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The Critical Role of Vitamin D in the Physiological and Immunological Response to a Covaid-19 Infection

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ABSTRACT: This research can contribute to elucidate this issue, in addition to subsidizing the clinical practice of health professionals and assist in the preparation of specific care protocols for this population, with peculiar clinical characteristics and little discussed in the scope of research. This study aimed to evaluate the results of studies that supplemented with vitamin D, impact of SOD on patients with CKD. Several studies have been done regarding the inhibitory effect of SARS. Mice without VDR expression have a 3-fold increased renin expression with a consequent increase in plasma angiotensin II and, clinically, arterial hypertension. In cell cultures of As 4.1 cells, with high renin synthesis, the administration of 1.25 (OH) 2D markedly decreased the activity of the promoter region of the VDR-mediated renin gene, resulting in a decrease in the respective messenger ribonucleic acid (mRNA). In mice lacking 1-hydroxylase expression, administration of 1.25 (OH) 2D was also effective in decreasing the activation of RAAS and in the treatment of arterial hypertension. An experimental study with partially nephrectomized animal models evaluated the renal effect of administering an ACE inhibitor, enalapril, a vitamin D analogue, paricalcitol, or both. It was found that in most of the parameters studied, the combination was more effective than either of the two drugs separately.

KEWWORDS: vitamin D, immunological response, covaid-19 infection

INTRODUCTION

Several studies have been developed in order to better understand the role of vitamin D in CKD in addition to phospho-calcium metabolism, considering it a possible therapeutic weapon with enormous potential given its intervention in multiple systems. Due to the lack of effective mechanisms for slowing the progression of CKD, its potential nephroprotective effect is very important. For nephroprotection, several beneficial mechanisms of vitamin D are considered such as suppression of RAAS, decreased systemic inflammation, and decreased proteinuria. The effects of vitamin D on blood pressure and cardiovascular anti-mitogenic effects in chronic kidney patients are also a consequence of these mechanisms, having high importance given their contribution to disease progression and mortality in these patients.

Vitamin D can be acquired in two ways, through diet (vitamin D2) or through endogenous synthesis induced through the action of ultraviolet B (UVB) radiation in the skin tissue (vitamin D3). However, in the kidneys, due to the action of the 1-α-hydroxylase enzyme, this metabolite is transformed into the active hormonal form of vitamin D, to 1.25 (OH) D or calcitriol. In recent years, vitamin D has been widely researched and it has been noted that its role goes far beyond its role in bone and calcium metabolism, observing its influence on the cardiovascular and immune systems and on the modulation of pathological and physiological processes, acting in this way, in decreasing the risk of certain chronic diseases, such as some types of cancers. Clinical and translational studies do not establish the relationship between vitamin D supplementation in patients diagnosed with Vitamin D and CKD.

MATERIALS AND METHODS

On entering the service, an initial biological assessment was carried out including the determination of calcium level, phosphatemia, DFG MDRD, PTH, 25 (OH) D and 1,25 (OH) 2 D serum. All the assays were carried out at the Teaching Medical Hospital of Baghdad, either in the Human Biology Center (biochemistry laboratory, and bone and endocrine biology laboratory), or in the nuclear medicine laboratory. According to their needs, the patients follow a supplementation by ampoules of cholecalciferol according to the protocol of the Hospital. After supplementation, a final assessment is carried out in town 30 days after taking the last ampoule of the protocol. The MVDN protocol is broken down into three main stages: an initial biological

assessment, the prescription of cholecalciferol supplementation according to the Hospital protocol, and a final biological assessment at D + 30 after the last intake of cholecalciferol. To facilitate prescribing, a support document was given to the physicians in the department, as well as prescribing assistance tools comprising standard prescriptions to be given to patients upon discharge. All the patients were called in order to check that the protocol was properly followed. The information transmitted by the patient was verified by contacting the pharmacies of the patients in order to precisely determine the number of ampoules delivered in town. The city medical analysis laboratories have also been called in to ensure that no transmission is forgotten on their part. After four calls (separated by a week's interval) without a response from the patients, we considered them lost to follow-up.

