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Calcium Metabolism Markers and Their Effects on Mortality in COVID-19 Patients Admitted to Intensive Care Unit

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ABSTRACT

To evaluate the status of serum calcium, parathyroid hormone (PTH), and 25 (OH) vitamin D and their effects on mortality in patients with COVID-19 who were admitted to the intensive care unit (ICU).

This was a retrospective chart review. Laboratory data at ICU admission included serum creatinine, corrected calcium, phosphorus, albumin, magnesium, among others. Same laboratory measurements were repeated two more times during hospitalization. The length of ICU stay, mortality, and need for mechanical ventilation were also recorded. Hypocalcemia, hypomagnesemia, and hypovitaminosis D rates were examined along with independent predictors of in-hospital mortality.

A total of 100 patients were included. The median length of ICU stay was 11.0 days. Mortality rate was 52%. Rates of vitamin D insufficiency and deficiency were 29% and 52%, respectively. Thirty percent of patients had serum calcium levels less than normal. On admission, 11% of the patients had hypomagnesemia. Patients with hypomagnesemia had lower serum calcium levels compared to normomagnesemic patients (8.7±0.6 vs. 9.2±0.7 mg/dL, p=0.031). Serum PTH levels were significantly different between hypomagnesemic and normomagnesemic patients. In contrast, 25(OH)vitamin D levels were comparable in both hypomagnesemic and normomagnesemic patients. In multivariate analysis, only the need for mechanical ventilation remained as a significant predictor of in-hospital mortality.

Hypocalcemia was less common compared to the literature, whereas Vitamin D deficiency was widespread. Serum magnesium level appeared as an important modifier of serum calcium levels in these patients. Hypocalcemia was not associated with COVID-19 disease severity or mortality.

Keywords: Calcium; COVID-19; Magnesium; Parathyroid hormone; Vitamin D

Introduction

For almost 27 months after its onset in China, according to World Health Organization numbers, COVID-19 has claimed almost 6 million lives worldwide (1). Even though the lungs are the major target of SARS-CoV-2, COVID-19 affects much beyond that. The endocrine system is also among the targets of the virus (2).

Recently, some studies have identified an osteo-metabolic feature of COVID-19 that may affect the clinical outcome of the disease. This phenotype is characterized by diffuse acute hypocalcemia, chronic hypovitaminosis D, and often vertebral fractures (3). Hypocalcemia is a common finding in COVID-19 patients. It was discovered that hypocalcemia constituted an independent risk factor for hospitalization. Patients with hypocalcemia needed hospital stays and admissions to intensive care units that were considerably longer than those without hypocalcemia. Although the clinical consequences of a significant prevalence of asymptomatic hypocalcemia (and hypophosphatemia) in individuals with mild COVID-19 have not been established, hypocalcemia is common and the high incidence of silent fractures in these patients is indicative of more severe COVID-19 variants (3,4,5,6,7).

In up to two-thirds of hospitalized patients, low vitamin D levels and hypocalcemia have been recorded. These biochemical abnormalities, the need for mechanical ventilation, hospitalization in the intensive care unit gauge the severity of clinical disease and mortality, such as a number of in addition to the host's inflammatory response

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caused by the lack of response may be associated with compensatory parathyroid hormone (PTH) (8,9,10).

Several potential pathophysiological factors, such as calcium-dependent viral mechanisms of action, the high prevalence of hypovitaminosis D in the general population, chronic and acute malnutrition during critical illness, and high levels of unbound and unsaturated fatty acids in inflammatory responses, have been speculated to play a role in hypocalcemia in COVID-19 (11). When evaluated along with 25(OH) vitamin D and PTH levels, the appraisal of the independent impact of serum calcium gets more difficult (12). In contrast, another meta-analysis demonstrates that none of the analyzed outcomes indicated a clear and robust direction for a cause and effect link between vitamin D status and COVID-19-related health outcomes (13). Moreover, another meta-analysis revealed supplemental vitamin D did not lower significant health outcomes such as mortality, intensive care unit (ICU) admission rates, and mechanical ventilation (14). Mazziotti and colleagues found that among 348 patients hospitalized for COVID-19, that secondary hyperparathyroidism and, to a lesser extent, vitamin D deficiency are associated with a poor prognosis of SARS-CoV-2-associated pneumonia and support a comprehensive examination of vitamin D levels in hospitalized COVID-19 patients (15). In contrast to the findings of the latter study, di Filippo at al. in their trial 78 patients participated found that hypocalcemia rate of 67.9% among hospitalized patients with COVID-19. The authors reported a high frequency 69% of patients had a Vitamin D deficit, although only 20.5% of them had secondary hyperparathyroidism. They presumed that the highly widespread low Vitamin D is a substantial risk factor for hypocalcemia in COVID-19 patients, and that a dampened compensatory PTH response, which may be directly related to SARS-CoV-2 infection, may enable hypocalcemia in these individuals (9).

