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Afr. J. Biomed. Res. Vol. 27 (May 2024); 305- 308

Research Article

Plasma Levels of Vitamin D and C-Reactive Protein in Adult Nigerians with COVID-19

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ABSTRACT

Vitamin D deficiency (VDD) has been associated with increased mortality in patients with COVID-19. The anti-inflammatory properties of vitamin D are vital when considering the heightened inflammation in COVID-19 patients. Although avalanche of reports are available on the plasma levels of vitamin D and C-reactive protein (CRP) in COVID-19 patients, information on the dynamics of CRP level changes in different categories of vitamin D status is lacking. Therefore, the plasma levels of CRP were determined in COVID-19 patients with different vitamin D status. Forty nine adults comprising 31 COVID-19 patients and 18 apparently healthy participants who served as controls were enrolled into this study. The plasma levels of high sensitivity-CRP (hsCRP) and vitamin D were determined using ELISA. Vitamin D status was classified as deficient, insufficient, sufficient and intoxication as appropriate. None of the COVID-19 patients had vitamin D insufficiency while majority of the controls were vitamin D sufficient. The proportion of COVID-19 patients with vitamin D intoxication was significantly higher than in the controls. The median plasma levels of vitamin D and hsCRP were not significantly different between the COVID-19 patients and the controls. The median plasma level of hsCRP was slightly lower in the vitamin D intoxication group compared with the vitamin D sufficient group. Vitamin D intoxication is common among Nigerian patients with COVID-19 and the vitamin D intoxication is associated with low plasma hsCRP level. There is the need for public enlightenment on the dangers inherent in vitamin D supplement abuse in Nigerians.

Keywords: *Acute phase protein, Anti-inflammation, COVID-19, Vitamin D intoxication*

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Received: August 2022; Accepted: February 2023

DOI: https://doi.org/10.4314/ajbr.v27i2.14

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INTRODUCTION

Coronavirus disease (COVID-19) is a global pandemic caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). The high morbidity and mortality rate of the disease is associated with a number of factors (Richardson *et al.,* 2020). These factors include presence of co-morbidities, nutritional deficiencies and gender among others (Rozenberg *et al.,* 2020; Velavan and Meyer 2020; Zhou *et al.,* 2020; Li *et al.,* 2021; Arinola *et al.,* 2021).

Adequate and balanced nutrition are essential for proper body functioning and boosting of the immune system. Deficiencies of vitamins especially, vitamin D has been associated with increased mortality in critically ill patients (Matthews *et al.,* 2012; Gombart *et al.,* 2020).

Vitamin D is a pleiotropic hormone with critical role in maintenance of health (Lee 2011). It is involved in calcium and phosphorous homeostasis and also has antimicrobial and immunomodulatory properties (Amrein and Venkatesh 2012). In immune response, vitamin D plays important roles in the regulation of both the innate and adaptive immune system. Several cells of the immune system including neutrophil, monocytes, macrophages, B-cells and T-cells express vitamin D receptor (VDR) which directly or indirectly modulate their activities towards achieving immunity (Martens *et al.,* 2020). Vitamin D deficiency (VDD) is the most common vitamin deficiency which is seldom recognized even in the general population. Up to 50% of critically ill patients are vitamin D deficient and this has been shown to result in a 3-fold increase in mortality (Flynn *et al.,* 2012). Vitamin D possesses anti-inflammatory and immune supporting properties and has thus gained tremendous attention during the COVID-19 pandemic. VDD has been shown to decrease the immune defences against COVID-19 and cause progression to severe disease.

VDD was reported as the most prevalent micronutrient deficiency in COVID-19 patients as 76% and 24% of patients studied by Im *et al.,* (2020) had moderate and severe VDD respectively. This observation is further elucidated by the reported inverse links between vitamin D and COVID-19 infection as well as outcomes (Chandran *et al.,* 2020; Merzon *et al.,* 2020). Similar reports have also shown that low vitamin D levels are associated with high mortality rate in COVID-19 patients (D'Avolio *et al.,* 2020; Panagiotou *et al.,* 2020; Shakoor *et al.,* 2021).

The anti-inflammatory properties of vitamin D are vital when considering the heightened inflammation occasioned by cytokine storm in COVID-19 patients (Arinola 2020; Daneshkhah *et al.,* 2020). One of the important acute phase proteins usually used as a biomarker for acute and chronic inflammation is C-reactive protein (CRP) (Arinola, 2020). It is a non-specific acute phase protein whose high levels and bimodal distribution have been used in triage, diagnostics and prognostication of COVID-19 patients (Liu *et al.,* 2020; Stringer *et al.,* 2021). Ruan *et al.,* (2020) reported that CRP levels have a strong correlation with COVID-19 severity and prognosis. They showed that non-survivors had CRP levels about three-times higher than the levels in the survivors. Similarly, Sadeghi-Haddad-Zavareh *et al.,* (2021) showed that plasma CRP level is a good predictor of COVID-19 severity and progression.

