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Hypocalcemia as a Risk of COVID-19 Mortality: What Level of Hypocalcemia? Direct Measurement or Corrected Calcium Value?

COVID-19 Mortalitesinde Hipokalseminin Rolü: Direkt Ölçüm mü, Düzeltilmiş Kalsiyum mu?

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ABSTRACT

Objective: The negative effects of vitamin D deficiency and hypocalcemia on the frequency and severity of COVID-19 are quite striking. To determine the presence of hypocalcemia in hospitalized COVID-19 patients, the relationship between severity and outcome, and whether albumin correction is necessary to predict this relationship.

Material and Method: Hospitalized COVID-19 patients were grouped for lung involvement, need for intensive care, need for mechanical ventilation, long hospitalization (≥14 days), sepsis, and mortality. Blood calcium, albumin, 25OHvitamin D, parathyroid hormone, phosphorus, and magnesium values and albumin-corrected calcium data were evaluated retrospectively.

Results: The study included 129 hospitalized COVID-19 patients, 67 (51.9%) male and 62 (40%) female. Vitamin D was insufficient in 89.9% of the patients and no association was found with disease severity. Calcium levels were significantly lower in terms of need for intensive care, sepsis, mechanical ventilation and mortality (p: 0.00, 0.013, 0.021, 0.004, respectively). No association was found with corrected Ca (p>0.05 for all). A significant increase was found in the same cases when total Ca values were below 8 mg/dl (p: 0.00, 0.001, 0.005 and 0.001, respectively). In the logistic regression analysis, it was found that age, increase in CRP and decrease in Ca levels significantly increased the risk for mortality (p: 0.005, 0.001 and 0.013, respectively).

Conclusion: It is found that total Ca values obtained by direct measurement below 8 mg/dl in COVID-19 patients were significantly more likely to require intensive care, require mechanical ventilation, sepsis and mortality, and in the analysis we determined that a decrease in direct measured (incorrected with albumin) total Ca values, together with advanced age and high CRP, was an independent risk factor that increased mortality.

Keywords: *COVID-19, Calcium, Corrected calcium, Mortality*

ÖZET

Giriş: D vitamini eksikliği ve hipokalseminin COVİD-19'daki sıklığı ve ciddiyeti üzerine olumsuz etkileri oldukça çarpıcıdır. Hospitalize COVİD-19 hastalarındaki hipokalsemi varlığını, şiddet sonuç ilişkisini ve bu ilişkiyi öngörmede albümin düzeltmesinin gerekli olup olmadığını belirlemek.

Materyal ve Metot: Yatan COVİD-19 hastaları akciğer tutulumu, yoğun bakım ihtiyacı, mekanik ventilasyon ihtiyacı, uzun yatış (≥14gün), sepsis, mortalite için gruplandırıldı. Kan kalsiyum, albümin, 25OHvitamin D, parathormon, fosfor ve magnezyum değerleri ile albüminle düzeltilmiş kalsiyum verileri retrospektif değerlendirildi.

Bulgular: Çalışmaya 67 (51.9%) erkek ve 62 (40%) kadın hasta olmak üzere 129 hospitalize COVİD-19 hastası alındı. Hastaların 89.9%'unda D vitamini yetersizdi ve hastalık şiddeti ile bir ilişki saptanmadı. Kalsiyum düzeyleri; yoğun bakım ihtiyacı, sepsis, mekanik ventilasyon ve mortalitede anlamlı olarak düşük tespit edilmiştir (sırası ile p: 0.00, 0.013,0.021, 0.004). Düzeltilmiş Ca ile bir ilişki saptanmadı (hepsinde p>0.05). Total Ca değeri 8mg/dl altında yine aynı durumlarda anlamlı olarak artış tespit edildi (sırası ile p:0.00, 0.001, 0.005 ve 0.001). Logistik regresyon analizinde mortalite için yaş, CRP artışı ve Ca düzeylerinde düşmenin anlamlı risk artışı yaptığı saptandı (sırası ile p: 0.005, 0.001 ve 0.013).

