Original Research Article

VITAMIN D DEFICIT INCREASES THE RISK OF DEATH FROM COVID-19 IN BRAZIL

ABSTRACT

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Introduction: The disease caused by the new Coronavirus (SARS-CoV-2) was called COVID-19 and has currently been a public and emergency health concern in Brazil and other countries. **Aim:** This article aims to describe the statistical associations of a group of patients who progressed to death with COVID-19 and who had a low level of Vitamin D in the blood. **Methods:** This is an observational, case-control and clinical study involving 103 patients with COVID-19 and with severe symptoms that progressed to death. **Results:** A total of 92 patients (89.3%) infected with COVID-19 died and had serum vitamin D levels significantly lower than 30 ng/ml. However, a total of 80 (77.6%) patients had a Vitamin D level of less than 20 ng/ml. Compared with the control group, all-cell levels of inflammatory markers were significantly higher in blood serum when level with Vitamin D from COVID-19 patients in the treatment group (n ≤ 30 ng/ml). **Conclusion:** This study showed that patients with serum Vitamin D deficiency are more susceptible to the worsening of COVID-19 and it is generally associated with the release of cytokines as cellular markers mainly in the increase in pro-inflammatory cytokines.

Keywords: SARS-CoV-2, inflammatory, Vitamin D, COVID-19, Cytokines.

1. INTRODUCTION

Coronavirus (SARS-CoV-2) is a virus of the genus Beta coronavirus [02], subgenus Sarbecovírus and the family Coronaviridae that causes the disease known as COVID-19 [07]. In Brazil, this disease has a lethality rate of around 10% in infected patients [07, 13]. Although this disease in Brazil is a public and emergency health problem [33], specific vaccines that are able to control the spread with reduced risk of worsening symptoms have already been made available in the last months of 2022 [07, 18]. Clinical symptoms of COVID-19 have been reported as mild upper respiratory symptoms for non-life-threatening pneumonia or severe pneumonia with acute respiratory distress syndrome (ARDS) [03, 18]. In addition, patients who developed ARDS showed a worsening in a short period, being hospitalized in Intensive Care Unit beds and progressing to death [45].

Studies carried out during the pandemic period on Vitamin D have shown that Vitamin D has essential regulatory functions in the metabolism and nutrition of the human body [01, 04], being considered important to keep the immune system active and thus prevent and overcome the development of infectious processes in the body [03, 04]. Therefore, the Vitamin D deficit increases the individual's susceptibility to infection mainly by viruses such as COVID-19 [05, 06] and develops severe signs and symptoms with evolution to death [07, 08]. In fact, Brazilian patients who evolved with severe or critical signs and symptoms of COVID-19 had metabolic malnutrition [09,12].

A statistical study carried out by Northwestern University involving countries such as Italy [15, 16], Iran, South Korea, Spain, Switzerland, the United Kingdom and the United States showed that there is a strong correlation between vitamin D deficiency and increased mortality rates [14, 16]. In fact, vitamin D has many relevant roles in health maintenance [17], for example, Vitamin D performs metabolic actions through Vitamin D receptor (VDR) enzymes that are found in

practically all cells of the innate and adaptive immune system [20, 22]. Therefore, these cells act specifically in the potential anti-inflammatory and immunomodulatory properties with activities that help decrease SARS-CoV-2-induced lung injury [22]. Cells that undergo the immunomodulatory actions of Vitamin D are able to produce in locus the expression of CYP227B1[22, 23] and directly conduct the cycle through a regulatory method by a network of information immunoregulated by 1,25(OH)2D3 [19, 24] instead of allow homeostatic entry of calcium. Thus, this process reduces the severe impacts on the functional integrity of the immune system such as influencing the production of cytokines, since Vitamin D increases the cell's innate immunity by inducing antimicrobial peptides such as human cathelicidin LL-37 through the 1,25(OH)2D3 [20, 21] and beta-defensins, therefore, these cathelicidins develop direct antimicrobial activities against a spectrum of etiologic agents such as the COVID-19 virus [25, 33].

Based on the results of the research carried out by Vanegas-Cedillo et al (2022) in which they reported that the levels of Vitamin D are significantly lower when compared in fatal and non-fatal cases by COVID-19 (41), our study aimed to describe Brazilian patients who died from COVID-19 and had low levels of vitamin D in their blood with significant changes in inflammatory markers.

2. MATERIAL AND METHODS

2.1 Study Design and Patient Enrolment

This research was carried out between May 1, 2021 and December 31, 2021 at the Federal University of Mato Grosso do Sul at the Laboratory of Spectroscopy and Bioinformatics Applied to Biodiversity and Health of the Faculty of Medicine. The present study included 103 patients who were treated in the primary health care (PHC) of the Brazilian Unified Health System (SUS).

This is an observational, descriptive study which considered a group of 103 patients with COVID-19 and with severe symptoms that progressed to death. The classification of cases considered severe (Treatment Group) was performed according to the presence of the following criteria: Vitamin D deficiency, patients with Vitamin D level ≤ 30 ng/ml in the blood, with respiratory distress, with tachypnea ≥ 30 rpm were considered with oxygen saturation level ≤ 95% and with evidence of bilateral diffuse pneumonia on chest radiography or computed tomography (TC).

The health care units in Brazil that participated in this study performed the blood collections. However, the analysis of chain reaction tests (RT-PCR) were performed by laboratories accredited to the Brazilian Ministry of Health.

2.2 Measurement of Vitamin D

The determination of the value of Vitamin D (1,25(OH)2D3) was performed considering a marker of the status of Vitamin D (1,25(OH)2D3) and using liquid chromatography-mass spectrometry (LC-MS/MS). In addition, the blood dosage [33, 34] of Vitamin D was standardized according to the guidelines of Nature Reviews Endocrinology (2017), and the serum level of Vitamin D ≤ 30 ng/ml was considered as vitamin D deficiency [36, 37].

2.3 Measurement of Cytokines

The quantification of blood levels of cytokines was performed by enzyme immunoassay involving patients in treatment and control group (total 103 patients). The measurement was performed by enzyme immunoassays (ELISA - (Elabscience Biotechnology Co Ltd)).