Clinical and laboratory parameters and outcomes of confirmed COVID-19 cases admitted to four hospitals in Kathmandu were retrospectively analyzed. Admitted COVID-19 cases with recorded D-dimer and definitive outcomes were included consecutively. D-dimer was measured using immunofluorescence assay and reported in Fibrinogen Equivalent Unit (μg/ml). The receiver operating characteristic curve was used to determine the accuracy of D-dimer in predicting mortality, and to calculate the optimal cutoff value, based on which patients were divided into two groups and predictive value of D-dimer for mortality was measured. D-dimer levels were higher in COVID-19 patients and were related with markers of inflammation, and after treatments, D-dimer levels decreased which was synchronous with hsCRP levels in patients with good clinical prognosis. Also, the low correlation between Padua VTE score and D-dimer levels weakened the role of D-dimer in the prediction of thrombosis .The abnormal changes of D-dimer and inflammatory factors suggest that aggressive anticoagulant therapy might be needed.

RESULT AND DISCUSSION

Results of present study showed there is significant differences ($p<0.05$) among age groups and BMI levels of patients. The age groups 21-40 and >40 years scored highest percentage (55.0% and 32.5%), compared to 1-20 years that scored least percentage (12.5%).The overweight and obese patients scored highest percentage (50.0% and 20.0%) compared to underweight that scored least percentage (12.5%) (table 1).

Table 1. frequency and percentage of age groups and BMI level of patients were calculated by chi-square test.

groups		Count	Percent	P value
Age (years)	1-20	5	12.5%	P<0.001***
	21-40	22	55.0%	
	>40	13	32.5%	
Body mass index	Under weight	5	12.5%	P<0.001***
	Normal weight	7	17.5%	
	Over weight	20	50.0%	
	Obese	8	20.0%	

Results of present showed there is significant differences ($p<0.05$) between calcium, vitamin-d3, CRP, ferritin, D-dimer, LDH, and study groups. The mean levels of calcium and vitamin-d3 were decreases in patients (5.34± 1.30 and 11.35±4.21) than healthy. In contrast, the mean levels of CRP, ferritin, D-dimer, LDH were increased in patients (91.45± 29.95 , 402.20±145.96, 727.25± 241.67 and 426.28±201.22) than healthy (table 2 and figure 1).

Table 2. Comparative of vitamin D3, CRP, and biochemical parameters between study groups were calculated by student T test.

Groups		N	Mean	SD	P value
Calcium	Patients	40	5.34	1.30	P<0.001***
	Healthy	20	9.80	2.38	
Vitamin_D3	Patients	40	11.35	4.21	P<0.001***
	Healthy	20	53.25	19.65	

CRP	Patients	40	91.45	29.95	P<0.001***
	Healthy	20	6.25	2.85	
Ferritin	Patients	40	402.20	145.96	P<0.001***
	Healthy	20	41.50	18.43	
D_dimer	Patients	40	727.25	241.67	P<0.001***
	Healthy	20	257.50	121.78	
LDH	Patients	40	426.28	201.22	P<0.001***
	Healthy	20	166.75	67.00	

