enhances macrophage production of antimicrobial peptides like cathelicidin which is an important component of the immune response to combat mycobacterial infections (6). Vitamin D deficiency results in increased respiratory infections (including mycobacterial tuberculosis infection) and airway microbe colonisation which could theoretically lead to COPD progression. Vitamin D deficiency also results in increased airway remodelling, fibroblast proliferation (and hence collagen synthesis) and alteration in gene expression resulting in smooth muscle proliferation and contraction promoting the airway changes characteristic of COPD.

1.4 Vitamin D and other respiratory illnesses

Vitamin D deficiency has been associated with other respiratory conditions including tuberculosis (7), sarcoidosis (8), childhood asthma, cystic fibrosis (6) and recently COVID-19 (9).

1.5 Vitamin D deficiency definition and normal levels

There is some heterogeneity in the values quoted for vitamin D deficiency. This variability may occur because vitamin D levels required for the calcaemic effects (prevention of rickets and osteomalacia) are better established by robust data than for non-calcaemic effects of vitamin D; as well as variation in assays used to ascertain levels. The Endocrine Society defines vitamin D deficiency as 25[OH]D levels less than 20 ng /ml, and vitamin D insufficiency as levels between 21-29 ng/ml (10). The Institute of Medicine (IOM) (11) recommended that a level of 25[OH] vitamin D exceeding 20 ng/ml is adequate.

1.6 Vitamin D and airflow/ spirometry parameters

A post hoc cross-sectional review of the Baltimore Longitudinal Study of Ageing (12) failed to demonstrate an association between vitamin D deficiency and airflow limitation. An important limitation of this study is that airflow limitation was not objectively assessed but based on self-reported patient diagnoses. Airflow limitation encompassed COPD, non-smoker COPD and asthma.

The majority of studies reviewed demonstrated lower FEV₁ (Forced Expiratory Volume in the first second) correlated with vitamin D deficiency (13–19). Most of these studies were in Europe (15,17-19) and South Korea (13,16) with one study (14) conducted in the United States of America (USA). The majority of participants were of Caucasian (14,15,17-19) or Asian (13,16) ethnicity. Three studies also showed no vitamin D FEV₁ association (12,20,21). The majority of participants in all three studies were Caucasian and from Europe (20,21) or the USA (12).

Jolliffe *et al.* (15), Mekov *et al.* (19) and Gawron *et al.* (21) found no association between FEV₁/FVC (Forced Vital Capacity) and vitamin D. Jung *et al.* (13) in a South Korean longitudinal study found higher FEV₁/FVC ratios in non-deficient vitamin D patients, as well as a trend towards lower exercise capacity in the deficient group.

A small COPD Turkish study (22) with a high prevalence of vitamin D deficiency (60%) found lung function parameters (FEV₁/FVC; FEV₁; FVC and total lung capacity [TLC]) were lower in COPD vitamin D deficient patients. Hand grip and isokinetic knee strength were lower in the vitamin D deficient subgroup.

1.7 Vitamin D deficiency prevalence and associations in COPD patients

A Bulgarian study (19) looked at vitamin D deficiency in COPD patients admitted to the hospital. Owing to the study design this study had patients with more severe disease – Global Initiative on COPD (GOLD) class C and D. This study demonstrated a high prevalence of vitamin D deficiency - < 20 ng/ml (83.6%) which is significantly higher than other studies where participants were not experiencing an exacerbation (31-77% prevalence). There existed a correlation between vitamin D and Quality of Life (QOL) indices (e.g. Modified Medical Research Council [MMRC] dyspnoea scale). No correlation existed between vitamin D level and comorbidities (Diabetes Mellitus, hypertension, metabolic syndrome and the number of exacerbations). Vitamin D deficiency was associated with a longer hospital stay.

Kentson *et al.* (17) in a single-centre case-control study found no difference in the prevalence of vitamin D deficiency in long term oxygen therapy (LTOT) COPD patients versus non-LTOT users. Since this study wanted to stratify patients based on oxygen therapy a sicker cohort was selected. The sample included majority Caucasians. Peak annual vitamin D levels were measured in summer and early autumn as seasonal variation in Sweden may be significant. Lower 25 [OH] vitamin D levels were found in the COPD group compared with controls. 25[OH]D levels did correlate with MMRC score and oxygen saturation.

Joliffe *et al.* (15) in an extensive multicentre cross-sectional study in the United Kingdom (UK) explored if an association between various lifestyle factors and COPD severity markers correlated with vitamin D levels. Lifestyle factors associated with vitamin D deficiency included high Body Mass Index (BMI), lower socio-economic status, lack of vitamin D supplementation, winter sampling and absence of a summer holiday. No role of vitamin D pathway genetic polymorphisms or inhaled corticosteroid dose and vitamin D deficiency was demonstrated.

1.8 Vitamin D and exacerbations in COPD patients

The literature is conflicting regarding exacerbations. Jung *et al.* (13); Gawron *et al.* (21); Kunisaki *et al.* (23) and Persson *et al.* (18) found no association between exacerbations and vitamin D status. A primary care setting study in Switzerland, Netherlands (24) with milder COPD phenotype (predominantly GOLD II) patients also found no such association. However, there was a trend toward vitamin D sufficient participants (25[OH]D > 30 ng/ml)

having fewer exacerbations.

Malinovschi *et al.* (20) found an odds ratio of being a frequent exacerbator if having severe vitamin D deficiency was 18.1 (95% confidence interval [CI] 4.98 -65.8). The authors did concede that association does not infer causality as exacerbation history was retrospective (exacerbations in the year preceding vitamin D measurement). Burkes *et al.* (14) also found that vitamin D deficient participants had more exacerbations (including severe exacerbations) in the year preceding recruitment. Hyun *et al.* (16) also found that there was a higher rate of severe exacerbations in patients with low vitamin D and high fibrinogen levels. A 2016 meta-analysis of 21 studies (25) showed no association between vitamin D levels and exacerbation frequency.

1.9 COPD treatment and vitamin D associations

Janssen *et al.* (26) and Gawron *et al.* (21) found no association between corticosteroid usage and vitamin D levels. Persson *et al.* (18) found an association between vitamin D and the use of inhaled corticosteroids in univariate, but not multivariate analysis. Jolliffe *et al.* (15) also showed no correlation between vitamin D status and inhaled corticosteroid dose. These findings call into question the idea that corticosteroids enhance vitamin D catabolism and thus contribute to deficiency in the COPD cohort.

1.10 Effect of vitamin D supplementation on COPD clinical parameters

Several studies explored the effect of vitamin D supplementation on clinical outcomes amongst COPD patients. Martineau *et al.* (27) did a double-blinded Randomised Control Trial (RCT) with the intervention being vitamin D supplementation in COPD. Vitamin D supplementation had no effect on time to first moderate or severe exacerbation or upper respiratory tract infection. However in the vitamin D deficient subgroup (< 20 ng/ml) there was an improvement in time to the first moderate/ severe exacerbation with supplementation.

An Iranian RCT (28) found that supplementation with vitamin D led to a marked reduction in exacerbation rates and significant FEV₁ improvement. Limitations of this study include that only severe and very severe COPD patients were selected, which may reflect selection bias. This may make findings less generalisable. In addition, no baseline vitamin D levels were measured in intervention or control groups, but the authors note a high prevalence of vitamin

D deficiency in Iran.

A meta-analysis (29) found that moderate to severe exacerbations were reduced in patients supplemented with vitamin D where baseline vitamin D was < 10 ng/ml. The meta-analysis was based on 4 trials. Raw data were re-analysed where available and queries were submitted to the primary author for clarification. One trial had no data set available. The small sample size of this component study would unlikely have a major impact on the results.

1.11 Mechanisms postulated to explain COPD vitamin D associations

Zhu *et al.* (25) provided plausible mechanisms for some of the associations. Lower vitamin D levels in the COPD group may be due to increased illness and indoor hours (poorer functional status) resulting in reduced sunlight exposure. Additional postulated mechanisms that could be contributing to lower vitamin D levels in COPD include older age and smoking reducing the vitamin D activation in the dermis; enhanced vitamin D catabolism owing to corticosteroid usage and reduced storage capacity of vitamin D in COPD patients.

In this meta-analysis (25), lower latitudes tended to have lower vitamin D levels but there was heterogeneity in this finding, contrary to what might be expected from sunlight exposure. This may be explained by the fact that many developed countries at higher latitudes fortify food with vitamin D; in addition, sunlight hours (based on latitude) may only be a significant contributory factor during the winter season at higher latitudes.

Zhu *et al.* (25) also postulate mechanisms for older patients (including older COPD patients) having higher vitamin D deficiency rates. Older patients may have atrophic skin and hence reduced cutaneous vitamin D activation. In addition, they may have reduced mobility and sunlight exposure and a less nutrient-rich diet.

It may be expected that individuals of African/black ethnicity may have reduced vitamin D levels owing to increased skin melanin content and reduced cutaneous vitamin D activation when compared with Asian/ Indian or Caucasian patients. George *et al.* (3) however, found higher rates of vitamin D deficiency in Asian/Indian participants in Johannesburg compared to Black/African participants. The authors attribute this to dietary differences and cultural dressing practices with reduced skin exposure amongst Indian/Asian participants.

The precise mechanism responsible for glucocorticosteroid administration resulting in vitamin D deficiency, in some studies, has not been elucidated. Skversky *et al.* (30) provide a convincing argument that glucocorticosteroids result in increased vitamin D catabolism accounting for the association. Akeno *et al.* (31) in 2000 demonstrated in an animal (mouse) model that the administration of dexamethasone increased the expression of the enzyme vitamin D-24-hydroxylase in mouse renal cells which resulted in increased degradation of vitamin D metabolites 25[OH]D and 1,25[OH]₂D.

