Vitamin D plasma levels in patients with COVID-19: a case series

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SUMMARY

Vitamin D deficiency has been associated to respiratory tract infections. We aimed to investigate vitamin D plasma levels in patients with chest infection with and without COVID-19 in a hospitalized population during the second pandemic wave. A prospective study was conducted in a Mediterranean tertiary center referring to 80 patients suffering from chest infection, who were divided into two groups according to a positive test for SARS-CoV-2 infection. The hospitalized COVID-19 patients had a high prevalence of vitamin D deficiency, and these patients also exhibited higher levels of plasma inflammatory markers. Intensive research is required to identify the role and mechanisms of vitamin D in patients with SARS-CoV-2 infection and its possible role as a prognostic factor of the disease.

Keywords: Plasma vitamin D, SARS-CoV-2 infection.

INTRODUCTION

The SARS-CoV-2 induced disease 2019 (COVID-19) outbreak, which began in China in late December 2019, spread rapidly all over the world, putting the health authorities on high alert. Epidemiological and clinical characteristics of patients with COVID-19 have been reported but risk factors for mortality, illness severity and length of hospitalization are still under investigation. Among other potential factors, vitamin D may play a role in the pathophysiological process of COVID-19. Vitamin D is a steroid with various immunomodulatory, anti-inflammatory, anti-fibrotic, and anti-oxidant actions. Specifically, calcitriol, the active form of vitamin D, plays an important role in immune function. It inhibits the expression of inflammatory cytokines (IL-1a, IL-1b, TNF-a) and its lack is associated with over-expression of Th1 cytokines [1].

There are growing evidence that vitamin D may play a role in the pathophysiological processes of COVID-19. In several meta-analyses with COVID-19 positive patients, low vitamin D level was significantly associated with poorer outcome and prognosis. It seems that vitamin D deficiency plays an independent causative role in the manifestation of disease severity [2].

The implications of vitamin D deficiencies are more pronounced among elderly patients who are more susceptible in developing SARS-CoV-2 infection and have a higher mortality rate. Briefly, in elderly population, the production of naïve T and B cells decreases, and the function of innate immune cells is impaired [3]. That might worsen due to a concomitant deficiency of vitamin D, which is very common in these individuals as a result of a lower intake [4, 5].

The aim of this study was to evaluate the plasma levels of vitamin D in patients with COVID-19 in comparison to non-COVID-19 patients who also suffered from chest infection, and to identify any potential relation with outcome. The study also aimed to assess any potential correlation between vitamin D level and inflammatory markers.

PATIENTS AND METHODS

This is a prospective study conducted in the University Hospital of Patras, Greece, during Novem-

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ber 1st, 2020 to December 31st, 2020. Participants were patients with chest infections who were admitted to the Department of Internal Medicine of the University Hospital of Patras. All patients were initially treated in an isolated sub-ward of the Department, modified according to all the necessary precautions, as results from the SARS-CoV-2 test were available after some hours. Patients were divided into two groups, *i.e.* group 1: patients whose Real-Time Polymerase Chain Reaction (RT PCR) forward SARS-CoV-2 infection confirmed positive (n=21) and group 2: patients whose RT PCR for SARS-CoV-2 infection was negative (n=59). Written informed consent was obtained from all enrolled patients or a relative when necessary. The Ethics Committee of the Hospital approved the study protocol. Demographic characteristics (age, gender, BMI), laboratory findings and final outcome of the enrolled patients are shown in Table 1. The laboratory parameters included White Blood Cells (WBC), Absolute Lymphocyte Count (ALC), coagulation parameters (PLTs, fibrinogen, d-dimers), C-reactive protein, lactic acid plasma levels, lactate dehydrogenase (LDH) and creatinine kinase (CPK), and 25 OH-Vitamin D plasma levels. According to the Hospital's Laboratory referral values, normal values of the vitamin were >30 ng/mL, levels 21-29 ng/mL were considered as vitamin D deficiency, and levels <20 ng/mL were considered as severe deficiency. We excluded any patient who was on Vitamin D supplements treatment.