The complex and intertwined association of serum calcium, vitamin D, and PTH makes it harder to understand the individual roles of these parameters on the prognosis of patients hospitalized with COVID-19. Add to this the conflicting results reported in the literature; we aimed to evaluate the status of serum calcium, PTH, and 25(OH) vitamin D and the trend of change in serum calcium, magnesium and other laboratory parameters both on admission and then throughout the hospitalization and their effects on mortality in patients with COVID-19 who were admitted to the intensive care unit in an hospital in Turkey.

Materials and Methods

Patients and Setting: This was a retrospective chart review study that was carried out in a teaching and research hospital in Turkey. The primary aim of the study was to evaluate the association between COVID-19 severity and calcium metabolism laboratory parameters, including serum calcium, PTH, and 25(OH) vitamin D. We retrospectively screened the patients who were admitted to the intensive care unit due to COVID-19 for eligibility for inclusion in the study between January 1st and October 31st, 2021. The inclusion criteria to be enrolled in the study were as follows: having severe enough COVID-19 requiring ICU admission. These patients usually had pulmonary involvement in CO-RADS category 3 or higher. Patients who had primary parathyroid disorders had thyroid disease or were receiving thyroid medications, and history of blood transfusion during hospitalization were excluded from the study. Although it directly affects calcium metabolism, we did not exclude patients with acute kidney injury or chronic kidney disease. Because these patients constitute a sizeable proportion of all ICU admissions and we aimed to evaluate the alterations in calcium metabolism in these patients as well.

Data Collection: Age, sex, and chronic medical conditions such as hypertension, diabetes mellitus, and congestive heart failure were recorded for each participant. Laboratory data studied at ICU admission included serum urea, creatinine, calcium (corrected), phosphorus, albumin, lactate dehydrogenase, magnesium, C-reactive protein (CRP), D-dimer, troponin I, procalcitonin, and ferritin. Serum urea, creatinine, calcium (corrected), phosphorus, albumin, magnesium, CRP, D-dimer, troponin I, procalcitonin, and ferritin levels during the course of the hospitalization were also collected as second and third laboratory measurements of the respective tests. Serum PTH measurements were performed by chemoilluminence method, whereas serum 25(OH) vitamin D levels were measured by antibody competitive immuntest method via an autoanalyzer (Advia Centaur XP, Siemens).

Vitamin D levels were determined as normal (>20 ng/mL), inefficient (10-20 ng/mL), and deficient (10 ng/mL) (16). The Modification of Diet in Renal Disease formula (MDRD) was used to

compute the estimated glomerular filtration rate (17). Patients with an eGFR less than 60 mL/min/1.73 m2 were diagnosed with chronic renal disease. Each participant's body mass index (BMI) was computed by dividing their body weight in kilograms by their height in meters squared.

The patient outcome, either being discharge or exitus, was also recorded from patient charts. The length of ICU stay was calculated for each study participant. Patients who were administered pulse prednisone as part of the COVID-19 treatment regimen and patients who needed intubation and mechanical ventilation were also recorded.

Statistics: The power value, as determined by the post-hoc power analysis using the repeated measured Calcium data, was 0.9987. The sample size (100), the mean correlation value between repeated measurements (0.465), the significance level (0.05), and the effect size (0.2270) were used for the calculation of this power value.

The Shapiro Wilks test histogram and Q-Q plot were used to check the normality of the continuous variables. Continuous variables were expressed as mean±standard deviation or median (min-max) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages %. To compare the categorical variables between the groups, the Chi-square test was used. The Independent t-test and the Mann-Whitney U test were used to compare two-group comparisons for numeric variables. The one-way analysis of variance (ANOVA) and the Kruskal Wallis test were used to compare three groups comparison for numeric variables depending on whether the data is normally distributed or not. Since the repeated measures ANOVA assumptions could not be drawn, the Friedman test with posthoc Durbin-Conover test were used to compare repeated measures of the whole cohort. The Spearman correlation and the point biserial correlation test were used to show the correlation.

We performed univariate and multivariate logistic regression analyses to determine the independent associates of mortality. According to the univariate logistic regression, among those with a p-value less than 0.050, only the variables that had clinical significance were included in the multivariate logistic regression analysis test.

The data from the study were analyzed using the SPSS 25.0 software package (IBM, Armonk, NY, USA). A probability value of $p<0.05$ was considered statistically significant, and other statistics were calculated using two-tailed p-values.

Results

A total of 100 patients (65% males) were included in the study. The mean age of the whole patient group was 68.5±14.2 years (Table1). The median length of ICU stay was 11.0 [min-max: 1.0-57.0] days. The frequencies of chronic conditions were as follows: hypertension 45%, diabetes mellitus 27%, chronic obstructive pulmonary disease 16%, coronary artery disease 15%, Alzheimer's 10%, epilepsy 4%, congestive heart failure 3%, and others 7%. Overall, 67% of the patients required tracheal intubation and mechanical ventilator support at some point during their hospitalization. Three-quarters of the patients were administered pulse prednisone during their hospital stay. Mortality rate was 52% at the end of the study period. The number of patients who had at least one comorbidity was 72 (72%). Almost half of all patients (47%) had eGFR values lower than 60 mL/min/1.73 m2.

