https://doi.org/10.31925/farmacia.2022.3.17 **ORIGINAL ARTICLE**

THE EVALUATION OF VITAMIN D DEFICIENCY AS A RISK FACTOR IN THE CASE OF PATIENTS WITH MODERATE COVID-19

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Abstract

The paper aimed to evaluate the role of vitamin D in patients with moderate COVID-19. A total number of 128 patients, divided into two groups based on their clinical outcome, were evaluated. The group of patients with a positive outcome consisted of 82 patients (POG), while the group with a negative outcome consisted of 46 patients (NOG). We determined at two different moments (on the patients' admission and their discharge) the plasma level of vitamin D (25-hydroxy vitamin D) along with the levels of inflammatory markers in COVID-19 as C reactive protein (CRP), lactate dehydrogenase (LDH), ferritin, fibrinogen, the total leukocyte count and total cholesterol. The level of 25-hydroxy vitamin D was significantly lower in the NOG group when compared to the POG group, while levels of LDH and CRP in the NOG group were significantly higher than those found in the POG group. The levels of CRP and fibrinogen decreased in the POG group during hospitalization. The levels of CRP, as well as the total leukocyte count were inversely correlated with the levels of 25-hydroxy vitamin D. This study brings new information on the interaction between vitamin D and pro-inflammatory markers and highlights the role of this vitamin in the modulation of the immune response in patients with moderate COVID-19.

Rezumat

Studiul și-a propus evaluarea rolului vitaminei D la pacienți diagnosticați cu COVID-19, formă moderată. Au fost incluși 128 de pacienți care au fost împărțiți, în funcție de evoluția clinică, în două grupuri. Grupul cu evoluție favorabilă (POG) a inclus 82 de pacienți, iar grupul cu evoluție nefavorabilă (NOG) a inclus 46 de pacienți. S-a determinat în două momente diferite (la admisia și la externarea pacienților) nivelul plasmatic al vitaminei D (25-hidroxi-vitamina D), împreună cu cel al markerilor inflamatori precum proteina C reactivă (PCR), lactat-dehidrogenaza (LDH), feritina, fibrinogenul, numărul total de leucocite și colesterolul total. Nivelul plasmatic al 25-hidroxi-vitaminei D a fost semnificativ mai scăzut în NOG comparativ cu POG, în timp ce nivelul LDH-ului și al PCR-ului în NOG au fost semnificativ mai mari comparativ cu cel găsit în POG. Nivelurile PRC-ului și al fibrinogenului au scăzut semnificativ în grupul POG pe timpul spitalizării. Nivelul plasmatic al PCR-ului, precum și numărul total de leucocite s-au corelat invers proporțional cu cel al 25-hidroxi-vitaminei D. Acest studiu aduce noi informații cu privire la interacțiunea între vitamina D și markerii pro-inflamatori și evidențiază rolul acestei vitamine în modularea răspunsului imun în infecția COVID-19, formă moderată.

Keywords: vitamin D, 25-hydroxy vitamin D, moderate COVID-19, C reactive protein, LDH

Introduction

Coronavirus disease 2019 (COVID-19) is the second pandemic of the twenty-first century [47]. The culprit for this new disease is a virus named Severe Acute Respiratory-Distress Syndrome Coronavirus-2 (SARS-CoV-2), a novel strain belonging to the *Coronaviridae* family. SARS-CoV-2 was first isolated, after a cluster of outbreaks, in Wuhan (China) [27]. This virus is a single stranded, positive-sense RNA virus with the main transmission way through respiratory droplets and fomites. COVID-19 has an unpredictable outcome, ranging from asymptomatic manifestation to severe

acute respiratory distress (ARDS) and even death [42, 48].

Several risk factors were identified for the unfavourable progression of COVID-19 as: older age, gender (men are more sensitive to SARS-CoV-2), associated pathologies (diabetes, cardiovascular disease, malignancy, chronic renal disease, diseases which cause immunodeficiency, autoimmune diseases) [34], elevated inflammation markers [20] and low levels of vitamin D [3].