Statistical analysis

Statistical analysis of data was performed using the SPSS-25 statistical software. The minimum value of the level of statistical significance (*p*-value) was set at 0.05. The Pearson correlation was used for studying relationships between variables. Chi-Square and the Mann-Whitney test were used to investigate the differences of a continuous variable to two different and independent population groups.

RESULTS

Eighty patients were enrolled in the study. The mean age of the participants was 64.9±18.3 years; 38% (n=31) were female and 62% (n=49) were male. The mean concentration of vitamin D was 23.53±10.6 ng/mL in the group of patients with SARS-CoV-2 infection (group 1) and 21.86±11.7 ng/mL in the group of patients with a negative test for SARS-CoV-2 infection (group 2). The difference between the two groups (group 1 *vs* group

Table 1 - Epidemiological data, laboratory findings and outcome of the two groups of patients.

	COVID-19 patients	*Non-COVID-19 patients*	*p value*
Gender (n) (M/F)	12/9	37/22	0.879
Number (n) of patients with Vitamin D >30mg/dl	6 (out of 21)	15 (out of 59)	
Age (years)	61.8±16.6	66.9±18.7	0.031
BMI	30.9±7	28.2±6.7	0.131
WBC (K/μL)	6651±3120.8	9516.8±3872.9	0.015
Lymphocytes (K/μL)	1074±679.6	1659.1±1351.1	0.044
Fibrinogen	581.8±197.7	576.35±384.4	0.486
d-dimer	1485.6±1748.4	2032.1±2719.2	0.457
LDH (U/L)	356.6±171.3	263.3±111.8	0.010
CPK(U/L)	179.2±224.8	218.8±458.4	0.347
CRP (mg/dL)	8.38±7.7	9.8±8.6	0.720
Lactate	1.38±0.8	1.31±0.54	0.850
Vitamin D (mg/dL)	23.53±10.6	21.86±11.7	0.486
Length of hospitalization (days)	4.9±4.3	3.6±3.2	0.759
Outcome (n) (survival/death)	15/6	55/4	0.004

2) was not statistically significant (p= 0.486). A total of 21 patients (26.25%) were diagnosed with COVID-19 (group 1). The mean age in this group was 61.8±16.6 years; 57.9% were male and 42.1% were female. The prevalence of vitamin D deficiency (21-29 ng/mL) in group 1 was 52.4%. From the eleven patients diagnosed with SARS-CoV-2 infection and vitamin D deficiency, 9 (42.8%) were found to be severely deficient (levels <20 ng/mL). Among them, 4 were critically ill and 2 died. In group 2, 19 from 59 patients (32.2%) were vitamin D deficient.

Among COVID-19 patients, we found statistically significant differences between those who had vitamin D deficiency and severe vitamin D deficiency in relation to the clinical outcome (patients who were clinically improved and discharged *vs* those who died) (27±3 *vs* 15.4±6, p=0.002).

LDH was statistically significant higher in the group of COVID-19 patients with vitamin D deficiency compared with those patients with normal vitamin D levels (388.2±183.9 *vs* 294.5±80.3, p=0.049). Further analysis revealed higher BMI among group 1 patients with vitamin D deficiency (<30 ng/mL) when compared with those COVID-19 patients with normal vitamin D levels (31.32±12 *vs* 27.75±5.15, p=0.006).

