C-terminal cross-linking telopeptide levels in COVID-19 patients: A prospective case-control study

Mehmet Ali Gul[1,A,C,D], Nezahat Kurt[2,C], Fatma Betul Ozgeris[3,B], Neslihan Yuce[4,C], Omer Faruk Kocak[5,C], Emine Parlak[6,B,C]

[1] Department of Medical Biochemistry, Faculty of Medicine, Amasya University, Turkey

[2] Department of Medical Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Turkey

[3] Department of Nutrition and Dietetics, Faculty of Healthy Sciences, Ataturk University, Erzurum, Turkey

[4] Department of Medical Biochemistry, Faculty of Medicine, Ataturk University, Erzurum, Turkey

[5] Department of Chemical Technology, Erzurum Vocational Training College, Ataturk University, Turkey

[6] Department of Infectious Diseases and Clinical Microbiology, Faculty of Medicine, Ataturk University, Erzurum, Turkey

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Abstract

Background. Coronavirus disease 19 (COVID-19) is a viral infection mediated by coronavirus-2 that causes severe acute respiratory syndrome (SARS-CoV-2). The disease may affect biochemical parameters and electrolytes. C-terminal cross-linking telopeptide (CTX-I) is released during mature bone resorption and is a biomarker for predicting bone resorption.

Objectives. As the pandemic progressed, understanding the effects of COVID-19 disease remained critical. Inflammatory responses triggered by the virus can result in a bone metabolism regulation imbalance. As such, this study aimed to analyze serum levels of CTX-I, calcium (CA), phosphorus (P), magnesium (Mg), C-reactive protein (CRP), and alkaline phosphatase (ALP) in COVID-19 patients to investigate the relationship between bone resorption and the disease.

Materials and methods. The study included 56 individuals with COVID-19 (divided into mild, moderate and severe subgroups depending on disease severity) and 25 healthy adults as a control group. Serum CTX-I concentrations were measured with enzyme-linked immunosorbent assay (ELISA). In addition, CRP, Ca, Mg, P, and ALP levels were measured using an automated clinical chemistry analyzer.

Results. Serum CTX-I levels were significantly higher in COVID-19 patients than in the control group ($p < 0.05$). Furthermore, a positive weak relationship was detected between CRP and CTX-I ($r = 0.303$, $p < 0.05$).

Conclusions. Increased serum CTX-I levels in the patient group caused COVID-19-driven bone degradation, though serum CTX-I levels did not differ according to disease severity.

Key words: bone, COVID-19, CTX-I

2

M. Gul et al. COVID-19 increases bone resorption

Background

Coronavirus disease 19 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection, was first detected in December 2019 in Wuhan, China, and spread rapidly over almost the entire globe.[1,2] Coronaviridae is a family of single-stranded ribonucleic acid (RNA) viruses, including SARS-CoV-2, that affect many animals, while other coronaviruses could infect humans.[3] Subjective clinical findings are considered more reliable with the concomitant biomarkers, which provide measurable data during the progression of biological processes.[4] With the rapid spread of COVID-19 and increased mortality in extreme cases, a better understanding of clinical features, determining its effects on metabolism and finding accurate laboratory markers are required to elucidate the disease process. Indeed, its influence on metabolism is not fully known, and there is a need for studies on the effects of COVID-19 on various human organs and tissues, such as muscle and bone.

The bone organic matrix comprises around 90–98% collagen I, which is proteolytically degraded by osteoclasts during bone turnover, releasing small C-terminal cross-linking telopeptide (CTX-I) peptide fragments.[5–7] Serum CTX-I levels correlate with histomorphometric measurements of bone resorption.[8] Although different biomarkers are available for measuring the metabolic products of bone resorption, serum CTX-I stands out for its high correlation with bone turnover rate and its immediate response to changes in bone metabolism, making it a sensitive marker of bone resorption.[5,9]

Objectives

Studies show that inflammation and bone metabolism are associated with various biological and clinical mechanisms and suggest measuring bone metabolism markers, such as CTX-I, in COVID-19 patients.[10] To investigate the effect of COVID-19 on bone, the current study examined the impact of COVID-19 on serum levels of the bone resorption marker CTX-I.

Materials and methods

Patients

This prospective observational research, conducted in June and July 2020, included 56 patients diagnosed with COVID-19 and 25 individuals with no health problems. The Clinical Research Ethics Committee of Ataturk University (Erzurum, Turkey) approved this research prior to experiments (approval No. B.30.2.ATA 0.01.00/369).