2.4 Statistical Analysis

To perform the general statistics in this study, the “Chi-square test” was used to perform the statistical analysis. Univariate regression analysis was used to assess the level of Vitamin D (1,25(OH)2D3) and clinical responses to COVID-19 infection compared to responses mediated by specific immune defense cells. Statistical results with a p value less than 0.05 from the univariate analysis were dependent in the multivariate regression model. Multivariate analysis was adjusted considering gender profile, signs and symptoms, vitamin D deficit ≤ 30 ng/ml in blood serum, respiratory distress, tachypnea ≥ 30 rpm, oxygen saturation level ≤ 95%, and evidence of pneumonia bilateral diffuse in chest radiography or computed tomography (TC) and length of stay ≥ 15 days in an intensive care unit bed. The statistical significance in this study for the p value was $p \leq 0.05$. All statistical analysis was performed using R Studio (Version 4.1.0).

3. RESULTS AND DISCUSSION

The epidemiological clinical data associated with the Vitamin D levels of the patients in each group are presented in **Table 1.** and **Table 2**.

Table 1. Data of (1,25(OH)2D3) levels associated with death and clinical profile by COVID-19.

Patient Characteristics of Study Population	n ≤ 30 ng/ml in blood serum (Treatment)	n ≥ 30 ng/ml in blood serum (Control)	p – value
Women	69	9	0,05
Men	23	2	0,05
O2 saturation < 95%.	63	40	0,001
Respiratory Discomfort.	92	11	0,001
Tachypnea ≥ 30 beats/min.	83	20	0,001
Length of stay ≥ 15 days in ICU beds.	92	11	0,001
Evidence of bilateral diffuse pneumonia.	76	27	0,001

Source: Performed by the authors, P-value – obtained from statistical method.

According to **Table 1**. the highest death rate from COVID-19 was identified in patients who had serum vitamin D levels less than 30 ng/ml, where totaled 92 patients (89.3%). However, 89.3% of the patients who died, 80 patients (77.6%) had a Vitamin D level below 20 ng/ml (**Table 2**.). In fact, **Table 1.** shows the prevalence of females in the sample studied with significant differences for Vitamin D deficit (p=0.05). In this case, Vitamin D deficiency is associated with length of stay in intensive care beds for more than 15 days.

Table 2. Mortality frequency according to the level of Vitamin D in patients with COVID-19.

Levels of (1,25 (OH)2D3)	n (103 patients)	f (%)
10- 20 ng/ml	80	77,66
21-30 ng/ml	12	11,65
31-50 ng/ml	9	8,73
> 50 ng/ml	2	1,94

Source: Performed by the authors.

The serum vitamin D range from 10 ng/ml to 30 ng/ml was adopted as a severe vitamin D deficiency. According to this study, 92 patients were severely vitamin D deficient and evolved with a higher percentage of signs of aggravation to death (Tables 1-3). However, only two patients had a mean level of Vitamin D above 50 ng/ml with the presence of mild signs of worsening (**Table 1**.). In **Table 2**. the percentage mortality rate in the Treatment Group (n ≤ 30 ng/ml in the blood serum of Vitamin D) was 23.5% (92 deaths) while in the Control Group (n ≥ 30 ng/ml in the blood serum of Vitamin D) was 4.75% (11 deaths).

Table 3. Hospitalization period, serum Vitamin D level and inflammatory cell type.

Levels of (1,25 (OH)2D3)	Interval of hospitalization time (days)	Cell types with the highest percentage.	Total Patients Vaccinated with COVID-19	Total Patients.
10- 20 ng/ml	4	IL-10	21	87
20-30 ng/ml	24	IL-10	3	9
30-50 ng/ml	16	IL-8	2	5
> 50 ng/ml	35	IL-4	1	2

Source: Performed by the authors.

As shown in **Table 3**. the average length of stay of patients in the intensive care bed was 16 days, in which there was an increase in the production of IL-10 (76.6% increase in cell production in patients hospitalized with level of vitamin D less than 30 ng/ml). However, in the mean interval of 24 days of admission to intensive care beds, patients with a mean level of 20-30 ng/ml (9 patients) developed a significant increase in IL-10 (9%). In this case, the difference in length of hospital stay was statistically significant (p=0.001) in relation to the increase in IL-10 production. In **Table 3**. it was shown that patients with an average blood level of Vitamin D (20-30 ng/ml) remained in an intensive care bed for an average of 24 days and showed an increase in the production of inflammatory cells such as IL-10. Moreover, patients with a Vitamin D level greater than 50 ng/dl were hospitalized for an average of 35 days; however, they had only an increase in the production of IL-04 inflammatory marker cells and they did not die from COVID-19, they died related to pre-existing diseases such as cardiac alterations. In **Table 3**, there was an increase in the production of inflammatory cells IL-8 in the range specifically in the blood level of Vitamin D (30-50 ng/ml) and these patients had an average period of hospitalization in an intensive care bed equivalent to 16 days.

Table 4. Analysis of Vitamin D dosage and cell marker types in patients with COVID-19 for n ≤ 30 ng/ml in blood serum.

