



## Investigation of hypovitaminosis D in patients infected with SARS-CoV-2 and its relationship with clinical worsening: a cross-sectional observational retrospective clinical study

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### Abstract

Numerous studies have demonstrated the profile of infection and symptoms related to COVID-19 that occur with pulmonary manifestations (such as Acute Respiratory Syndrome), digestive symptoms, anosmia, and ageusia. Many comorbidities have been associated with deaths and severe cases of the disease, such as diabetes, hypertension, obesity, and heart disease. However, many questions remain unanswered, especially the association of disease severity with hypovitaminosis D. Vitamin D deficiency is widely found in patients in Intensive Care Units, and recent studies have shown that it has been suggested that patients with severe manifestations of COVID-19 have hypovitaminosis D. Therefore, the present study aims to associate the presence of hypovitaminosis D in patients with the acute respiratory syndrome, positive for SARS-COV-2, admitted to the José Alencar Regional Hospital in Uberaba/Brazil and the evolution of the disease (days of hospitalization, hospitalization in the ICU, discharge and death) through the analysis of vitamin D (25(OH)D) hospitalized patients clinical records. The incidence of hypovitaminosis D among the patients was also assessed. The results may contribute to the understanding of the disease, as well as the need for vitamin supplementation.

**Keywords:** SARS-CoV-2. Vitamin D. COVID-19 evolution. Hypovitaminosis.

### Introduction

In December 2019, in China, the COVID-19 pandemic began as an outbreak of severe pneumonia, and transmission of the infection has grown substantially since then [1, 2]. Numerous studies have demonstrated the profile of infection and symptoms related to the disease, which evolves with pulmonary manifestations, such as SARS, digestive, gastric, anosmia and ageusia. Many comorbidities have been associated with deaths and severe cases of the disease, such as diabetes, hypertension, obesity, and heart disease. However, many questions remain unanswered, mainly regarding the association of disease severity with intrinsic host factors, such as in patients with hypovitaminosis D [3].

In this context, hypovitaminosis D is related to changes in bone metabolism and has been associated with respiratory diseases, autoimmune diseases, sarcopenia, insulin resistance, and neoplasms [4]. The risk groups for vitamin D deficiency are individuals with low exposure to UVB light, elderly people over 60 years of age, obese, individuals with increased skin pigmentation, sarcopenia, people with malabsorption, diabetics, osteoporosis, chronic kidney disease, people with autoimmune diseases, neoplasms, with liver failure, pregnant and lactating women [4-8].

Also, vitamin D is a pre-hormone and its synthesis begins with exposure of the skin to UVB light, which

causes the conversion of 7-dehydrocholesterol into pre-vitamin D. Via VDBP (vitamin D binding protein), it is transported to the liver where it will be metabolized into 25-hydroxycholecalciferol. In the kidneys, 25-hydroxycholecalciferol is hydroxylated to its active form 1,25(OH)<sub>2</sub>D, by the action of the parathyroid hormone (MARQUES et al., 2010). In food, vitamin D is found in the form of cholecalciferol (D<sub>3</sub>) and ergocalciferol (D<sub>2</sub>), but these amounts do not meet daily recommendations for a healthy adult. The main food sources are meat, fish, seafood, eggs, milk, and dairy products [9].

Besides, observational studies show that low serum concentrations of 25(OH)<sub>2</sub>D would predispose to acute respiratory tract infections [10]. This would happen through the induction of antimicrobial peptides in response to viral and bacterial stimuli [11]. Vitamin D metabolites would also induce innate antimicrobial mechanisms, including autophagy and the synthesis of reactive nitrogen and oxygen intermediates [12]. It is also described to exert immunomodulatory and anti-inflammatory functions that significantly benefit the treatment of viral infections [13].

There is a high prevalence of hypovitaminosis D in patients admitted to Intensive Care Units (ICU), showing an inverse relationship between serum levels of vitamin D and mortality, presence of nosocomial infections, sepsis, increased hospital costs, and length of stay [13-15]. Hu et al. (2013) [17] performed an observational study with 216 patients admitted to the ICU, in which serum levels of 25-hydroxyvitamin D were determined within 24 hours of admission to the unit. A level of 0 to 19.9 ng/mL (0-49.9 nmol/L) was defined as a deficiency, 20-29.9 ng/mL as insufficiency, and values above 30 ng/mL as sufficiency. The results showed that 44% of the patients had a vitamin deficiency. Patients with vitamin D deficiency had higher Acute Physiology and Chronic Health Evaluation II (APACHE II) scores, a higher rate of positive blood cultures, a higher incidence of multiorgan dysfunction syndrome, and a higher 90-day mortality rate than patients with vitamin D deficiency. vitamin D insufficiency or sufficiency [17].