Sonuç: COVİD-19 hastalarında direkt ölçüm ile elde edilen total Ca değerinin 8 mg/dl nin altında olmasını yoğun bakım ihtiyacı, mekanik ventilasyon ihtiyacı, sepsis ve mortalitede anlamlı olarak daha fazla bulduk ve yapılan analizde direkt ölçüm (albumin ile düzeltilmemiş) total Ca değerinde düşmenin, ileri yaş ve CRP yüksekliği ile birlikte mortaliteyi artıran bağımsız bir risk faktörü olduğunu belirledik.

Anahtar kelimeler: *COVİD-19, Kalsiyum, Düzeltilmiş kalsiyum, Mortalite*

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INTRODUCTION

The COVID-19 pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) that started in December 2019 has spread rapidly globally. 761,402,282 people have been infected with this virus worldwide. It is estimated that 1-20% of the world's population has been exposed to COVID-19. The number of infected people is beyond estimates, as many patients are asymptomatic and have not been tested. There has been a great loss of life due to COVID-19. As of March 19, 2023, the number of deaths reported to the World Health Organization is 6,887,000 (WHO, 2023). Various studies have been published to determine the risk factors for poor prognosis of this devastating viral disease (Carpagnano, 2020; Di Filippo, 2020; Lippi, 2020; Ponti, 2020; Ahmet, 2021). The negative effects of vitamin D deficiency and hypocalcemia on the frequency and severity of this disease are quite striking.

Vitamin D has been shown to play an important role in immune system activation and prevention of infections. Vitamin D receptors are found in many cells of the immune system, such as macrophages, dendritic cells, and T and B cells. It has been shown that there is an increase in respiratory system diseases, influenza, and M. tuberculosis infections in those with vitamin D deficiency (Mao, 2020). In a retrospective observational study conducted in Italy and including patients with severe COVID-19, it was found that patients presenting with severe vitamin D deficiency had a significantly higher short-term mortality risk (Carpagnano, 2020).

It has been reported that calcium is used by the virus by participating in the replication mechanisms in SARS-CoV-2 infection, also known as COVID-19, just as it has been shown in Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Ebola virus infections (Millet, 2018; Nathan, 2020; Straus, 2020). In line with this, hypocalcemia has been a prominent finding in COVID-19 patients with pneumonia (Crespi, 2020). Diez et al. have shown that a decrease in calcium levels causes an increase in the need for mechanical ventilation, the need for intensive care, hospital mortality and the risk of combined outcome (Diez, 2022).

In our study, we specifically investigated the presence of hypocalcemia in COVID-19 patients, its relationship with the clinical course of COVID-19, and whether albumin correction was necessary to predict this relationship. We also checked the blood levels of 25-OH vitamin D, which plays a role in Ca metabolism and is an immune system regulator and anti-inflammatory hormone, and PTH, P and Mg, which also affect and are affected.

MATERIAL and METHOD

The study was conducted as a retrospective cohort on the records of 129 patients who were hospitalized in the wards and intensive care units of Van Yuzuncu Yil University Medical Faculty Hospital due to COVID-19 between 01.10.2021-01.02.2022. Samples were taken from upper respiratory tract samples (nose or throat) with a swab and studied with RT-PCR in Van YYU Hospital Clinical Microbiology Laboratory. Patients who were diagnosed with COVID-19 and had positive RT-PCR or antibody tests were included in the study.

Laboratory markers (creatinine, albumin, Ca, Mg, P, vitamin D, PTH, ferritin, CRP), vital signs (fever, O_2 saturation, blood pressure, pulse, respiratory rate) and lung Computed Tomography and direct radiography images important in the prognosis of COVID-19 were obtained from the hospital's computer system.