1.12 The rationale for the current study

COPD secondary to smoking and biomass fuel exposure is a common medical diagnosis at Chris Hani Baragwanath Academic Hospital (CHBAH). This tertiary facility services a large catchment population including the largest township in South Africa, Soweto, and thus patients may be a fairly representative sample of the South African population demographics. There was scope for a study to explore vitamin D status in COPD patients and its clinical correlates at CHBAH in Johannesburg, where seasonal sunlight exposure is more consistent, and to determine if vitamin D status findings conform to those reported in the developed world. This study may serve as an epidemiological precursor to further intervention studies involving vitamin D supplementation in COPD patients in Johannesburg.

CHAPTER 2

METHODOLOGY

2.1 Objectives

The primary objective was to determine the prevalence of vitamin D deficiency ($25 [\text{OH}]\text{D} \leq 20 \text{ ng/ml}$) and insufficiency ($25[\text{OH}]\text{D} = 21\text{-}29 \text{ ng/ml}$). Secondary objectives included to investigate for an association between vitamin D and demographic/lifestyle factors; lung function parameters; markers of COPD severity and corticosteroid type and dosage.

2.2 Study design

This was a prospective, cross-sectional study of COPD patients.

2.3 Study population

Patients with spirometry-confirmed COPD were included, as per GOLD 2019 guidelines (31). For inclusion, participants had to have a post-bronchodilator FEV_1/FVC of less than 70% combined with at least one of the classical symptoms of COPD – chronic cough, dyspnoea or chronic sputum production. Spirometry was the most recent lung function in the patient's file or repeated on the date of the interview if none could be traced in patient records. Patients had to be over 18 years of age. Patients were excluded if had reversible airflow limitation, current active pulmonary tuberculosis; concomitant diagnosis of asthma; active malignancy; malabsorption; history of pancreatic insufficiency or if already on vitamin D supplementation.

2.4 Study setting

The study was conducted at a tertiary academic hospital in Johannesburg. The hospital serves as a major referral centre in the area. Patients were recruited from the outpatient clinic and inpatient wards. The majority of patients were recruited from a specialist respiratory clinic. Johannesburg is at a latitude of 26.2°S of the equator.

2.5 Sample size

Based on previous studies, normal vitamin D levels among COPD patients range from 7% upwards. Using an estimate of 7%, a precision of 5% and a confidence level of 95%, a sample size of 100 was initially aimed for. Unfortunately, due to COVID-19 spirometry limitations

and COVID-19 clinic patient number curtailments a sample size of 76 patients was reached. Consecutive patients between November 2020 and July 2022 were recruited.

2.6 Data collection

Data were collected by the investigators using a data sheet. Spirometry was performed if none was available in the records. Pulmonary functions were performed using a JAEGER © Vyntus SPIRO PC spirometer with a calculation of % predicted FVC and FEV₁ values according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations. A 400 µg dose of salbutamol was administered for post-bronchodilator spirometry. Patient demographic/ lifestyle factors; clinical and COPD severity markers; spirometry findings, laboratory data and treatment information were collected.

A 3-5 ml cuffed venous sample of blood was taken in an acid citrate dextrose tube from participants. Samples were transported under cold chain conditions to the laboratory.

2.7 Definitions

Ethnicity

The ethnicity was recorded as self-reported by the participant.

Season of sample collection

The date of blood sample collection/ interview was categorised into seasons. Seasons were defined as follows: summer – December to February; autumn – March to May; winter – June to August and spring – September to November.

Sunlight exposure

The sunlight exposure was self-reported by the participant and based on recall in the week preceding the interview. Categories included: 1-4 hours/week; 5-6 hours/week; 1-2 hours/day; 3-5 hours/day and ≥ 6 hours/day

Exacerbation

An exacerbation was based on patient-reported figures/ file notes and included episodes requiring oral/intravenous (IV) corticosteroids; antibiotics; casualty visit or medical

practitioner consultation; hospital admission or worsening of cough, dyspnoea, or sputum production (exceeding 2 days).

Smoking status

A patient was regarded as an ex-smoker if they quit smoking for over 3 months. Never-smoker was defined as less than 100 cigarettes consumed throughout life.

Systemic corticosteroids

Included the use of IV/ oral corticosteroids in the year preceding interview.

Low dose inhaled corticosteroids

Included low and medium dose inhaled corticosteroids (fluticasone ≤ 250 mcg/day, budesonide 160 mcg/day, beclomethasone 200-400 mcg/day)

High dose inhaled corticosteroids

Included fluticasone ≥ 500 mcg/day, budesonide 320 mcg/day, Beclomethasone ≥ 400 mcg/day

2.8 Vitamin D testing and definition

25[OH]D was measured using a double sandwich immunoassay using a chemiluminescent label at a South African National Accreditation System (ISO15189) approved laboratory. The instrument used is the Abbott © Architect. This method is traceable to the reference method, namely liquid chromatography-mass spectrometry (LC-MS) and meets the required standards for clinical testing (32). There is some variation in levels quoted for vitamin D deficiency and insufficiency in the literature. Definitions used in this study for 25[OH]D were: deficiency: ≤ 20 ng/mL; insufficiency: 21-29 ng/mL; adequate: ≥ 30 ng/ml (10). For statistical comparison 25[OH]D ≤ 20 ng/ml indicated vitamin D deficiency and non-deficiency was defined as 25[OH]D > 20 ng/ml.

2.9 Statistical analysis

All data obtained were entered onto a spreadsheet on Microsoft Excel and then entered into Statistica v13.3 (StatSoft, United States [US]). The prevalence data was provided as a percentage with 95% CI. The distribution of data was determined from histograms, the Shapiro Wilk and Lilliefors's tests. Categorical variables were presented as counts (n) and

percentages and comparisons were made in vitamin D deficient and non-deficient groups using the Chi-square test. Continuous variables were summarised as means with standard deviations for normally distributed data and medians with interquartile ranges for non-standard distributed data. Independent variables were compared in deficient vs non-deficient groups using Mann Whitney U test for independent medians and Student's T-test for independent means.

For multivariate analysis, 8 variables were used to predict the presence of vitamin D deficiency or insufficiency. There were 6 continuous variables; age in years, BMI, waist circumference, smoking pack history in years, FEV₁ in liters, MMRC dyspnea score, and two categorical variables; sunlight exposure (< 1 hour/day vs. ≥ 1 hour/day), and race (black vs. non-black). Six variables with a $p < 0.2$ on the univariate model were selected for the final multivariate model

2.10 Ethical considerations

Ethics approval was obtained by the Human Research Ethics Committee (Medical) at the University of the Witwatersrand (ethics reference number M200112) before the study commencement. Written informed consent was obtained from each participant before recruitment. National Health Research Database submission has been completed and is pending approval (reference [GP 202210 034](#))

CHAPTER 3

RESULTS

3.1 Sample description

A total of 80 patients met the inclusion criteria. 4 patients declined to participate in the study. 76 patients were included in our analysis. The study patient characteristics are summarised in Table 3.1. There were 55 males (72 %). The ethnicity profile consisted of black/ African ethnicity - 48 (63%); coloured (mixed race) – 17 (22%); Indian/Asian – 8 (11%); Caucasian – 3 (4%). The mean corrected calcium in our study population was 2.25 mmol/l (standard deviation [SD] 0.15) while the median alkaline phosphatase (ALP) was 92 U/l (interquartile range [IQR] 76-121). Figure 3.1 shows the sunlight exposure of study participants.