All participants provided written informed consent, and the study conformed to the principles outlined in the Declaration of Helsinki.

Participants provided nasopharyngeal and oropharyngeal samples for real-time polymerase chain reaction (PCR) COVID-19 detection, with those testing positive stratified to the COVID-19 group and those testing negative to the control group. Those diagnosed with COVID-19 (n = 56) were divided into mild, moderate and severe subgroups based on disease severity, with 25 healthy individuals used as a control group. Power analysis was performed before determining sample size. Subgroups included a non-complicated group (NCG), with mild fever and cough present/no and no radiological pulmonary findings, a moderate group (MG) with fever-cough, radiological uni/bilateral lung involvement and oxygen saturation $SpO_2 > 93\%$, and a severe group (SG) displaying lung radiological imaging with uni/bilateral involvement and $SpO_2 < 93\%$. Exclusion criteria encompassed being aged less than 18 years, the presence of diseases affecting bone metabolism, undergoing chemotherapy or immunosuppressive drug treatment, and using calcium, magnesium or calcitriol supplements. The MG and SG included some patients with hypertension, diabetes mellitus and chronic obstructive pulmonary disease (COPD).

Blood sampling

Participants provided fasting blood on the first day of hospital admission, with venous blood drawn using a 10 mL syringe and transferred to gel-containing vacutainers. After 30 min, samples were centrifuged at $1,500 \times g$ for 10 min, and serum was stored at $-80°C$ until the day of measurement.

Measurement and data collection

Serum CTX-I concentrations were analyzed using a human-specific CTX-I sandwich enzyme-linked immunosorbent assay (ELISA) (cat. No. CSB-E11224h; Cusabio Technology, Wuhan, USA). The kit detection range was 0.625–40 ng/mL, with an intra-assay coefficient of variance (CV%) <8%, inter-assay precision CV% of <10% and sensitivity of 0.156 ng/mL. The ELISA measurements used serum samples (100 µL) according to the manufacturer's instructions, with samples analyzed using an XS PowerWave multi-plate spectrophotometer (Agilent Technologies, Santa Clara, USA). Other biochemical parameters were measured using compatible kits and detected on a cobas 8000 modular analyzer (Roche Diagnostics, Basel, Switzerland), with C-reactive protein (CRP) immunoturbidimetrically detected in the e801 module, and calcium (Ca), magnesium (Mg), phosphorus (P), and alkaline phosphatase (ALP) in the c502 module. These results were obtained from existing patient files. Analysis was carried out at the Faculty of Medicine of Ataturk University.

Statistical analyses

Data analysis employed IBM SPSS 20.0 (IBM Corp., Armonk, USA). Shapiro–Wilk tests evaluated numerical data distribution, and F test/Brown–Forsythe tests confirmed the homogeneity of variances. Normally distributed data were calculated as mean ± standard deviation (SD). Student's t-test was used to compare 2 groups and analysis of variance (ANOVA) 3 groups. Data with a non-normal distribution were calculated as median and interquartile range (Me (IQR)), with Mann–Whitney U tests used for paired group comparisons and Kruskal–Wallis post hoc with Dunn's test used for 3 group comparisons. Spearman's correlation coefficient was employed to evaluate the monotonic component in the relationship between the data. A $p < 0.05$ was considered significant, and $p < 0.001$ very significant. Supplementary tables (https://doi.org/10.5281/zenodo.10719329) provide confirmation of parametric test assumptions and related data for the control group, COVID-19 patient group and COVID-19 subgroups (NCG, MG and SG).

Results

Table 1 shows the clinical and demographic characteristics of COVID-19 patients. While 30 of the 56 COVID-19 patients were female and 26 were male, the control group contained 11 women and 14 men. There was no statistical difference between group mean age ($p > 0.05$).

Serum CTX-I levels were higher in COVID-19 patients than in the control group ($p < 0.001$), as were CRP levels ($p < 0.001$). On the other hand, serum Ca, Mg, P, ALP, creatinine, and blood urea nitrogen (BUN) levels were similar in both groups ($p > 0.05$). Table 2,3 show the biochemical tests measured in both groups, while Fig. 1A–H provides box-whisker graphics of the group comparison.

When the COVID-19 subgroups were compared and evaluated, the highest serum CTX-I levels were found in the NCG, but this difference was not significant ($p > 0.05$). While CRP was significantly higher in the SG compared to the other groups ($p < 0.05$), no difference was observed between other parameters ($p > 0.05$). Table 4,5 show the COVID-19 subgroup's serum biochemical tests, and Fig. 2A–H presents box-whisker graphics of the group comparisons.