Median serum 25(OH) vitamin D concentration on admission was 9.9 ng/mL [4.2-132]. When patients were evaluated in terms of vitamin D sufficiency status, only 19% of them had normal levels. Rates of vitamin D insufficiency and deficiency were 29% and 52%, respectively. The vast majority of the patients had an abnormal vitamin D level. Mean corrected serum calcium level on admission was 8.2±0.6 mg/dL, whereas the mean serum phosphorus level was 3.6±1.1 mg/dL. Serum calcium levels tended to increase during hospitalization; however, this did not reach statistical significance. Albumin and CRP values significantly decreased, whereas eGFR and d-dimer significantly increased throughout the hospitalization.

On admission, 11% of the patients had hypomagnesemia. Ten percent of the patients had hypermagnesemia. Mean serum magnesium level did not change significantly compared to baseline values during the course of the hospitalization. Patients with hypomagnesemia had significantly lower serum corrected calcium levels compared to normomagnesemic patients (8.7±0.6 vs. 9.2±0.7 mg/dL, p=0.031). Serum PTH levels were also significantly different between hypomagnesemic and normomoagnesemic patients (149.6 pg/mL [min-max: 35.5-397.0] vs 76.5 pg/mL [min-max: 11.4-880.0], p=0.038). In contrast, 25(OH) vitamin D levels were comparable in both hypomagnesemic and normomagnesemic patients

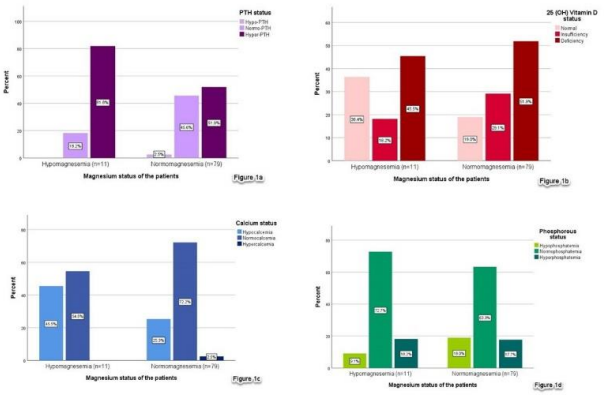

Fig.1. a, b, c, d. Clustered bar chart graphs of status of parathyroid hormone (a), 25(OH) vitamin D (b), corrected calcium (c) and phosphorus (d) in patient groups with normomagnesemia and hypomagnesemia

(Figure-1). Median estimated glomerular filtration rates were also similar in normomagnesemic and hypomagnesemic patients (p=0.829). Table2 summarizes the clinicodemographic characteristics and laboratory data of the entire patient group.

We grouped all study cohort into patients with hypocalcemia and patients with normocalcemia (Table3). Thirty patients (30%) had serum corrected calcium levels less than normal on admission. Hypocalcemic patients had significantly higher serum PTH, phosphorus, and lower eGFR values, whereas lower 25(OH) vitamin D levels compared to normocalcemic counterparts. Serum magnesium levels were similar in both groups. Mortality rate was similar between the two groups.

We compared features and findings of patients who were grouped based on 25(OH) vitamin D levels (Table4). Duration of ICU stay was significantly shorter for patients with normal vitamin D status compared to patients with vitamin D insufficiency and deficiency. Despite this difference, admission PTH and corrected calcium levels were not significantly different between the groups. In addition, admission serum magnesium level was significantly lower in patients who had normal serum 25(OH) vitamin D values compared with patients who had inadequate vitamin D levels. Estimated GFR levels were comparable between the groups.Likewise, there was no mortality difference between the groups.

When we stratified patients as eGFR below and over 60 mL/min/1.73 m2 (Table5), patients with lower eGFR were significantly older. Serum PTH value was significantly higher in patients who had chronic kidney disease, while serum 25(OH) vitamin D values were comparable. While mean serum phosphorus level was significantly higher among patients with chronic kidney disease, calcium levels were not different between the groups. The mortality rate was significantly higher in the chronic kidney disease patient group relative to non-chronic kidney disease patient group.

In-hospital Death and its Determinants: Overall, 52 patients died during the hospitalization. Deceased patients were significantly older (72.1 vs 64.6 years, p=0.009). In terms of admission levels of serum corrected calcium, 25(OH) vitamin D, PTH, phosphorus, and magnesium, there was no difference between decedents and surviving patients. Expectedly, mechanical ventilatory support was needed more frequently in the deceased group. Serum levels of procalcitonin and troponin I were significantly higher, whereas serum albumin was significantly lower in decedents compared to survivors at the study outset. Estimated GFR values were also significantly lower in deceased patients (65.5 [8.0-104] mL/min vs 85.5 [19.0-160] mL/min, p=0.046).