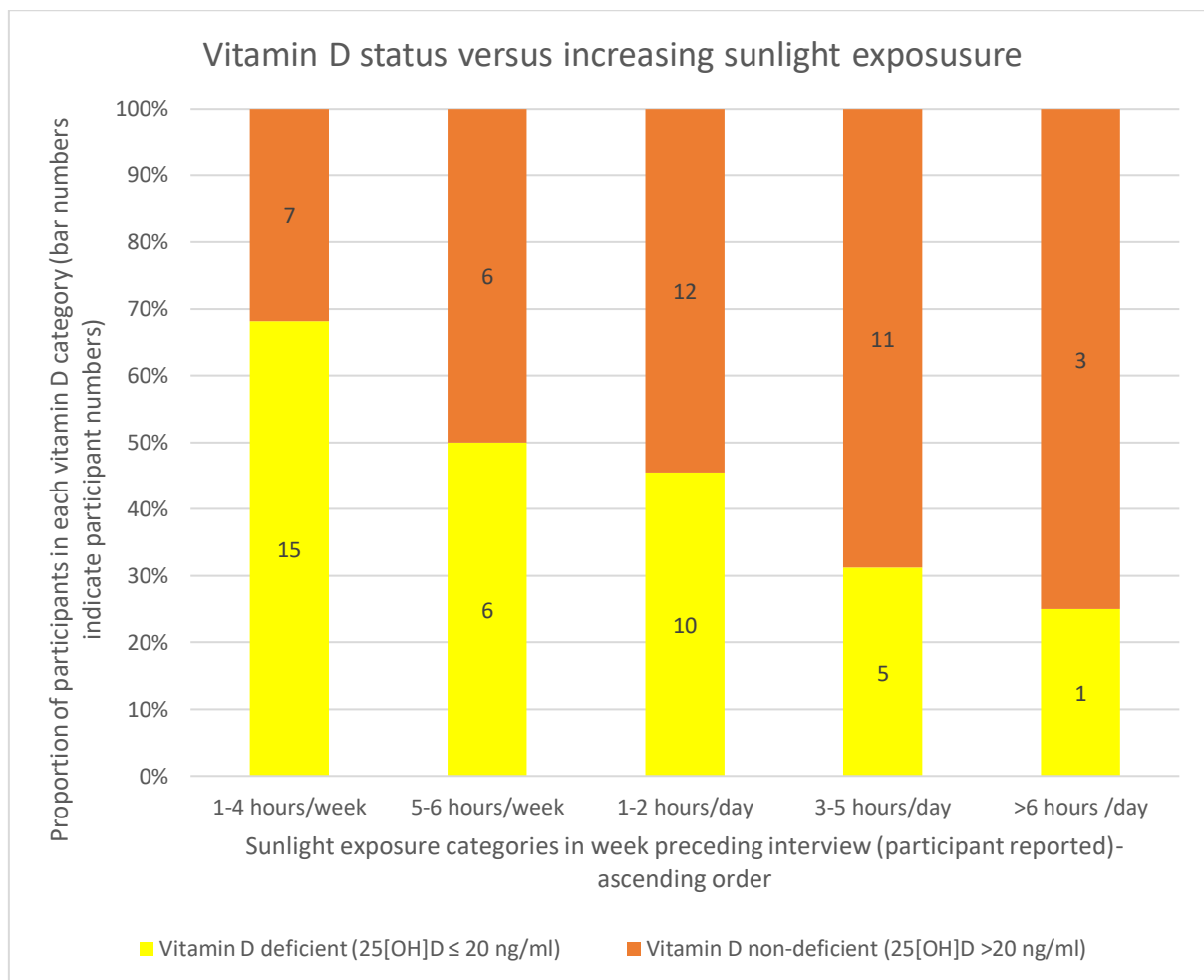


Figure 3.1: Vitamin D Status versus increasing sunlight exposure

Table 3.1: Study patient characteristics *

Variable	N = 76
Median 25[OH]D, ng/ml (IQR)	21 (14.5 – 26.5)
Mean age, years, (SD)	62 (10)
Median BMI, kg/m² (IQR)	21 (18-25)
Median waist circumference, cm (IQR)	83 (73-92)
Median smoking pack years, (IQR)	19 (5-37)
Season of blood collection, n (%)	
Summer (December to February)	24 (32)
Autumn (March to May)	30 (39)
Winter (June to August)	12 (16)
Spring (September to December)	10 (13)
Lung function parameters, median (IQR)	
FEV ₁ (l)	1.1 (0.8 - 1.5)
FEV ₁ (% predicted)	41.9 (31.1 - 65.2)
FVC (% predicted)	86.1 (59.9 - 99.9)
FEV ₁ /FVC	44.6 (35.6 -55.5)
Clinical characteristics	
Number of exacerbations last year, median (IQR)	2 (2-3)
MMRC dyspnoea scale, n (%)	
MMRC 0	1 (1)
MMRC 1	16 (21)
MMRC 2	39 (51)
MMRC 3	15(20)
MMRC 4	5 (7)
GOLD** grade, n (%)	
Grade 1	7 (9)
Grade 2	23 (30)
Grade 3	30 (39)
Grade 4	16 (21)
GOLD** group, n (%)	
Group A	7 (9)
Group B	33 (43)
Group C	9 (12)
Group D	27 (36)

*- data summarised as numbers (n) and %, for normally distributed data - mean and standard deviation (SD), for non-normally distributed data median and interquartile range (IQR); ** GOLD 2019 (31)

3.2 Primary objective

The prevalence of vitamin D deficiency and insufficiency (25[OH]D <30ng/ml) was 84% (CI 80-88). Table 3.2 summarises the proportion/percentages and median vitamin D level in each category. The median 25[OH]D in our sample was 21 ng/ml (IQR 14.5 – 26.5).

Table 3.2: The prevalence of Vitamin D deficiency and insufficiency among COPD study population

Vitamin D status	Prevalence (Confidence Interval)	n/ total for the group	Median 25[OH]D, ng/ml (IQR)
Vitamin D deficiency and insufficiency (<30 ng/ml)	0.84(0.80-0.88)	64/76	18 (12.5 – 23.5)
Vitamin D deficiency (≤ 20ng/mL)	0.48(0.42-0.54)	37/76	14 (11 – 17)
Vitamin D insufficiency (21-29ng/mL)	0.35 (0.30-0.41)	27/76	25 (22-27)
Adequate Vitamin D levels (≥ 30ng/mL)	0.16 (0.12-0.20)	12/76	37.5 (31.5 – 38.5)

n – number of participants, IQR – Interquartile range

3.3 Secondary objectives

3.3.1 Univariate analysis

The differences in lifestyle/ demographic factors between vitamin D deficient and non-deficient groups are provided in Table 3.3. There was a relative risk of vitamin D deficiency for patients with daily sunlight exposure of less than 1 hour/day compared with ≥ 1 hour/day of 1.62 (CI 1.02 -2.57), Figure 3.1. There was no difference in vitamin D deficiency between black/African and non-black/ African ethnicity, $p = 0.11$. The smoking status (never, ex-smoker, current) was not compared in the deficiency versus non-deficiency as 2 groups with a similar number of participants to facilitate meaningful comparison could not be constituted. The majority of participants were ex or current smoker: 68 (89%) and only 8 (11%) had never smoked.

Table 3.4 illustrates the spirometry differences in deficiency and non-deficiency groups. There were no significant differences in spirometry parameters (FEV_1 , FEV_1 % predicted, FVC and FVC % predicted, FEV_1/FVC).

Table 3.5 summarises the severity features and therapeutic differences between the vitamin D deficient and non-deficient groups. The MMRC dyspnoea scale of ≥ 2 was associated with a relative risk of 1.34 (CI 1.05-1.7) for vitamin D deficiency when compared with an MMRC dyspnoea scale of <2 . No significant difference in the deficiency versus non-deficiency

groups was noted in the number of exacerbations in the preceding year; GOLD grade; GOLD group; use of inhaled, systemic corticosteroids in the preceding year and inhaled corticosteroid dosage.

Table 3.3: Vitamin D levels and demographic/lifestyle factors (univariate analysis) *

	Vitamin D deficiency n= 37	Vitamin D sufficiency /Insufficiency n= 39	P
Mean age (years) (SD)	64 (11)	61 (8)	0.10
Gender male, n/ total in group (%)	27/37 (73)	28/39 (72)	0.91
Median weight - kg (IQR)	58 (53-72)	58 (47 – 68)	0.44
Mean height - m (SD)	1.66 (0.08)	1.66 (0.09)	0.90
Median BMI - kg/m ² (IQR)	20 (19 -26)	21 (17 – 23)	0.56
Median waist circumference - cm (IQR)	83 (76 – 94)	83 (72 - 90)	0.44
Median smoking pack years (IQR)	24 (7.5 – 40)	17 (5 – 27)	0.13

*- data summarised as numbers (n) and %, for normally distributed data - mean and standard deviation (SD), for non-normally distributed data median and interquartile range (IQR)

Table 3.4: Vitamin D levels and lung function parameters (univariate analysis)

	Vitamin D deficiency n=37		Vitamin D sufficiency/Insufficiency n=39		
Variable	Median	IQR	Median	IQR	P
FEV ₁ (l)	0.94	0.69-1.49	1.11	0.77-1.75	0.18
FEV ₁ (% predicted)	41.20	28.50-53	42.90	32.2-70	0.22
FVC (l)	2.39	1.71-3.01	2.56	1.95-3.32	0.22
FVC (% predicted)	82	57.10-97.30	89	66.14-100.40	0.22
FEV ₁ /FVC	42	36.27-55.50	47.98	34.14-55.56	0.72

IQR – interquartile range, FEV₁ – Forced Expiratory Volume in 1st second, FVC – Forced Vital Capacity

3.3.2 Multivariate analysis

We used 8 variables to predict the presence of vitamin D deficiency or insufficiency. The choice of these variables was based on pathophysiological plausibility and prominence in the literature review regarding their effect on vitamin D levels. There were 6 continuous variables; age in years, BMI, waist circumference, smoking pack history in years, FEV₁ in liters, MMRC dyspnea score, and two categorical variables; sunlight exposure (< 1 hour/day vs. ≥ 1 hour/day), and race (black vs. non-black). Six variables with a p<0.2 on the univariate model

were selected for the final model. Only sunlight exposure was an independent predictor of vitamin D deficiency or insufficiency (Odds ratio 2.4, CI 1.3 -4.5).

Table 3.5: Vitamin D status and COPD severity markers, therapies (univariate analysis)

Vitamin D status (ng/ml)	Deficiency (≤ 20) n=37	Non-deficiency (> 20) n=39	X² test
MMRC dyspnoea scale			Relative risk of deficiency (CI)
MMRC < 2	4/17 (24%)	13/17 (76%)	
MMRC ≥ 2	33/59 (60%)	26/59 (44%)	1.34 (1.05-1.7)*
Exacerbations in last year			
≤ 1	21/48 (44%)	27/48 (56%)	P=0.26
> 1	16/28 (57%)	12/28 (43%)	X ² = 1.27
GOLD** grade			
GOLD 1	3/7 (43%)	4/7 (57%)	GOLD 1,2 vs 3,4
GOLD 2	10/23 (43%)	13/23 (57%)	p= 0.45
GOLD 3	14/30 (47%)	16/30 (53%)	X ² = 0.57
GOLD 4	10/16 (63%)	6/16 (38%)	
GOLD** group			
GOLD A	2/7 (29%)	5/7 (71%)	GOLD A,B vs C,D
GOLD B	18/33 (55%)	15/33 (45%)	p= 0.8
GOLD C	1/9 (11%)	8/9 (89%)	X ² = 0.06
GOLD D	16/27 (59%)	11/27 (40%)	
Use of inhaled corticosteroids last year			
Yes	33/65 (51%)	32/65 (49%)	p=0.38
No	4/11 (36%)	7/11 (64%)	X ² = 0.06
Use of systemic corticosteroids last year			
Yes	21/42 (50%)	21/42 (50%)	p=0.80
No	16/34 (47%)	18/34 (53%)	X ² = 0.07
Dose of inhaled corticosteroids			
Low [†]	8/13 (62%)	5/13 (38%)	p=0.39
High [‡]	25/52 (48%)	27/52 (52%)	X ² = 0.75

MMRC – Modified Medical Research Council; GOLD – Global Initiative for Chronic

Obstructive Disease; IV – intravenous; * - statistically significant; [†] - includes low and

medium dose inhaled corticosteroids (fluticasone ≤ 250 mcg/day, budesonide 160 mcg/day, beclomethasone 200-400 mcg/day); [‡] - high dose inhaled corticosteroid includes fluticasone

≥ 500 mcg/day, budesonide 320 mcg/day, beclomethasone ≥ 400 mcg/day *** GOLD 2019

(31)

CHAPTER 4

DISCUSSION

The main finding from this study is the high prevalence of vitamin D deficiency (48%) and insufficiency (35%) in this study population. In an analysis of the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) cohort (14), with a large sample size of 1609, 20.55% and 33.2% of participants were vitamin D deficient and insufficient respectively. The same definitions for vitamin D levels were used in this study. The deficiency proportion is significantly lower than in the present study. Some reasons that may account for this discrepancy in findings include a higher proportion of milder COPD phenotype patients (majority GOLD 2 participants) and majority Caucasians (lower skin melanin content) in the USA study. In addition, our sample may reflect a selection bias representing a sicker cohort of COPD patients owing to recruitment predominantly from a referral centre respiratory (specialist) clinic and a small sample size.

