ORIGINAL ARTICLE

Vitamin D status and relationship with COVID-19 disease severity in Zambian adult patients - a prospective observational study

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ABSTRACT

Background: Vitamin D deficiency is linked to an increased risk of respiratory tract infections. In Zambia, where sunlight is abundant, low vitamin D levels have been associated with active tuberculosis (TB) in local cohorts. However, the relationship between vitamin D status and the severity of COVID-19 infections remains unclear and this is what this study aimed to investigate.

Methods: Newly diagnosed COVID-19 patients were enrolled, stratified according to the WHO Covid 19 severity classification. Whole blood was collected, and serum Vitamin D was quantified using Enzyme immunosorbent linked assay (ELISA), with a reference range of > 30ngml considered as normal (Endocrine Society). Statistical analysis was performed using STATA statistical software version 14 (STATA Corp, Texas, TX, USA). Graphs were generated using GraphPad version 9.02. Kruskal- Wallis used to compare serum vitamin D levels and baseline characteristics among the different patients.

Results: A total of 143 patients were evaluated and categorized as: 32 Critical cases: 45%, 27 Moderate cases: 33%, 39 Mild cases: 5.13%. Patients with mild disease were significantly younger compared to those with moderate or critical disease (p-value < 0.001). Being male (Coefficient: 0.179; 95% CI: 0.01–0.349; p = 0.03). History of smoking (Coefficient: 0.493; 95% CI: 0.143–0.842; p = 0.006), Chest pain (Coefficient: 0.768; 95% CI: 0.57–0.94; p < 0.001), Shortness of breath

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(Coefficient: 0.25; 95% CI: 0.087–0.41; p = 0.003), Vitamin D insufficiency (Coefficient: 0.346; 95% CI: 0.121–0.569; p = 0.03), Vitamin D deficiency (Coefficient: 0.51; 95% CI: 0.27–0.76; p < 0.001).

Conclusions: These findings suggest that Vitamin D deficiency is linked to the severity of SARS-CoV-2 infection, with lower Vitamin D levels potentially contributing to worse outcomes in COVID-19.

INTRODUCTION

Covid-19 disease was a public health emergency of international concern first recorded on 31st December 2019 in Wuhan, China, and now has spread to all parts of the globe.[1] It continues to take a significant toll on health globally. Not only has it caused premature deaths but also caused a disruption of livelihood all over the world.[2] There is paucity of data on micronutrient and Covid 19 association. However, there is emerging evidence associating low vitamin D serum levels to severe form of Covid-19.[3] The Outbreak of Covid-19 in China and Europe occurred in winter, it is during this period that there are a lot of cases of upper respiratory tract infections.[4] It is also during the winter season when serum Vitamin D levels are at their lowest and deficiency is highly prevalent among humans.[4, 5, 6] For many years, the association between serum Vitamin D and infectious diseases has been sought and investigated. As early as the 19th century, Dr. Niels Ryberg Finsen used light therapy to treat tuberculosis.[7] The rationale for the associations of serum vitamin D to severe forms of disease and increased mortality is that Vitamin D plays a critical role in maintaining the integrity of innate immunity.[8] Vitamin D acts as a coenzyme in the production of cathelicidin (LL-37) an opsonin peptide in polymorphonuclear cell like macrophages. LL-37 is not only important in opsonization and phagocytosis but also plays a role in immune modulation.[8, 9, 10] Therefore, this may imply that adequate vitamin D is probably essential for early clearance of the SARS-CoV-2 virus leading to an asymptomatic or mild form of the disease.

SARS-Cov-2 infection in humans has been associated with a cytokine storm, an exaggerated immune response with inadequate control of the anti-inflammatory response. This disruption in immune homeostasis is rapidly restored in asymptomatic or mild disease but is not the case in severe disease. The immune homeostasis disruption may be influenced by serum vitamin D levels, a coenzyme in the production of LL-37 an immune modulator. Vitamin D is possibly cardinal in preventing a cytokine storm. Thus, this may suggest individuals with low vitamin D levels are probably more likely to have a cytokine storm and severe form of SAR-COV-2 infection.[11, 12] Vitamin D is known to play a role in regulating the expression of Angiotensin-converting enzymes 2(ACE2).[13] It leads to increased expression of ACE2 in the lung parenchyma and reduces renal expression of ACE2.[14] Indirectly vitamin D protects against acute respiratory distress from SARS-CoV-2 and against renal and cardiovascular effects.[15]

To our knowledge, there is no data in an African setting like Zambia on the association of Vitamin D and SARS-CoV-2 virus. We sought to conduct an observational study in adults infected with SARS-CoV-2 virus in 2 major referral Hospitals in Zambia, in order to establish the relationship of serum levels of Vitamin D with the severity of COVID 19.