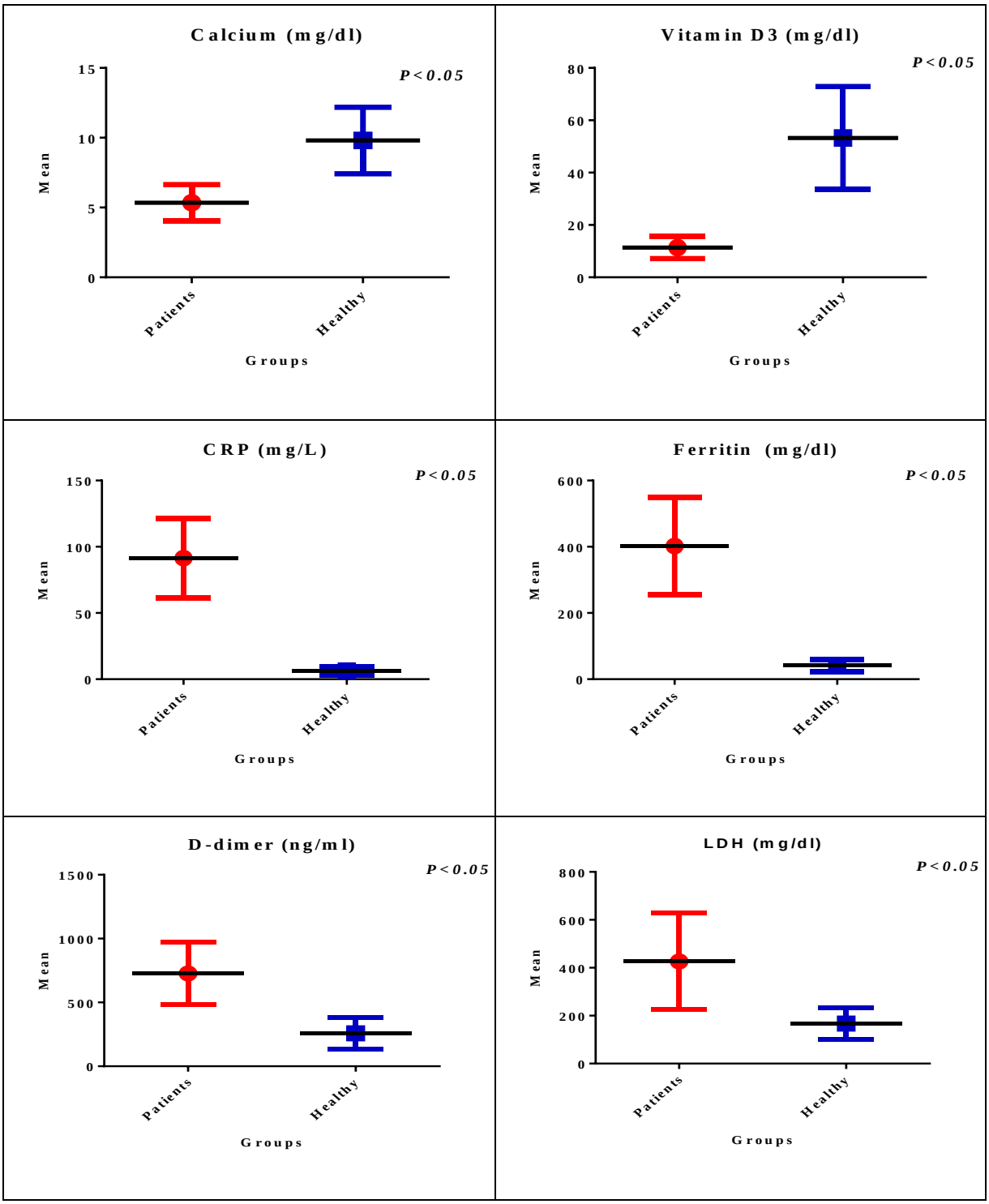

Figure 1. comparative of vitamin D3, CRP, and biochemical parameters between study groups.

Results of present study showed there is significant positive and negative correlations among variables ($p<0.05$). calcium is no significant negative correlate with D-dimer (r=-.023) and LDH (r=-.141), whereas calcium is significant negative correlate with CRP (r=-.453**). Vitamin_D3 is no significant positive correlate with Calcium (r=.110) and negative with CRP (r=-.126), D-dimer (r=-.005), and LDH (r=-.056). fructose (r=.359*). Ferritin is positive significant correlate with CRP (r=.700**), D-dimer (r-=.740**), and LDH (r.656**), but ferritin is negative no significant correlate with calcium (r=-.041). Finally, LDH is positive significant correlate with CRP (r=.656**) and D-dimer (r= .528**), but LDH is negative no significant correlate with calcium (r=-.141) (table 5).

Table 3. Correlation relationship among of vitamin D3, CRP, and biochemical parameters were calculated by pearson correlation.

		Calcium	CRP	D_dimer	LDH
Calcium	Pearson coefficient	1	-.453**	-.023	-.141
	Significant		.003	.886	.387
Vitamin_D3	Pearson coefficient	.110	-.126	-.005	-.056
	Significant	.498	.439	.974	.732
Ferritin	Pearson coefficient	-.041	.700**	.740**	.656**
	Significant	.802	.000	.000	.000
LDH	Pearson coefficient	-.141	.656**	.528**	1
	Significant	.387	.000	.000	

Results of present study showed no sign`ificant ($p>0.05$) between vitamin D3 and age groups and BMI levels of patients (table 4).

Table 4. Comparative of vitamin D3 with age groups and BMI level of patients were calculated by F test.