Deficiency of vitamin D is considered a public health concern worldwide, with an estimate of one billion individuals being deficient and ~ 50% of the population having insufficient levels [31]. Several risk factors that lead to vitamin D deficiency can be pointed out, the main ones being age, geographic latitude and skin pigmentation [36]. Low vitamin D levels were associated with increased inflammation (due to the increased secretion of inflammatory cytokines) as well as increased risk of pneumonia and viral upper respiratory tract infections [29]. In COVID-19, low levels of vitamin D were associated with a higher incidence of thromboembolism [45] and acute respiratory failure [4]. The crucial role that this vitamin plays in stimulating immune response has been well demonstrated [21]. Vitamin D can stimulate both the adapted and innate immune system, due to its binding capability on the vitamin D receptor (VDR) of different immune cells [23]. VDR is found on activated T lymphocytes (CD4 and CD8) and also on macrophage membranes [24]. One of the main defences against viral infection and uncontrolled inflammation is provided by T regulatory lymphocytes. The subtype of lymphocytes was reported to be low in most COVID-19 and way increase after vitamin D supplementation [45].

C-reactive protein (CRP) is a non-specific, acute-phase marker of infection or inflammation. CRP was found to correlate with the severity of the disease as well as treatment response in several infectious and non-infectious conditions [7]. CRP levels were found elevated in patients with COVID-19 and were found to be correlated with the severity and disease progression [5, 17, 37].

Serum ferritin is generally considered a biomarker of iron-deficiency state, one of the main causes of anaemia. In the case of COVID-19 patients, ferritin can be regarded as an acute-phase protein and its plasmatic level can be elevated in the setting onset of inflammation [21]. High plasma levels of this protein were pointed out in many inflammatory states that involve activation of macrophage, such as sepsis or antiphospholipid syndrome. Ferritin was well correlated with the cytokine response, being considered a modulator of cytokine synthesis and a trigger factor for the cytokine storm [12]. Lactate dehydrogenase (LDH) is the catalyst in the last step of glycolysis [25]. LDH was found to be a potential prognosis marker in patients with COVID-19 [28].

The relationship between vitamin D and total cholesterol has already been pointed out in several studies; vitamin D was demonstrated to have an inverse relationship with total cholesterol [13]. Moreover, some studies show that supplementation with vitamin D in the case of cardiac patients could prove beneficial due to the reduction of the plasma level of total cholesterol [10].

Currently, limited data is available on the role vitamin D plays in moderate COVID-19. Due to this reason, the aim of this study was to assess the role that vitamin D, cholesterol and several inflammatory markers play in the progression of moderate COVID-19. We evaluated the intricate correlations between these markers at different moments. We also compared vitamin D, total cholesterol and the inflammatory markers between a positive outcome group and a negative outcome group, as well as at two different moments within the favourable outcome group.

Materials and Methods

Study population

A number of 128 patients (admitted to the Emergency County Hospital of Arad, Romania) with moderate COVID-19 symptoms were included in this study, between January and April 2021. The inclusion criteria were represented by a positive Polymerase Chain Reaction (PCR) test against SARS-CoV-2 as well as the signs and symptoms of moderate COVID-19. We define moderate COVID-19 as a patient who presents fever, evidence of lower respiratory disease during clinical assessment or imaging but has an oxygen saturation above or equal to 94% on atmospheric air [9, 39].

All the patients received medical care in accordance to the internal hospital guidelines with minor alterations. The treatment administered consisted of: dexamethasone 8 mg given intravenously every 12 hours, vitamin C given intravenously 750 mg every 12 hours, metamizole 1 g intravenously every 8 hours, ceftriaxone 1 g given intravenously every 12 hours, acetaminophen 500 mg given orally every 12 hours, rosuvastatin 20 mg was given orally every 12 hours, silymarin 5 mg given orally every 12 hours, enoxaparin 0.4 mg subcutaneous every 12 hours, aminophylline 125 mg intravenously every 12 hours and favipavir 600 mg orally every 12 hours. No vitamin D supplements were administered during the hospitalization.