Although reports are abound on the plasma levels of vitamin D and CRP in COVID-19 patients, information on the dynamics of CRP level changes in different categories of vitamin D status is lacking. This thus serves as the basis for this study.

MATERIALS AND METHODS

Study Design: This study was a case-control study.

Study Population: A total of 49 participants were enrolled into this study. They comprised 31 patients with COVID-19 (not in severe or critical stage) and 18 age- and gender-matched apparently healthy participants who served as controls. All the COVID-19 patients were confirmed positive while the controls were certified negative using the real-time reverse-transcriptase polymerase-chain reaction (RT-PCR) assay following WHO guideline (WHO, 2020). The COVID-19 patients were followed up from the point of diagnosis till discharge from the Isolation Centre.

Sample collection: Venous blood (10 mL) was obtained from each SARS-CoV-2 infected patient upon diagnosis and at discharge and at enrolment only in controls. The blood samples were dispensed into lithium heparin containing sample bottles and plasma samples obtained were kept at -20 ^{o}C until analyzed.

Ethical consideration*:* Ethical approval was obtained from the University of Ibadan/University College Hospital (UI/UCH) Joint Ethics Review Committee. Also, informed consent was obtained from the study participants.

Laboratory analyses: The plasma levels of vitamin D and hsCRP were determined using ELISA following the Manufacturer's instruction (Calbiotech Inc., USA).

Vitamin D classification: The plasma vitamin D level was classified as reported by Kader *et al.* (2019). Vitamin D levels of <10 ng/mL, 10 – 30 ng/mL, 30 – 100 ng/mL and >100 ng/mL were considered as deficiency, insufficiency, sufficiency and intoxication respectively.

Statistical Analysis

Statistical analysis was carried out using Mann Whitney U, Fisher's Exact test and Spearman rank correlation as appropriate. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

The vitamin D status of the study participants are shown in Table 1. None of the study participants had vitamin D deficiency. Also, no COVID-19 patient had vitamin D insufficiency whereas 11.1% of the controls had vitamin D insufficiency. The proportion of COVID-19 patients with vitamin D intoxication was significantly higher than in the controls. Unlike the COVID-19 patients, majority of the controls were vitamin D sufficient (Table 1).

Table 1:
Distribution of the vitamin D status of the study participants. Data shown are frequency; n (%).

Category	COVID-19	Control	*N*	*P*-value
Deficiency	0 (0.0)	0 (0.0)	0	0.038*
Insufficiency	0 (0.0)	2 (11.1)	2	
Sufficiency	15 (48.4)	12 (66.7)	27	
Intoxication	16 (51.6)	4 (22.2)	20	

*Significant at $P<0.05$

The median levels of vitamin D were not significantly different between the COVID-19 patients and the controls (*P*-value = 0.059). Similarly, the median levels of hsCRP were similar between the 2 groups (*P*-value = 0.173) (Table 2).

Table 2:
Baseline plasma levels of vitamin D and high sensitivity C-reactive protein (hsCRP) in COVID-19 patients and the controls

Parameters	COVID-Diagnosis (n = 31)	Controls (n = 18)	*P*-value
Vitamin D (ng/mL)	100.39 (66.48 - 134.49)	72.49 (47.23 - 98.34)	0.059
hsCRP (mg/L)	1.56 (0.54 - 7.62)	2.09 (1.46 - 5.79)	0.173

There were no significant changes in the median plasma levels of vitamin D and hsCRP at diagnosis compared with the levels at discharge (Table 3).

Table 3:
Plasma levels of vitamin D and hsCRP in COVID-19 patients at diagnosis and at discharge

Parameters	COVID-Diagnosis (n = 31)	COVID-Discharge (n = 31)	*P*-value
Vitamin D (ng/mL)	100.39 (66.48 - 134.49)	91.93 (68.56 - 124.02)	0.695
hsCRP (mg/L)	1.56 (0.54 - 7.62)	1.39 (0.63 - 5.77)	0.975

The correlation between vitamin D and hsCRP is shown in Figure 1. Vitamin D had an insignificant inverse correlation with CRP (r-value = -0.095; P-value = 0.471).