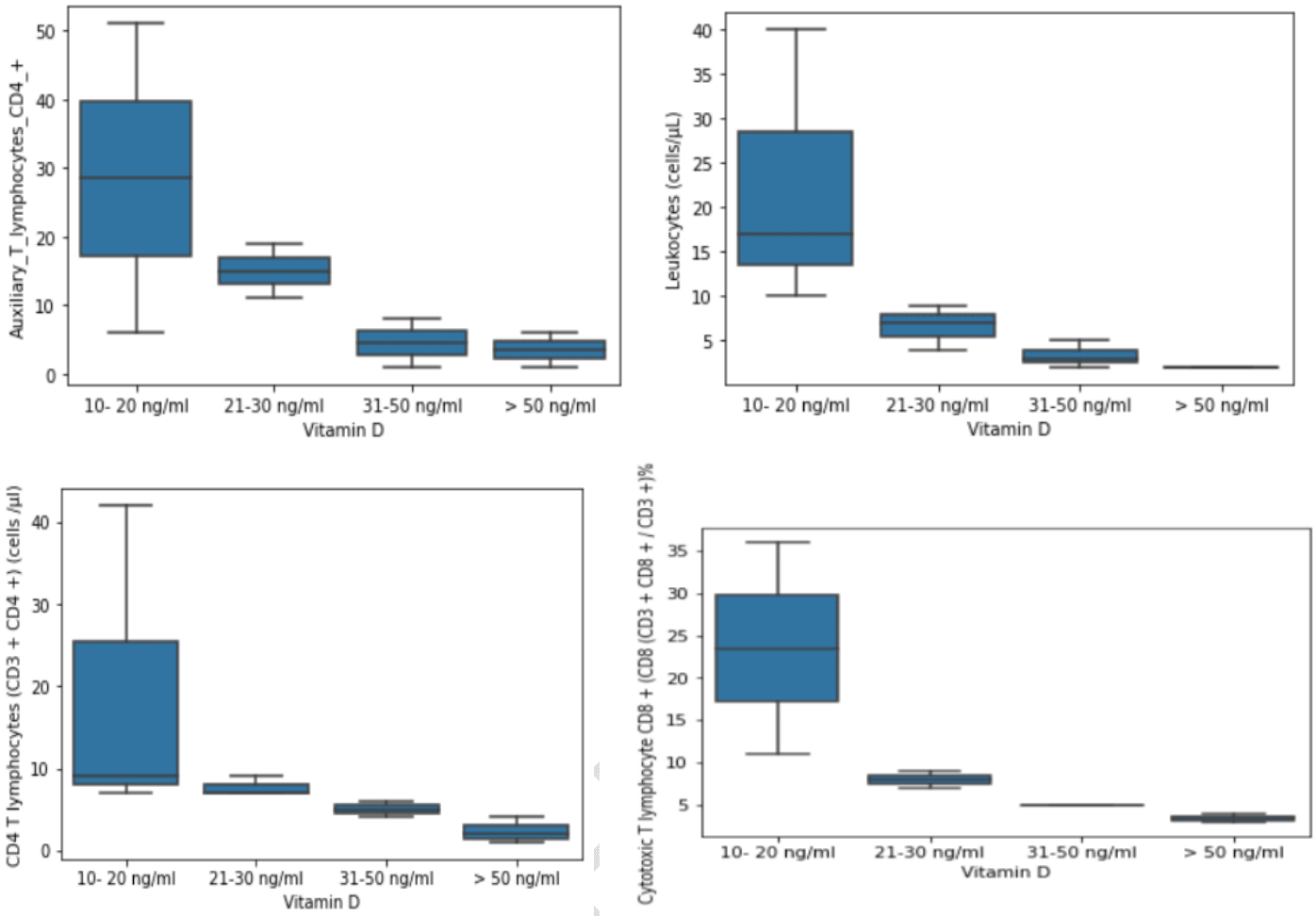
Source: Performed by the authors.

Types of Inflammatory Cell Markers.	n ≤ 30 ng/ml in the blood serum. (Treatment)	n ≥ 30 ng/ml in blood serum (Control)	OR	95% Confidence interval (CI)
Auxiliary T lymphocytes CD4+ (%)	57	30	0,66	[0.55 - 0.75]
CD3 %	75	16	0,82	[0.73 - 0.90]
CD4 T lymphocytes (CD3 + CD4 +)	58	23	0,72	[0.60 - 0.81]
CD8 T lymphocytes (CD3 + CD8 +)	64	20	0,76	[0.66 - 0.85]
Cytotoxic T lymphocyte (CD8 +)	47	16	0,75	[0.62 - 0.85]
Interleukin 10 (IL-10) (pg/mL)	75	16	0,82	[0.73 - 0.90]
Interleukin 4 (IL-4) (pg/mL)	79	12	0,87	[0.78 - 0.93]
Interleukin 8 (IL-8) (pg/mL)	69	17	0,80	[0.70 - 0.88]
Natural Killer cells (CD56 + CD16)	68	16	0,81	[0.71 - 0.89]
Neutrophils	69	17	0,80	[0.70 - 0.88]
Total Leukocytes	67	20	0,77	[0.67 - 0.85]

Vitamin D deficiency was significantly associated with increased production of Inflammatory Cell Markers as shown in Table 4. Compared with the control group, the levels of all inflammatory marker cells were significantly higher in the group n ≤ 30 ng/ ml in the blood serum of Vitamin D from patients with COVID-19 (**Table 4**). According to the blood dosage of Vitamin D, a total of 103 patients with COVID-19 were distributed in groups n ≤ 30 ng/ml in blood serum (Treatment) and n ≥ 30 ng/ml in blood serum (Control), concentrations of inflammatory marker cells (auxiliary T lymphocytes CD4+ (0.66[0.55 - 0.75])), total leukocytes (0.77, [0.67 - 0.85]), CD4 T lymphocytes (0.72, [0.60 - 0.81]) and cytotoxic CD8+ T lymphocytes (0.75, [0.62 - 0.85]), natural killers (0.81, [0.71 - 0.89]), CD8 T lymphocytes (0.76, [0.66 - 0.85]), CD3% (0.82 , [0.73 - 0.90]) and interleukin 10 [0.82, [0.73 - 0.90]), interleukin 4 (0.87, [0.78 - 0.93]), neutrophils (0.80, [0.70 - 0.88]) and interleukin – 8 (0.80, [0.70 - 0.88]))), all of them were represented in **Graphs 1**, **Graphs 2** and **Graphs 3** respectively and according to **Table 4**. As analyzed in **Graph 1**, when the group of patients with COVID-19 with n ≤30 ng/ml in blood serum was compared with the control group, in auxiliary T lymphocytes CD4+ there was an increase followed by CD4+, T lymphocytes and Total leukocytes respectively (**Table 4**. and **Graph 1**).