Kunisaki *et al.* (23) in another large USA study, found 40.4% of participants to be vitamin D deficient and 33.1% of participants to be insufficient. This is not dissimilar to our study findings, as the population matched our cohort's COPD disease severity (majority GOLD grade 3 and had a high exacerbation risk). The marginally lower deficiency prevalence may be accounted for by ethnicity (majority Caucasian participants).

Gawron *et al.* (21) in a small Polish case-control study found the highest reviewed rates of vitamin D deficiency (90.2%) in COPD patients. Controls also had similarly high vitamin D deficiency rates and the above may be accounted for by winter only, nadir vitamin D, sampling in a temperate location. These findings bring into question whether COPD patients do truly have higher vitamin D deficiency rates when contrasted with matched participants in the general population. Our study was not designed as a case-control study and hence cannot accurately elucidate this. It may however be useful to compare prevalence rates of vitamin D deficiency in a healthy cohort versus COPD patients.

Holick (33) in a review article quoted vitamin D deficiency prevalence as 40 to 100% in elderly non-institutionalised healthy people in the USA and over 50% of postmenopausal osteoporotic women (without COPD) having suboptimal ($25[\text{OH}]\text{D} < 30 \text{ ng/ml}$) vitamin D. A

large African meta-analysis (34) found a population prevalence of 59% for combined vitamin D deficiency and insufficiency, compared to 84% in our COPD study. Similar findings were noted in case-control studies (17,18,26). These general population vitamin D deficiency/insufficiency prevalence estimates appear lower than in the COPD population.

Some of the postulated mechanisms for higher vitamin D deficiency prevalence in COPD patients include a sicker phenotype hence less sunlight exposure; a poorer diet; smoking resulting in pigmentary skin changes and decreased cutaneous previtamin D₃ activation; possible increased vitamin D catabolism due to corticosteroid usage and lower BMI and hence fat/muscle stores of the vitamin (6).

Sunlight exposure was significantly associated with vitamin D deficiency in univariate analysis and remained the only independent predictor in the multivariate model. This is evident in many studies in Europe. Jolliffe *et al.* (15) in a multicentre cross-sectional study in London with 278 participants also showed that the absence of a recent sunny holiday correlated with vitamin D deficiency in a COPD cohort. Kentson *et al.* (17) in a Swedish case-control study with 38 COPD patients also found vitamin D deficiency associated with a lower Ultraviolet score (UVS). The UVS was a composite measure of seasonality and sunlight (UV) exposure.

This study found a trend toward an association between vitamin D deficiency and greater smoking pack years, lower FEV₁ and older age. Malinovski *et al.* (20), in an Italian cohort, found no association between vitamin D level and age or smoking history. A Belgian study (26) also found no age, current smoking and vitamin D association. Burkes *et al.* (14) found the inverse trend to our study, with younger age associated with vitamin D deficiency. This trend from our study may be explained physiologically as age and disease severity may limit mobility resulting in decreased outdoor sunlight exposure. Also, with aging, skin pigmentation can become darker, decreasing cutaneous vitamin D UV activation. Burkes *et al.* (14) and Persson *et al.* (18) (in multivariate analysis) also showed an association between vitamin D levels and smoking status.

The majority of reviewed studies showed an association between a lower FEV₁ and vitamin D deficiency (13–19). Three studies also showed no vitamin D FEV₁ association (12,20,21). The

relationship between FEV₁ and vitamin D deficiency in our study may not have reached significance due to disparate sampling times and a limited sample size.

Our data showed an MMRC dyspnoea scale of ≥ 2 was associated with a higher risk of vitamin D deficiency. Kentson *et al.* (17) also demonstrated the association between higher symptom scores (which include dyspnoea as a component) and vitamin D deficiency (COPD Assessment Test [CAT] and MMRC dyspnoea score if not on vitamin D supplementation). Kunisak *et al.* (23) made a similar association but with the St. George's Respiratory Questionnaire (SGRQ). Persson *et al.* (18) in a large Norwegian case-control study also found the same association between MMRC dyspnoea score and vitamin D status in univariate analysis, but not the multivariate analysis, similar to our data. Hyun *et al.* (16) in a South Korean study also found high fibrinogen and low vitamin D associated with higher MMRC dyspnoea scores. The outlier in the literature (15) found no correlation between vitamin D and SGRQ (with dyspnoea as a component).

Strengths of this study include a South African context (where sparse data exist) and a wide variety of factors investigated. Additionally, a diverse sample of COPD severity was included. Vitamin D samples were taken over multiple seasons in the study population, as a whole (on the date of interview for each patient) which may be more representative of vitamin D deficiency prevalence as opposed to vitamin D nadir (winter/ spring) only sampling. The majority of patients were recruited from an OPD setting reducing the confounding of acute illness. The same investigator conducted surveys and measured parameters, hence negating the effects of inter-investigator inconsistency/ variability.

Limitations of this study include a smaller sample size due to COVID-19 clinic number curtailments and spirometry restrictions. This is a cross-sectional study and vitamin D levels were only measured at a single time/ season in each patient. There may be seasonal variations in vitamin D levels in each patient. No inferences about causality can be made as vitamin D deficient subjects were not followed up prospectively. No analysis of the comorbidities (including Human Immunodeficiency Virus [HIV]) of participants was done owing to the small sample size and this could be a confounder. A single-centre tertiary hospital study may limit the transferability of findings to different COPD populations.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

Conclusion

A high prevalence of vitamin D deficiency and insufficiency exists within this COPD study population. A higher MMRC score was associated with an increased risk of vitamin D deficiency while sunlight exposure was the only independent predictor of vitamin D deficiency.

Recommendations

There is scope for case-control studies to evaluate vitamin D deficiency prevalence among healthy/hospitalised African participants compared with their COPD counterparts. Given the high prevalence of vitamin D deficiency in COPD patients, routine testing among high risk groups may be valuable. These findings need to be validated in a milder phenotype COPD population. Future studies should focus on the clinical impact of vitamin D replacement among deficient patients.

CHAPTER 6

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CHAPTER 7

APPENDICES

Appendix 1 – Research protocol

PROTOCOL INTRODUCTION

Epidemiology

Globally more than 170 million people are affected by Chronic Obstructive Pulmonary Disease (COPD) and COPD accounted for approximately 3.2 million deaths in 2015 (1). Low and middle-income countries bear a significant burden of COPD mortality. Limited data on the epidemiology of COPD in Africa is available. The BOLD study (2) found a prevalence exceeding 20% in Cape Town. It should be noted that the demographics of Cape Town differ significantly from the remainder of South Africa and it would be inaccurate to infer that these estimates represent a national prevalence.

There is an increasing interest in lifestyle and dietary factors that may impact disease severity in the COPD population. There have been a large number of studies investigating the role of Vitamin D deficiency in COPD and its association with markers of disease severity. From the review of the available literature, there is a paucity of data available from South Africa or Africa in general concerning this subject. George et al (3) in a healthy cohort in Johannesburg found a prevalence of vitamin D < 30 nmol/l ranging between 5.1% (in black patients) to 28.6 % (in Asian/ Indian patients) emphasising the impact of race/ethnicity on vitamin D level.

Vitamin D - role in calcium and phosphate homeostasis

Vitamin D₃ is predominantly synthesised in the skin. Ultraviolet (UVB) radiation converts previtamin D₃ to vitamin D₃ (cholecalciferol). This step is influenced by melanin content in the skin and sunlight exposure. The other less significant source of vitamin D is dietary intake. Ergocalciferol (in plant material) and vitamin D₃ (from animal sources or fortification) can be absorbed from the gut and incorporated into chylomicrons and transported into the lymphatics and then on to blood. This vitamin D₃ is transported bound to vitamin D Binding Proteins (VBPs) in the blood to the liver where it is hydroxylated to 25 hydroxy-vitamin D (25 [OH] D). This is the major storage form and is used to ascertain

vitamin D status in populations. Vitamin D then undergoes further hydroxylation in the kidney and other tissues to become 1,25-dihydroxy vitamin D ($1,25\text{ [OH]}_2\text{ D}$). This is the active form of vitamin D and interacts with vitamin D receptors (VDRs). Vitamin D enhances gastrointestinal absorption of calcium and phosphate and also results in osteoclastic bone resorption (4).

Non-calcaemic vitamin D effects and role in COPD

Vitamin D exerts pleiotropic effects unrelated to its role in calcium and phosphate homeostasis. It is now evident that vitamin D plays a significant immunomodulatory effect. Antigen presenting cells can be inhibited by vitamin D. Vitamin D inhibits the innate and adaptive immune response through VDRs on inflammatory cells. Vitamin D deficiency fails to restrain macrophage and dendritic cell maturation through major histocompatibility class II restriction. Vitamin D also plays an important function in T cell differentiation. Vitamin D inhibits the Th1 type response and its associated cytokines i.e. interleukin (IL)-2, Granulocyte Monocyte Colony Stimulating Factor (GM-CSF) and interferon γ (IFN γ) in favour of Th2 type and regulatory T cell (T reg) responses. Thus, vitamin D deficiency leads to dysregulation of the immune and inflammatory response resulting in chronic lung inflammation and structural lung changes promoting the onset and progression of COPD (4).

Vitamin D also enhances macrophage production of antimicrobial peptides like cathelicidin which is an important component of the immune response to combat mycobacterial infections (4). Vitamin D deficiency results in increased respiratory infections (including mycobacterial tuberculosis infection) and airway microbe colonisation leading to COPD progression.