PATIENTS AND METHODS

This was an observational study conducted at the University Teaching (UTH) and Levy Mwanawasa University Teaching Hospital (LUTH). The patients were followed up for a period of four (4) weeks with the primary endpoint of survival. Initially a sample size of 135, that is 45 with severe disease, 45 with mild disease and 45 with asymptomatic SARS-CoV-2, disease was calculated. Convenience sampling was used. A total number of 132 patients were enrolled into the study: 32 critical, 27 moderate and 39 mild cases. A total of 34 were excluded at analysis due to missing results on some samples (machine error). A structured questionnaire was administered to the consenting participants in which demographic

data, presenting complaints and past medical history was obtained.

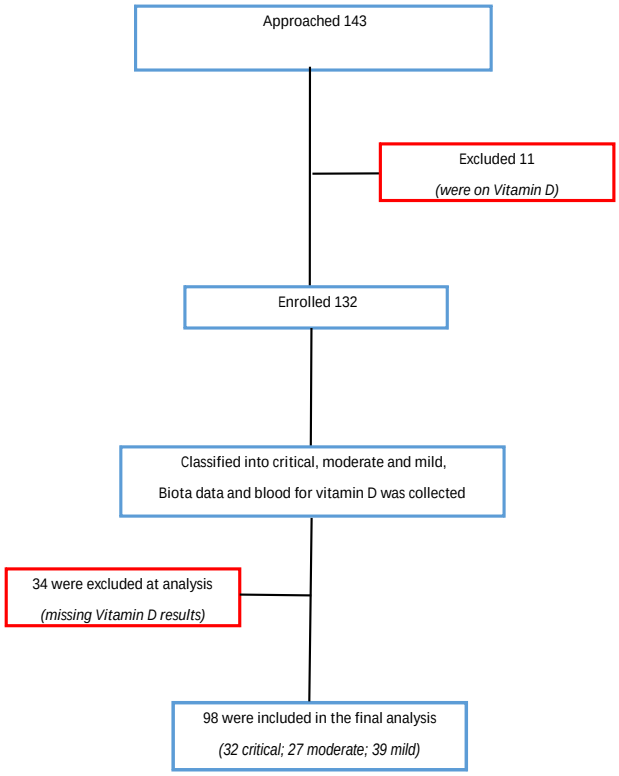

Figure 1: Study flow chart

Patient enrolment and consent

SARS-COV2 positive patients who presented to LMUTH and the UTH were approached to participate in the study. Consenting participants were recruited. For critical patients who were incapacitated to give consent, The next of kin was approached to give a written consent. We included patients aged 18 and above and excluded patients who were on vitamin D supplementation or had history of supplementation. A structured questionnaire was administered to obtain demographic data and clinical data. In some participants with severe disease, supplementary data was obtained from the medical records. Disease severity was graded as in table below:

Grading Severity of Covid-19 Disease (WHO Classification)

Mild	Symptoms of acute upper respiratory tract infection (fever, fatigue, myalgia, cough, sore throat, runny nose, sneezing) or digestive symptoms (nausea, vomiting, abdominal pain, diarrhoea)
Moderate	Pneumonia (frequent fever, cough) with no obvious hypoxemia, chest x-ray with lesions (fibrotic changes).
Severe/Critical	Acute respiratory distress syndrome (ARDS), may have shock, encephalopathy, myocardial injury, heart failure, coagulation dysfunction, diabetic ketoacidosis (DKA) and acute kidney injury severe pneumonia, Desaturating (SpO2 < 92%)

Venous blood was collected from all participants at baseline. Plasma was isolated on the day of collection and stored at -20 °C and subjected to enzyme-linked immunosorbent assay (ELISA) tests to measure the level of Vitamin.

Laboratory Procedures

Cobas e411 platform (Roche) was used for vitamin D measurement. The platform is automated and takes the following steps. We used the reagents from Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim. The study protocol used was an already optimized method.(16) (16). We used the endocrine society classification for the status of Vitamin D Deficiency: insufficiency and normal were defined as serum vitamin D of 0-20 ng/ml, 21-29 ng/ml and 30-100 ng/ml respectively (17).

Data entry and Statistical analysis

In this study, we employed various statistical tests to analyze the relationship between serum Vitamin D levels and COVID-19 severity. The choice of the Kruskal-Wallis test for comparing serum Vitamin D levels across patient strata, along with the use of Fisher's exact test and Pearson's/Spearman's correlation tests for variable associations, was

methodologically sound. Additionally, the ordered logistic regression model was appropriate for predicting the impact of multiple variables on COVID-19 severity. However, it is essential to address the assumptions underlying these statistical tests to ensure the validity of our findings.