A recent study involving the association between hypovitaminosis D and COVID19 infection demonstrated that in Spain and Italy, infected patients had low mean concentrations of 25(OH)<sub>2</sub>D, high rates of vitamin D deficiency, and higher rates of infection and death compared to the rest of Europe [18]. While in Norway, Finland, and Sweden, where infection and mortality rates were lower, 25(OH)<sub>2</sub>D levels were higher and there were fewer people with vitamin D deficiency [18]. Other observational studies found that the serum 25(OH)<sub>2</sub>D level was lower in critical cases and higher in mild cases [19], and the relative risk of testing positive for COVID-

19 was 1.77 times higher for patients with probable vitamin D deficiency compared to patients with probably sufficient vitamin D status [20].

In the context of the COVID-19 pandemic, the role of vitamin D in the function of immune cells and the modulation of the inflammatory response has been discussed [16]. The vitamin D receptor is present in B and T lymphocytes, modulating the proliferation and differentiation of these immune cells [18]. In experimental models, hypovitaminosis D has been associated with higher levels of interleukin 6 (IL-6), which could lead to a greater inflammatory response in SARS [21].

Because of this, serum levels of 25(OH)<sub>2</sub>D in patients admitted to Hospital Regional José Alencar (HRJA) with a confirmed diagnosis of COVID-19 were analyzed and may provide us with information on the evolution of patients with SARS-associated hypovitaminosis D. Therefore, the present study aimed to investigate the relationship between SARS-CoV-2 infection, disease progression and hypovitaminosis D.

## Methods

### Participants

Data were obtained from 462 patients with the acute respiratory syndrome, who tested for SARS-CoV-2 between August 3 and December 16, 2020, admitted to Hospital Regional José Alencar (HRJA), located in Uberaba, Minas Gerais, Brazil, and diagnosed positive for SARS-CoV-2. All of these patients had the diagnosis of COVID-19 confirmed by polymerase chain reaction (PCR) test or by serological assay (ELISA) to detect anti-SARS-CoV-2 IgG. Positive patients were considered to have moderate to severe COVID-19. In addition, blood samples from 379 patients were collected for measurement of their serum 25-hydroxyvitamin D levels.

### Ethical Approval

Approved by the ethics committee of the University of Uberaba, number: 4.240.362. Who considered the research relevant in the current context of the Coronavirus - SARS-COV2 pandemic, with a waiver of the Free and Informed Consent Term due to the urgency of collecting data during the pandemic. For disease prevention and due to social distancing, patient and family access were restricted or prohibited.

This study included patients over 18 years of age, residing in Uberaba and the region. The study did not present risks to the participants, as it was an analysis of data from medical records. The name and any identification (voice, photo, etc.) of the patients are

confidential information and only those responsible for the research had access to the information, guaranteeing the professional commitment to its absolute secrecy. The study was funded by the hospital's resources.

### Measurements

Samples for serum 25-hydroxyvitamin D dosage were collected within 48 hours of patient admission and the degree of disease evolution was analyzed according to three parameters: 1) days of hospitalization, counted from the period of hospitalization to discharge or death; 2) admission to the intensive care unit (ICU) for patients who needed it and 3) discharge or death. All data were obtained from the HRJA electronic medical record. The test requested for the analysis of vitamin D levels was the serum dosage of 25-hydroxyvitamin D (25(OH)D), measured by Chemiluminescence (CLIA) and analyzed by the Laboratory of Clinical Analysis of the Hospital Universitário Mário Palmério, which serves the José de Alencar Regional Hospital and some health units in the city. The presence or absence of comorbidities was not included in this study.

### Statistical Analysis

The number of participants was chosen based on available resources and it is a cross-sectional descriptive study using basic descriptive statistics for the analysis of variables.

## Results

### Presence of hypovitaminosis D in patients with SARS-CoV-2

During the study period, 462 patients with a positive diagnosis of the new coronavirus were admitted to the José de Alencar Regional Hospital, of which 379 had their serum levels of vitamin D measured. The results obtained were: 13.98% of the patients had 25(OH)D levels  $<$  or  $=$  10 ng/mL; 29.29% had levels from 10.1 to 20 ng/mL; 32.72% levels between 20.1 and 30 ng/mL; 13.72% from 30.1 to 40 ng/mL; 5.28% from 40.1 to 50 ng/mL and 5.01%  $>$  or  $=$  to 50.1 ng/mL (mean value: 23.83 ng/mL).

According to the Brazilian Society of Endocrinology (SBEM), serum concentrations of 25(OH)D above 30 ng/mL are desirable and these values should be encouraged for populations at higher risk. Most of the patients evaluated in the study had 25(OH)D levels below 30 ng/mL (75.99%), and for the prevention of chronic diseases, the Brazilian Association of Nutrology (Abran) considers a higher level desirable at 40 ng/mL, a serum level between 30 ng/mL and 60 ng/mL is

considered sufficient, as insufficient between 21 ng/mL and 29 ng/mL, and deficient for values below 20 ng/mL [4].