Patients were divided into two groups based on their outcome after twelve days of hospitalization. If the patient presented markedly symptoms, amelioration and was discharged from the hospital, he was placed in the Positive Outcome Group (POG). Two determinations of the laboratory tests (25-hydroxy vitamin D, total cholesterol, total number of leukocytes, ferritin, CRP, LDH and fibrinogen) were performed in the POG group: the first blood drawn (POG1) of on the second day of admission and the second on the day of the

discharge (POG2). If the patient's general status deteriorated and the disease progressed to severe COVID-19 resulting in the patient being transferred to the Intensive Care Unit (ICU) or succumbing to their illness, they were placed in the NOG (Negative Outcome Group). For the NOG group a single determination of the laboratory parameters was made, on the second day since admission to hospital.

Patients with severe cardio-vascular disease (advanced heart failure, acute myocardial ischemia or severe arterial hypertension), diabetic ketoacidosis, morbid obesity (Body Mass Index (BMI) > 40), autoimmune diseases and cancers were excluded from this study.

Informed Consent and Ethics

This study was approved by the Ethics Committee of Emergency County Hospital of Arad, Romania. All participants were over 18 years old and signed a written informed consent.

Serum samples

Venous blood samples were collected using standard venepuncture techniques into serum separator clot activator tubes and EDTA Blood Collection Tubes between 9 a.m. and 10 a.m. while the patients were in a fasted state (due to overnight fasting). The filled separator clot activator tubes were further centrifuged at 2000x g for 10 min within 1 hour of collection, then immediately processed on the following platforms: Cobas 6000's module e601 and module c501 (Roche Diagnostics, Mannheim, Germany), STA-Compact-Max 3 analyser (Stago, Asnieres sur Seine, France) and Sysmex® XN-1000 (Sysmex Corporation, Kobe, Japan). We excluded from this study lipaemic, haemolytic and icteric sera, samples with insufficient volume and samples collected in inadequate test tubes.

Biochemical assays

The level of 25-hydroxy vitamin D was determined using electrochemiluminescence on Cobas e411 (Roche Diagnostics, Mannheim, Germany). Total number of leukocytes was determined using the haematology analyser Sysmex® XN-1000 (Sysmex Corporation, Kobe, Japan). Ferritin was determined using ECLIA on Cobas e601. Fibrinogen was determined using STA-Compact-Max3 analyser (Stago, Asnieres sur Seine, France). Total cholesterol, CRP and LDH were determined using Cobas c501 analyser. All determinations were in accordance with manufacturer's instructions and internal laboratory standards.

Statistical analysis

Data was collected using Microsoft Excel, version 2011 (Microsoft Corp., Redmond, WA, USA). Statistical analyses were performed using free and open software JAMOVI 1.6.23 (retrieved from https://www.jamovi.org). Shapiro-Wilks's test was used in order to find the distribution of the values in each group. Mann-Whitney U statistical test was used for comparison of the non-parametric distribution values of the analysed groups. The Wilcoxon U statistical test was used for comparisons of non-parametric distributions within the same group. Student's t test was used for parametric distributions. The Pearson coefficient and the p value were calculated in case of each correlation, $p < 0.05$ was considered of statistical significance.

Results and Discussion

A total number of 128 patients with a positive PCR test and moderate COVID-19 signs and symptoms were included in this study. The mean age of the patients included in the study was 66 ± 13 years old ranging from 33 to 89 years old. Regarding gender, 74 (57.8%) were females and 54 (42.2%) were males. In terms of area of residence, 92 (71.9%) were residing in urban areas while 36 (28.1%) were residing in rural areas.