Groupings made in our study:

1-) Female and male

2-) Hospitalized patients' need for ward or intensive care

3-) Patients' survival status according to whether they are alive or dead

4-) Patients' sepsis and non-sepsis according to qSOFA criteria

5-) Patients' need for mechanical ventilation and non-sepsis

6-) Hospitalization period of 14 days and above and those who are not

7-) According to 25OHvitamin D level; sufficient (≥30ng/ml), insufficient (20-29ng/ml), deficient (10-19ng/ml) and profoundly deficient (<10ng/ml)

8-) According to calcium level; hypocalcemia (<8.5mg/dl), significant hypocalcemia (<8mg/dl) 'qSOFA criteria' are criteria used in the bedside assessment of adult patients with suspected infection for sepsis in out-of-hospital, emergency department or general hospital settings.

There are 3 parameters.

1- Tachypnea(>22/min)

2- Hypotension(<100mmHg)

3- Mental changes

The presence of 2 or more of the 3 criteria suggests sepsis (Vincent, 2016).

25-OH vitamin D measurement was performed by a chemiluminescence method using 25-OH D commercial kit (Elecsys) in our hospital. Results are given as ng/ml. The normal value of serum 25-OH D was accepted as ≥30 ng/ml (Chauhan, 2023).

Calcium levels were recorded by direct measurement and corrected Ca calculation. The normal value range of calcium level is 8.5 to 10.3 mg/dL. It is free, ionized extracellular calcium that affects all physiological functions. It was calculated with the formula Corrected calcium (mg/dl) = (4-serum albumin) x0.8 + measured total calcium (mg/dl) (Benabel, 1994). The normal value range of magnesium level is 1.7 to 2.3 mg/dL. The normal value range of phosphorus level is 2.5-4.5 mg/dl (0.81-1.45 mmol/l). The normal value range for PTH levels is 15-65 pg/mL.

Statistical analysis

In calculating the sample size of this study, Power (Test Power) was determined for each variable by taking at least 80% and Type-1 error as 5%. Kolmogorov-Smirnov (n>50) and Skewness-Kurtosis tests were used to check whether the continuous measurements in the study were normally distributed, and since the measurements were normally distributed, parametric tests were applied. Descriptive statistics for the variables in the study were expressed as mean, standard deviation, minimum, maximum, number (n) and percentage (%). Chi-square test was used to compare measurements according to "categorical groups", and "Independent T-test" was used to compare parametric groups. Logistic Regression analysis was used to determine (estimate) the effect of some variables on "mortality". Pearson correlation coefficients were calculated to determine the relationship between measurements. In the calculations, the statistical significance level (á) was taken as 5% and SPSS (IBM SPSS for Windows ver. 25) statistical package program was used for the analysis

RESULTS

Of the 129 patients included in the study, 67 (51.9%) were male and 62 (40%) were female. In 116 of 128 patients (89.9%), 25OH vitamin D levels were found to be below 30ng/ml. Magnesium and creatinine were significantly lower in women than in men (p: 0.025, 0.001, respectively). (Table 1)

Table 1. Comparison of parameters between genders

	Gender				
	Female (N:62)48.1%		**Male (N:67) 51.9%**		***p**
	Mean	**Std. Dev.**	**Mean**	**Std. Dev.**	
Age	60.69	15.69	59.79	13.93	.730
25 OH vitamin D (ng/ml)	16.16	22.61	14.76	15.39	.680
PTH (pg/ml)	124.7	76.47	104.56	70.18	.143
Ca (mg/dl)	7.93	.71	7.75	.64	.138
Mg (mg/dl)	1.96	.31	2.07	.23	.025
P (mg/dl)	2.92	.82	2.77	.65	.267
Albumin (g/l)	4.23	5.76	3.19	.56	.151
Ferritin (ng/ml)	834.09	1841.90	1127.77	1203.72	.282
Crp (mg/l)	57.03	67.87	59.05	54.46	.853
Creatinine (mg/dl)	.73	.21	.85	.22	.001
O_2 Saturation (%)	89.68	6.86	89.18	7.57	.697
Duration Covid (days)	13.81	7.63	10.81	9.42	.050
Corrected Ca	8.59	.56	8.44	.59	.142