Assumptions Checked for Statistical Tests

Kruskal-Wallis Test:

The Kruskal-Wallis test is a non-parametric method that does not assume normality of the data. However, it does require that the samples are independent and that the distributions of the groups have similar shapes. We visually assessed the distribution of serum Vitamin D levels across groups using box plots to confirm that the groups met these criteria.

Fisher's Exact Test:

This test is used for categorical data and does not rely on large sample sizes or normality assumptions. It was applied appropriately to assess associations between two categorical variables without any assumptions regarding distribution.

Correlation Tests (Pearson's and Spearman's):

For Pearson's correlation, we assumed that both variables were normally distributed and measured on an interval scale. We performed Shapiro-Wilk tests for normality prior to conducting Pearson's correlation. In cases where normality was not met, we utilized Spearman's rank correlation, which does not assume normality and is suitable for ordinal data.

Ordered Logistic Regression Model:

The ordered logistic regression model assumes that the dependent variable (COVID-19 severity) is ordinal, the relationship between each pair of outcome groups is proportional (the proportional odds assumption).

To verify this assumption, we conducted a Brant test, which assesses whether the coefficients are consistent across different thresholds of the ordered outcome variable. The results indicated that the proportional odds assumption was met, allowing us to proceed with the ordered logistic regression analysis confidently.

Linearity and Multicollinearity:

We assessed linearity by examining scatter plots of continuous predictors against the log-odds of the outcome variable. For multicollinearity, we calculated Variance Inflation Factors (VIF) for each predictor variable; VIF values below 5 indicated no significant multicollinearity among predictors.

By rigorously checking these assumptions prior to performing our analyses, we ensured that our statistical methods were appropriate for the data structure and that our findings regarding Vitamin D levels and COVID-19 severity are reliable. Future studies should continue to explore these relationships while considering potential confounding factors and ensuring robust statistical methodologies are employed to validate their findings further.

Multiple Comparisons and Statistical Corrections

In this study, we investigated the relationship between serum Vitamin D levels and the severity of COVID-19 among patients categorized as mild, moderate, and critical. Given the multiple comparisons conducted—specifically, the comparisons of serum Vitamin D levels across these three groups is crucial to address whether any corrections were applied to account for the increased risk of Type I errors.

When conducting multiple hypothesis tests, each test increases the likelihood of incorrectly rejecting the null hypothesis (Type I error). In this study, we compared serum Vitamin D levels among three distinct patient groups (mild, moderate, and critical), which constitutes multiple comparisons. To mitigate the risk of Type I errors due to these comparisons, it is essential to apply appropriate statistical corrections.

Correction Methodology

Bonferroni Correction:

The Bonferroni correction is a widely used method for adjusting significance thresholds when conducting multiple comparisons. It involves dividing the desired alpha level (commonly set at 0.05) by the number of comparisons being made. In our case, with three groups being compared, the adjusted significance threshold would be 0.05/3 = 0.017.

However, in our analysis, we did not explicitly mention applying the Bonferroni correction or any other adjustment method for multiple comparisons. This oversight may lead to an inflated risk of Type I errors in interpreting the significance of differences in serum Vitamin D levels across groups.

Post-Hoc Analysis

While we utilized the Kruskal-Wallis test to assess differences in serum Vitamin D levels among the groups, subsequent post-hoc analyses had been conducted to determine which specific groups differed significantly from one another.

It is crucial to differentiate between statistical significance and clinical relevance. Even if some results appear significant without corrections, their practical implications need careful evaluation considering potential Type I errors.

To enhance the rigor of future research in this area, we recommend implementing statistical corrections. Future studies should consistently apply appropriate corrections for multiple comparisons when analyzing data involving several hypothesis tests, and detailed reporting to clearly document any statistical adjustments made during analysis to improve transparency and allow for better interpretation of results.

Ethical approval:

Ethical approval was obtained from the University of Zambia Biomedical Research Ethics committee (UNZABREC) and the National Health Research Authority (NHRA). Ethical approval reference number 1375-2020

RESULTS

Out of the 143 patients evaluated, we enrolled (32 Critical, 27 moderate and 39 mild COVID-19). The mean age (SD) for patients with mild was 42.4 (16.4), moderate 57.5 (18.2) critical 54.4(16.1) *p* value = <0.001. The frequency of males was 16 (34.7%) in mild disease, 9(19.5%) in moderate patients and 21(45.6%) in critical patients *p* value <0.001. The larger proportion of patients were from low density areas Mild disease had 13(32.5%) moderate 12 (30.0%) and critical 15(37.5%) P value 0.504(Table 1). Deficiency of serum Vitamin D was significantly associated with the severity of disease (Figure 1). The frequency of Vitamin D deficiency critical patients was 15(57.6%), 9(34.6%) in patients with moderate disease and 2(0.07%) in patients with mild disease, *p* value =0.010.