Out of the 128 patients, 86 (67.19%) were included in the POG group and 42 (32.81%) were included in the NOG group. Out of the 42 patients included in the NOG, 18 (42.86%) were transferred to the ICU and 24 (57.14%) succumbed.

Data analysis between POG1 and NOG groups

The levels of 25-hydroxy vitamin D in the POG1 group were statistically significantly higher than the levels of 25-hydroxy vitamin D in NOG ($p = 0.025$) (Figure 1A). No statistically significant levels were found when the total cholesterol levels were compared between POG1 and NOG groups ($p = 0.632$) (Figure 1B).

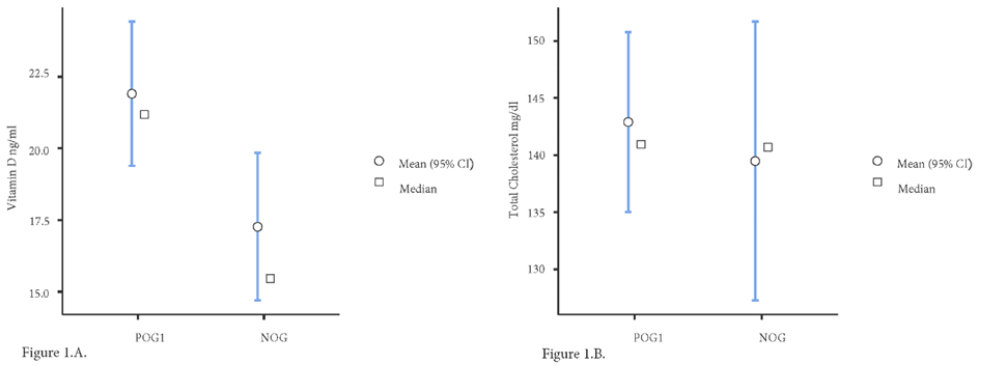

Figure 1.
25-hydroxy vitamin D, and total cholesterol in POG1 and NOG

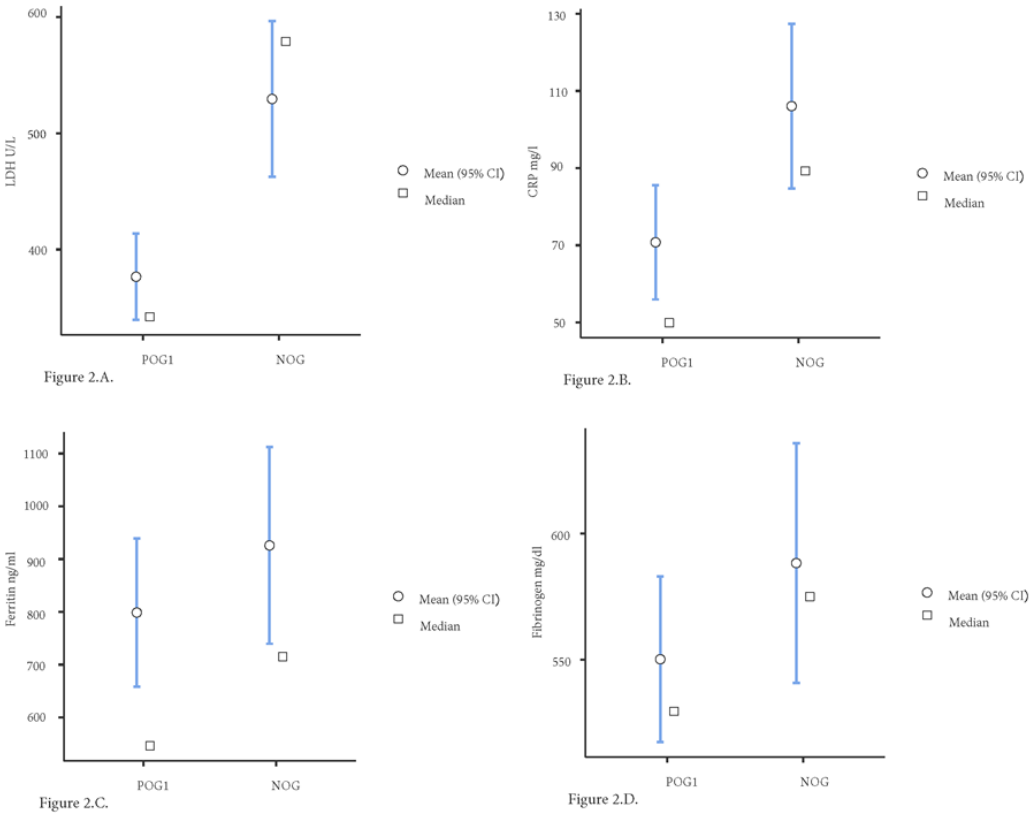

Figure 2.
LDH, CRP, ferritin and fibrinogen in POG1 and NOG groups

When inflammatory markers were compared, NOG patients presented statistically significant higher levels of LDH ($p < 0.001$) (Figure 2A) and CRP ($p = 0.003$) (Figure 2B) compared to POG1 patients.

No statistically significant difference was found in case of ferritin ($p = 0.156$) (Figure 2C) and fibrinogen ($p = 0.189$) (Figure 2.D.).

Data analysis between POG1 and POG2 groups

POG2 patients had a statistically significant higher total cholesterol levels compared to POG1 group ($p = 0.037$). The only inflammatory markers which showed a significant statistical decrease were CRP ($p < 0.001$) and fibrinogen ($p = 0.044$) No significant changes were observed in leukocytes, LDH and ferritin between POG1 and POG2 groups. No significant difference was registered between the levels of vitamin D ($p = 0.054$).