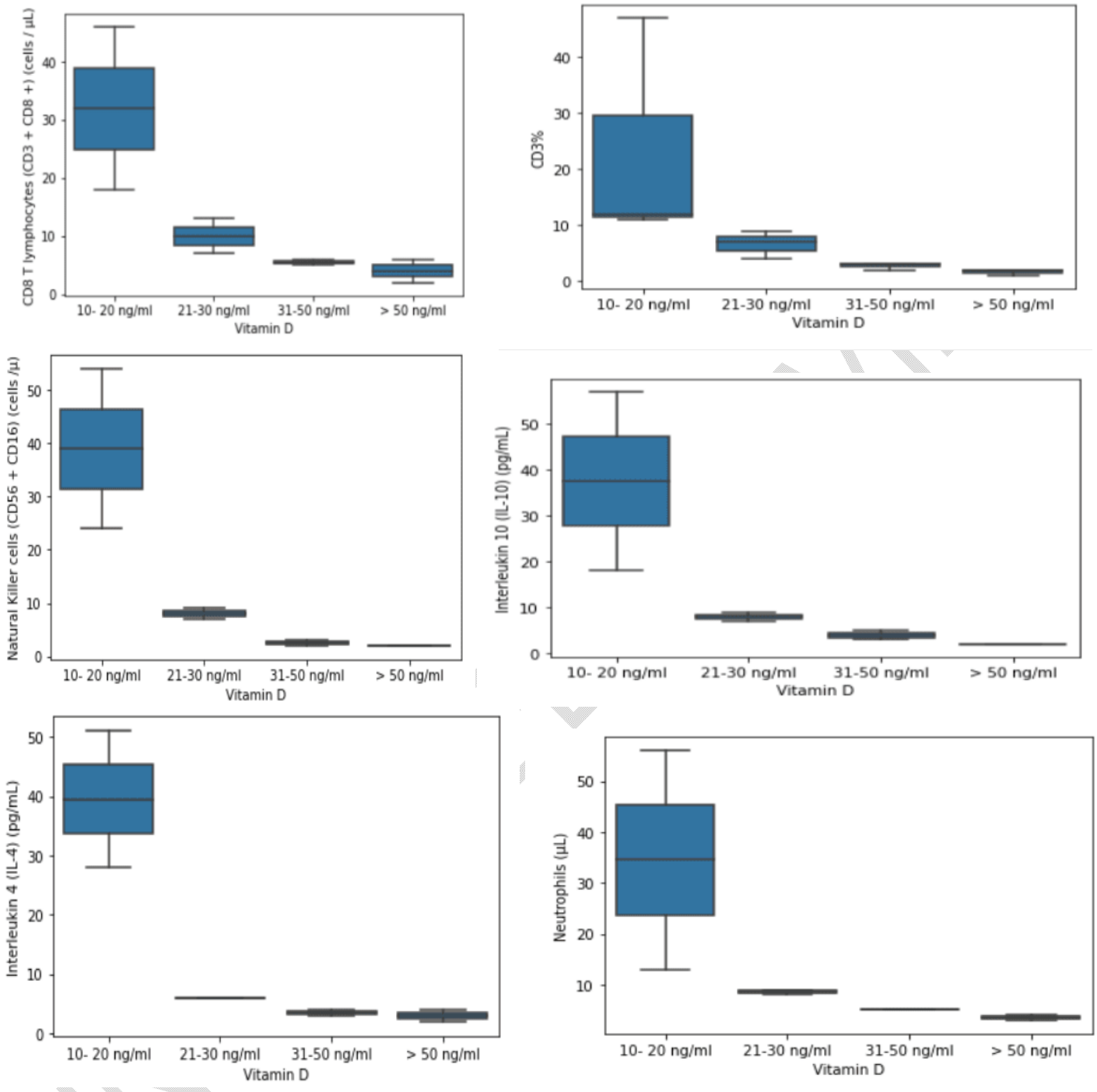
According to **Table 4**, we can say that in this study there is a negative correlation (p value = 0.001) of the deficit of Vitamin D in blood, and the production of pre-inflammatory and post-inflammatory cell markers that were visualized in **Graph 1**, **Graph 2** and **Graph 3**. According the results of this study, a profile of inflammatory marker cells was established that included individual levels of serum cytokines with proportions of anti-inflammatory and pro-inflammatory cytokines and then a profile of serum cytokines as a function of serum Vitamin D was droped (**Graph 1**, **Graph 2** and **Graph 3**). Thus, according to the use of univariate analyzes included through multivariate and retroactive regression models of the Stepwise type, it was possible to predict the Vitamin D status of patients with values lower than 30 ng/ml in relation to length of stay, O2 saturation < 95 %, tachypnea ≥30 beats/min and evidence of bilateral diffuse pneumonia (**Table 1.**, **Graph 1**, **Graph 2** and **Graph 3** and **Figure 2**.).

Graphs 1. The level of Vitamin D and the inflammatory markers cells in the sample.



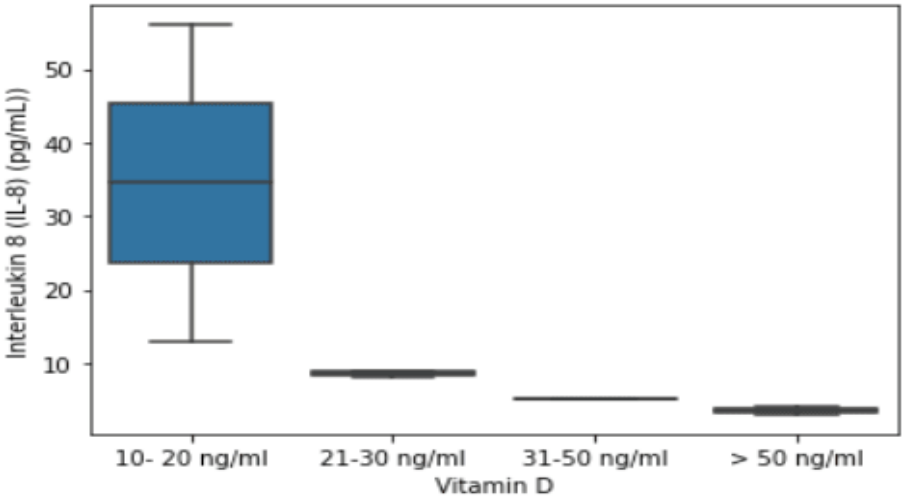
In Graph 01, patients with Vitamin D deficit in the range 10 – 30 ng/ml had an increase in the production of inflammatory cells (Auxiliary T lymphocytes CD4+ of (30.0% – 51.1%)) however, in the range of 21 – 30 ng/ml of Vitamin D in CD4 T lymphocytes (CD3+, CD4+) cells, a change in (51.2 % – 52.2%). 15 patients with Vitamin D Deficiency in the range 21 – 30 ng/ml had a significant decrease in the production of inflammatory cells such as Leukocytes (9.801 – 10.801 cells/µL).

Graphs 2. The level of Vitamin D and the inflammatory markers cells in the sample.