Vitamin D3	N	N	Mean	SD	P value
Age groups	1-20	5	10.67	1.15	P>0.05
	21-40	22	11.34	1.15	
	>40	13	11.80	1.64	
Body mass index	Under weight	5	11.25	0.96	P>0.05
	Normal weight	8	10.00	0.00	
	Over weight	20	11.40	0.84	
	Obese	7	11.46	1.38	

DISCUSSION

This study distributed of COVID-19 patients according to age groups. These results clarified statistically significant differences between COVID-19 patients among age groups, the higher frequency in age group 21-40 years (55%) this accepted with other Iraqi study (Lami, 2021) and other study (Liu and Li., 2020), this percent may be due to being the working group and most susceptible to transmission of infection. Additionally, these finding is similar with many studies which belief that age is a significant risk factor for evaluation covid-19 or for its severity, just like the study of Liu and Li., (2020), whose results demonstrated that age can be considered as a death-associated risk factor in this pandemic because elderlies tend to have a higher prevalence of chronic diseases like cardiovascular diseases and diabetes. Further reduced production of B and T cells in primary lymphoid organ and decline function of mature lymphocytes in secondary lymphoid tissues have been associated with aging (Lami, 2021).

Study finding seems to correspond with more recent studies that there is a positive correlation between age and the COVID-19 infection (Bi *et al.,* 2020). Reduced ACE-2 expression in the children's nasal epithelium may be responsible for reduced SARS-CoV-2 susceptibility, therefore the COVID-19 infection is little or disappear in children (Jakhmola et al., 2021). An exponential increase

with age is a good model to describe and analyze both COVID-19 and all-cause mortality above 40 years old, where almost all COVID-19 deaths occur. Recently reported the incidence rates which confirmed an increased disease incidence in men > 60 years (Robert Koch Institute, 2020). The prevalence COVID-19 infection is occurring high risk in elderly individuals and that may be impaired immune response, chronic diseases, malnutrition, high ACE-2 expression, and organ dysfunction. In Kirkuk city, it was concluded that the infection in men are more, especially those over the age of 61 years of them and those who have other comorbidities such as high blood pressure, heart diseases and diabetes (Faiq et al., 2021). In contrast to adults with COVID-19, the majority of children and adolescents infected with the SARS-CoV-2 exhibited milder disease and also presented with nasal congestion, rhinorrhea, pharyngeal erythema, diarrhea, and vomiting. Those young patients showed an adequate treatment response and a short duration to COVID-19 resolution (Zheng et al., 2020).

Body Mass Index (BMI) is used to classify obesity. BMI is calculated as the ratio of weight in kilograms to the square of height in meters, expressed in units of kg/m2. According to WHO, BMI was classified into six groups: underweight (<18.5 kg/m2), normal (18.5–24.9 kg/m2), pre-obesity (25–29.9 kg/m2), obesity class I (30–34.9 kg/m2), obesity class II (35–39.9 kg/m2), and obesity class III (>40 kg/m2) (WHO, 2020). The worldwide prevalence of overweight (BMI ≥ 25 kg/m2) and obesity has increased significantly and extent that nearly a third of the world population.

This meta-analysis study showed that both higher BMI and obesity were associated with related poor outcomes (ICU admission, ARDS, severe COVID-19, use of mechanical ventilation, hospital admission, and mortality) in COVID-19 adult patients. Furthermore, subgroup analysis showed that both higher BMI and obesity were not associated with ICU admission but still associated with other poor outcomes. ICU admission criteria may be difficult to define clearly. Since it addressed not only administrative guideline but also ethical and medico-legal aspect of patient care. In contrast, effect of obesity was less in male patients. SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE2) expressed in the lungs and other tissues, as the main entry point into the cells (Kimura ET AL., 2020).

Previous data showed that obesity was present in nearly one-third of hospitalizations and fatal cases during the 2009H1N1 pandemic and recognized as an independent risk factor for severity, hospitalization, increased risk of transmission, and mortality of influenza during the H1N1 pandemic in 2009 (Honce and Schultz-Cherry, 2019). Therefore, it is not surprising that higher BMI and obesity as is also associated with poor outcome in SARS-CoV-2 infection as well.

Obesity has been identified as a significant risk factor for severe disease following lower respiratory tract infections (Kulcsar et al., 2019). Obesity appears to be associated with a higher chance to developing respiratory complication and need for ICU admission and mechanical ventilation (Severin et al., 2020). COVID-19 can lead to potential airways threatening and result in ARDS and respiratory failure (Honce and Schultz-Cherry, 2019). Respiratory muscle strength decreases and oxygen demand increase more than three-fold due to increased airway resistance and chest wall mechanics (Sood et al., 2009). Increased oxygen consumption can lead to respiratory failure and predispose the need for more oxygen support. In addition, due to anatomical factor obese patients have a higher incidence difficult mask ventilation compared to non-obese patients (Moon et al., 2019).