Vitamin D deficiency also results in increased airway remodelling, fibroblast proliferation (and hence collagen synthesis) and alteration in gene expression resulting in smooth muscle proliferation and contraction promoting the airway changes characteristic of COPD. The immune dysregulation also explains the association between vitamin D deficiency and autoimmune disease.

Vitamin D and other respiratory illnesses

Vitamin D levels have shown an association with certain respiratory illnesses. Kamphuis et al (5) in a retrospective review in the Netherlands found a negative correlation between vitamin D levels and sarcoid disease activity. In a multicentre case-control study (6) including low and middle-income countries investigating antiretroviral therapies (ART) among majority black participants, vitamin D deficiency at ART initiation was associated with a higher incidence of tuberculosis (TB) at 96 week follow up. A meta-analysis (7) demonstrated the beneficial effect of vitamin D supplementation in reducing the incidence of acute respiratory tract infections. A greater beneficial effect of the supplementation was seen in the subgroup of vitamin D deficient patients ($25[\text{OH}]\text{D} < 25 \text{ nmol/l}$).

Definitions of vitamin D deficiency and vitamin D normal values

There is some heterogeneity in the values quoted for vitamin D deficiency. This may arise from the fact that vitamin D levels required for the calcaemic effects (prevention of rickets and osteomalacia) are better established by robust data than for levels necessary to maintain non-calcaemic effects of vitamin D, as well as variation in assays used to ascertain levels. The Endocrinology Society (8) defines vitamin D deficiency as $25[\text{OH}]\text{D}$ levels less than 50 nmol/l , and vitamin D insufficiency as levels between 52.5 and 72.5 nmol/l . The Institute of Medicine (IOM) (9) recommended that a level of $25[\text{OH}]\text{D}$ exceeding 50 nmol/l as adequate.

Vitamin D and association with airflow parameters

Hansen et al (10) found an association between vitamin D level and Forced Expiratory Volume in 1 second [FEV_1] (especially in the vitamin D deficient subgroup) in third-generation and offspring of the original Framingham Heart Study participants. Importantly this study looked at healthy individuals (not those with COPD). Other limitations included a single vitamin D measurement and no data collected on vitamin D supplementation.

A post hoc cross-sectional review (11) of the Baltimore Longitudinal Study of Ageing failed to demonstrate an association between vitamin D deficiency and airflow limitation. An important limitation of this study is that airflow limitation was not objectively assessed but based on self-reported patient diagnoses. Airflow limitation encompassed COPD, non-smoker

COPD and asthma.

Vitamin D deficiency prevalence and associations in COPD patients

A Bulgarian study (12) looked at vitamin D deficiency in COPD patients admitted to the hospital. Owing to the study design this study had patients with more severe disease – Global Initiative on COPD (GOLD) class C and D. This study demonstrated a high prevalence of vitamin D insufficiency - <50 nmol/l (83.6%) which is significantly higher than other studies where participants were not experiencing an exacerbation (31-77% prevalence). There existed a correlation between vitamin D and Quality of Life (QOL) indices (e.g. Modified Medical Research Council [MMRC] dyspnoea scale) as well as certain pulmonary function parameters (Forced Vital Capacity [FVC], FEV_1 , Forced Expiratory Volume in 6 seconds [FEV_6]) but not the FEV_1/FVC ratio. No correlation existed between vitamin D level and comorbidities (Diabetes Mellitus, hypertension, metabolic syndrome and the number of exacerbations). Vitamin D deficiency was associated with a longer hospital stay.

Kentson et al (13) in a single centre case-control study found no difference in the prevalence of vitamin D deficiency in long term oxygen therapy (LTOT) COPD patients versus non-LTOT users. Since this study wanted to stratify patients based on oxygen therapy a sicker cohort was selected. The sample included majority Caucasians. Peak annual vitamin D levels were measured in summer and early autumn as seasonal variation in Sweden may be significant. Lower 25 [OH] vitamin D levels were found in the COPD group compared with controls. 25[OH]D levels did correlate with disease severity indices i.e. FEV_1 % predicted, MMRC score and oxygen saturation. Other lifestyle factors such as a Mediterranean diet and omega-3 consumption correlated with vitamin D levels. The study designed a useful tool to quantify UV exposure (UV score) which incorporated the quarterly average UV index and sunlight exposure hours into a single value. Sunlight exposure time categories used in this study may be useful to employ in my proposed study.

A Korean prospective study (14) demonstrated a correlation between 25[OH]D levels and FEV_1/FVC in COPD. In keeping with other studies (15), vitamin D deficiency was also associated with lower FEV_1 and FEV_1 % predicted. Vitamin D deficiency was also associated with reduced 6-minute walk distance.

Changhwan et al (16) assessed the decline in 6-minute walk tests annually in COPD patients and a decline exceeding 17 m/year was defined as rapid exercise decline. An association was established between low vitamin D levels and rapid exercise decline especially in the severe vitamin D deficient group ($<25\text{nmol/l}$). Important study limitations include that majority of subjects had some degree of vitamin D deficiency. The study was retrospective and there was no documentation of environmental exposure (e.g. use of vitamin D supplements and sunlight exposure) and this could skew the data.

Joliffe et al (15) in an extensive multicentre cross-sectional study in the UK explored if an association between various lifestyle factors and COPD severity markers correlated with vitamin D levels. Lifestyle factors associated with vitamin D deficiency included high Body Mass Index (BMI), lower socio-economic status, lack of vitamin D supplementation, winter sampling and absence of a summer holiday. No role of vitamin D pathway genetic polymorphisms or inhaled corticosteroid dose and vitamin D deficiency was demonstrated.

A small Turkish group (17) demonstrated a high prevalence of vitamin D deficiency (60%) in COPD patients. Lung function parameters (FEV_1/FVC ; FEV_1 ; FVC and total lung capacity [TLC]) were lower in COPD vitamin D deficient patients. Hand grip and isokinetic knee strength were lower in the vitamin D deficient subgroup.

Vitamin D and COPD acute exacerbation/ hospitalisation rates

Malinovschi et al (18) in a small retrospective case study found no significant association between vitamin D status and FEV_1 decline as well as other lung function parameters. Notably they measured vitamin D levels at a nadir (winter) and had a very high prevalence of vitamin D deficient subjects. A negative correlation was demonstrated between vitamin D levels and acute exacerbation and hospital admission rates. The researchers did concede the relationship did not necessarily imply causality. The investigators measured vitamin D levels at a time point and then retrospectively reviewed the medical records of participants for the preceding year concerning exacerbations and admissions. Therefore, a conclusion cannot be drawn that low vitamin D levels led to more exacerbations as the exacerbations were not recorded prospectively from the time of vitamin D level measurement. The PRECOVID randomised control trial (RCT) (19), currently underway, might provide insight into vitamin

D supplementation's effect on acute exacerbations.

Zhu et al (20) in a meta-analysis of 21 studies showed that COPD patients (especially the severe COPD subgroup) had lower vitamin D levels than control patients. Lower vitamin D levels were also associated with an increased risk of COPD and markers of disease severity but not associated with increased exacerbations. In addition, lower vitamin D levels were found in exacerbation patients when compared with stable COPD patients. Individual study results had significant disparity in findings which was partly due to the vitamin D assays employed.

Mechanisms postulated to explain COPD vitamin D associations

Zhu et al(20) provided plausible mechanisms for some of the associations. Lower vitamin D levels in the COPD group may have been accounted for by increased illness and hence indoor hours (poorer functional status) and therefore reduced sunlight exposure. Additional postulated mechanisms that could be contributing to lower vitamin D levels in COPD include increased age and smoking reducing the vitamin D activation in the dermis; enhanced vitamin D catabolism owing to corticosteroid usage and reduced storage capacity of vitamin D in COPD patients. Lower latitudes tended to have lower vitamin D levels but there was heterogeneity in this finding, contrary to what might be expected from sunlight exposure. This may be explained by the fact that many developed countries at higher latitudes fortify food with vitamin D; in addition sunlight hours (based on latitude) may only be a significant contributory factor during the winter season at higher latitudes.

The exact mechanism responsible for glucocorticosteroid administration resulting in vitamin D deficiency has not been precisely elucidated. Skversky et al (21) provide a convincing argument for the role of glucocorticosteroids resulting in increased vitamin D catabolism accounting for the association. Akeno et al (22) in 2000 demonstrated in an animal (mouse) study that the administration of dexamethasone increased expression of the enzyme vitamin D-24-hydroxylase in mouse renal cells which resulted in increased degradation of vitamin D metabolites 25[OH]D and 1,25[OH]₂D.

Vitamin D supplementation in COPD

Martineau et al (23) did a double-blinded RCT with the intervention being vitamin D supplementation in COPD. Vitamin D supplementation had no effect on time to first moderate or severe exacerbation or upper respiratory tract infection. However, in the vitamin D deficient subgroup (<50nmol/l) there was an improvement in time to the first moderate/severe exacerbation with supplementation.

An Iranian RCT (24) found that supplementation with vitamin D led to a marked reduction in exacerbation rates and significant FEV₁ improvement. Limitations of this study include that only severe and very severe COPD patients were selected, which may reflect selection bias. This may make findings less applicable to the general COPD population. In addition, no baseline vitamin D levels were measured in intervention or control groups but authors note a high prevalence of vitamin D deficiency in Iran.

A meta-analysis (25) found that moderate to severe exacerbations were reduced in patients supplemented with vitamin D where baseline vitamin D was < 25nmol/l. The meta-analysis was based on 4 trials where raw data was re-analysed where available and queries were submitted to the primary author for clarification. One trial had no data set available. The small sample size of this component study would unlikely have a major impact on results.

Vitamin D and COPD prognosis

Few studies involving COPD prognosis in relation to vitamin D could be identified. A longitudinal study (26) based on long term follow up of patients with either defined clinical or spirometric COPD was conducted. It demonstrated that COPD with reduced vitamin D levels had an increased risk of all-cause mortality. There was the suggestion made that this may be attributable to respiratory and other cause mortality (although this was not statistically significant). Limitations of this study include the use of prebronchodilator spirometric measures which do not conform to international COPD definition as well as delay in processing vitamin D specimens.