In **Graph 2**, patients with Vitamin D deficit in the range of 10 – 30 ng/ml had higher production of interleukin-10 with an equivalent increase in interleukin-10 with a total of 72.8% (5.2 – 8.1 pg/mL) in patients in the Treatment Group and this result was highly significant, as it presented a $p<0.001$. In this study, it was observed that in the range of 21 – 30 ng/ml, a decrease in the production of inflammatory marker cells begins to occur after 15 days of hospitalization in intensive care. In Graphs 3 was described of the level of Vitamin D (1,25(OH)2D3) compared to the level of production of inflammatory marker cells of patients with COVID-19 (Interleukin-04, Neutrophils, Interleukin-8) of COVID-19.

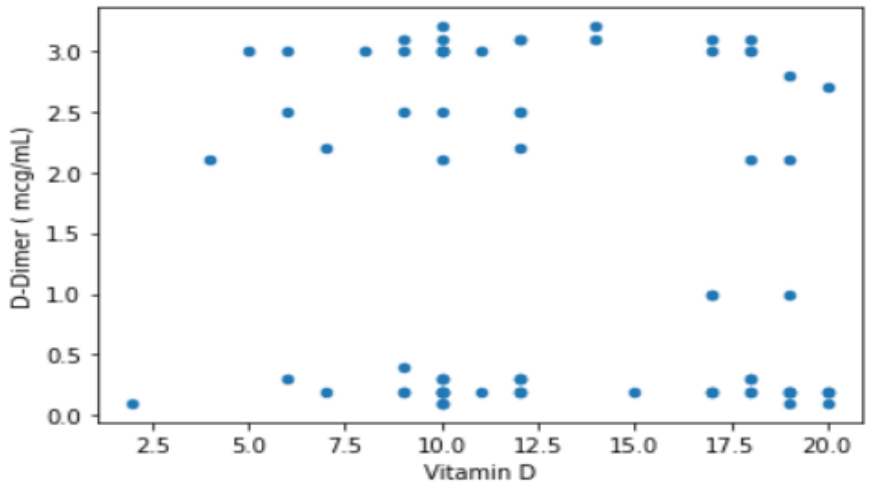
Graphs 3. The level of Vitamin D and the inflammatory markers cells in the sample.



In **Graph 3**, patients with Vitamin D deficiency produced interleukin-8 mainly patients with COVID-19 and Vitamin D deficiency in the range of 10 - 30 ng/ml and had a 52.8% increase in production of interleukin – 8 (32 – 85 pg/mL) and this result was significant ($p<0.001$).

Graph 1, Graph 2, Graph 3 and **Figure 2** proved that the worsening of signs and symptoms in COVID-19 occurred due to a progressive decrease in Vitamin D levels (10 – 30 ng/ml), that is, the increase in the production of pre-inflammatory and pro-inflammatory marker cells inflammatory reactions in COVID-19 was stimulated in the patients analyzed in the Treatment Group due to the progressive deficit of Vitamin D (10 – 30 ng/ml), the patients got worse with the signs and symptoms and progressed to death (**Figure 2**). For example, the increase in the production of Interleukin-8 and neutrophils occurred in 66.9% of the patients analyzed in the Treatment Group and presented a $p<0.001$ considered significant and the natural Killers cells showed an increase in production in 66% of the patients verified in the Treatment Group (**Graph 2)**.

Graph 4. Blood dispersion of Vitamin D and D-dimer in the worsening of COVID-19 patients.