Other factor that probably explains the association between obesity and poor outcome of COVID-19 is that obesity has a negative effect on the immune system and host defense mechanism . Adipose tissue involved in many physiologic and metabolic processes as well as a reservoir for T lymphocytes and macrophages. Excess body fat reduces the response to antiviral agents through poor T cell and macrophage functions (Soeroto et al., 2020).

Afshan et al., (2020) show low levels of vitamin D3 in conid-19 patients than controls and these results compatible to our results. The COVID-19 pandemic has spurred renewed interest in vitamin D to address viral replication and hyperinflammation that have a major role in the pathogenesis of severe COVID-19. In addition to known antimicrobial and anti-inflammatory effects, vitamin D metabolites also have direct action on angiotensin-converting enzyme 2 (ACE2), which serves as the cell surface entry receptor for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Vitamin D metabolites upregulate ACE2 expression in pulmonary microvascular endothelial cells in animal models of acute lung injury (Xu et al., 2017). Although enhanced expression of ACE2 could theoretically increase viral entry into cells, it may paradoxically have beneficial effects in patients who are already infected because SARS-CoV-2–mediated downregulation of ACE2 may perpetuate lung injury (Leaf and Ginde, 2021).

Vitamin D3 can also induce the anti-viral immunity which is of prime importance nowadays considering the global COVID-19 pandemic. Vitamin D3 is also responsible for inducing autophagy as a result of both anti-bacterial and anti-viral activity (Afshan et al., 2020). There is emerging evidence revealing the promising role of vitamin D in preventing cytokine storm and, consequently, determining outcomes of SARS-Cov2 (Silberstein, 2020). Vitamin D insufficiency has been shown to be related to latitude, obesity, diabetes, hypertension, ethnicity, and sex, and it is a condition associated with the increased susceptibility for SARS-Cov2 infection and mortality. It has also been suggested that the different gender-related susceptibilities involve testosterone levels associated with vitamin deficiency in men (Calder et al., 2020). Authors evaluated in which repertoire of effector cells and molecules of the immune response vitamin D could intervene to counteract against SARS-COV2 infection. This examination highlighted the extreme

complexity of mechanisms used by vitamin D for modulating the immune response following a viral invasion . Some clinical trials revealed that vitamin D supplementation was effective to prevent infection both in the early and in the hyperinflammatory stage of the disease, since it modulated efficaciously the immune response against SARS-COV2 (Panagiotou et al., 2020). It can be noted that vitamin D, in severe SARS-CoV2 stage, should be administered with caution because it could stimulate or inhibit some cellular functions that could induce infectious tolerance . The proposed guidelines for the treatment of COVD19 recommended the use of glucocorticoids. Therefore, the suggested treatment with glucocorticoids in combination with vitamin D supplementation (Entrenas et al., 2020) could be an interesting perspective to be taken into account. Since vitamin D has multiple cellular and intracellular targets, additional studies are needed to determine the consequences of the interaction of vitamin D in the immune-response against SARS-CoV2 in order to achieve a significant vision into prophylactic and therapeutic strategy for the prevention of this viral infection (Malaguarnera, 2020).

Oral supplementation of D3 is the easiest means to prevent deficiencies. A frequent argument against supplementation of vitamin D3 is that an increased intake could lead to a vitamin D toxicity, also called hypervitaminosis D (Orme et al., 2016). This again can cause hypercalcemia, which is the buildup of calcium in the blood leading to vascular calcification, osteoporosis, and kidney stones. However, it has been reported that the reason for hypercalcemia rather lays in a vitamin K2 deficiency (Flore et al., 2013), as K2 activates the bone gamma-carboxyglutamic acid-containing protein (osteocalcin) through carboxylation. Activated osteocalcin deposits calcium in the bones, whereas non-activated osteocalcin inhibits calcium absorption by the bones. As the osteocalcin synthesis rate is increased by higher 25(OH)D serum levels, K2 is required as a natural antagonist (Dofferhoff et al., 2020). Study findings showed bolus vitamin D3 supplementation during or just before COVID-19 was associated in frail elderly with less severe COVID-19 and better survival rate (Annweiler et al., 2020).