Conclusion

From the current literature search, it is evident that vitamin D deficiency is more prevalent in the COPD population. There exists some association with vitamin D level and lung function parameters (FEV_1 % predicted, FEV_1/FVC ratio) as well as lifestyle factors e.g. (sunlight exposure and dietary intake) in COPD. Some of these associations can be explained by the physiology and immunomodulatory role of vitamin D. There is inconsistency in the data on the association of vitamin D deficiency and exacerbation rates in COPD. The majority of published reports are from developed countries with significant seasonal sunlight exposure variation. There were no available studies on COPD and vitamin D status in South Africa or Africa.

COPD secondary to smoking and biomass fuel exposure is a common medical diagnosis at Chris Hani Baragwanath Academic Hospital (CHBAH). This tertiary facility services a large catchment population including the largest township in South Africa, Soweto, and thus patients may be a fairly representative sample of the South African population demographics. There is scope for a study to explore vitamin D status in COPD patients at CHBAH in Johannesburg, where seasonal sunlight exposure is more consistent, and to determine if vitamin D status findings conform to those reported in the developed world. This study may serve as an epidemiological precursor to further intervention studies involving vitamin D supplementation in COPD patients in Johannesburg.

STUDY OBJECTIVES

The primary objective of this study is

1. To determine the prevalence of vitamin D insufficiency (25-62.5 nmol/l) and deficiency (<25 nmol/l) in the COPD population at CHBAH

Secondary objectives include:

1. To determine if vitamin D levels have an association with demographic/lifestyle factors: age, gender, ethnicity, body mass index (BMI), waist circumference, smoking status, smoking pack year history and sunlight exposure in COPD patients
2. To determine the association between vitamin D levels and lung function parameters (FEV_1 , FVC, FEV_1 % predicted, FEV_1/FVC) in COPD patients.
3. To determine if any association exists between vitamin D levels and markers of

COPD severity (MMRC dyspnoea scale, number of exacerbations in the last year, GOLD grade or GOLD group)

4. To determine if an association exists between vitamin D level and inhaled corticosteroid dosage (low/medium, and high) or oral/intravenous corticosteroid usage in COPD patients in the preceding year

METHODS

Study design

Prospective cross-sectional, descriptive study

Study population

Inclusion criteria:

1. Patients that have spirometric confirmed COPD will be included, as per GOLD 2019 guidelines. Patients who have a post-bronchodilator FEV₁/FVC less than 70% combined with at least one of the classical symptoms of COPD – chronic cough, dyspnoea or chronic sputum production.
2. Patients will have to be over 18 years of age.

Exclusion criteria:

1. Patients with current active pulmonary tuberculosis or a concomitant diagnosis of asthma or active malignancy
2. Patients with malabsorption or a history of pancreatic insufficiency or disease
3. Patients already on vitamin D supplementation

Study setting

The study will be conducted at Chris Hani Baragwanath Academic Hospital respiratory outpatient department (Respiratory OPD), a tertiary hospital in Johannesburg part of the University of the Witwatersrand academic circuit. Patients may also be recruited from the

Medical outpatient department (MOPD). If the sample size is difficult to reach from these locations additional patients will be recruited from the medical wards. Baragwanth Hospital is found at a latitude of 26.2 °S of the equator. Convenience sampling will be used.

Sample size

Based on previous studies, normal vitamin D levels among COPD patients range from 7% upwards. Using an estimate of 7%, a precision of 5% and a confidence level of 95%, a sample size of 100 is required.

Data collection

Patients will sign informed consent (Appendix 2) after being given a study information leaflet (Appendix 3) and verbal information regarding the study. Patients will have the choice to join the study. Data will be collected from the managing clinician or investigators at the listed locations using a datasheet (Appendix 4). An identifier list will issue a random study number which will be linked to the patient's hospital number, cellular number and name. This study number will appear on the data sheet and no personal identity information of the patient will be included on the sheet. The identifier list will only be accessible to the principal investigator and supervisors. Spirometry will be traced in the patient records and if not yet done will be done on the date of the visit. The machine used is JAEGER © Vyntus SPIRO PC spirometer (CareFusion GmbH, Hoechberg, Germany) with the calculation of % predicted FVC and FEV₁ values according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations. A 400 µg dose of salbutamol will be administered for post-bronchodilatory spirometry.

Data collected in the datasheet will include the date; managing clinician name and cellular number (to convey vitamin D deficiency results); study number; age; gender; ethnicity (self-reported by the patient); BMI; waist circumference; smoking status; smoking pack year history; average sunlight exposure in the preceding week (as per patient recall); lung function parameters(as per patient file or new spirometry) such as FEV₁, FEV₁% predicted, FEV₁/FVC; MMRC dyspnoea scale (as reported by the patient); GOLD grade and GOLD group and number of exacerbations in the last year. An exacerbation will be based on patient-reported

figures and will include episodes requiring oral/intravenous (IV) corticosteroids; antibiotics; casualty visit or medical practitioner consultation; hospital admission or worsening of cough, dyspnoea, or sputum production (exceeding 2 days). A patient will be regarded as an ex-smoker if quit smoking for over 3 months. Never smoker will be defined as less than 100 cigarettes consumed throughout life. Data about inhaled /IV or oral corticosteroid treatment usage in the preceding year will be obtained based on patient recollection or file data where available.

A 3-5 ml cuffed venous sample of blood will be taken from the cubital fossa by the clinician/ investigator or phlebotomist in an acid citrate dextrose (ACD)/ yellow top tube. The sample will be accompanied by a requisition form and stored in the respiratory OPD fridge at a temperature of 4-8 °C. When convenient for the investigator, with a delay not exceeding 1 month, samples will be taken to Global Labs offices in Johannesburg.

Global labs measure 25[OH]D using a double sandwich immunoassay using a chemiluminescent label. The instrument used is the Abbott © Architect.

The reference range used by the laboratory is as follows:

1. Severe deficiency- <10 ng/ml (< 25 nmol/l)
2. Mild to moderate deficiency- 10-24 ng/ml (25-60 nmol/l)
3. Normal population- 25-80 ng/ml (62.5-200 nmol/l)

Laboratory testing is ISO 15189 compliant.

Vitamin D levels will be obtained from Global Labs and added to the datasheets for relevant patients. If the patient is found to be vitamin D deficient, the managing clinician will be telephoned and informed of the result. A recommendation will be made that the patient be initiated on vitamin D 50 000 IU orally weekly, as recommended CHBAH endocrinology department. If the managing clinician is not reachable telephonically, the patient will be informed telephonically of the result and sent a text message with patient details and the vitamin D result and the vitamin D supplementation recommendation, and a follow up

consultation will be arranged at the patient's clinic (MOPD or ROPD). The patient will be requested to show the text message to the clinician.

Where available, on NHLS Labtrack the most recent (if done within last 3 months) calcium and alkaline phosphatase (ALP) will be obtained and added to the datasheet. If additional funding is procured a calcium and ALP level may be added to the blood request form.

STATISTICAL ANALYSIS

Once data is obtained it will be entered into an Excel spreadsheet, and it will be anonymised. From there it will be entered into a statistical program- Statistica. The patient demographics will be summarised using descriptive statistics. For dependent variables that are normally distributed means and standard deviations will be obtained. For comparisons between normally distributed variables comparing means between 2 groups students t-test will be employed. For comparing means for more than 2 groups ANOVA test will be used. If data is not normally distributed then the Mann-Whitney or Kruskal-Wallis test will be used. For correlations between continuous variables (e.g. FEV₁ and vitamin D) regression modelling will be used and the Pearson correlation coefficient calculated. For statistical purposes, 95% confidence interval with $p < 0.05$ will be considered significant.

ETHICS

The study participants will sign informed consent (Appendix 2) after being granted study information pamphlets (Appendix 3) and in addition have a full explanation of the study role, risks and benefits. They will sign an informed consent form and joining the study will be completely voluntary. Ethics application will be done through the Human Research Ethics Committee. Written consent will also be obtained from the Head of Department (HOD) of Baragwanath Hospital and the HOD of pulmonology. An identifier list will issue a random study number which will be linked to the patient's hospital number, cellular number and name. This study number will appear on the datasheet and no personal identity information of the patient will be included on the sheet. The identifier list will only be accessible to the principal investigator and supervisors, thus maintaining anonymity. All data will only be accessible to the principal investigator and supervisors and kept on a password-protected personal laptop computer. In the instance that the patient is vitamin D deficient, the managing clinician or patient will be informed and supplementation recommended (as elaborated on in

the data analysis section).

TIMING

	J U N 19	J U L	A U G	S E P	O C T	N O V	D E C	J A N 20	F E B	M A R	A P R	M A Y	J U N	J U L	A U G	S E P	O C T	N O V	D E C	J A N 21
Literature review																				
Preparing Protocol																				
Protocol Assessment																				
Ethics Application																				
Funding approval																				
Collecting Data																				
Data Analysis																				
Writing up – Thesis																				
Writing up – Paper																				

FUNDING

Basic project budget

Item	Cost per item	Number of items	Total Cost
Vitamin D test	R130	100	R13 000
VAT on test	R19.50	100	R1 950
Printing and paper costs			R400
Transport			R600
			R 15 950

Consumables such as needles, syringes, specimen bags, webcols and gloves will be provided by Global Laboratories complimentary and included in the per specimen test fee.

Funding will be applied for from the Wits Pulmonology fund or postgraduate funding initiatives. If funding is delayed or difficult to secure Dr M and Mrs Z Kola have given a generous offer to sponsor the research project. The printing and transport costs will be financed by the investigator.

LIMITATIONS

1. It is often time the case that patients seen in general medical wards with COPD are followed

up at MOPD or discharged to local clinics if uncomplicated and hence Respiratory OPD patients may represent a sicker cohort.