** Significance levels according to Independent T-test results*

Of the 129 patients, 47 (36.4%) were intensive care patients and 82 (63.5%) were ward patients. Of the intensive care patients, 26 (61.9%) were male and 16 (38%) were female. When intensive care patients were compared with ward patients; COVID-19 disease duration was significantly longer and the mean age was higher in the intensive care unit (p: 0.001, 0.031, respectively). Oxygen saturation of intensive care patients was significantly lower (p: 0.000). Ferritin and CRP were significantly higher in intensive care patients (p: 0.012, 0.000, respectively). PTH levels of intensive care patients were significantly higher than ward patients (p: 0.000). Ca levels of intensive care patients were significantly lower than ward patients (p: 0.000). (Table 2)

Table 2. Comparison of ward and intensive care patients

	Group				
	Ward (N:82 63,6%)		Intensive Care (N:47 36,4%)		*p
	Mean	Std. Dev.	Mean	Std. Dev.	
Age	58.11	14.21	63.91	15.09	.031
25 OH vitamin D	14.75	19.36	16.60	18.81	.601
PTH	95.44	58.05	145.33	86.29	.000
Ca	7.99	.63	7.56	.66	.000
Mg	2.02	.27	2.01	.29	.762
P	2.88	.75	2.78	.73	.496
Albumin	3.86	4.21	3.38	3.66	.521
Ferritin	729.34	879.78	1435.51	2225.17	.012
Crp	43.22	44.88	84.55	76.07	.000
Creatinine	.81	.20	.77	.26	.338
O_2 Saturation	91.23	4.64	86.26	9.53	.000
Duration Covid	10.40	5.73	15.47	11.68	.001
Corrected Ca	8.51	.55	8.51	.63	.981

** Significance levels according to Independent T-test results*

When patients with pulmonary involvement (n: 101, 78%) were compared with those without (n: 23, 22%), only CRP was found to be significantly higher in those with involvement (p: 0.02 63.5 mg/l vs 31.5 mg/l). In the statistical analysis performed between genders, no significant difference was found in terms of pulmonary involvement, mortality and sepsis (p: 0.105, 0.567, 0.478, respectively). When we compared those with a hospital stay of 14 days (n:42) or more (n:87) with those without, age, 25OH vitamin D, PTH, Ca, Mg, albumin, ferritin, CRP, creatinine and O_2 saturation levels were found to be similar (p: 0.34, 0.42, 0.80, 0.90, 0.11, 0.83, 0.66, 0.06, 0.35 and 0.14, respectively). Albumin-corrected Ca and P were found to be significantly higher in those with a longer hospital stay (p: 0.025, 0.006, respectively).

According to qSOFA criteria, when patients with sepsis (n: 57) were compared with those without (n: 72), PTH was significantly higher and Ca was significantly lower in sepsis (p: 0.024, 0.013, respectively). (Table 3)

Table 3. Comparison of laboratory parameters between sepsis and non-sepsis patients

	qSOFA (Sepsis)				
	No (N:72, 55,8%)		Yes (N:57, 44.18%)		p*
	Mean	Std Dev.	Mean	Std Dev.	
Age	58.46	14.71	62.46	14.63	.127
25-OH vitamin D	16.28	21.14	14.37	16.35	.575
PTH	100.32	63.12	130.78	82.31	.024
Ca	7.97	.53	7.67	.80	.013
Mg	2.04	.26	1.99	.30	.339
P	2.93	.74	2.73	.73	.136
Corrected Ca	8.48	.44	8.56	.73	.464