We ran univariate and multivariate regression analyses to identify the independent predictors of in-hospital mortality. Age, need for mechanical ventilation, and serum albumin were found to be independent associates of mortality in univariate analysis. However, in multivariate analysis, the only need for mechanical ventilation remained as a significant predictor of in-hospital mortality (Table6).

Correlation Analysis: Serum calcium level was positively correlated with CRP (r:0.212, p=0.034) and 25(OH) vitamin D (r=0.26, p=0.09) and negatively correlated with serum PTH (r=-0.227, p=0.024).

Discussion

The most significant findings of this investigation were as follows: (i) Hypocalcemia among COVID-19 patients admitted to ICU was less common (30%) compared to reported in the pertinent literature. (ii) Overall, 81% of all patients had either deficiency or insufficiency of 25(OH) vitamin D. This rate was 96.7% among hypocalcemic patients. (iii) On admission, 11% of the patients had hypomagnesemia. Hypomagnesemic patients both had significantly lower calcium and higher serum PTH values compared to patients with normal serum magnesium. (iv) Presence or severity of

Table 1. Demographic and clinical features of the whole study cohort (n=100)

	Patients
Age (years)	68.5 (14.2)
Sex	
Male	65 (65%)
Female	35 (35%)
Body mass index (kg/m2)	27.8 (18.9- 45.0)
Length of hospital stay (days)	11.0 (1.0- 57.0)
Need of mechanical ventilation	67 (67%)
Pulse steroid administration	73 (73%)
In-hospital mortality	
Decedents	52 (52%)
Survivors	48 (48%)
25(OH) vitamin D (ng/ml) (on admission)	9.9 (4.2- 132)
25(OH) vitamin D status	
Normal	19 (19%)
Insufficiency	29 (29%)
Deficiency	52 (52%)

Continuous variables were expressed as mean±standard deviation or median (min-max) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages (%)

hypocalcemia was not associated with COVID-19 severity or mortality.

Hypocalcemia frequency was relatively less in our study compared to reported by similar studies conducted earlier. There is hypocalcemia in 59% of COVID-19 patients, according to clinical data from 12 articles. The results of the meta-analysis demonstrated a strong association between hypocalcemia and disease severity mortality in COVID-19 patients, hospital days, and intensive care unit hospitalization. This rate was only 30% in our study on admission. Actually, in two studies involved in this meta-analysis hypocalcemia frequencies were 37.2 (18) and 35.8% (19), respectively.

The specific etiology of hypocalcemia in COVID19 patients in critical condition is unknown, although the following processes have been proposed (20). Hypocalcemia can be caused by old age and persistent starvation that resulted in vitamin D insufficiency (21). Plasma albumin binds the majority of calcium. Low serum albumin concentrations can cause hypocalcemia (22). In individuals with COVID19, proinflammatory cytokines impede PTH secretion and produce calcium imbalance (23). Patients with COVID19 have been observed to have elevated amounts of unsaturated lipids, as unsaturated fatty acids can bind to calcium and produce hypocalcemia (24). Several interrelated factors such as hypovitaminosis D, the suppressive effect of increased inflammatory mediators on PTH release, and decreased serum albumin levels might affect the level of serum calcium in patients with severe COVID-19 (25). In our study, we used corrected serum calcium to negate the effect of critical illness-related hypoalbuminemia on serum calcium levels. Nevertheless, broad changes in these numerous factors might be responsible for the discrepant hypocalcemia frequencies observed in different studies.

Most studies found significant untoward effects of hypocalcemia on clinical outcomes and survival of COVID-19 patients (7,19). Bennouar and coleagues indicate the significant prevalence of hypocalcemia and hypovitaminosis D in patients with severe COVID-19 and gives additional evidence of their possible association with a poor short-term prognosis (19). Liu J and his group showed that at the time of admission, nearly two-thirds of severe COVID-19 patients had hypocalcemia. Patients with hypocalcemia were admitted with a more severe illness and had poorer outcomes (7). However, some studies failed to demonstrate such an asociation. Doedatus and colleagues (26) in their study, showed plasma calcium concentrations were lower in COVID-19-positive patients compared to COVID-19-negative patients, but multivariate analysis revealed no correlation with disease

Table 2. Laboratory parameters of the whole study cohort (n=100)