2. If sampling is done from the medical wards this may imply that the patient is admitted with an acute exacerbation which may impact on the vitamin D level. All attempts will be made to obtain patients from the respiratory OPD/MOPD except if the time allocated to data collection is expiring and the sample size is not reached.
3. Private funding can be problematic and delay study but a generous offer has been made by private donors if research funding through Wits cannot be secured.
4. Vitamin D levels will be sampled through several seasons and hence not necessarily represent annual peak /trough or average. However, compared to centres with significant seasonal sunshine hour variability where most current data is sourced from, Johannesburg has consistent sunlight throughout the year and hence seasonal variation will be less important.
5. No data regarding vitamin D supplementation doses and comorbidities will be obtained.
6. No calcium and PTH levels will be measured with vitamin D levels and these could impact vitamin D levels. Where available, the most recent calcium and ALP levels will be traced on NHLS Labtrack and added to the datasheet, but may not be available for all patients. If additional funding is available calcium and ALP may be added to the blood request form.
7. Another problem may be the delay in vitamin D sample processing but from a review of available data 25[OH]D is stable for a prolonged period if refrigerated.

LIST OF ABBREVIATIONS (USED IN PROTOCOL)

1,25[OH]₂D – 1.25-dihydroxy vitamin D
25[OH]D – 25 hydroxy-vitamin D
ALP – Alkaline Phosphatase
ANOVA – Analysis of Variance
ART – Antiretroviral Therapy
CHBAH – Chris Hani Baragwanath Academic Hospital
COPD – Chronic Obstructive Pulmonary Disease
FEV₁ – Forced Expiratory Volume in 1 Second
FEV₆ – Forced Expiratory Volume in 6 Seconds
FVC – Forced Vital Capacity
GMCSF –Granulocyte Monocyte Colony Stimulating Factor
GOLD – Global Initiative on COPD
IFN γ – Interferon γ
IL – Interleukin
IOM – Institute of Medicine
IV – Intravenous
LTOT – Long Term Oxygen Therapy
MMRC – Modified Medical Research Council
MOPD – Medical Outpatient Department
OPD – Outpatient Department
PTH – Parathyroid Hormone
QOL – Quality of Life
RCT – Randomised Control Trial
ROPD – Respiratory Outpatient Department
T reg – regulatory T cell
TB – tuberculosis
TLC – Total Lung Capacity
UV – Ultraviolet
VDBP – Vitamin D Binding Proteins
VDR – Vitamin D Receptor

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Appendix 2 – Patient informed consent form

Informed consent

Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital	
Principal investigator:	I Kola
Principal investigator's title:	Dr
Telephone number of principal investigator:	011 933 8000 0727399437
Supervisors:	Dr S.A. Van Blydenstein Prof S. Omar

Informed consent

I hereby confirm that I have been informed by the investigator/ managing clinician, Dr

.....

about the nature, benefits and risks of the study. I have also received, read and understood the above information (patient information leaflet) about this study

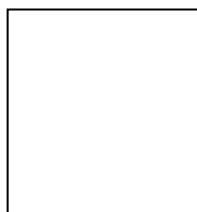
I am aware that the results of the trial including personal details regarding my sex, age, initials will be anonymously processed into a study report.

I may at any stage without prejudice withdraw my consent and participation from this study. I have had sufficient opportunity to voice my queries and, of my own free will, declare myself prepared to participate in the study.

Patient's name.....

Patient's signature.....

If patient illiterate, thumbprint or mark



I, Dr..... hereby confirm that the above patient has been fully informed about the nature, conduct and risks of study.

Investigator's name.....

Investigator's signature.....

Date.....

Witness's name.....

Witness's signature.....

Date:.....

Appendix 3 – Patient information leaflet

Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital
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Principal investigator:	I Kola
Principal investigator's title:	Dr
Telephone number of principal investigator:	011 933 8000 0727399437
Supervisors:	Dr S.A. Van Blydenstein Prof S. Omar

Good day

You are invited to participate in a research study being done at Chris Hani Baragwanath Hospital by myself, Dr I Kola and my supervisors.

You will find a brief summary of the research study below. You should take time to read the summary and voice any questions. You should not feel in any way pressured to take part in the project. You can if you like, discuss with family, friends before agreeing to take part in the project. This process is termed 'informed consent'.

You are in no way or form forced to join the study. If you opt to not participate, your medical management will not be impacted at all. You can at any time choose to leave the study without any disclosing of reason.

There will be no direct benefit to you if you choose to participate in the study. You will be asked a series of questions that will not take more than 5 minutes and require you to take a blood sample.

Your confidentiality will be strictly maintained during the study. Your name, hospital number and cellular number will be linked to a study number on an identifier list. This identifier list will only be available to the principal investigator and his supervisors and kept on a password protected computer. Hence your data sheet will have no identifiable data.

Why is this study being done?

Chronic lung disease, often as a result of smoking (COPD) is common in South Africa and likely to be increasing. There are studies from other countries showing that vitamin D (from food and sunlight) may be lower in COPD patients and giving them extra vitamin D may help their condition

I would like to find out what the vitamin D levels are in COPD patients are and if this has any link to your medication, disease severity, lung function tests.

I, Dr I Kola am conducting the study with the help of my supervisors. The results of the study may be published in academic journal or presented at a conference. The study will help to find out what vitamin D levels are in COPD patients and how to better take care of COPD patients in future.

Why I am being asked to join study?

You are included in the study because your symptoms and lung tests demonstrate that you have this chronic lung disease called COPD.

How will the study be done?

The study will only start once ethical approval has been obtained. Once this has been obtained COPD patients at respiratory clinic, medical clinic or medical wards will complete a questionnaire with the help of your treating doctor/ the investigator. A random study number linked to your name, hospital number and cell number will be issued on an identifier list. Thus the data sheet will have no personal identity data. If you have not already had lung function tests, these will be done. These would have to be done even if you choose not to participate in the study. After this is done you will be requested to have a blood sample taken for a vitamin D level. The study will not require you to make additional visits and not impact your standard clinic care in any way. Your information will remain completely confidential. If you are found to have low vitamin D your managing doctor will be informed and vitamin D supplementation recommended.

What will the benefit be?

If your vitamin D level is low, you will find this out and likely get supplemented. Additional questions may be asked by your doctor allowing him/her to see your COPD fully and its effects on your life and thus improve care. The long term benefits of the study may improve care of COPD patients, like you in the future.

What is the cost or risk?

There will be no financial cost to you. The study will pay for the blood test. Most of the information in the form will be sourced from your file although you may be asked a few additional questions prolonging consultation time slightly. You may need to have lung function test if not yet done. These would be necessary even if you don't take part in the study. You will have to feel a small amount of pain having a blood sample drawn.

Is the study private/ confidential?

Once you choose to participate in the study, a random study number will be allocated to you based on your name, hospital number and cell number on an identifier list. This identifier list

will only be accessible to principal investigators and supervisors. The data collection sheet will have none of your identity information on it. The results of the study may be published in medical journals or presented at conferences. In these papers no information regarding patient identity will be used. You are free to request a final report once study is done to confirm that strict privacy/ confidentiality is maintained.

Where can I get additional information from?
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If you have any further questions regarding the study or would like to opt out you can contact the investigator Dr I Kola on 0727399437 or email: imraankola@gmail.com

Appendix 4 – Data collection sheet

Kindly issue study number and fill out identifier list then complete below questions (except laboratory results). Also ensure patient has blood sample taken_for vitamin D levels and consents to study. Appendices are attached for all scoring systems

Date:					
Managing clinician:					
Clinician cell number:					
Patient details					
1. Study number:					
2. Age:					
3. Gender:	Male			Female	
4. Ethnicity:	Black	White	Coloured	Indian	Other
5. Weight (kg)					
6. Height (m)					
7. BMI (weight/height ²)					
Lifestyle factors					
1. Smoking status	Never	Ex smoker	Current		
2. Smoking pack years (no. Cigarettes on a day / 20 * smoking years)					
3. Sunlight exposure (recall for last week)	1-4h/ week	5-6h/ week	1-2h/ day	3-5h/ day	≥6h/ day
Lung function parameters – if not done please do FLOOP					
1. FEV1					
2. FVC					
3. FEV1 % predicted					
4. FEV1/ FVC (absolute value)					
Treatment					
Use of inhaled corticosteroids in prior 1 year	Yes			No	
Oral or IV corticosteroids prior 1 year	Yes			No	
Dose of inhaled corticosteroid					

COPD severity					
MMRC dyspnoea scale – appendix 5	0	1	2	3	4
No. Of exacerbations per year	0	1	2	≥3	
GOLD grade (based FEV1% predicted) – Appendix 6	1	2	3	4	
Gold group – Appendix 7	A	B	C	D	
Laboratory results (not for completion at Respiratory OPD/MOPD)					
25[OH]D (ng/ml)		Ca (mmol/l)		ALP (U/l)	

Appendix 5 – MMRC dyspnoea scale

▶ MODIFIED MRC DYSPNEA SCALE ^a		
PLEASE TICK IN THE BOX THAT APPLIES TO YOU ONE BOX ONLY Grades 0 - 4		
mMRC Grade 0.	I only get breathless with strenuous exercise.	☐
mMRC Grade 1.	I get short of breath when hurrying on the level or walking up a slight hill.	☐
mMRC Grade 2.	I walk slower than people of the same age on the level because of breathlessness, or I have to stop for breath when walking on my own pace on the level.	☐
mMRC Grade 3.	I stop for breath after walking about 100 meters or after a few minutes on the level.	☐
mMRC Grade 4.	I am too breathless to leave the house or I am breathless when dressing or undressing.	☐

https://goldcopd.org/wp-content/uploads/2018/11/GOLD-2019-POCKET-GUIDE-FINAL_WMS.pdf