** Significance levels according to Independent T-test results*

The number of patients requiring mechanical ventilation was 30 and the number of patients not requiring mechanical ventilation was 99. In the statistical analysis, the mean age was 64 in those intubated and 59 in those not (p: 0.095). Again, PTH was higher in those requiring mechanical ventilation, but it was not statistically significant (mean: 135 vs 107pg/ml, respectively, p: 0.085). 25OHvitamin D, Mg, P and corrected Ca values were also found to be statistically insignificant (p: 0.84, 0.34, 0.96, 0.97, respectively). Calcium levels were significantly lower in those requiring mechanical ventilation than in those not (mean: 7.58 vs 7.91 mg/dl, respectively, p: 0.021)

Thirty-eight (80.8%) of the intensive care patients and 1 (1.2%) of the ward patients were COVID-19 mortal. When the deceased (n: 39) and surviving (n: 90) patients were compared; age, disease duration, CRP level and PTH level were found to be significantly

higher in mortality (p: 0.002, 0.000, 0.000, 0.003, respectively), while oxygen saturation and Ca level were found to be significantly lower (p: 0.000, 0.004, respectively). (Table 4)

Table 4. Comparison of data of mortal and surviving patients

	Alive (N:90 69,76%)		Mortal (N:39 30,24%)		*p
	Mean	Std. Dev.	Mean	Std. Dev.	
Age	57.56	14.41	66.38	13.80	.002
Vitamin D	15.74	21.57	14.72	11.91	.781
PTH	100.93	64.39	143.74	84.95	.003
Ca	7.95	.65	7.57	.67	.004
Mg	2.02	.26	2.01	.31	.982
P	2.87	.73	2.79	.77	.615
Albumin	3.76	4.05	3.52	3.97	.762
Ferritin	848.37	1496.01	1305.68	1626.10	.123
CRP	43.99	44.57	91.43	80.03	.000
Creatinine	.78	.19	.81	.28	.611
O_2 Saturation	91.11	4.80	85.51	9.94	.000
Duration-Covid	10.52	5.84	16.23	12.33	.000
Corrected Ca	8.52	.55	8.49	.65	.812

** Significance levels according to Independent T-test results*

In the correlation analysis, a significant, inverse and low-level relationship was found between CRP and Magnesium in ward patients (r=-0.249). This relationship was also present in intensive care patients (r=-0.044). In addition, in this patient group, a significant, positive relationship was found between phosphorus and magnesium levels (r=0.333) and a significant, negative relationship was found between CRP and vitamin D (r=-0.038).

All patients were grouped as profound deficiency (<10ng/ml), deficiency (10-19ng/ml), insufficiency (20-29 ng/ml) and sufficient (≥30ng/ml) according to their 25OH vitamin D levels. Mortality was 46.1% at vitamin D levels ≥30ng/ml, 36.3% in the range of 20-29 ng/ml, 26.8% in the range of 10-19 ng/ml and 28.57% below 10 ng/ml and it was not found statistically meaningful (p: 0.56). In the statistical analysis, although the numbers of the sufficient and insufficiency groups were low, no significance was found for the presence of pulmonary involvement and sepsis. The same result was reached in the comparison of the deficiency and profound deficiency groups with sufficient numbers for the same parameters (p: 0.49, 0.51, 0.25, respectively). (Table 5)

Table 5. Comparison of groups according to 25OH vitamin D levels for pulmonary involvement, mortality, sepsis and need for mechanical ventilation

		<10ng/ml	10-19ng/ml	20-29ng/ml	≥30ng/ml	Total	p *
Pulmonary involvement	**No**	13	8	2	0	23	0.33
	Yes	47	33	8	13	101	
Mortal	**No**	45	30	7	7	89	0.56
	Yes	18	11	4	6	39	
qSOFA(sepsis)	**No**	33	25	7	6	71	0.68
	Yes	30	16	4	7	57	
Need for mechanical ventilation	**No**	49	33	8	8	98	0.54
	Yes	14	8	3	5	30	
Hospitalization period≥14 days	**No**	43	31	7	5	86	0.099
	Yes	20	10	4	8	42	