	On admission (1st)	Second (2nd)	Third (3rd)	*P-value
Corrected Calcium (mg/dL)	8.2 (0.6)	9.0 (0.6)	8.9 (7.0- 10.2)	.268*
Phosphorus (mg/dL)	3.6 (1.1)	4.3(7.9)	3.2 (1.5- 6.9)	.040*
Magnesium (mg/dL)	2.2 (0.4)	2.2 (1.2- 3.3)	2.0 (1.5- 2.9)	.878*
Parathyroid hormone (PTH) (pg/mL)	85.0 (11.4- 880)	107 (19.3- 677)	67.3 (17.6-609)	.210*
Procalcitonin (ng/mL)	0.3 (0.0- 100)	0.2 (0.0- 100)	0.2 (0.1- 21.7)	.088*
Ferritin (ng/mL)	424 (13.7- 1650)	435 (38.6- 1650)	457 (72.0-1650)	.879*
Troponin I (ng/L)	50.5 (6.0- 19702)	48.5 (6.0- 5690)	16.0 (6.0-4890)	.052*
D-dimer (mg/L)	1684 (347- 34960)	2184 (304-34960)	2158 (229-25039)	.022*
C-reactive protein (mg/L)	144.3 (92.1)	62.6 (1.2- 611)	48.0 (1.6- 283)	.003*
Lactate dehydrogenase (U/L)	474 (129- 2517)	421 (166- 1737)	426.8 (183.6)	.088*
Albumin (g/dL)	2.94 (4.2)	2.57 (1.91- 28.9)	2.53 [1.66-29.2)	.001*
Urea (mg/dL)	61.5 (14.0- 216)	74.0 (24.0- 295)	71.1 (41.0)	.022*
Creatinine (mg/dL)	1.1 (0.6- 4.0)	1.0 (0.5- 5.1)	0.9 (0.4- 3.7)	.001*
eGFR (mL/min)	61.0 (27.9)	68.0 (8.0- 160)	92.0 (14.0-110)	.001*

Continuous variables were expressed as mean±standard deviation or median (min-max) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages (%).
eGFR: Estimated glomerular filtration rate, pg/mL:picogram/milliliter, ng/ml:nanogram/milliliter, ng/l: nanogram/liter, mg/l: milligram/liter, U/L:units/liter, g/dL: grams/deciliter, mg/dL: milligram/deciliter.
* The Friedman test
Signifianct differences according to the posthoc Durbin-Conover test: C-reactive protein (1^{st}) - C-reactive protein (2^{nd}) (*P*=.004), C-reactive protein (1^{st}) - C-reactive protein (3^{rd}) (*P*=.001), Albumin (1^{st}) - Albumin (2^{nd}) (*P*<.001), Albumin (1^{st}) - Albumin (3^{rd}) (<.001), Phosphorus (1^{st}) - Phosphorus (2^{nd}) (P=.018), Phosphorus (1^{st}) - Phosphorus (3^{rd}) (*P*=.042), eGFR (1^{st}) - eGFR (2^{nd}) (*P*=.008), eGFR (1^{st}) - eGFR (3^{rd}) (*P*<.001). D-dimer (1^{st}) - D-dimer (2^{nd}) (*P*=.007). Phosphorus (1^{st}) - Phosphorus (2^{nd}) (*P*=.018), Phosphorus (1^{st}) - Phosphorus (3^{rd}) (*P*=.042), eGFR (1^{st}) - eGFR (2^{nd}) (*P*=.008), eGFR (1^{st}) - eGFR (3^{rd}) (P<.001). D-dimer (1^{st}) - D-dimer (2^{nd}) (*P*=.007). Urea (1^{st}) -Urea (2^{nd}) (*P*=.006), Creatinine (1^{st}) – Creatinine (3^{rd}) (*P*<.001), Creatinine (2^{nd}) – Creatinine (3^{rd}) (*P*<.001)

severity. Our results also did not show any substantial relationship between hypocalcemia and severe COVID-19 and mortality. The negative result in our study might be due to relatively low number of participants and low frequency of hypocalcemia compared to previous studies.

To the best of our knowledge, most research evaluating the frequency and prognostic significance of hypocalcemia in COVID-19 patients have relied on a single serum calcium value, typically the value measured upon admission. (4,5,7,9,25). However, we evaluated the trend of change in serum calcium and other laboratory parameters both on admission and then throughout the hospitalization. This enabled us to contribute novel data onto the already known literature. Serum corrected calcium levels tended to increase during the hospitalization, albeit statistically insignificantly.

di Filippo at al. (9) in their 78 patients trial participated reported patients with vitamin D deficiency had considerably lower total Ca levels, and regression analysis revealed a positive association between vitamin D and total Ca. They demonstrated for the first time that hypocalcemia predominated in COVID-19 patients with significant hypovitaminosis D that was not fully compensated by secondary hyperparathyroidism, which may be directly related to SARS-CoV-2 infection and may facilitate the development of hypocalcemia in these patients (9). They identified three study limitations: the relatively small patient enrollment due to stringent inclusion and exclusion criteria; the evaluation of uncorrected

Table 3. Comparison of clinical features and laboratory data between groups stratified according to Calcium levels