Spearman's correlation analysis indicated a positive weak monotonic correlation between CTX-I and CRP in all

Table 1. Demographic data of all participants

Variables	COVID-19				Control (n = 25)
	non-complicated group (n = 19)	moderate severe group (n = 25)	severe group (n = 12)	total COVID-19 (n = 56)	
Age [years], mean ±SD	40.5 ±15.9	52.6 ±18.0	58.7 ±13.3	49.9 ±17.8	48.2 ±13.3
Sex (female/male), mean ±SD	6/12	14/11	4/9	30/26	11/14

mean ±SD – mean ± standard deviation.

Table 2. Biochemical parameters of the coronavirus disease 19 (COVID-19) and control groups

Variables	COVID-19 mean ±SD (n = 56)	Control mean ±SD (n = 25)	F	Sig.	t	df	Mean difference	Standard error difference	95% CI of the difference		p-value
									lower	upper	
Ca [mg/dL]	8.9 ±0.4	9.1 ±0.4	0.331	0.567	−1.788	79	−0.1833	0.1025	−0.3874	0.0208	0.780
Mg [mg/dL]	2 ±0.2	2.1 ±0.2	2.644	0.108	−1.674	79	−0.07424	0.04434	−0.16250	2.644	0.980
P [mg/dL]	3.2 ±0.6	3.1 ±0.4	10.554	0.002	0.205	79	0.0282	0.1378	−0.2461	0.3026	0.838

Ca – calcium; Mg – magnesium; P – phosphorus; mean ±SD – mean ± standard deviation; df – degrees of freedom; 95% CI – 95% confidence interval. The t-test was used for Ca, Mg, and P.

Table 3. Biochemical parameters of the coronavirus disease 19 (COVID-19) and control groups

Variables	COVID-19 Me (IQR) (n = 56)	Control Me (IQR) (n = 25)	Mann–Whitney U	Wilcoxon W	Z	p-value
CTX-I [ng/mL]	3.81 (2.18)	2.94 (0.76)	239.00	564.000	−4.714	0.001*
ALP [U/L]	79.5 (32)	99 (58)	501.50	2097.500	−2.032	0.095
CRE [mg/dL]	0.8 (0.3)	0.8 (0.2)	420	612.000	−0.59	0.552
CRP [mg/L]	5.5 (28.3)	3.0 (2)	204.00	529.000	−5.094	0.001*
BUN [mg/dL]	14.9 (5.4)	15 (8.1)	643.5	968.5	−0.578	0.563