**Chi square test*

Patients with hypocalcemia were divided into two groups as Ca value <8mg/dl and <8.5mg/dl and analyzed for gender, age, clinical need, long hospitalization period (≥14 days), lung involvement, mortality, sepsis and mechanical ventilation need. A significant increase in the need for intensive care, mortality, sepsis and mechanical ventilation need was found only in the group with significant hypocalcemia (Ca <8mg/dl) (p: 0.000, 0.001, 0.001, 0.005, respectively). The mortality rate was 32% in Ca <8.5mg/dl and 41.6% in <8mg/dl. No relationship was found with age in either the Ca <8.5mg/dl or <8mg/dl groups (p: 0.92, 0.27, respectively). Additionally, when we analyzed the hypocalcemia and profound hypocalcemia groups for the under 50 age group and the over 50 age group, no significance was found (p: 1 and 0.83, respectively). (Table 6)

When we made the same grouping with albumin-corrected Ca values, although it was not significant when the value was below 8 mg/dl, a result close to significance was obtained in need of intensive care, need for mechanical ventilation and mortality (p: 0.068, 0.075, 0.056, respectively). No significance was found in the analysis for age, gender, need for intensive care, sepsis, lung involvement, mortality, mechanical ventilation, and prolonged hospitalization in the corrected calcium group of threshold value 8.5 (p: 0.35, 0.36, 0.85, 0.71, 0.63, 0.56, 0.4 and 0.44). No significance was found in the analysis for age, gender, sepsis, lung involvement and prolonged hospitalization in the corrected group of threshold value 8 (p: 0.63, 0.14, 0.45, 0.75 and 0.6, respectively).

Table 6. Comparison of groups with calcium values below 8.5 mg/dl and 8 mg/dl

		Ca<8mg/dl (56.25%)		P*	Ca<8.5mg/dl (82.8%)		P*
		Yes (n)	No (n)		Yes (n)	No (n)	
Gender	**Female**	30	32	0.059	48	14	0.059
	Male	42	24		58	8	
Long hospital stay	**No**	48	39	0.849	73	14	0.849
	Yes	24	17		33	8	
Clinical need	**Ward**	36	46	0.000	65	17	0.000
	Intensive Care	36	10		41	5	
Pulmonary Involvement	**No**	11	12	0.364	16	7	0.364
	Yes	59	42		87	14	
Mortal	**No**	42	48	0.001	72	18	0.001
	Yes	30	8		34	4	
qSOFA (sepsis)	**No**	31	41	0.001	58	14	0.001
	Yes	41	15		48	8	
Need for mechanical ventilation	**No**	49	50	0.005	81	18	0.005
	Yes	23	6		25	4	

**Chi square test*

In Model 1, established for the dependent mortality rate, age and CRP levels have a significant effect in distinguishing mortality. It can be said that increasing age and CRP levels increase the probability of mortality.

In Model 2, age, CRP and calcium levels have a significant effect in distinguishing the mortality situation. It can be said that increasing age and CRP levels increase the probability of mortality. Low calcium also increases the probability of mortality (B=-0.934). (Table 7)

Table 7. Determination of variables that may have an impact on "mortality frequency"

			S.E.	Wald	p.	Odds	95% Safe Search	
							Bottom	Top
Step 1	**Age**	.043	.018	5.465	.019	1.044	1.007	1.082
	PTH	.003	.004	.570	.450	1.003	.996	1.010
	Ca	-.793	.419	3.590	.058	.452	.199	1.028
	CRP	.015	.004	11.756	.001	1.015	1.007	1.024
	Steady	1.393	3.384	.169	.681	4.027		
Step 2	**Age**	.048	.017	7.956	.005	1.049	1.015	1.085
	Ca	-.934	.378	6.119	.013	.393	.187	.824
	CRP	.015	.004	11.603	.001	1.015	1.007	1.024
	Steady	2.484	3.063	.658	.417	11.990		

**Logistic Regression Analyse.*

DISCUSSION

Calcium ion is one of the most common molecules involved in signal transduction in almost all cellular processes. Again, in viral infections, it is an ion used in almost every stage of the virus life cycle, such as the formation of the virion structure, entry of the virus into the cell, viral gene expression, formation of virus proteins, virion maturation and release from the cell (Zhou, 2009). In our study, calcium levels of hospitalized COVID-19 patients were found to be significantly lower in the group requiring intensive care, the group with sepsis, the group requiring mechanical ventilation and the group with a mortal course.