	Patients with hypocalcemia (n=30)	Patients with normocalcemia (n=68)	P-value
Age (years)	69.5 (15.8)	68.1 (13.6)	.675*
Sex			
Male	20 (66.7%)	45 (66.2%)	.999**
Female	10 (33.3%)	23 (33.8%)	
Parathyroid hormone (pg/mL)	118.4 (35.5-880.0)	75.2 (11.4-674.0)	.001χ
25(OH) vitamin D (ng/mL)	8.1 (4.2-27.2)	11.7 (4.9-132.0)	.002 χ
25(OH) vitamin status			
Normal	1 (3.3%)	17 (25.0%)	
Insufficiency	5 (16.7%)	24 (35.3%)	.001**
Deficiency	24 (80.0%)	27 (39.7%)	
Albumin (g/dL)	30.4 (4.0)	29.1 (4.2)	.152*
Magnesium (mg/dL)	2.2 (0.5)	2.2 (0.4)	.998*
Phosphorus (mg/dL)	4.0 (1.4)	3.4 (0.9)	.042*
Urea (mg/dL)	70.0 (22.0-216.0)	58.5 (14.0-191.0)	.043 χ
Creatinine (mg/dL)	1.3 (0.6-3.6)	1.0 (0.6-4.0)	.003 χ
eGFR (mL/min/1.73 m2)	51.1 (26.5)	66.7 (26.7)	.010*
Glomerular filtration rate			
<60	17 (56.7%)	28 (41.2%)	.231**
≥60	13 (43.3%)	40 (58.8%)	
APACHE score	17 (5-30)	15 (3-41)	.379 χ
In-hospital mortality			
Decedents	14 (46.7%)	36 (52.9%)	
Survivors	16 (53.3%)	32 (47.1%)	.724**

Continuous variables were expressed as mean±standard deviation or median (min-max) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages (%).
eGFR: Estimated glomerular filtration rate, pg/mL:picogram/milliliter, ng/mL:nanogram/milliliter, g/dL: grams/deciliter, mg/dL: milligram/deciliter.
*The Independent t-test, ** Chi-square test, χ Mann-Whitney U test

total Ca levels due to the absence of albumin levels (although ionized calcium levels were analyzed); and the absence of information on magnesium levels (9).

One important aspect of evaluation in patients with hypocalcemia is the need for evaluating serum magnesium levels. Unfortunately, serum magnesium levels were measured in only a few of the studies examining hypocalcemia and its impact on prognosis in COVID-19 patients (6,24). Since hypomagnesemia can lead to hypocalcemia (27) and is common in critically ill patients (28), we studied serum magnesium levels in our patient cohort. Torres et al. (24) found within 72 hours of hospital admission, 63% of patients had hypocalcemia that is common in hospitalized patients infected with SARS-CoV-2 and may identify individuals with a poor prognosis and immediate need for intensive care. The frequency of hypomagnesemia in the whole cohort was 17%. However, there was no difference between hypocalcemic and normocalcemic patients in terms of hypomagnesemia frequency. Pal and colleagues (6) conducted a case-control study in which they evaluated nonsevere COVID-19 patients retrospectively. Although the authors measured serum magnesium levels in COVID-19 patients, they did not present any data regarding the percentage of the patients with hypomagnesemia. Our results showed that around 10% of the patients had hypomagnesemia on admission. These patients had significantly lower serum corrected calcium and higher serum PTH values compared to patients with normal serum magnesium levels. On the other hand, vitamin D levels were similar in both groups. Despite similar vitamin D levels, patients with hypomagnesemia had significantly lower serum calcium values. One interesting observation was also that hypomagnesemic patients had deeper hypocalcemia although their median PTH levels were significantly higher compared to normomagnesemic patients. These higher PTH values could not increase serum calcium levels

Table 4. Comparison of Clinical Features and Laboratory Data Between Groups Stratified According To Vitamin D Status