An inverse correlation was obtained between the levels of 25-hydroxy vitamin D, CRP ($r = -0.201$, $p = 0.023$) and the total number of leukocytes ($r = 0.251$, $p = 0.004$) within POG1 group of patients. No correlations were found between 25-hydroxy vitamin D and fibrinogen ($r = -054$, $p = 0.542$), ferritin ($r = 0.042$, $p = 0.635$) as well as LDH ($r = -0.082$, $p = 0.357$). Two highly significant positive correlations were obtained between CRP and fibrinogen ($r = 0.523$, $p < 0.001$) and between CRP and LDH ($r = 0.289$, $p < 0.001$) in POG1 group. Also, for the same group of patients, a highly significant positive correlation was obtained between fibrinogen and ferritin ($r = 0.262$, $p = 0.003$) but not between fibrinogen and LDH ($r = 0.154$, $p = 0.082$). A statistically significant positive correlation was also obtained between LDH and ferritin ($r = 0.261$, $p = 0.003$).

Regarding the POG2 group of patients, no statistically significant correlations were obtained between vitamin D and CRP ($r = 0.1$, $p = 0.372$), fibrinogen ($r = -0.008$, $p = 0.947$), ferritin ($r = 0.149$, $p = 0.181$), LDH ($r = 0.175$, $p = 0.116$) and total number of leukocytes ($r = -0.077$, $p = 0.491$). A statistically significant positive correlation was noticed between ferritin and CRP ($r = 0.246$, $p = 0.026$) as well as LDH ($r = 0.249$, $p = 0.024$). No correlation was found between CRP and fibrinogen ($r = 0.113$, $p = 0.314$). No correlation was found between fibrinogen and ferritin ($r = 0.104$, $p = 0.352$) as well as LDH ($r = 0.205$, $p = 0.064$). No correlation was found between ferritin and LDH ($r = 0.213$, $p = 0.055$).

We also found an additional inverse correlation in this group of patients between the value of vitamin D and the level of total cholesterol ($r = -253$, $p = 0.022$).