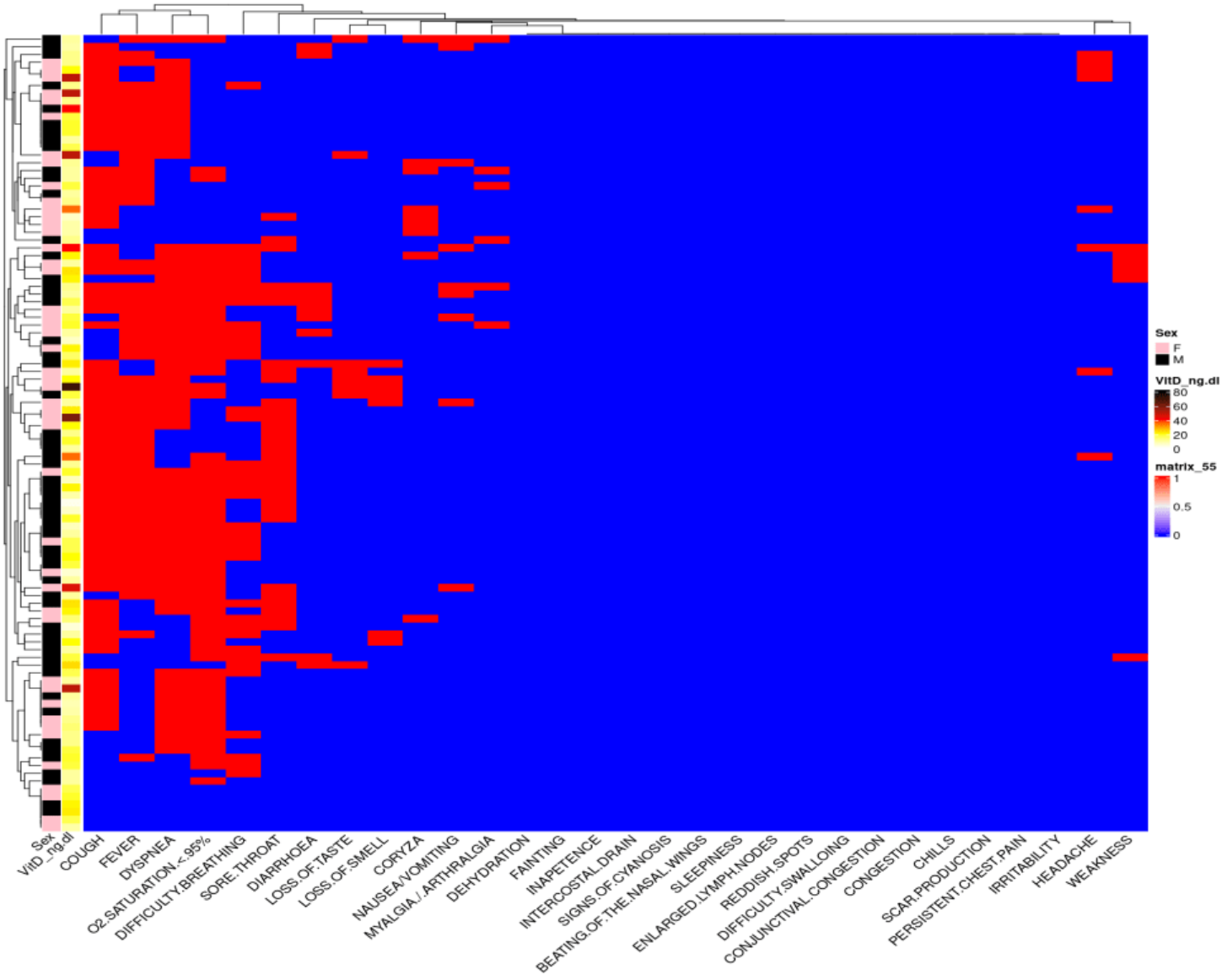


From **Graph 4** and **Figure 2**, it is observed that there is a significant decrease in the concentration of doses of Vitamin D (10 – 20 ng/ml) in the serum of patients included in the treatment group and with tail infection by COVID-19. In fact, these results showed a strong (two-tailed) correlation between the concentration of Vitamin D and the severity of the COVID-19 infection (**Table 1**) ($p<0.05$). On the other hand, the statistical correlation between D-Dimer in the groups with ranges lower than 30 ng/ml of Vitamin D in serum ($p<0.05$) was significantly higher compared to groups with a lower dose of Vitamin D in serum at 30 ng/ml of Vitamin D when compared to the group with a dose of Vitamin D **≥** 30 ng/ml (**Table 2** and **Table 3**).

Graph 1, Graph 2, Graph 3 and **Graph 4** compared to **Figure 2** helped to prove the (two-tailed) correlation between the concentration of Vitamin D and the increase in the production of inflammatory marker cells (**Graph 1**, **Graph**

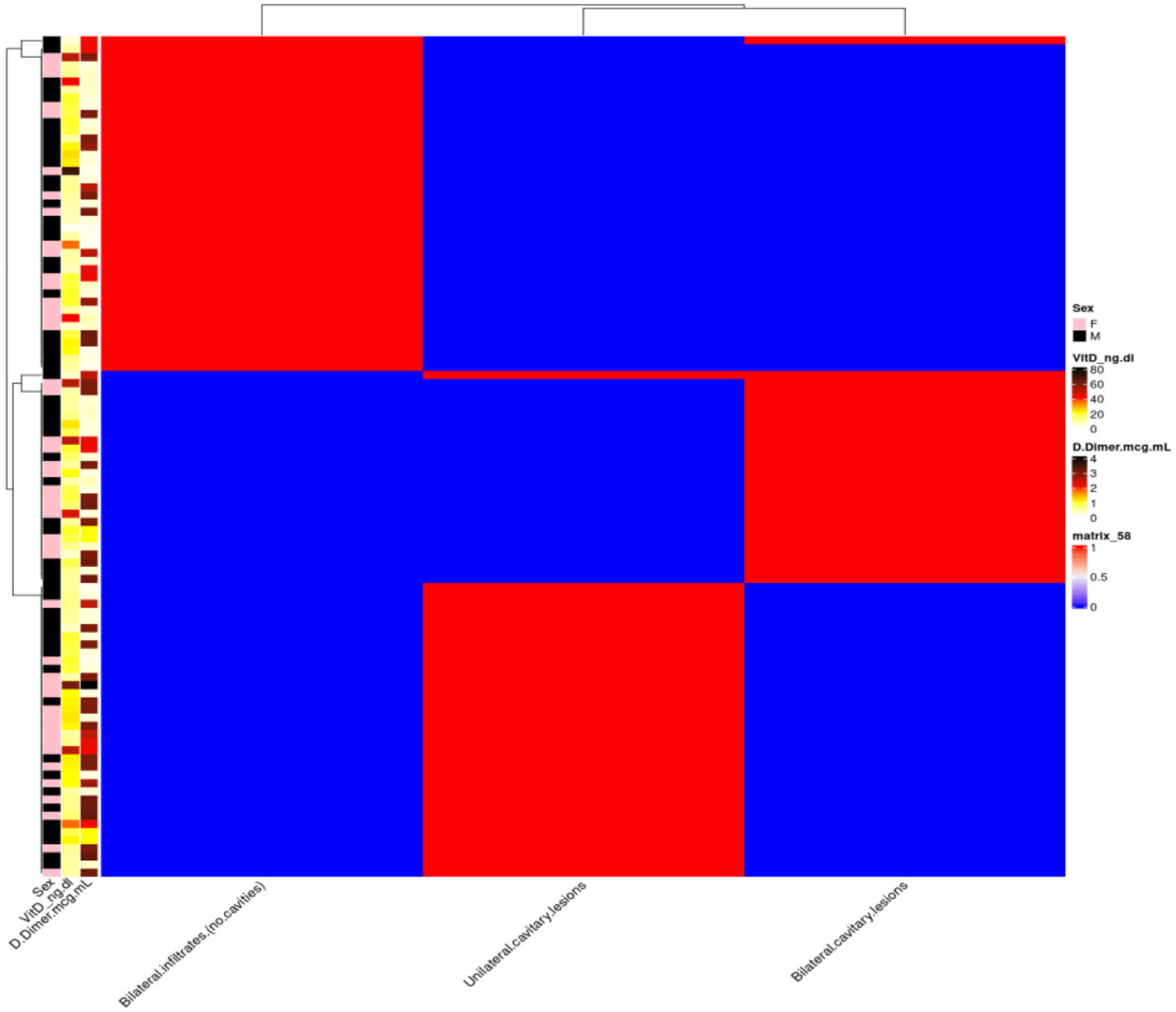
2 and **Graph 3**). The significance of the two-tailed correlation of the concentration of Vitamin D and the increase in the production of inflammatory marker cells is significantly elevated in parallel with the increase of D-Dimer in the infectious process caused by the SARS-CoV-2 virus, that is, mainly in the group with interval less than 30 ng/ml of Vitamin D in serum ($p<0.05$) of patients who developed significant lung lesions (**Figure 2**).

Figure 1. Heat Maps illustrating Vitamin D deficiency (Treatment Group) correlated with increasing symptoms with worsening. (A) Thermal shifts, which indicate an increase in symptoms with worsening Vitamin D deficit, were identified from the symptoms (30 variables). (B) A higher matrix_55 value represents stronger correlation affinity.