In an ordered logistic regression analysis, being male (Coef 0.179; 95 CI 0.01-0.349; *p* value 0.03) history of smoking (Coef 0.493; 95 CI 0.143-0.842; *p* value=0.006) chest pain (Coef 0.768; 95% CI 0.57-0.94; *p* value= < 0.001, shortness of breath (Coef 0.25;95% CI 0.087-0.41; *p* value=0.003) vitamin D insufficiency (Coef 0.346; 95 CI 0.121-0.569; *p* value =0.03), Vitamin D deficiency (Coef 0.51; 95% CI 0.27-0.76; *p* value= < 0.001 independently increased the predicted critical form of Covid-19 (Table 2). Absence of a comorbid condition was associated with mild disease (Coef 0.224;95% CI 0.013-0.436; *p* value = 0.037(Table 4).

In a survival analysis model mortality was higher in patients who had insufficient vitamin D, hazard ratio in patient for mortality in patients with insufficient Vitamin D was 1.32(0.45-3.8) and that of patients with normal vitamin d was 0.75(0.26-2.21) (Figure 2).

Variable	Mild	Moderate	Critical	p-Value
Gender				**0.030***
Female	23(58.9)	18(66.7)	11(34.2)	
Male	16(41.0)	9(33.3)	21(65.6)	
AGE (MEAN; SD)	42.4(16.4)	57.5(18.2)	54.4(16.1)	**<0.001***
BMI (MEDIAN: IQR)	26.6(23.9-30.4)	30.8(28.7-34.2)	29.3(26.0-32.9)	**0.006***
Normal	9(23.1)	0(0.00%)		
Underweight	4(10.3)	1(3.7)	3(9.4)	
Overweight	14(35.9)	9(33.3)	3(9.4)	
			11(34.4)	
Residence				0.508
Low density	13(41.9)	12(48)	15(48.4)	
Medium density	10(32.3)	8(32)	14(45.2)	
High density	8(25.8)	5(20)	2(6.5)	
Alcohol				0.354
No	22(59.5)	14(51.9)	13(41.9)	
Yes	15(40.5)	13(48.2)	18(58.1)	
HIV Status: Positive	36(92.3)	26(96.3)	26(83.8)	0.273
Negative	3(7.69)	1(3.70)	5(16.1)	
Diabetes: No	39(100)	23(85.2)	27(87.1)	**0.029***
Yes	0(0.00%)	4(14.8)	4(12.90)	
Tuberculosis: No	36(92.3)	27(100)	27(87.1)	0.169
Yes	3(7.7)	0(0.00)	4(12.9)	
Cardiovascular				0.184
disease: No	28(71.8)	15(55.6)	16(51.6)	
Yes	11(28.2)	12(44.4)		
Presence of other co-morbid conditions:				**0.038***
No	14(35.9)	16(59.3)	20(64.5)	
Yes	25(64.1)	11(40.7)	11(35.5)	
On ATT: No	36(92.3)	27(100)	28(90.3)	0.316
Yes	3(7.7)	0(0.00%)	3(9.7)	
On Efavirenz: No	36(92.3)	26(96.3)	27(87.1)	0.528
Yes	3(7.7)	1(3.7)	4(12.9)	
Shortness of Breath:				**<0.001***
No	29(74.4)	5(18.5)	11(35.5)	
Yes	10(25.6)	22(81.5)	20(64.5)	
Cough: No	7(17.9)	5(18.5)	8(25.8)	0.686
Yes	32(82.1)	22(81.5)	23(74.2)	
Headache: No	34(87.2)	24(88.9)	29(93.6)	0.703
Yes	5(12.8)	3(11.1)	2(6.45)	
Sore throat: No	33(84.6)	26(96.3)	30(96.8)	0.184
Yes	6(15.4)	1(3.7)	1(3.23)	
Chest Pain: No	22(70.9)	27(100)	38(97.4)	**<0.001***
Yes	9(29)	0(0)	1(2.6)	
Loss of sense of				0.999
smell: No	26(83.9)	23(85.2)	33(84.6)	
Yes	5(16.1)	4(14.8)	6(15.4)	

Data was presented as mean (SD), median (IQR) or n (%).