It has also been observed that D3 supplementation led to an increase in anti-inflammatory and immunoregulating interleukin 10 (IL-10) cytokines and reduced frequency in Th17 cells , which in turn leads to a decrease in IL-17 and the proinflammatory cytokine TNFα production, decreasing inflammatory effects in the host (Ferreira et al., 2020). Also, Zheng et al. (2014) reported that TNFα promotes pathogenic Th17 cell differentiation. On the other hand, IL-10 reduces the activity of the TNF-α-converting enzyme (TACE) (Gray et al., 2008). Gray et al. (2008) also observed that lipopolysaccharides (LPS) in the bloodstream enhanced TNFα values.

Whereas Th1 and Th17 cells are proinflammatory, regulatory T-cells (Tregs) have anti-inflammatory effects. Prietl et al., (2010) proclaim that vitamin D3 supplementation showed an increase in regulatory Tregs and a more tolerogenic immunological status in general. A chronic D3 deficit would shift the T-cell ratio towards the inflammatory pathway. Given the fact that an abundance of Th17 cells are highly associated with autoimmune diseases (Yasuda et al., 2019), it is therefore unsurprising that many fatal cases showed comorbidities.

A further interesting point is represented by the potential differences in serum level of 25(OH)D among men and women. Sanghera et al., (2017) observed a significantly reduced level of 25(OH)D in both men and women with obesity that represents a further important risk factor for COVID-19. In previous study, 25(OH)D level remains consistently lower in obese men than in obese women (Sanghera et al., 2017). On the contrary, in another study, Mucogiuri and et al., (2019) stratifying the sample population according to sex and body mass index (BMI), found that 25(OH)D concentrations were significantly higher in males compared to females in all BMI classes and decreased along with the increase of BMI values. Although these contrasting data seem to not assign to 25(OH)D a clear role in determining sex differences in obese COVID-19 patients, we think that attention could be paid to 25(OH)D levels in the context of this comorbidity.

Interestingly, sex differences have been observed in the immunomodulatory and anti-inflammatory effects of Vitamin D3 in some autoimmune diseases. In particular, a study of Correale et al., (2010) showed that Vitamin D3 induces a stronger inhibition of the production of pro-inflammatory cytokines and a higher increase of anti-inflammatory cytokines in lymphocytes from multiple sclerosis female patients in comparison to those from male patients. Interestingly, Spanier and et al., (27) suggested that Vitamin D3 acts in an estrogen-dependent manner in controlling T regulatory cell differentiation. Moreover, estrogen seems to increase the expression of the nuclear vitamin D receptor (VDR) gene in CD4+ T cells (Spanier et al., 2015) and to decrease the expression of CYP24A1, the cytochrome P450 component of the 25-hydroxyvitamin D(3)-24-hydroxylase enzyme which inactivates Vitamin D3. In turn, Vitamin D3 exerts tissue-specific effects on peripheral estrogen metabolism (Pagano et al., 2020). Hence, the sex-related immunomodulatory effects of Vitamin D3 suggest that it is possible to speculate that also in COVID-19, Vitamin D3 could play a role in the outcome and lethality.

Crespi and Alcock, (2021) show low levels of calcium in conid-19 patients than controls and these results compatible to our results.

Authors found that the abnormalities of some systemic indexes including lymphocytes counts, AST, LDH, D-dimer, PaO2, oxygenation index, CRP, serum Ca, serum P, and ESR could indicate the severity of COVID-19 (Li et al., 2020). These laboratory

findings demonstrated that patients with COVID-19 also may be at high risk of cardiac, liver, hematological and cellular immune system dysfunction as previously reported (Frater et al., 2020). Of note, serum Ca in combination with oxygenation index or lymphocytes could increase diagnositic performances and favor early identification of the severe/critical COVID-19 patients, which would be helpful in clinical practice to prioritize medical resources to help the COVID-19 patients at different risks of life-threatening situations (Li et al., 2020).

Previous study showed the clinical virtue of hypocalcemia in the diagnosis of different severity of COVID-19 patients (Yang et al., 2021). Compared with moderate infected cases, severe COVID-19 patients were more likely to have hypocalcemia even after adjustment by age and comorbidities. Therefore, hypocalcemia may be an indicator for patients likely turning severe. Additionally, the specificity of hypocalcemia was highest among different key clinical parameters. Recently, the high incidence of hypocalcemia among COVID-19 patients has been reported by several other articles (Cappellini et al., 2020). Moreover, a Italian study of 531 patients found that hypocalcemia could predict the need for hospitalization of COVID-19 patients (Di Filippo et al., 2020).