The analysis of the serum level of inflammatory markers, in the group of COVID-19 patients, revealed several differences. Absolute lymphocyte count was lower in patients with vitamin D deficiency (985±377.1 *vs* 1032.9±913.9, p=0.325). Fibrinogen was higher, but not statistically significant, in the group of COVID 19 patients with vitamin D deficiency when compared with those who had normal vitamin D levels (673.4±205.2 *vs* 502.6±157.8, p=0.09) while d-dimers were lower in the same group of patients (1682.5±2228.1 *vs* 2963.75±5001.1, p=0.36). Lactic acid, ferritin, and CRP were higher in COVID 19 patients with vitamin D deficiency when compared with those with normal vitamin D levels but the differences were not statistically significant (1.56±1.0 *vs* 1.2±0.45 p=0.556, 1143.8±940.2 *vs* 825±602 p=0.670 , 10.8±7.9 *vs* 6.5±6.8 p=0.093). All measurements are shown in Table 2.

DISCUSSION

In this study we found that serum 25(OH) vitamin D deficiency is more prevalent in hospitalized

Table 2 - Comparison of patients with and w/o Vitamin D deficiency in each group of patients.

	COVID-19 patients			*Non COVID-19 patients*		
	Vitamin D-deficient patients	*Vitamin D-non-deficient patients*	*P value*	*Vitamin D-deficient patients*	*Vitamin D- non-deficient patients*	*P value*
Gender (n) (M/F)	6/6	4/5	0.075	31/13	9/6	0.645
Age (years)	60.5±17.9	61.1±16.6	0.94	67.34±17.8	70.0±22.4	0.5
BMI	31.32±12	27.75±5.15	0.006	29.13±6.7	23.75±2.4	0.580
WBC (K/μl)	9346.3±5239.3	6631±3120.8	0.121	9752.06±3044.8	9292.7±5679.7	0.525
Lymphocytes (K/μL)	985±377.1	1032.9±913.9	0.325	1586.12±1201.6	1442±964.6	0.329
Fibrinogen	673.4±205.2	502.6±157.8	0.09	617.07±483.6	475.4±148.9	0.274
d-dimer	1682.5±2228.1	2963.75±5001.1	0.36	2069.33±2474	2260.7±3752.6	0.349
LDH (U/L)	388.2±183.9	294.5±80.3	0.049	270.6±129.7	285.7±148.07	0.457
CPK (U/L)	261,3±298.9	478.75±1053.3	0.653	128.4±148.3	392.3±826.1	0.560
CRP (mg/dL)	10.8±7.9	6.5±6.8	0.093	16.73±10.2	7.5±7.8	0.651
Lactate	1.56±1.0	1.2±0.45	0.556	1.46±0.46	1.18±0.4	0.820
Length of hospitalization (days)	10	1	0.134	4	3	0.777
Outcome (n) (survive/death)	8/4	6/3	0.503	42/2	11/4	0.759

COVID-19 patients when compared to patients with non-COVID-19 chest infection during the same period of hospitalization. It is well known that low vitamin D levels are likely sufficient to optimize skeletal effects in mankind and such effects of vitamin D are detectable when serum vitamin D levels are >20 ng/mL, whereas the extra-skeletal effects of vitamin D (as immune function modulation and increase of immune response activity) are observed when serum vitamin D levels are >30 ng/mL [6]. Moreover, it is already known that low plasma levels of vitamin D are associated with increased susceptibility to several infections [7, 8]. Vitamin D is a hormone with a pleiotropic role and there is great evidence of an epidemiological association between low serum levels of 25(OH) vitamin D and human viral infections such as influenza, HIV, hepatitis virus. The interaction between vitamin D and viral infection is of great interest. Immunomodulatory effects, induction of autophagy and apoptosis, have been reported as antiviral effects of the hormone [9]. Additionally, vitamin D plays a significant role in the immune system by inhibiting the adaptive immune system and by promoting the innate immune system. It interferes with the majority of cells of the immune system such as macrophages, B and T lymphocytes, neutrophils and dendritic cells. Both band of lymphocytes can form the active metabolites of vitamin D, which inhibits T lymphocytes proliferation and activation. Moreover, vitamin D inhibits the production of pro-inflammatory cytokines and enhances the production of anti-inflammatory cytokines [1].