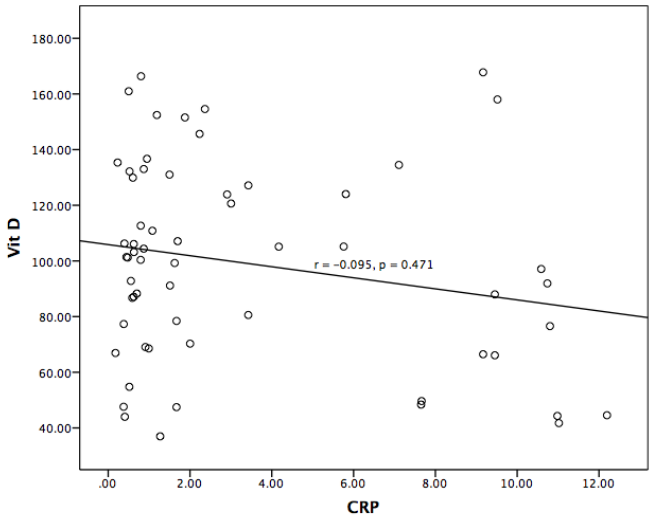

Figure 1:
Correlation between the plasma level of vitamin D and hsCRP in COVID-19 patients

To further understand the relationship between the vitamin D status and the plasma levels of hsCRP in COVID-19 patients, the hsCRP levels between the vitamin D sufficient and vitamin D intoxication groups were compared. It was observed that the median plasma level of hsCRP was nonsignificantly slightly lower in the vitamin D intoxication group compared with the vitamin D sufficient group (*P*-value = 0.633) (Table 4).

Table 4:
Plasma levels of CRP in different classes of vitamin D

Parameter	Vitamin D sufficient (n = 14)	Vitamin D intoxication (n = 16)	*P*-value
hsCRP (mg/L)	1.65 (0.52 - 10.69)	1.29 (0.56 - 2.85)	0.633

DISCUSSION

Vitamin D and CRP play important but opposing roles in immune responses. Vitamin D possesses anti-inflammatory properties while the CRP is an acute phase reactant involved in the inflammatory responses. The observed high proportion of COVID-19 patients with vitamin D intoxication is a source of concern. This observation might not be unconnected with indiscriminate use of various drugs and supplements such as vitamin D upon noticing signs and symptoms of SARS-CoV-2 infection by the study participants with COVID-19. This act could be occasioned by poor knowledge about COVID-19 and prevailing apprehensive situations with a view to boosting immunity against SARS-CoV 2 infection especially, during the early phase of the pandemic. Our observation is of public health importance as there is the need for public enlightenment on self-medication in future epidemics or pandemics. It is well known that vitamin D toxicity causes hypercalcemia, formation of calcium stones and kidney injury. The resulting hypercalcemia can also have profound effects on cardiac, renal and central nervous system functions (Waterbury 2018; Lim and Thadhani 2020). Effects on the aforementioned organs can result in direct and/or indirect impacts on the immune response since physiological organ cross-talk is essential in maintaining equilibrium and homeostasis (Peesapati *et al.*, 2020).

Reduced levels of vitamin D have been reported in COVID-19 patients and deficiency has been associated with high mortality rate in the patients (Chandran *et al.*, 2020; Merzon *et al.*, 2020; D'Avolio *et al.*, 2020; Panagiotou *et al.*, 2020; Shakoor *et al.*, 2021). In this study, a contrast observation was made as there was no significant difference in the plasma levels of vitamin D in COVID-19 patients compared with the controls. Our observation could be linked to the observed prevailing vitamin D intoxication in the studied COVID-19 patients. This could also explain the observed similar levels of CRP between the two groups. Although reports have shown that COVID-19 is associated with hyperinflammation which is reflected in CRP elevation (Liu *et al.*, 2020; Ruan *et al.*, 2020; Sadeghi-Haddad-Zavareh *et al.*, 2021; Stringer *et al.*, 2021), the COVID-19-associated hyperinflammation could have been masked by the expected heightened anti-inflammatory activities of vitamin D occasioned by high proportion of vitamin D intoxication in COVID-19 patients.

Downward trend in CRP level has been reported in hospitalized patients with COVID-19 (Sharifpour *et al.*, 2020). Similar observation was observed in this study as the median level of hsCRP was lower albeit insignificant; in COVID-19 patients at diagnosis compared with the level at discharge. This observation, in conjunction with other reports, further confirms the use of CRP in monitoring and prognostication of COVID-19.

The observed inverse correlation between vitamin D level and hsCRP level is not surprising. This observation corroborates the anti-inflammatory and immune supporting properties of vitamin D which usually results in hyperinflammation dampening. These attributes of vitamin D could explain the observed lower median level of hsCRP in the vitamin D intoxication group compared with the vitamin D sufficient COVID-19 patients.

It could be concluded from this study that vitamin D intoxication is common among Nigerian patients with vitamin D. Also, there was reduction in hsCRP level at discharge compared with the level at diagnosis in COVID-19 patients. There is the need for public enlightenment on the dangers

inherent in indiscriminate use of vitamin D as supplements by Nigerians as the expected benefit could be marred by the dangers associated with the toxicity of the drugs and supplements.

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