Appendix 6 – Gold grade of COPD based on FEV1 % predicted

Classification of COPD by impairment of lung function

	COPD stage	Spirometry (postbronchodilator)
GOLD 1	Mild	FEV ₁ ≥80% predicted FEV ₁ /FVC <0.7
GOLD 2	Moderate	50% ≤ FEV ₁ < 80% predicted FEV ₁ /FVC <0.7
GOLD 3	Severe	30% ≤ FEV ₁ < 50% predicted FEV ₁ /FVC <0.7
GOLD 4	Very severe	FEV ₁ <30% predicted FEV ₁ /FVC <0.7

FEV₁: Forced expiratory volume in 1 second; **FVC:** Forced vital capacity; **GOLD:** Global Initiative for Chronic Obstructive Lung Disease

Classification of COPD severity should be undertaken with care in patients with comorbid diseases or other possible contributors to shortness of breath

Postbronchodilator forced expiratory volume in 1 s (FEV₁) to forced vital capacity (FVC) ratio less than 0.7 is required for the diagnosis of COPD to be established

Reference: Modified from GOLD Global strategies for the diagnosis, management, and prevention of chronic obstructive pulmonary disease updated 2014

Appendix 7 – Gold groups based on exacerbation and symptom scores

Exacerbation history	Symptoms	
	mMRC 0–1 CAT <10	mMRC ≥2 CAT ≥10
≥2 or ≥1 leading to hospital admission	C	D
0 or 1 (not leading to hospital admission)	A	B

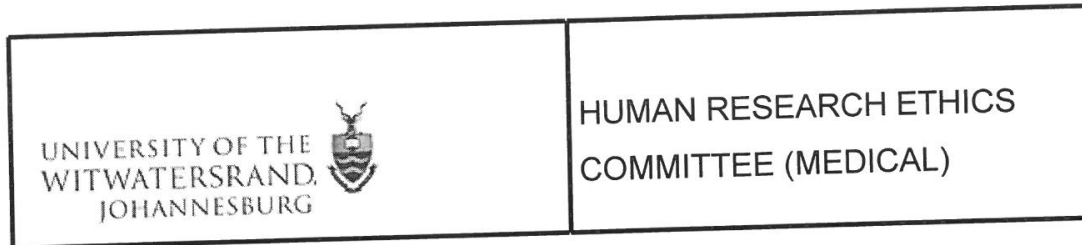
GOLD group A: Low symptom severity, low exacerbation risk.

GOLD group B: High symptom severity, low exacerbation risk.

GOLD group C: Low symptom severity, high exacerbation risk.

GOLD group D: High symptom severity, high exacerbation risk

Appendix 8 – Ethics clearance certificate



Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr I Kola
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine
Chris Hani Baragwanath Academic Hospital

E-mail: imraankola@gmail.com

CC: Supervisor: Dr S van Blydenstein and Professor S Omar
<savanblydenstein@gmail.com>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2020/03/26

REF: R14/49

PROTOCOL NO: M200112 (This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)

PROJECT TITLE: *Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.



MSWorks2000/Iain0007/Clearscan.wps

R14/49 Dr I Kola

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M200112

NAME: Dr I Kola
(Principal Investigator)
DEPARTMENT: School of Clinical Medicine
Department of Medicine
Division of Internal Medicine
Chris Hani Baragwanath Academic Hospital

PROJECT TITLE: Vitamin D status in COPD patients at Chris Hani
Baragwanath Hospital

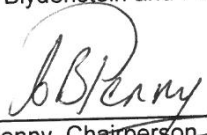
DATE CONSIDERED: 2020/01/31

DECISION: Approved unconditionally

CONDITIONS:

SUPERVISOR: Dr S van Blydenstein and Professor S Omar

APPROVED BY:


Dr CB Penny, Chairperson, HREC (Medical)

DATE OF APPROVAL: 2020/03/26

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

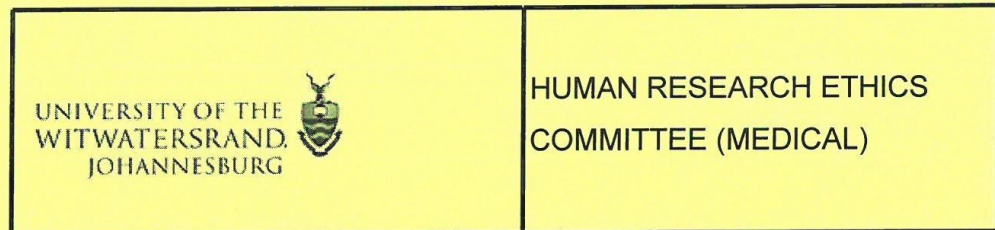
To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the 3rd Floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to submit details to the Committee. I **agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **January** and will therefore reports and re-certification will be due early in the month of **January** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

PLEASE QUOTE THE CLEARANCE CERTIFICATE NUMBER IN ALL ENQUIRIES

Appendix 9 – Ethics approval of covid-19 modifications



2020/08/31

Dr I Kola
School of Clinical Medicine
Department of Medicine
Division of Internal Medicine
Chris Hani Baragwanath Academic Hospital

Sent by e-mail to: imraankola@gmail.com

Dear Dr Kola

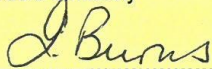
Re: Protocol Ref No: M200112
Protocol Title: *Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital*
Principal Investigator: Dr I Kola

I refer to a letter of 2020/05/11 from Professor Wong, forwarded to us by your M Med supervisor Dr van Blydenstein.

I confirm that we have noted and approve of the suggestion that the staff of the Respiratory Outpatient Clinic should assist you in the recruitment of patients and the taking of blood samples, to minimise contact and the possible spread of COVID-19.

Thank you for keeping us informed.

Yours Sincerely


.....
Mr I Burns
For the Human Research Ethics Committee (Medical)


.....
Dr CB Penny, Chairperson, Human Research Ethics Committee (Medical)

Appendix 10 – CHBAH Medical Advisory Committee permission letter



GAUTENG PROVINCE

HEALTH
REPUBLIC OF SOUTH AFRICA

MEDICAL ADVISORY COMMITTEE

CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL

PERMISSION TO CONDUCT RESEARCH

Date: 28th November 2019

TITLE OF PROJECT:

Vitamin D status in COPD patients at Chris Hani Baragwanath Academic Hospital.

UNIVERSITY: University of the Witwatersrand

Principal Investigator: Dr I Kola

Department: Internal Medicine

Supervisor : Dr SA van Blydenstein

Permission Head Department (where research conducted): Yes

The Medical Advisory Committee recommends that the said research be conducted at Chris Hani Baragwanath Academic Hospital. The CEO / management of Chris Hani Baragwanath Academic Hospital is accordingly informed and the study is subject to:-

- **Permission having been granted by the Committee for Research on Human Subjects of the University of the Witwatersrand.**
- The Hospital will not incur extra costs as a result of the research being conducted on its patients within the hospital
- The MAC will be informed of any serious adverse events as soon as they occur
- Permission is granted for the duration of the Ethics Committee Approval.


.....
Recommended
(On behalf of the MAC)
Date: 28/11/2019


.....
Approved/Not Approved
Hospital Management
Date: 29/11/2019

Appendix 11 – CHBAH Internal medicine Head of Department (HOD) permission letter



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Chris Hani Baragwanath Academic Hospital
Department of Medicine
P.O. Bertsham
2013
Tel: +27 11 933 2040
Fax: +27 11 938 1454

4 May 2015

Dr Tsitsi
Head of Department of Internal Medicine
Chris Hani Baragwanath Academic Hospital

Dear Dr Tsitsi

re: PERMISSION FOR DR I KOLA TO CONDUCT A MMED STUDY ENTITLED "VITAMIN D STATUS IN COPD PATIENTS AT CHRIS HANI BARAGWANATH ACADEMIC HOSPITAL "

As the principal investigator, I would like to request your permission and the permission of the CHBAH authorities to conduct a MMed study: Vitamin D Status in COPD Patients at Chris Hani Baragwanath Academic Hospital.

Permission will also be obtained for the University of the Witwatersrand Ethics Committee.

Thanking you in anticipation.

Yours sincerely

DR. SA VAN BLYDENSTEIN

Pulmonology Fellow

Chris Hani Baragwanath Academic Hospital

Signature of approval by Dr Tsitsi

Date 05/may 2019

Appendix 12 – CHBAH Respiratory HOD permission letter

Date: 06 October 2019

Permission to conduct research at Chris Hani Baragwanath Academic Hospital (CHBAH), Respiratory Unit

Title: Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital

Investigators: Dr Imraan Kola; Dr SA van Blydenstein; Prof S Omar

Permission is hereby granted.

The respiratory HOD understands the following:

Prerequisite approval from both University (Wits) Ethics Committee (HREC) and
Hospital Chris Hani Baragwanath Academic Hospital

The study is anonymous – families and patients cannot be linked to data after
collection.

The hospital is not liable for any costs related to the study

The study will not disadvantage any Chris Hani Baragwanath patient/staff member.


Professor M Wong

Head of Respiratory Unit

Appendix 13 – Plagiarism/ Turnitin report



GAUTENG PROVINCE
HEALTH
REPUBLIC OF SOUTH AFRICA

Chris Hani Baragwanath Academic Hospital
Department of Medicine
P.O. Bertsham
2013
Tel: +27 11 933 8940
Fax: +27 86 553 3527
savanblydenstein@gmail.com

16/05/2023

Re: TurnItIn Similarity Report for MMED Student Imraan Kola, 362488

MMED: Vitamin D status in COPD patients at Chris Hani Baragwanath Hospital

To whom it may concern

The above student has completed her MMED and is submitting it for examination.

We have noted that he has run it through TurnItIn, and it has an 29% similarity index. The similarities identified are related to medical definitions found in multiple sources, and from standard layout. There is no evidence of plagiarism. This letter serves to confirm that we have reviewed his MMED with the TurnItIn report and approve for submission.

Sincerely

Dr Alex van Blydenstein
Supervisor
Specialist Physician, Pulmonologist
Chris Hani Baragwanath Hospital
savanblydenstein@gmail.com

Prof Shahed Omar
Supervisor
Critical Care Specialist, Intensivist
Chris Hani Baragwanath Academic Hospital
Shahed.omar@wits.ac.za

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