It is important to highlight that some groups considered at risk for COVID-19 are also groups considered at risk for hypovitaminosis D: elderly people over 60 years old, obese, diabetics, chronic kidney patients, patients with liver failure, cancer patients, and pregnant women [9, 22]. Although further population studies are needed to identify and quantify the presence of hypovitaminosis D in the population, some studies have shown that the incidence of individuals with vitamin D deficiency is increasing in Brazil [23-25].

### Vitamin D serum level and disease progression: death and discharge

Among the patients in the study, information on disease progression to discharge or death could be analyzed for 370 patients. Nine patients were transferred to other hospitals, and it was not possible to collect this information. There were 101 deaths among the analyzed patients and 269 discharges. Patients with different 25(OH)D levels were stratified by the in-hospital outcome.

Of the patients with a serum 25(OH)D level less than or equal to 20 ng/mL ( $n = 158$ ), the percentage of people who died ( $n = 57$ ; 27.30%) was higher than those who were discharged ( $n = 101$ ; 15.40%). For those with serum levels above 20 ng/mL ( $n = 212$ ), this picture was reversed and the percentage of patients who evolved to discharge ( $n = 168$ ; 45.40%) was greater than deaths ( $n = 44$ , 11.90%).

A decrease in serum vitamin D levels from the range between 10.1 ng/mL and 20 ng/mL to the range with levels  $\leq 10$  ng/mL was associated with a 52% greater chance of the disease progressing to death (Odds Ratio = 1,52). The chance of evolution to death for patients with serum vitamin D levels in the range between 10.1 and 20 was 65% higher compared to those patients stratified in the range between 20.1 ng/mL and 30 ng/mL (Odds Ratio = 1.65). Note that no adjustments were made for other factors in this study, such as age or race. Consequently, this is only a relative risk approximation and cannot be used to guarantee the incidence of the observed result. Other factors besides the serum level of vitamin D may have affected the result, such as the existence of previous comorbidities [26,27]. The same must be considered for the next results.

### Vitamin D serum level and need for ICU

For a more in-depth analysis of the association

between disease severity and vitamin D concentrations, information on the need for hospitalization of patients in the Intensive Care Unit (ICU) was also collected. Only patients who were discharged from the hospital were analyzed, to remove those who died before being taken to the ICU. Of the 269 patients analyzed, only 27 went to the ICU. Most patients who required ICU had a serum level of 25(OH)D less than or equal to 20 ng/mL (n = 14; 51.89%).

### **Vitamin D Serum Level and Days of Hospitalization**

For the 269 patients who were discharged from the hospital, the average length of stay was 7.24 days. Thus, patients were divided into two groups to determine another indicator of severity for COVID-19: patients with hospital stays longer than seven days (n = 130) and patients with hospital stays shorter than seven days (n = 139). Again, patients who died were retained from this sample, so that patients who died within a few days of hospitalization would not influence the results. This analysis considered all hospitalization days, including ICU days.

Of the patients with a serum level of 25(OH)D less than or equal to 10 ng/mL (n = 28), the percentage of people who required hospitalization for a period longer than 7 days (n = 20; 71.42 %) was higher than those who required hospitalization for less than 7 days (n = 8; 5.76 %). For those with serum levels in the range of 10.1 ng/mL to 30 ng/mL, the percentage of people who required hospitalization for a period equal to or greater than 7 days was 46.04% (n=64) and the percentage of those who needed it for less than 7 days was 53.96% (n=75). For those with serum levels above 30 ng/mL (n = 74), the percentage of those who required hospitalization for a period greater than or equal to 7 days decreased to a percentage of 35.14% (n = 26) while the percentage of patients who needed it for less than seven days increased to 64.86% (n = 48).

### **Conclusion**

This work provides an observational analysis relating the presence of hypovitaminosis D and a worse prognosis for patients with COVID-19. For the three severity indicators evaluated: hospital discharge/death, need for ICU, and days of hospitalization, patients with lower serum levels of 25(OH)D had worse disease progression. In addition, most patients (75.99%) had hypovitaminosis (< 30 ng/mL). However, further studies are needed to verify whether there is any causal relationship between hypovitaminosis D and a worse evolution of the disease and also whether measuring the

serum levels of vitamin D in patients can be used as a possible marker of severity.

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José Alencar Regional Hospital of Uberaba, Minas Gerais, Brazil.

### **Ethics approval**

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### **Informed consent**

Not applicable.

### **Data sharing statement**

The data that support the findings of this study are available.

### **Conflict of interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The Ph.D. researchers who helped in the development of this work had their individual research funded by Brazilian funding agencies in their original research areas. However, due to the extraordinary pandemic situation, they volunteered for this work and none of the authors has any personal relationship with organizations that could inappropriately influence this study.

### **Similarity check**

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