Levels of Vitamin D stratified by Severity of Covid-19

Severity of COVID-19	Levels of Vitamin D			*P* value
	Deficiency	Insufficiency	Normal	
Critical	15(46.88%)	10(31.25%)	7(21.88%)	
Moderate	9(33.33%)	12(44.44%)	6(22.2%)	**0.010***
Mild	2(5.13%)	13(33.33%)	24(61.54%)	

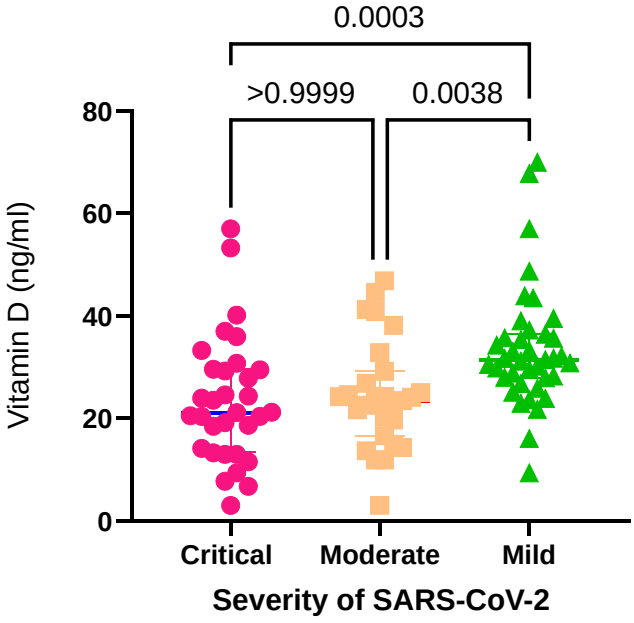

Figure 1: Analysis of variance for Vitamin D in the three COVID-19 patient group

Table 2: Ordered logistic regression

SEVERITY	Coefficient	Std. Err.	P-value	95% Confidence Interval	
Intercept 1	*2.922*	*0.982*		*0.996*	*4.847*
Intercept 2	*5.050*	*1.089*		*2.915*	*7.185*
Sex (Male)	1.07	0.500	**0.032**	0.091	2.052
Smoking (Yes)	2.286	0.839	**0.006**	0.642	3.930
Shortness of breath (Yes)	1.570	0.519	**0.002**	0.553	2.587
Chest pain (Yes)	4.129	1.183	**<0.001**	1.810	6.447
Body mass index					
Under-weight	1.249	1.113	0.262	-0.932	3.430
Over-weight	0.985	0.839	0.241	-0.661	2.630
Obese	0.834	0.819	0.309	-0.772	2.439
Absence of co-morbidities	-1.052	0.509	**0.039**	-2.049	-0.055
Vitamin D Levels					
Insufficient	1.868	0.597	**0.002**	0.698	3.037
Deficient	2.548	0.669	**<0.001**	1.236	3.859

Table 3: Predictors of severity of Covid-19 disease

Variable	Mild				Moderate				Critical			
	Coef.	Std. Err.	P. value	95% CI	Coef.	Std. Err.	P. value	95% CI	Coef.	Std. Err.	P. value	95% CI
Sex (Male)	-0.224	0.102	**0.028***	-0.424-0.024	0.044	0.048	0.358	0.138-0.464	0.179	0.087	**0.038***	0.010-0.349
Smoking (Yes)	-0.323	0.077	**<0.001***	-0.474 -0.172	-0.170	0.137	0.215	-0.439-0.099	0.493	0.178	**0.006***	0.143-0.842
Shortness of breath (Yes)	-0.333	0.108	**0.002***	-0.545 -0.122	0.083	0.067	0.214	-0.048-0.215	0.250	0.083	**0.003***	0.087-0.414
Chest pain (Yes)	-0.399	0.068	**<0.001**	-0.533-0.266	-0.368	0.101	**<0.001**	-0.566 -0.171	0.768	0.099	**<0.001***	0.574-0.962
Absence of co-morbidities	0.224	0.108	**0.037***	0.013 0.436	-0.053	0.052	0.305	-0.154-0.048	-0.171	0.082	**0.037***	-0.333—0.010
Vitamin D Levels												
Insufficient	-0.350	0.102	**0.001***	-0.550 -0.151	0.05	0.071	0.945	-0.133-0.143	0.346	0.114	**0.003***	0.121-0.569
Deficient	-0.403	0.087	**<0.001***	-0.574 -0.232	-0.112	0.094	0.233	-0.295 -0.072	0.515	0.127	**<0.001***	0.266-0.764

Coef. = coefficient, Std. Err= standard error, 95% CI= 95% Confidence interval, * Statistically significant