The relation between vitamin D plasma level and SARS-CoV-2 infection and outcome remains a matter of debate. Vitamin D has a number of immunomodulatory effects that could potentially protect against SARS-CoV-2 infection or decrease the severity of the illness. The potential antiviral activity of vitamin D, due in general to its capability of modulating the cell plasmatic membrane fluidity and membrane lipid rafts. Some studies have suggested that the infectivity of viruses is stimulated by homeostatic control of cholesterol and regulation of fatty acid metabolism [10]. As reported in the studies, vitamin D alone or in association with tocilizumab is able to reduce IL-6 activity and to promote the generation of Foxp3+ T-cells and to counteract IL-17 production. These cells modulate the immune response and contribute to turn off the production of pro-inflammatory cytokines [11]. The role of vitamin D supplementation in the prevention or treatment of COVID-19 is yet not known [12]. The results from studies at the moment are conflicting; however, these are based on different studied populations. Some variables that should be considered are the vitamin D deficiency in the elderly and in specific populations such as the African American patients, and the close relation of vitamin D deficiency to several chronic diseases. In the study by Hernandez et al., 25-OH vitamin D levels were found lower in hospitalized COVID-19 patients than in population-based controls and these patients had a higher prevalence of deficiency. No relationship between vitamin D concentrations or vitamin deficiency and the severity of the disease was found [9]. Vitamin D deficiency has been found also to occur more frequently in diabetic patients and obese population and these conditions are related to higher COVID-19 mortality rates. It is reasonable to consider giving vitamin D supplements to these populations in order to decrease the impact of the pandemic [13]. In the prospective cohort study by Baktash et al. it is concluded that older adults with vitamin D deficiency and COVID-19 may demonstrate worse morbidity outcomes [14]. Vitamin D status may be a useful prognostic factor. In the narrative review by Rhodes et al., it is indicated a substantial link between the vitamin D deficiency and COVID-19 severity [15]. In the report by Murdaca et al., key points mention the role of vitamin D as a factor which increases the expression and concentration of ACE2, MasR and Ang-(1-7) leading to a potential protective role against acute lung injury and acute respiratory distress syndrome. However, despite the potential effectiveness of vitamin D as an antiviral, more clinical studies are needed to define the best cut off levels and, finally, the optimal dosage [16].

Another finding of our study is the potential significant association between low vitamin D levels in COVID-19 patients and the common measured inflammatory markers. COVID-19 patients with vitamin D deficiency exhibited higher levels of serum markers of inflammation, such as WBC, fibrinogen, LDH and CRP compared to non-deficient vitamin D COVID-19 patients. This finding mentions that vitamin D deficiency could contribute to the possibility of having more severe disease after infection with SARS-CoV-2. All above

could direct research towards further investigation of the role of vitamin D in patients with SARS-CoV-2 infection.

This study was conducted in a western Greek tertiary center during the second wave of COVID-19 pandemic covering a period of 2-monthtime. It gives some information in regards to vitamin D deficiency and COVID-19 referring to a Mediterranean population in a country where lockdown measurements gave sufficient results, decreasing the spread of SARS-CoV-2 infection. Our study has some limitations. At first, it is a single-center study with a small number of patients. Secondly, the assessed plasma parameters were referring only to the admitted to hospital patients, which may impact upon inflammatory markers due to the disease severity. More multicenter studies are warranted to further objectively confirm conclusions about the role of vitamin D in COVID-19 infection.

In conclusion, the role of Vitamin D status in COVID-19 patients is a matter of debate. Whether vitamin D plays a role in COVID-19 severity, needs to be confirmed with prospective randomized multi-center trials based on the specific limitations of the levels of the vitamin among populations and underlying diseases. The supplementation of vitamin D aiming to reach normal plasma levels, should be carefully evaluated as a preventing factor of the COVID-19.

Conflict of interest

The authors declare no competing interests.

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