Hypocalcemia is common in critically ill patients and is considered to be associated with multiple organs dysfunction and poor prognosis of critically ill patients (Kelly and Levine, 2013). The causes of hypocalcemia may include endocrine disorders and medication side effects, such as insufficient parathyroid hormone (PTH) secretion or resistance to PTH action, vitamin D deficiency, decreased diet, hypoproteinemia, hypomagnesemia drug interactions (Zivin et al., 2001).

Study finding indicates that calcium balance is a primal hit of COVID-19 and a biomarker of clinical severity at the beginning of symptom onset. Calcium is closely associated with virus-associated multiple organ injuries and the increase in inflammatory cytokines. Additionally, these results provide a new, important indicator of COVID-19 patients from mild/moderate to severe/critical: serum calcium (Zhou et al., 2020).

Kaushal et al., (2022) show high levels of ferritin in conid-19 patients than controls and these results compatible to our results. Meta-analysis showed that an elevated serum CRP, PCT, D-dimer, and ferritin were associated with a poor outcome in COVID-19 (Huang wt al., 2020).

Hyperferritinaemia is observed across a range of inflammation driven disorders and it serves as a validated biomarker across different disease domains e.g. rheumatologic disorders, different cancers and inflammatory conditions (Kernan and Carcillo, 2017). Many of the important SARS-CoV-2 regulatory and functional proteins use iron. Excess iron can also induce fibrin polymerization and induce a pro-coagulant state (Colafrancesco et al., 2020). Previous literature reported occurrence of coagulopathy among severe COVID-19 patients. While one hypothesis states that infection with SARS-CoV-2 results into direct interaction with haemoglobin (thus facilitates removal of iron), however this was later refuted by subsequent authors. Again, the "role of iron chelation" in the context of COVID-19 is yet controversial (DeMartino et al., 2020).

Authors showed high serum ferritin level was found to be associated with more severe disease and negative/poor outcome in COVID-19. Thus serum ferritin level can serve as an important predictive biomarker in COVID-19 management and in triag (Kaushal et al., 2022).

Huang et al., (2020) show high levels of CRP and D dimer in conid-19 patients than controls and these results compatible to our results.

In the systemic hyperinflammation phase of COVID-19 proposed by Siddiqi and Mehra, (2020) there is a significant elevation of inflammatory cytokines and biomarkers, such as interleukin (IL)-2, IL-6, IL-7, granulocyte-colony stimulating factor, macrophage inflammatory protein 1-α, tumor necrosis factor-α (TNF-α), CRP, ferritin, PCT, and D-dimer. This stage consists of the most severe manifestation of the cytokine storm, in which excessive hyperinflammation may lead to cardiopulmonary collapse and multi-organ failure (Zhang et al., 2020).

CRP is an acute phase inflammatory protein produced by the liver that may be elevated in several conditions, such as inflammation, cardiovascular disease, and infection (Sproston and Ashworth, 2018). A study showed that an elevated serum CRP level was associated with a 30-day mortality rate, while other studies showed otherwise (Koozi et al., 2020). These inconsistencies might be caused by the different cutoff values used. Liu *et al.*(2020) proposed a cutoff value of ⩾41.8 mg/L to predict severe COVID-19. The distributional characteristics of CRP indicated an optimal cut-off of ≥40 mg/L was associated with mortality. This threshold may assist clinicians in using CRP as an early trigger for enhanced observation, treatment decisions and advanced care planning (Stringer et al., 2021). The time period for serum CRP measurement was critical in light of the timely manner of serum CRP increment, which culminates 72 h after the initial insults. Despite its value in predicting a poor outcome in COVID-19, it should be noted that various factors could affect serum CRP levels, including age, gender, smoking status, weight, lipid levels, blood pressure, and liver injury. These factors should be taken into account while interpreting the serum CRP level. In addition, recent evidence has shown that serum CRP level could also be used in monitoring the progression and improvement of patients with COVID-19 (Li et al., 2020).