	Groups			
	Normal (n=19)	Insufficiency (n=29)	Deficiency (n=52)	P-value
Age (years)	70.4 (14.7)	63.3 (13.5)	70.7 (14.0)	.063*
Sex				
Male	10 (52.6%)	22 (75.9%)	33 (63.5%)	.242**
Female	9 (47.4%)	7 (24.1%)	19 (36.5%)	
Body mass index (kg/m2)	25.7 (18.9- 39.1)	29.3 (20.5- 37.1)	27.8 (19.5- 45.0)	.411 χ
Length of hospital stay (days)	6.0 (1.0- 25.0)	14.0 (2.0- 50.0)	11.5 (3.0- 57.0)	.015 χ
Need of mechanical ventilation	14 (73.7%)	20 (69.0%)	33 (63.5%)	.695**
Pulse steroid administration	10 (52.6%)	23 (79.3%)	40 (76.9%)	.082**
Parathyroid hormone (PTH) (pg/mL)	82.9 (11.4- 377)	74.0 (20.8- 674)	106 (20.5- 880)	.224 χ
25(OH) vitamin D (ng/ml)	33.8 (20.9- 132.0)	13.9 (10.0- 19.7)	7.5 (4.2- 10.0)	.001 χ
Procalcitonin (ng/mL)	0.4 (0.0- 31.0)	0.3 (0.0- 100.0)	0.4 (0.0- 68.3)	.522 χ
Ferritin (ng/ml)	402 (43- 1650)	418 (35- 1650)	480 (14- 1650)	.801 χ
Troponin I (ng/L)	46.0 (6.0- 4197)	31.0 (6.0- 19702)	56.5 (6.0- 10357)	.552 χ
D-dimer (mg/L)	1717 (482-21494)	1045 (347- 34400)	2004 (393-34960)	.258 χ
C-reactive protein (mg/l)	140.0 (83.1)	135.5 (119.0)	150.8 (78.6)	.776*
Lactate dehydrogenase (U/L)	408 (129- 2517)	459 (207- 1055)	497 (213- 1054)	.662 χ
Albumin (g/dL)	2.75 (0.41)	3.05 (0.41)	2.94 (0.41)	.057*
Corrected Calcium (mg/dL)	9.3 (0.9)	9.2 (0.5)	8.9 (0.6)	.051*
Magnesium (mg/dL)	2.0 (0.4)	2.2 (0.4)	2.2 (0.4)	.040*
Phosphorus (mg/dL)	3.2 (0.8)	3.7 (0.8)	3.7 (1.3)	.071*
Urea (mg/dL)	70.0 (26.0- 174)	55.0 (23.0- 190)	64.0 (14.0- 216)	.263 χ
Creatinine (mg/ dL)	1.0 (0.6- 3.1)	1.0 (0.7- 3.3)	1.1 (0.6- 4.0)	.782 χ
eGFR	58.9 (33.4])	67.5 (28.0)	58.2 (25.5)	.336*
eGFR groups				
<60 mL/min/1.73 m2	9 (47.4%)	11 (37.9%)	27 (51.9%)	.481**
≥60 mL/min/1.73 m2	10 (52.6%)	18 (62.1%)	25 (48.1%)	
APACHE score	18 (8-33)	12 (3-24)	15.5 (5-41)	.002*
In-hospital mortality				
Decedents	11 (57.9%)	16 (55.2%)	25 (48.1%)	.704**
Survivors	8 (42.1%)	13 (44.8%)	27 (51.9%)	

Continuous variables were expressed as mean±standard deviation or median (min-max) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages (%).
eGFR: Estimated glomerular filtration rate, pg/mL: picogram/milliliter, ng/ml: nanogram/milliliter ng/l: nanogram/liter, mg/l: milligram/liter, U/L: units/liter, g/dL: grams/deciliter, mg/dL: milligram/deciliter.
*The one-way analysis of variance (ANOVA) test, ** Chi-square test, χ The Kruskal Wallis test.

back to normal. This might reflect the occurrence of peripheral resistance to the effects of PTH due to hypomagnesemia. Median estimated glomerular filtration rates were also similar in normomagnesemic and hypomagnesemic patients. Similar kidney function also negates the discriminative effect of chronic kidney disease on serum PTH levels. Thus, we think that serum magnesium levels should be a component of the evaluation of hypocalcemia in patients hospitalized with COVID-19.

Serum 25(OH) vitamin D levels have been studied in COVID-19 patients relatively well. Several meta-analyses evaluated the prevalence and effects of serum 25(OH) vitamin D on prognosis in COVID-19 patients. A very recent meta-analysis by Pereira et al. (12) support the significant frequency of vitamin D deficiency in COVID-19 patients, particularly the elderly. It's important to note that COVID-19 infection was not linked to vitamin D deficiency. However, they found a link between vitamin D i
illness severity. From
blood vitamin D levels may be taken ınto account in medical professionals' clinical practice (12). In a similar meta-analysis Wang et al. (29) reported

Table 5. Comparison of Clinical Features and Laboratory Data Between Groups Stratified Based on eGFR Values