CTX-I – C-terminal cross-linking telopeptide; ALP – alkaline phosphatase; BUN – blood urea nitrogen; CRP – C-reactive protein; CRE – creatinine; Me (IQR) – median (interquartile range); *statistically significant. Mann–Whitney U test used for CTX-I, ALP, BUN, CRE, and CRP.
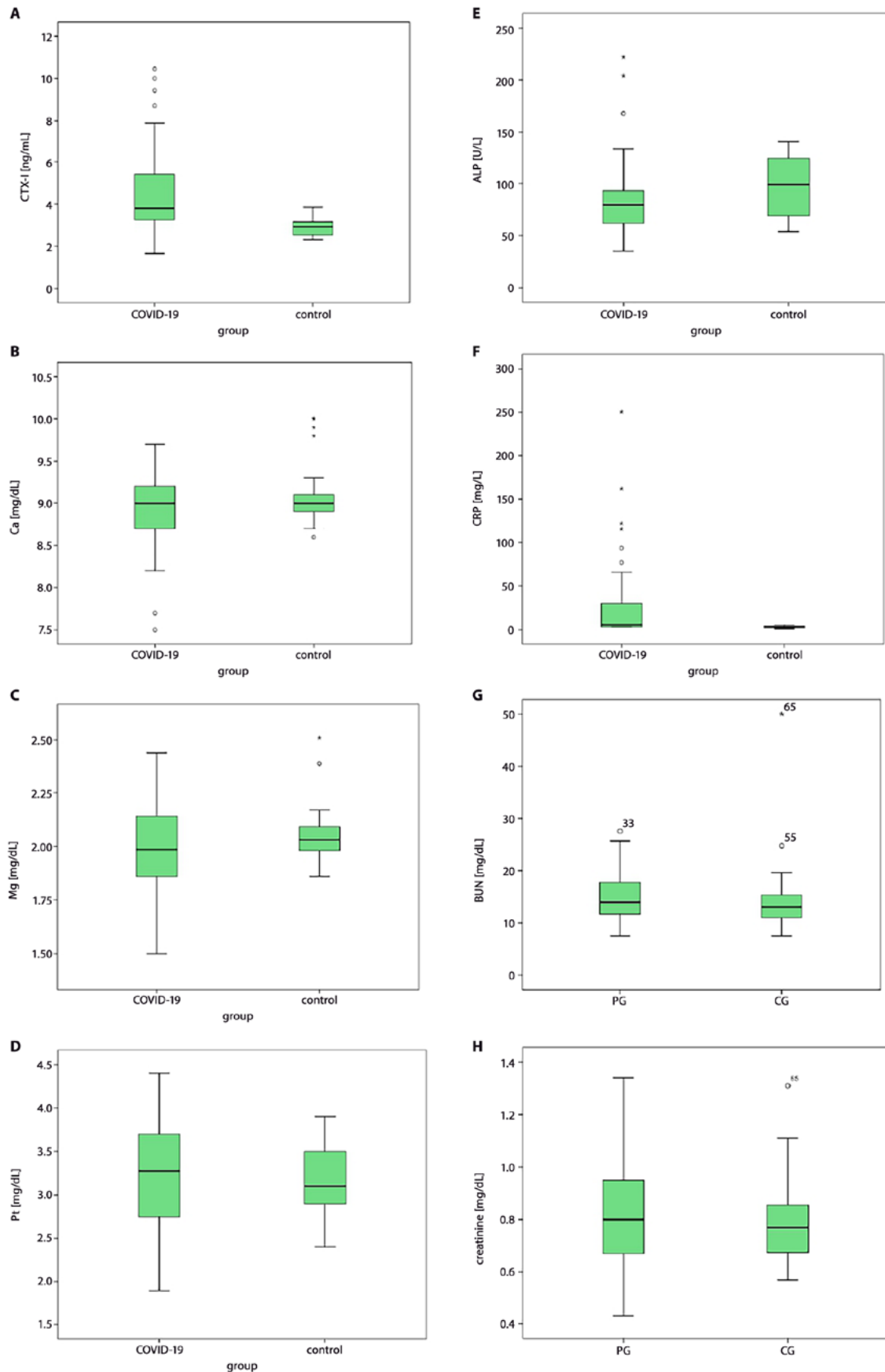
Fig.1. A. Comparison of serum CTX-I in COVID-19 and control groups; B. Comparison of serum Ca in COVID-19 and control groups (p > 0.05); C. Comparison of serum Mg in COVID-19 and control groups (p > 0.05); D. Comparison of serum P in COVID-19 and control groups (p > 0.005); E. Comparison of serum ALP in COVID-19 and control groups (p > 0.05); F. Comparison of serum CRP in COVID-19 and control groups (p > 0.05); G. Comparison of serum BUN in COVID-19 and control groups (p > 0.05); H. Comparison of serum CRE in COVID-19 and control groups (p > 0.005). COVID-19 group n = 56 and control group n = 25. The lowest and last stem ends show the minimum and maximum values. The bottom and top edges of the box indicate the IQR, and the line in the middle of the box indicates the median. A t-test was used for Ca, Mg and P, and a Mann–Whitney U test was used for CTX-I, ALP, BUN, CRE, and CRP