The level of plasma CRP was positively correlated to the severity of COVID-19 pneumonia. Study findings could assist to discern patients of moderate to severe COVID-19 pneumonia from the mild ones depend on CRP levels patients for intense care unit transfer (Chen ert al., 2020). Previous study revealed the maximal level of IL-6, followed by CRP level, was highly predictive of the need for mechanical ventilation. This suggests the possibility of using IL-6 or CRP level to guide escalation of treatment in patients with COVID-19–related hyperinflammatory syndrome (Herold et al., 2020).

The common COVID-19 abnormal hematological indexes on admission included hyperfibrinogenemia, lymphopenia, the elevation of D-dimer, and leukopenia, which were significantly different between the mild/moderate and severe COVID-19 groups. Furthermore, the dynamic change of neutrophil-lymphocytes ratio and D-dimer level can distinguish severe COVID-19 cases from the mild/moderate (Fu et al., 2020). D-dimer on admission greater than 2.0 μg/mL (fourfold increase) could effectively predict in-hospital mortality in patients with Covid-19, which indicated D-dimer could be an early and helpful marker to improve management of Covid-19 patients (Zhang et al., 2020).

The Blood parameter shows that the ferritin level will increase in second week after affecting with Covid-19, also other parameters will be changed according to the normal range, such as D-Dimer and ESR. Depend on previous case study, these levels will be change because they got Paracetamol vial 500 ml daily twice in hospital that affect the liver function (Hussein et al., 2021).

Mo et al., (2021) show high levels of LDH in conid-19 patients than controls and these results compatible to our results.

Lactate dehydrogenase (LDH) is an intracellular enzyme involved in anaerobic glycolysis that catalyzes the oxidation of pyruvate to lactate (Komolafe et al., 2017). Serum LDH is routinely tested in various diseases clinically. It has been reported that elevated serum LDH levels are associated with poor prognosis in various diseases, especially in tumors and inflammation (Van Wilpe et al., 2020). To date, studies have shown that patients with severe COVID-19 have elevated serum LDH levels (Mo et al., 2021), and these results matched with present study that showed high levels of LDH in COVID-19 patients than healthy. Li et al., (2020) revealed that serum LDH at admission was useful in evaluating the disease severity and in-hospital mortality among patients with COVID-19.

As suggested by comparison of laboratory indicators, there were significant differences in the levels of LDH between nonsevere and severe groups. The differences in these indicators were very similar to those reported by Huang et al. (Huang ET AL., 2020). Notably, LDH showed a powerful correlation with the other indexes by Pearson correlation analysis, which suggested that LDH was a significant factor associated with the severity of patients with COVID-19. When the body experiences acute hypoxia or inflammation, the level of LDH in serum will rise significantly. COVID-19, caused by SARS-Cov-2 infection, mainly involves in the lungs, as well as other tissues and organs, leading to hypoxia, thrombogenesis, inflammation and organ injury. Theoretically, elevated serum LDH is an important laboratory indicator for evaluating COVID-19 (Guan ET AL., 2020). Data results suggested that LDH is a potentially useful follow-up parameter in COVID-19 pneumonia, which might assist in recognition of disease progression and thus help in risk stratification and early intervention (Wu et al., 2020). Han et al., (2020) showed that LDH could be identified as a powerful predictive factor for early recognition of lung injury and severe COVID-19 cases. Study findings showed Our findings suggest that elevation of plasma LDH activity in patients with COVID-19 is not associated to a specific release of LDH-3 into the bloodstream, and do not support the use of LDH as a specific biomarker for lung affectation in patients with COVID-19 (Serrano-Lorenzo et al., 2021).

CONCLUSION

In our study, we also found that an elevated D-dimer was associated with an increased composite poor outcome, especially mortality and severe COVID-19. This finding supports the hypothesis that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection could induce the dysfunction of the hemostatic system, leading to a hypercoagulable state. Recent evidence of lung pathology dissection has shown occlusion and micro-thrombosis formation in pulmonary small vessels of patients critically ill with COVID-19. However, the etiology of elevated serum D-dimer level is multifactorial and the optimal cutoff value of elevated D-dimer in patients with COVID-19 remains to be established. It is clear that COVID-19-associated coagulopathy warrants distinct emphasis and special treatment. According to the International Society of Thrombosis and Hemostasis (ISTH) guideline, a markedly elevated serum D-dimer level (which is still poorly defined as a three- to four-fold increase) implies an increased thrombin production.

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