In the case-control studies of Cappellini et al., a significant relationship was reported between COVID-19 positivity, male gender, advanced age and low calcium (Cappellini, 2020). In our study, we did not find a relationship between age and calcium. However, in male gender, we found calcium below 8 mg/dl at a level close to significance (p: 0.059).

Many studies have shown the presence of hypocalcemia during the course of COVID-19 infection (Lippi, 2019; Di Filippo, 2020, Crespi, 2020; Diez, 2023). In most of the existing studies, the hypocalcemia threshold value was taken as 8.8 mg/dl (2.2 mmol/l), 8.6 mg/dl (2.15 mmol/l) or 8.5 mg/dl (2.12 mmol/l) by correction with albumin or direct measurement, and the prevalence of hypocalcemia was reported between 62.6% and 74.7% according to these threshold values (Cappellini, 2020; Sun, 2020; Tezcan, 2020; Torres, 2020; Wu, 2020; Zhou X, 2020; Bennouar, 2021; Hernandez, 2021; Liu, 2020; Pal, 2021). In our study, where we took the Ca threshold value as 8.5 mg/dl, we detected a higher rate of hypocalcemia in cases hospitalized due to COVID-19, with 82.8%. This rate was 79.2% in ward patients and 89.1% in intensive care patients. This situation can be explained by the use of Ca by the virus, as well as vitamin D deficiency, impaired intestinal absorption, hypoalbuminemia and increased fatty acids due to stress.

In a study where the hypocalcemia threshold value was taken as 2.2 mmol/l (8.8 mg/dl), its relationship with the need for long hospitalization was reported, while in another study where the same threshold value was used, hypocalcemia was seen significantly more in COVID-19 patients with severe clinical picture compared to mild-moderate clinical picture (84% vs. 49%) and this was reported to be related to increased interleukin-6 (Wu, 2020; Zhou 2020). We took 8.5 mg/dl (2.12) as the hypocalcemia threshold for grouping and below 8 mg/dl, which we can interpret as significant hypocalcemia. Although we did not detect any relationship in this direction in the hypocalcemia group with the threshold value of 8.5 mg/dl, we found that this situation increased at a level close to significance in the group with lung involvement (p: 0.06).

In a retrospective study by Sun et al., it was observed that the incidence of multiple organ failure, septic shock, and 28-day mortality were significantly higher in those with calcium levels below 2 mmol/l (8.02 mg/dl). The authors did not correct serum calcium levels according to serum albumin levels (Sun et al., 2020). In line with this study, we found that there was a significant increase in the need for intensive care, the need for mechanical ventilation, mortality, and sepsis in the hypocalcemia group below 8 mg/dl, which we detected by direct measurement. We also found that a decrease in calcium was a significant risk factor for mortality.

In a case-control study, albumin-corrected Ca was found to be significantly lower in COVID-19 patients than in controls, but its relationship with disease severity was not reported (Hernandez, 2021). Again, Tezcan et al. detected hypocalcemia with a corrected Ca value below 8.5 mg/dl in 21.5% of severe COVID-19 cases and did not report it as a risk for poor prognosis (Tezcan, 2019). In another study, hypocalcemia was detected in 35% of cases with a corrected Ca value below 2.05 mmol/l and was reported to be an indicator of poor prognosis (Bennouar, 2021). Liu J et al. also detected hypocalcemia with a corrected Ca value below 2.15 mmol/l in 62.6% of severe COVID-19 patients and reported that hypocalcemia was an important risk factor for poor prognosis for any of the outcomes of mechanical ventilation, need for intensive care or mortal results (Liu, 2020). Torres et al. also studied hypocalcemia in hospitalized COVID-19 patients with corrected Ca measurement below 8.5 mg/dl and showed that this was only associated with the need for intensive care (Torres, 2020).