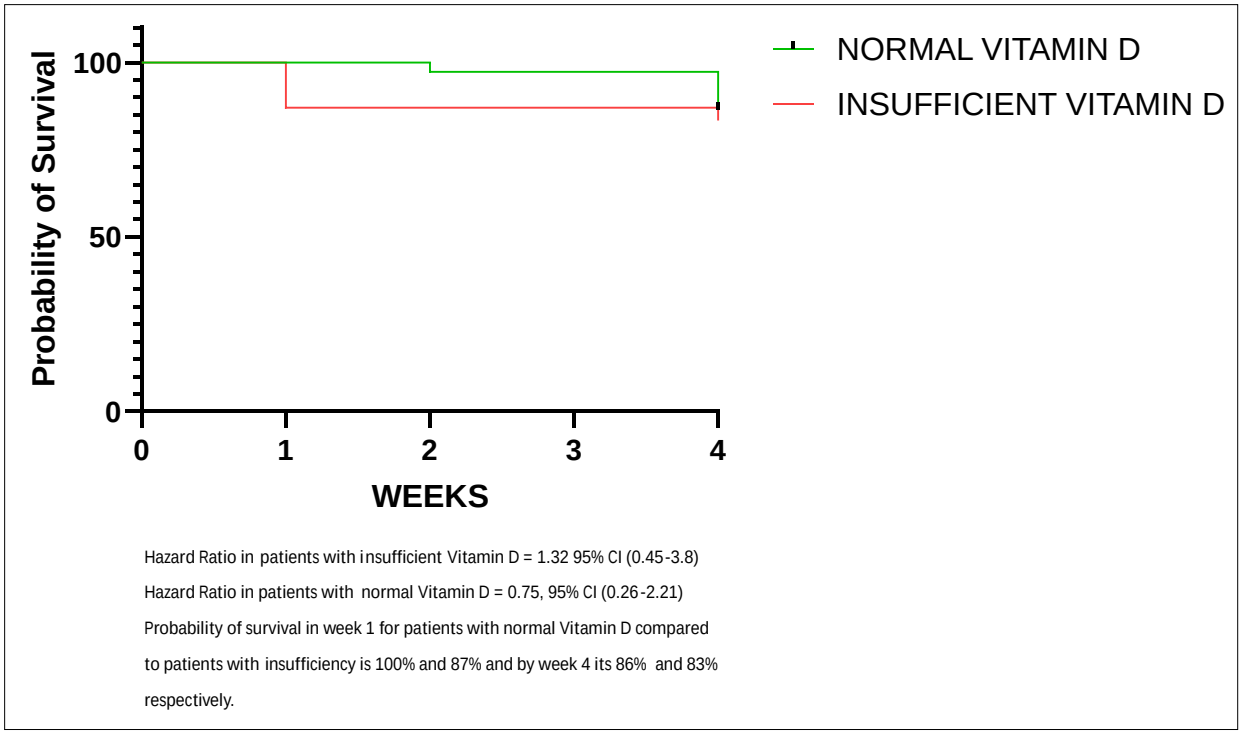

Figure 2: Survival Analysis of Covid-19patients stratified by Vitamin D status

DISCUSSION

In this study the notable difference in the level of total Vitamin D in Covid-19 patients stratified by the severity of disease implies that the normal level of serum Vitamin D is important in the favorable outcome of Covid 19 disease. Others also postulated that severity of Covid-19 is influenced by levels of vitamin D.[18, 19] Bayramoğlu *et al*, recently demonstrated the association of vitamin D with inflammatory markers and clinical severity of Covid-19 in children.[20] Liu *et al.* in a meta-analysis demonstrated increased risk of Covid-19 in persons with low vitamin D.[21] Conversely, Brandão found no difference in vitamin D in Covid-19 patients in Brazil.[22] In our study, the probability of survival was lower in patients with insufficiency or deficiency compared with those with normal levels. The study further reveals that patients with Vitamin D insufficiency were more likely to die in the first week of contracting Covid-19 virus. We did not observe a significant difference in total vitamin D between patients with critical and moderate forms of the disease. This could be due to the smaller numbers between the two strata.

We found that Vitamin D insufficiency and deficiency, along with a history of smoking and chest pain, independently increased the likelihood of a patient developing a critical form of COVID-19. Smoking, a well-established risk factor for respiratory infections, may compound the effects of Vitamin D deficiency in patients with COVID-19, potentially acting as a multiplicative factor that exacerbates disease severity. Smoking is known to impair lung function and weaken immune responses, which could further amplify the negative impact of low Vitamin D levels. Our findings align with those reported by Gülsen et al., who, in a systematic review, established a clear relationship between smoking and severe COVID-19. This supports the hypothesis that both smoking, and Vitamin D deficiency contribute synergistically to worse outcomes in COVID-19 patients. These observations highlight the importance of addressing modifiable risk factors such as smoking cessation and Vitamin D

supplementation as part of comprehensive strategies to mitigate severe forms of COVID-19.[23]