The role of vitamin D as a marker of prognosis in COVID-19 patients was demonstrated by the difference obtained in the values between the two groups (POG1 and NOG). This result is consistent with several other studies [1, 15] which found the values of 25-hydroxy vitamin D to be an important prognostic factor. No fluctuations were pointed out in the levels of 25-hydroxy

vitamin D in two weeks of hospitalization (between POG1 and POG2). Due to the lack of a second determination of 25-hydroxy vitamin D in NOG we cannot hypothesize that this maintenance of 25-hydroxy vitamin D plasma levels is one of the factors which indicates a favourable clinical evolution.

Total cholesterol levels were significantly higher in the POG2 when they were compared to POG1. Poor appetite was reported during the COVID-19 infection [32] and it is considered a symptom in moderate COVID-19 [35]. A possible explanation of the increase in total cholesterol may be the regain of appetite due to the amelioration of the underlying disease. Several studies [30, 33] have also reported the effects of stress on the total cholesterol levels. Considering that stress levels are higher in the general population during the COVID-19 pandemic [38] and that being hospitalized is a stressful situation, elevated total cholesterol levels may be considered a response to this situation. Further studies are needed to assess the mental status as well as the in-hospital diet of patients with moderate COVID-19.

The level of 25-hydroxy vitamin D appears to be in an inverse correlation with the levels of total cholesterol. Similar results were obtained by Glueck *et al*. [13] in hyperlipidaemic patients.

CRP was the only inflammatory marker found in this study to hold a significant importance as a favourable prognostic factor due to its statistically significant decrease between POG1 and POG2 groups of patients. We also found statistically significant higher values of CRP in NOG patients compared to POG1 group. These results are similar to those obtained by other researches [26, 40, 46]. CRP is the only inflammatory marker that inversely correlated with vitamin D levels, further supporting the idea that vitamin D plays a main role in attenuating the inflammatory response [11].

Our results pointed out the statistically significant higher levels of LDH between the POG and the NOG groups of patients. LDH was found to be higher in patients with a negative outcome in COVID-19, as pointed out by several other studies [16, 44]. Tests used to measure LDH in the blood are often used in order to monitor the tissue damage associated with a wide range of diseases such as interstitial lung disease [2]. LDH has also been identified to be an important marker for the activity and severity of idiopathic pulmonary fibrosis [22]. An increase in LDH levels in COVID-19 patients may suggest tissue and cell destruction and may be issued as a sign of lung damage (i.e. pneumonia) [2].

In our study, we did not recorded any statistically significant changes in the ferritin levels amongst the studied groups. A possible explanation may be that ferritin levels, as Vargas *et al*. pointed out, are increased only in severe and highly severe COVID-19 [43], while our study we only evaluated ferritin in moderate COVID-19 (on admission to hospital). Several other reports only considered ferritin on admission and found the values within normal range [14, 19]. Although ferritin proved a useful biomarker in severe COVID-19 [6], our study found no prognostic value at the moment of admission, in patients with moderate COVID-19.

Our research did not clearly demonstrate the possible role that fibrinogen may have as a prognostic factor. Our data indicates a decrease in the values of fibrinogen barely reaching statistical significance between POG1 and POG2 groups of patients. Fibrinogen may be considered in some cases an acute phase protein [41]. Evaluation of this parameter in COVID-19 patients is controversial, considering that most studies indicated the risk of microvascular thrombosis in these cases [8, 18].

We admit as a limitation of our study the back of evaluation of the values of cytokines such as interleukin 1 and 6, as well as not performing a second assay for the NOG group.

Conclusions

Deficiency of vitamin D assessed using 25-hydroxy vitamin D is one of the risk factors for infection in general and, based on our findings, SARS-CoV-2 does not appear to be an exception. This study revealed the association between vitamin D deficiency and an increased risk of more serious clinical outcomes for moderate COVID-19 infected patients. Data obtained also demonstrated that the level of 25-hydroxy vitamin D, besides CRP plasma level, could be considered a marker for the progression of this disease.

Supplementation with vitamin D may be a safe and efficient option in moderate cases of COVID-19 and may also play a role in the prevention of severe COVID-19.

Conflict of interest

The authors declare no conflict of interest.

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