In the study by Diez et al., both direct measurement Ca and corrected Ca values were compared in mechanical ventilation requirement, intensive care requirement, hospital mortality and combined outcome groups and while direct measurement was found to be significantly lower in each group, it was found that corrected Ca value was significantly lower only in mortality compared to direct measurement (p: 0.02 vs 0.001, respectively) (Diez, 2023). In our study, we also found that the value obtained with direct total calcium measurement was significantly lower in intensive care requirement, sepsis, mechanical ventilation requirement and mortality. However, we did not find any significance with corrected Ca value.

Regarding the 25OH vitamin D levels that should be checked in relation to calcium metabolism; Hernandez JL. et al. found vitamin D levels below 20 ng/ml in 82.2% of the patients in their retrospective case-control study and significantly lower than the control group, and did not report any relationship with disease severity except for prolonged hospitalization (Hernandez, 2021). Bennouar et al. also found hypovitaminosis D (<39 nmol/l-15.6 ng/ml) in 75% of severe COVID-19 cases in their study and reported that it was lower in men than in

women (Bennouar, 2021). In our study, we found vitamin D levels below 20 ng/ml in 82% of the cases, below 30 ng/ml in 89.8%, and below 10 ng/ml in 49%. Our rates are consistent with Hernandez's study. We found that the 25 OH vitamin D blood levels of intensive care patients and ward patients were statistically similar, and the results were similar for both genders, too. In a large meta-analysis including 1368 COVID-19 cases, it was reported that vitamin D deficiency was associated with poor prognosis (Munshi, 2020). In our study, when patients were grouped as sufficient, insufficient, deficient, and profoundly deficient according to their 25 OH vitamin D levels and compared statistically for pulmonary involvement, need for intensive care, need for long-term hospitalization, sepsis, and mortality, the levels were found to be insignificant in determining clinical severity. The fact that most of our patients had inadequacy in this result may be a factor.

We found PTH, another factor that plays an important role in calcium metabolism, to be high in 72.1% of our patients. In addition, PTH levels were significantly higher in sepsis, the need for intensive care, and mortality. However, when evaluated as a risk factor for death with logistic regression analysis, age, high CRP, and hypocalcemia were determined as significant risk factors. Finally, we did not find any significance in the evaluation of P and Mg levels. Both et al. showed that 53% of SARS-infected patients had hypophosphatemia, 57% had hypomagnesemia, and 70% had hypocalcemia (Booth, 2003). Our rates of hypomagnesemia and hypophosphatemia in the ward and intensive care patient groups were lower than in this study (13.4% vs. 14.2% and 32.9% vs. 31.9%, respectively), and hypocalcemia was higher.

The relatively limited number of cases in our study, the fact that it was a cross-sectional study and that healthy controls were not used are factors that weaken its power. A well-organized prospective study with replacement of deficiencies and a large number of cases may demonstrate the clinical benefit of this data.

In conclusion, we found that total Ca values below 8 mg/dl obtained by direct measurement without albumin correction in COVID-19 patients significantly increased the need for intensive care, the need for mechanical ventilation, sepsis and mortality, and in our analysis, we found that advanced age and high CRP values, along with a decrease in directly measured total Ca values, were independent risk factors that increased mortality. Our study suggests that correction with albumin may not be necessary when evaluating Ca levels in COVID-19 patients and that Ca replacement may be appropriate for directly measured Ca values below 8 mg/dl. In addition, these results can be taken into consideration in critically ill patients with other viral infections.

Conflict of interest: The authors have no conflicts of interest to report.

Ethical approval: This study was approved by the Van Yuzuncu Yil University Non-Interventional Clinical Research local ethics committee on 10.09.2021 with the decision number 2021/10-08.

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