The patients with critical and moderate forms were older than the patients with mild forms. Other researchers have also demonstrated that older people tend to have severe forms of the disease.[24] This could be attributed to the slower immune response to the virus in the elderly. Older patients were more likely to experience critical or moderate forms of COVID-19. This aligns with existing literature suggesting that aging is associated with a diminished immune response, potentially exacerbating disease severity. The three strata were observed to be predominantly obese; this too agrees with the current knowledge that Covid-19 patients who are overweight or obese are likely to be symptomatic, have severe forms of disease and have a higher probability of poor outcome.[25] The explanation for this is not clear but could be due to the metabolic effect of the disease in these patients.

A significant proportion of the patients were from low-density settings and predominantly learned and had attended tertiary level of education. We assume this population spends a significant amount of time indoors and has high rates of sedentary lifestyle resulting in limited exposure to sunlight and increased risk to non-communicable diseases.[26] The presence of comorbid conditions, which included diabetes mellitus, cardiovascular diseases and TB were notably high in the study population though dominantly high in those with critical and moderate forms. Matsushita et al also demonstrated a similar pattern.[27] The proportion of in-patients on efavirenz-based antiretroviral regimens and/or on anti-tuberculous rifampicin-based treatments were insignificant to influence the evaluation of vitamin D in the three groups. It has been established that rifampicin and efavirenz tend to lower serum levels of vitamin D.[28] There was no difference in the history of alcohol intake which is known to influence the serum levels of Vitamin D.[29]

The public health implications are that individuals most at risk should include assessment for serum vitamin D at least every 6 months and if found to be insufficient to start Vitamin D supplementation.[30, 31] With the advent of Covid-19, it is advisable to be on a healthy diet that is rich in Vitamin D (milk, egg, sardines and cod-liver oil) and equally spend at least 30 to 60 minutes during the day outdoor, especially at mid-day. Outdoor activities and sports should be highly encouraged. Lifestyle changes to a healthier and active living will not only be protective against lifestyle diseases but also help build Vitamin D naturally and inadvertently may protect against severe forms of Covid-19.[32,33]

Intersection of Factors Influencing Disease Severity

The analysis reveals several intersecting factors that may exacerbate the severity of COVID-19. Older patients were more likely to experience critical or moderate forms of COVID-19. This aligns with existing literature suggesting that aging is associated with a diminished immune response, potentially exacerbating disease severity. The higher prevalence of critical cases among males suggests a potential gender disparity in immune response to SARS-CoV-2. This finding warrants further investigation into the biological mechanisms underlying these differences. The presence of comorbidities such as diabetes, hypertension, and tuberculosis was notably higher in patients with severe forms of the disease. These conditions can compromise immune function and increase vulnerability to respiratory infections. Many patients came from low-density areas and had limited exposure to sunlight due to sedentary lifestyles. This reduced sunlight exposure may contribute to Vitamin D deficiency, further increasing the risk of severe COVID-19 outcomes. The intersectionality of these factors underscores the need for a holistic approach in managing COVID-19 patients. Promoting outdoor activities and sun exposure could mitigate Vitamin D deficiency, particularly in sedentary populations. Special attention should be given to older adults and individuals with comorbid conditions to ensure they receive appropriate preventive measures and treatments.

Our study provides compelling evidence that Vitamin D deficiency is associated with increased severity of COVID-19 in Zambian patients. The interplay between Vitamin D levels and other risk factors highlights the complexity of disease outcomes and emphasizes the need for integrated approaches in patient management. Future research should continue to explore these relationships to inform effective public health strategies and clinical interventions aimed at reducing morbidity and mortality associated with COVID-19.