	eGFR Groups		
	<60 mL/min/1.73 m2 (n=47)	≥60 mL/min/1.73 m2 (n=53)	P-value
Age (years)	76.3 (10.0)	61.5 (13.8)	.001*
Sex			
Male	25 (53.2%)	40 (75.5%)	.034**
Female	22 (46.8%)	13 (24.5%)	
Body mass index	27.5(20.2- 45.0)	27.8 (18.9- 42.5)	.912 χ
Length of hospital stay (days)	9.0 (3.0- 57.0)	12.0 (1.0- 50.0)	.399 χ
Need of mechanical ventilation	36 (76.6%)	31 (58.5%)	.088**
Pulse steroid administration	30 (63.8%)	43 (81.1%)	.086**
Parathyroid hormone (PTH)(pg/mL)	121 (20.5- 880)	61.4 (11.4- 785)	.001 χ
25(OH) vitamin D (ng/mL)	8.4(4.2- 76.3)	10.7 (5.3- 132)	.165 χ
25(OH) vitamin D status			
Normal	9 (19.1%)	10 (18.9%)	
Insufficiency	11 (23.4%)	18 (34.0%)	.481**
Deficiency	27 (57.4%)	25 (47.2%)	
Procalcitonin (ng/ml))	0.7 (0.1- 100)	0.3 (0.0- 2.2)	.001 χ
Ferritin (ng/ml)	376(23.0- 1650)	429 (13.7- 1650)	.641 χ
Troponin I (ng/L)	77.0(6.0- 19702)	21.0 (6.0- 2208)	.001 χ
D-dimer (mg/l)	2188 (480- 34400)	1109 (347- 34960)	.072 χ
C-reactive protein (mg/L)	157.1 (110)	133.0 (71.9)	.206*
Lactate dehydrogenase (U/L)	437 (207- 2517)	512 (129- 1055)	.054 χ
Albumin (g/dL)	2.93 (0.47)	2.95 (0.37)	.855*
Corrected Calcium (mg/dL)	9.1 (0.8)	9.0 (0.5)	.846*
Magnesium (mg/dL)	2.0 (0.4)	2.3 (0.4)	.005*
Phosphorus (mg/dL)	3.9 (1.3)	3.3 (0.9)	.014*
Urea (mg/dL)	80.0 (38.0- 216)	44.0 (14.0- 107)	.001 χ
Creatinine (mg/dL)	1.7 (1.0- 4.0)	0.9 (0.6- 1.4)	.001 χ
eGFR (mL/min/1.73 m2)	36.0 (15.7)	83.2 (14.0]	.001*
In-hospital mortality			
Decedents	31 (66.0%)	21 (39.6%)	.015**
Survivors	16 (34.0%)	32 (60.4%)	

Continuous variables were expressed as mean±standard deviation or median (min-max) depending on the distribution of the variable. Categorical variables were reported as numbers and percentages (%).
eGFR: Estimated glomerular filtration rate, pg/mL:picogram/milliliter, ng/ml:nanogram/milliliter ng/l: nanogram/liter, mg/l: milligram/liter, U/L:units/liter, g/dL: grams/deciliter, mg/dL: milligram/deciliter.
* The Independent t-test, ** Chi-square test, χ Mann-Whitney U test

compared to those without vitamin D insufficiency, vitamin D deficiency was associated with considerably greater death rates, higher hospital admission rates, and longer hospital stay. In our study, vitamin D deficiency and insufficiency combined were present in 81% of the patients, and this high prevalence was consistent with the published literature of COVID-19 patients. On the contrary, vitamin D deficiency/insufficiency was associated neither with COVID-19 severity nor with in-hospital mortality rates. This discrepancy with the previous studies might be due to the relatively small sample size, comorbidity distribution of the patients, and differences in COVID-19 treatment protocols between various centers.

We conducted univariate and multivariate regression analyses to determine the independent predictors of in-hospital mortality in the hospital setting. In the univariate analysis, age, the need for

Table 6. Univariate and Multivariate Logistic Regression Analyses To Determine Independent Predictors of In-Hospital Mortality

	Univariate			Multivariate		
Parameters	Hazard Ratio	95% Confidence Interval	P-value	Hazard Ratio	95% Confidence Interval	P-value
Age	1.040	1.009-1.071	.011	0.997	0.947-1.050	.951
Need of mechanical ventilation	0.022	0.005-0.102	.001	0.021	0.004-0.109	.001
Procalcitonin	1.062	0.996-1.133	.067	-	-	-
Troponin I	1.000	1.000-1.001	.228	-	-	-
Albumin	0.86	0.774-0.957	.006	0.867	0.750-1.003	.055
Urea	1.008	0.998-1.018	.107	-	-	
eGFR	0.985	0.971-1.000	.047	0.981	0.955-1.007	.151

eGFR: Estimated glomerular filtration rate.
Hosmer and Lemeshow test *P*=.145 (for multivariate regression)

mechanical ventilation, and serum albumin level were found to be independent associates of mortality. However, in the multivariate model, only the requirement for mechanical ventilation emerged as a significant determinant of in-hospital mortality. Due to the low number of patients and the retrospective design, the effects of other variables may not have been revealed.

There are a few limitations of this research that need addressing. First, since our review was retrospective, data correctness and completeness cannot be determined with absolute certainty. Second, we conducted a single-center study. Hence our sample size was relatively small compared to similar studies in the literature. We did not take into account the exact mortality date of the patients thus we could not perform Cox and survival analyses. Lastly, we included patients with eGFR<60 mL/min. Despite the potential impact of impaired kidney function on calcium metabolism, we thought our sample of COVID-19 patients in the ICU reflected a more real-life situation.

In conclusion, we demonstrated in a cohort of severe COVID-19 patients that hypocalcemia was less common compared to the literature. Vitamin D deficiency/insufficiency was widespread among patients. Importantly, we showed that serum calcium levels did not change throughout the hospitalization. In addition, serum magnesium level appeared as an important modifier of serum calcium levels in these patients. Hypocalcemia was not associated with COVID-disease severity or mortality. Particulary the role of magnesium in COVID-19 related hypocalcemia needs further attention of the researchers.

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