ALP – alkaline phosphatase; Ca – calcium; COVID-19 – coronavirus disease 19; CRE – creatine; CRP – C-reactive protein; CTX-I – C-terminal cross-linking telopeptide; BUN – blood urea nitrogen; IQR – interquartile range; Mg – magnesium; P – phosphorus.
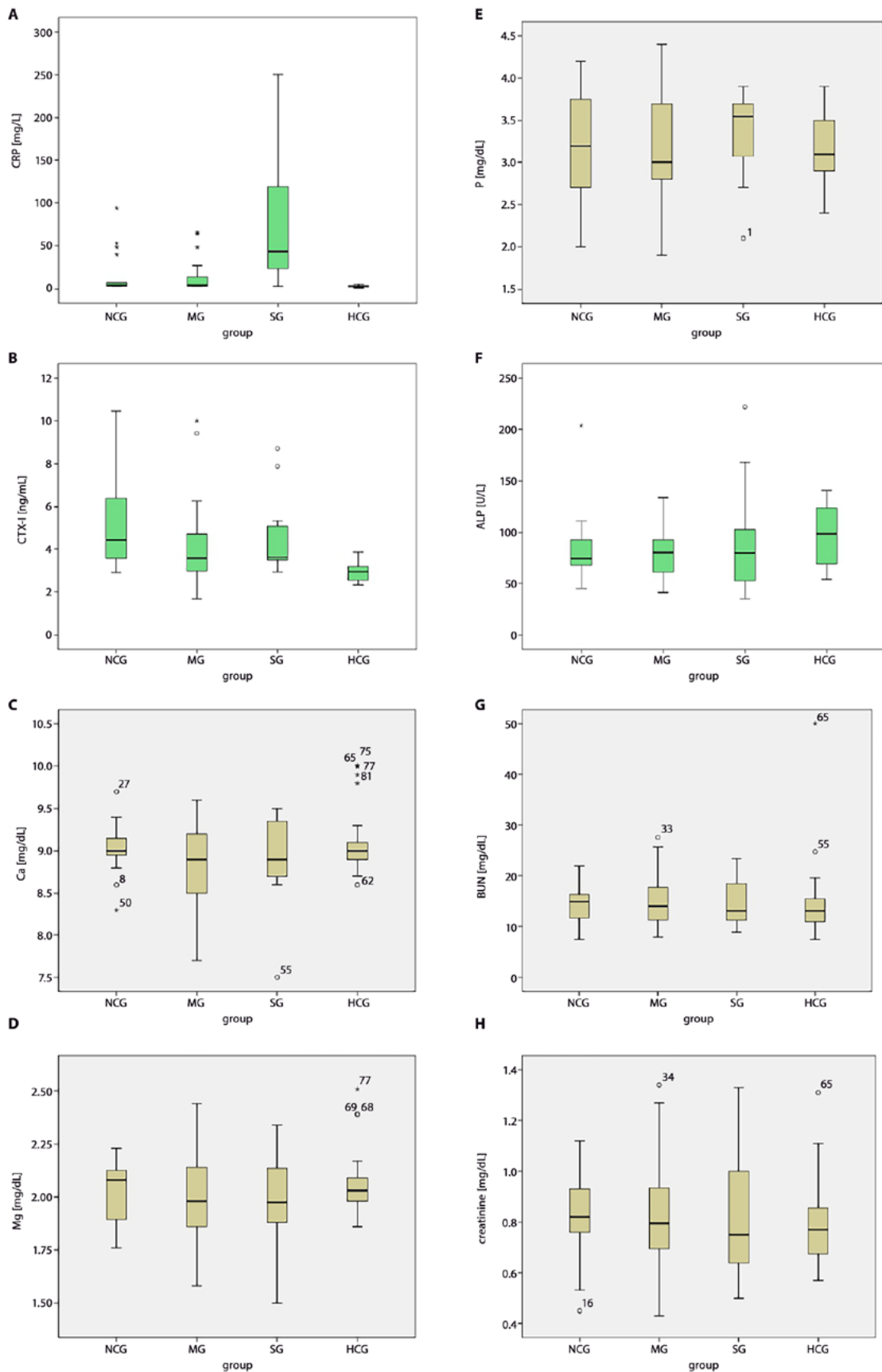
Fig. 2. A. Comparison of serum CRP of all groups (p < 0.200 for COVID-19 subgroups). B. Comparison of serum CTX-I of all groups (p > 0.005 for COVID-19 subgroups). C. Comparison of serum Ca of all groups (p > 0.005 for COVID-19 subgroups). D. Comparison of serum Mg of all groups (p > 0.005 for COVID-19 subgroups). E. Comparison of serum P of all groups (p > 0.005 for COVID-19 subgroups). F. Comparison of serum ALP of all groups (p > 0.005 for COVID-19 subgroups). G. Comparison of serum BUN of all groups (p > 0.005 for COVID-19 subgroups). H. Comparison of serum CRE of all groups (p > 0.005 for COVID-19 subgroups). Non-complicated group n = 19, moderate severe group n = 25, severe group n = 12, and control group n = 25. The lowest and last stem ends show the minimum and maximum values. The bottom and top edges of the box indicate the IQR, and the line in the middle of the box indicates the median. ANOVA was used for CA, Mg and P, and a Kruskal–Wallis test was used for CTX-I, ALP, CRE, BUN, and CRP

ALP – alkaline phosphatase; ANOVA – analysis of variance; Ca – calcium; COVID-19 – coronavirus disease 19; CRE – creatine; CRP – C-reactive protein; CTX-I – C-terminal cross-linking telopeptide; BUN – blood urea nitrogen; IQR – interquartile range; Mg – magnesium; P – phosphorus.