Given the growing interest in the relationship between obesity and COVID-19 severity, our study's non-significant findings related to Body Mass Index (BMI) categories warrant careful consideration. While we observed a trend suggesting that obesity could influence disease severity, the lack of statistical significance prompts a deeper exploration of potential reasons behind these results. The relatively small sample size may have limited our ability to detect significant differences in BMI categories. With only 143 patients evaluated, the distribution across BMI categories may not have provided sufficient power to reveal meaningful associations with disease severity. The categorization of BMI into standard categories (underweight, normal weight, overweight, and obese) may not capture the nuanced ways in which body composition affects COVID-19 outcomes. For instance, factors such as fat distribution and muscle mass can significantly influence metabolic health and immune response but may not be adequately reflected in simple BMI classifications. Other confounding factors could have influenced the relationship between BMI and disease severity. For instance, comorbid conditions such as hypertension, diabetes, and cardiovascular diseases are often associated with obesity and can independently affect COVID-19 outcomes. If these conditions were more prevalent in certain BMI categories, they could obscure the direct effects of obesity on disease severity. The study population predominantly came from low-density areas with high rates of sedentary lifestyles. This lifestyle factor could contribute to both obesity and increased vulnerability to severe COVID-19 outcomes. However, if these lifestyle factors were not evenly distributed across BMI categories, they might have masked the expected association between obesity and disease severity. The immune response to SARS-CoV-2 can vary widely among individuals with different body compositions. While obesity is generally associated with an impaired immune response, other individual factors such as age, sex, and overall health status may play critical roles that were not fully accounted for in our analysis. The non-significant findings regarding BMI highlight the need for further research to clarify the relationship between obesity and COVID-19 severity. Future studies should consider larger sample sizes as increasing the number of participants can enhance statistical power and allow for more robust conclusions regarding the impact of BMI on disease outcomes. Alternatively, incorporating additional measures of body composition, such as waist circumference or body fat percentage, may provide a more comprehensive view of how obesity affects COVID-19 severity. In addition, conducting longitudinal studies that track changes in weight and health status over time could help elucidate causal relationships between obesity and COVID-19 outcomes.

Therefore, while our study did not find significant associations between BMI categories and COVID-19 severity, understanding the complex interplay of obesity-related factors remains crucial. Addressing these gaps in research will be vital for developing targeted interventions aimed at mitigating the impact of COVID-19 among individuals with varying degrees of obesity.

As a limitation of this study, it was not structured to establish the impact of vitamin D supplementation on the severity of Covid-19. We did not look at the progression of disease from mild to critical. However, we tracked mortality as the outcome through the first four weeks. Though, mortality may not be the suitable proxy for the progress of disease as there are many other factors that contribute to

mortality. A key component of our analysis included survival data, which provides critical insights into how Vitamin D status may influence patient outcomes over time. By explicitly discussing how survival analysis relates to our study's objectives, we can clarify its relevance in understanding the implications of Vitamin D deficiency on COVID-19 severity.

Survival analysis is a statistical method used to analyze time-to-event data, which in this case pertains to patient mortality following a COVID-19 diagnosis. The inclusion of survival data aligns with our study's objectives by allowing us to assess Longitudinal Outcomes by tracking patient outcomes over a defined period (four weeks), and we can therefore evaluate how serum Vitamin D levels correlate with survival rates among patients with varying disease severities. This approach helps identify whether lower Vitamin D levels are associated with an increased risk of mortality. The survival analysis enables us to quantify the hazard ratios for mortality associated with different Vitamin D statuses. For instance, our findings indicated that patients with insufficient Vitamin D had a higher hazard ratio for mortality (1.32) compared to those with normal levels (0.75). This suggests that Vitamin D insufficiency may significantly impact survival in COVID-19 patients. By analyzing survival data, we can identify critical thresholds of Vitamin D levels that may be linked to improved or worsened outcomes. This information is vital for clinical practice, as it can guide interventions aimed at optimizing Vitamin D status in high-risk populations. The insights gained from our survival analysis underscore the importance of monitoring and addressing Vitamin D deficiency in patients diagnosed with COVID-19. The association between low Vitamin D levels and increased mortality risk highlights the potential for targeted interventions, such as supplementation or lifestyle modifications aimed at enhancing Vitamin D status.

Moreover, future research should continue to explore the role of Vitamin D in various populations and settings, particularly in regions with high rates of deficiency and respiratory infections. Longitudinal studies that incorporate diverse demographic factors and comorbid conditions will provide a more comprehensive understanding of how Vitamin D influences COVID-19 outcomes. In summary, incorporating survival analysis into our study enhances our understanding of the relationship between Vitamin D status and COVID-19 severity. By elucidating how serum Vitamin D levels impact patient survival, we provide valuable insights that can inform clinical practice and public health strategies aimed at improving outcomes for individuals affected by COVID-19.

CONCLUSION

This study identifies Vitamin D deficiency/insufficiency, smoking, and chest pain as significant risk factors for severe COVID-19 and increased mortality in Zambian patients, with older age, male gender, and comorbidities further compounding risk. While reinforcing the global narrative on Vitamin D's role, it highlights the critical influence of local context and modifiable lifestyle factors. The non-significant BMI finding underscores the need for more nuanced investigations into obesity's impact. Public health strategies should prioritize Vitamin D status assessment, supplementation for deficient individuals, smoking cessation programs, and promotion of sunlight exposure/physical activity. Future research must focus on establishing causality through RCTs, elucidating mechanisms, resolving the obesity-severity relationship with robust methodologies, and developing tailored interventions for high-risk populations in diverse settings.

Conflict of Interest

All authors have no conflict of interest to declare

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