The heat map used to assess risk factors for the worsening of COVID-19 was obtained considering sex, symptoms and vitamin D deficit (10 – 20 ng/ml), in addition, for the statistical calculations It´s 30 important variables in the worsening of COVID-19 were considered (**Figure 1**). In this study, it is possible to verify that the symptoms increased in samples with Vitamin D deficit from the patients included in the Treatment Group. That is, with a high level of Vitamin D deficit (10 – 20 ng/ml), the matrix_55 with value equivalent to 01, demonstrated a high affinity of stronger correlation between Vitamin D deficit (10 – 20 ng/ml) and the increase in signs and symptoms, which in turn progressed to worsening and death.

Figure 2. Heat Maps illustrating Vitamin D deficiency in the treatment group correlated with D-Dimer changes associated with lung disease. (A) Thermal displacements, which indicate an increase in D-Dimer (> 02 mcg.ml) in the Vitamin D deficit in the Treatment Group, were identified with an increase in the presence of pulmonary cavity lesions in patients who died. (B) A higher matrix_55 value represents stronger correlation affinity.



According to the heat map depicted in **Figure 2**, changes with D-Dimer were grouped into two categories based on serum Vitamin D deficit (<30 ng/ml). When the Matrix_58 thresholds was used to judge guidelines for damage caused by Vitamin D deficit with increasing cavity and non-cavity lung lesions, it was found that 72.8% of patients had cavity lung lesions with increased rates of D- Dimer, in this case, the cut-off value (Matrix_58) is equivalent to 01, that is, there is a significant connection with mortality related to Vitamin D deficit (10 – 20 ng/ml).

3.1 DISCUSSION

The observational and descriptive study results obtained in our study are comparable to those obtained in other COVID-19 related studies, in which female patients had an increased rate of contracting SARS-CoV-2 and being hospitalized for complications related to the disease [18]. AlQuaiz et al (2018) similarly described that women are at greater risk than men of developing Vitamin D deficiency (10 – 20 ng/ml) in the body; this may be due to the absence of daily

Vitamin D supplementation and the use of irregular diets on a daily basis [37], such as the consumption of cola-based soft drinks and the presence of aggravating factors such as obesity, considered a strong indicator for aggravation [03] (**Table 1**). Based on the results, our study showed that the clinical manifestations of COVID-19 were related to severe symptoms, that is, the critical illness was characterized by respiratory failure that justifies a long stay in an intensive care unit bed with death. Moreover, saturation (2) less than 95% and followed by tachypnea.

Holick et al (2008) highlighted that Vitamin D deficiency is associated with several adverse health outcomes such as hypertension, myocardial infarction, inflammatory bowel disease, leukemia, squamous cell carcinoma, infectious diseases and I diabetes mellitus [16, 18]. Thus, the patient with COVID-19 evolves with signs of aggravation and death correlated with Vitamin D deficit (10 – 30 ng/ml) [20 - 23]. In addition, Jain et al (2020) similarly in their study obtained results of the level of Vitamin D associated with the severity of viral diseases, that is, the authors when comparing the serum levels of Vitamin D among critically ill patients with COVID-19 with a serum level of less than 30 ng/ml observed that there is a significant difference [23]. Therefore, the results of Jain et al (2020) are agree with results in which we considered a case-control and cohort study (Table 3 and Table 4) (20 – 25) [24]. That is, we demonstrated in our study that a Vitamin D deficit of less than 30 ng/ml in serum is directly related to the incidence and evolution of worsening symptoms in COVID-19 that progress to death [25]. Similar to the results found in the study by Jain et al (2020), our study reinforced the evidence that patients hospitalized in intensive care units during the hospitalization period with an interval of days greater than 24 days had low levels of Vitamin D in the serum of patients with COVID-19 and with symptoms of worsening present.

In the results of our study found in Table 3, it was highlighted that the levels of Vitamin D in serum were lower than those found in the study by Jain et al (2020) [21, 23, 25]. Similarly, mean vitamin D levels of less than 30 ng/ml in serum produced a higher percentage of significant pre-inflammatory and pro-inflammatory cells in COVID-19 patients [24, 26]. That is, low levels of Vitamin D can cause a decrease in the physical defense barrier in the natural and adaptive cellular immunity in the body [25]. Thus, it appears that there is a similarity between the results found in our study and those obtained in the studies by Lim et al (2015)[28, 30].

The study carried out by Pletz and Cols (2014) and Arvinte et al (2020) found results similar to ours in proving that Vitamin D deficiency is regularly related to severity in the development of community-acquired pneumonia in cases of viral diseases, currently the COVID-19 (27-31). In addition, our results are also similar to the results of Pletz and Cols (2014) and Arvinte et al (2020), where levels below 30 ng/ml of Vitamin D in patients who died within a hospital stay of less than 24 days were more frequent when compared to the group with a Vitamin D level above 30 ng/ml [28, 31].

The studies by Huang et al (2020) (18) and Ramos et al (2020) described that the most common symptoms in COVID-19 infection are high fever (> 38^{o}C), shortness of breath (O2 saturation < 95percentage) and dry cough that normally appears after 4.2 days of virus incubation [32, 38]. Thus, such results presented by Huang et al (2020) (18) and Ramos et al (2020) are in agreement with the results found in our study considering 103 patients with COVID-19 (32-37). According to Huang et al (2020) (18) there is a 63% increase in lymphopenia in the laboratory findings of hospitalized patients with COVID-19, these results are similar to those shown in Graph 2, in which it appears that there is a 76% increase in lymphocytes in hospitalized patients with Vitamin D deficiency with a level of Vitamin D lower than 30 ng/ml in serum [38 - 41]. Our study demonstrated atypical findings such as diarrhea, nausea and drowsiness in critically ill patients with COVID-19 in the vitamin D deficiency group [38, 42], however, such findings are in agreement with the study by Wang et al (2019). Plasma concentrations of inflammatory markers among patients with Vitamin D deficiency and serum normality showed higher amounts of IL-04, IL-08 and IL-10 in this study considering 103 patients with COVID-19 [39, 41, 42].

The study by Zhou et al (2020) evaluated 191 patients with COVID-19 from two hospitals in Wuhan and reported that the cytokines IL-06, IL-08 and IL-10 were also increased in critically ill patients hospitalized with COVID-19. In addition, patients with Vitamin D deficiency had approximately twice the amount of inflammatory markers in serum because Vitamin D has effects on the adaptive immune system which includes cell-mediated alternation between cells (Th1) and Humoral immunity (Th2) [32, 33,45]. Vitamin D by cellular receptors of the regular type can develop a negative feedback to the TH1 immune response by inhibiting the production of type I pro-inflammatory cytokines such as: IL-12, IFN-γ, IL-6, IL-8 TNF- α and IL-9 [32, 44]. In a compensatory way, Vitamin D regulates the production of anti-inflammatory type two (Th2) cytokines, such as IL-4, IL-5 and IL-10 [11, 12, 39, 47], by means of positive feedback (38). Ramos et al (2020) (32) described that this cytokine regulation is specifically mediated by blocking the activation of NF-kB p65 through upregulation of the NF-kB inhibitor protein IkBa[39, 43, 45, 47]. Therefore, this study found similar results to the study by Zhou et al (2020), since the Vitamin D in the normal range with a serum level equal to 30 ng/ml is capable of positively regulating Th2 and tolerogenic DC cells, in addition to inhibiting the proliferation of Th1 cells) and inducing the production of regulatory T cells (Treg) [28, 32], in this case, the results obtained in our study are similar to those published in Refs. [40, 47].

This study is in agreement with research carried out by Zhou et al (2020) when describing the mortality of the 103 patients included in this study, proving that Vitamin D deficiency is considered a risk factor and influences the production of inflammatory cells on the immune system and this production is related to how some patients responded to SARS-CoV-2 [32], since Vitamin D has a modulating role in the adaptive and innate immune response and is proven in the current data of this study available in the clinical presentation of COVID-19 [40]. 19 with their relationship in the production of cytokines and similar cells [47] (Graph 1, Graph 2, Graph 3 and Figure 2). It is worth emphasizing that this immune response to SARS-CoV-2 occurs through the modulation of Vitamin D by performing a modular transformation from its initial form to calcitriol in the nuclear cell VDR in B cells and T lymphocytes, neutrophils, monocytes and dendritic cells[32] and this mechanism of immune and inflammatory modulation of conversion to calcitriol occurs through the positive feedback regulation of the enzyme 1-α-hydroxylase (CYP27B1) [25, 27, 38].

In addition, Vitamin D induces the production of antimicrobial peptides such as β2, β4 defensins and cathelicidin peptide (CAMP) antimicrobials through macrophages, monocytes, keratinocytes, epithelial, intestinal, lung and corneal cells [38]. Thus, with these immunological and inflammatory mechanisms, macrophages and monocytes develop chemotaxis, autophagy and phagolysoma fusion of immune cells [27, 32]. On the contrary, keratinocytes, epithelial, intestinal, pulmonary and corneal cells increase and reinforce the physical barrier function, that is, collectively this antimicrobial effect will increase the organism's defense against COVID-19 [32, 38].

And related to Vitamin D deficiency, our study was developed with 103 patients with COVID-19 who had an increased inflammatory reaction in the presence of SARS-CoV-2, which was proven through the increase in the production of inflammatory markers such as, IL-04, IL-08 and IL-10 [27, 38] in COVID-19 patients with serum vitamin levels in the range of 10 to 20 ng/ml in agreement with the study by Sassi et al (2018).

The results obtained in our study proved that non-survivors of COVID-19 had increased serum levels of D-Dimer and low levels of Vitamin D in serum when compared to surviving and hospitalized patients with normal levels of Vitamin D [20, 30]. Furthermore, our results are similar to those obtained by Infante et al (2021) and Vanegas-Cedillo et al (2022), in which they proved that severe complications in COVID-19 are related to an increase in immune response and mediated in part by increased pro-inflammatory cellular levels [30, 39]. Thus, cytokines such as IL-04, IL-08 and IL-10 through the immunomodulatory properties of Vitamin D are downregulated [39].

However, we can highlight in this study in **Graph 1**, **Graph 2**, **Graph 3** and **Table 4** that the innate immune system in patients with COVID-19 was the first and immediate line of defense against the invading virus and an immunological cascade alliance mainly in the components of the host microbiota was developed for the defense of the organism [39, 40], that is, the defense of the host was composed of several components, such as cellular defense such as an increase in the production of mast cells, dendritic cells, macrophages, neutrophils and natural killer cells [38, 39], and it also activated cell receptors that are capable of recognizing pathogens, for example, Toll-like receptors, antimicrobial peptides and proteins such as defensing and cathelicidins [47, 42]. And Biesalski (2020) describes in a previous retrospective study that the binding of SARS-CoV-2 to ACE2 works as a proposed trigger of inflammation related to acute lung damage and this study brought [44, 48] similar results in **Figure 2**.

Other evidence among the results to be highlighted is that the results obtained by Biesalski (2020) are similar to our clinical data. That is, Figure 2 of our study proves that the Vitamin D deficit favors lung inflammation, in this case, which may be caused by lower levels of ACE2 [06, 48], also evidenced in the study by Zhou et al (2020) by the increase levels of IL-08 and Natural Killers (NK) cells [44, 48]. **Graphs 1, 2, 3** and **Figure 2** proved that the most severe cases of COVID-19 had high levels of inflammatory cell markers. In addition, the influx of cytokines helps to increase the pulmonary [32] inflammatory responses and thus causes parenchymal damage with an increase in progression to develop SARS, therefore, in this study computed tomography (TC) with severe alteration scans were verified in patients with COVID-19 [33, 48].

4. CONCLUSION

Our study showed that patients with vitamin D deficiency in blood serum with a dose of less than 30 ng/ml are more susceptible to the worsening of COVID-19 and the aggravation is associated with the release of cytokines as cellular markers mainly in the increase in pro-cytokines. -inflammatory.

However, it was possible to verify that Vitamin D is able to modulate several types of cellular markers mainly of the components of the innate immune system.

Therefore, the search for alternatives for the immunomodulatory capacity of Vitamin D should be explored considering that there is previous evidence regarding its specific action against COVID-19.

CONSENT

As per international standard or university standard, patient (s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

This study was carried out according to the ethical criteria established in the "Declaration of Helsinki" and had the approval of the research ethics committee provided by the Federal University of Mato Grosso do Sul (CEAA: 42969320.0.0000.0021).This research was partially supported by the Brazilian Research Council (CNPq) (CNPq: Process No 310621/2020-8).

Findings
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