6

M. Gul et al. COVID-19 increases bone resorption

Table 4. Biochemical parameters of the coronavirus disease 19 (COVID-19) subgroups

Variables	Non-complicated group mean ±SD (n = 19)	Moderate severe group mean ±SD (n = 25)	Severe group mean ±SD (n =12)	Sum of squares	df	Mean square	F	p-value
Ca [mg/dL]	9.0 ±0.3	8.8 ±0.5	8.9 ±0.5	0.40	2	0.20	1.05	0.358
Mg [mg/dL]	2 ±0.1	1.9 ±0.2	1.9 ±0.2	0.02	2	0.01	0.22	0.799
P [mg/dL]	3.14 ±0.7	3.11 ±0.6	3.34 ±0.53	0.45	2	0.23	0.54	0.586

Ca – calcium; Mg – magnesium; P – phosphorus; mean ±SD – mean ± standard deviation; df – degrees of freedom. The analysis of variance (ANOVA) was used for CA, Mg and P.

Table 5. Biochemical parameters of the coronavirus disease 19 subgroups

Variables	Non-complicated group Me (IQR) (n = 19)	Moderate severe group Me (IQR) (n = 25)	Severe group Me (IQR) (n = 12)	χ^2	df	p-value
CTX-I [ng/mL]	4.45 (2.97)	3.56 (2.09)	3.6 (1.73)	3.21	2	0.200
ALP [U/L]	74 (29)	80 (33)	79.5 (61)	0.14	2	0.933
CRE [mg/dL]	0.82 (0.27)	0.80 (0.26)	0.75 (0.39)	0.183	2	0.991
CRP [mg/L]	4.2 (4.4)	4.1 (11.7)	43.5 (92)	13.68	2	0.001*
BUN [mg/dL]	14.9 (4.7)	14.5 (8.3)	13.1 (7.9)	0.302	2	0.860

CTX-I – C-terminal cross-linking telopeptide; ALP – alkaline phosphatase; CRP – C-reactive protein; CRE – creatinine; BUN – blood urea nitrogen; Me (IQR) – median; df – degrees of freedom; *statistically significant. Kruskall–Wallis test used for CTX-I, ALP, CRE, BUN, and CRP.

Table 6. Spearman's correlation analyses between CTX-I and other tests

Parameter		CRP	Ca	Mg	P	ALP	BUN	CRE
CTX-I	r-value	0.303*	−0.104	0.201	−0.083	−0.020	0.023	−0.025
	p-value	0.006	0.357	0.073	0.460	0.858	0.841	0.827

CTX-I – C-terminal cross-linking telopeptide; Ca – calcium; Mg – magnesium; P – phosphorus; ALP – alkaline phosphatase; BUN – blood urea nitrogen; CRP – C-reactive protein; CRE – creatinin; *statistically significant.
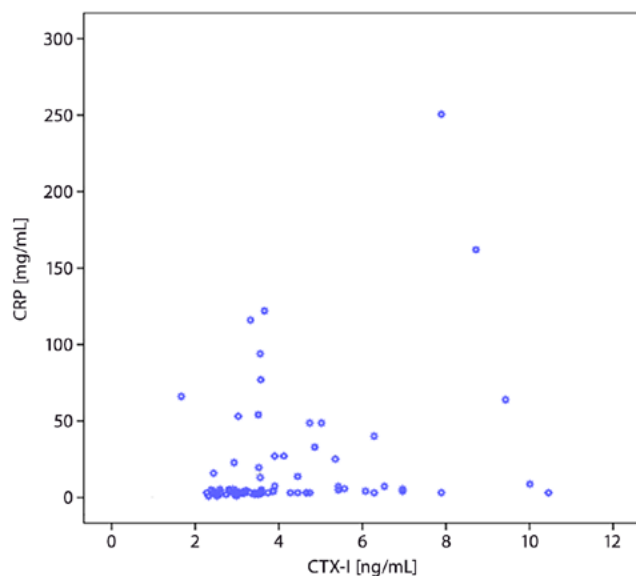
Fig. 3. Relationship between C-reactive protein (CRP) and C-terminal cross-linking telopeptide-I (CTX-I) for all participants

participants (r = 0.303, p < 0.05). Table 6 shows the analysis of the monotonic component of the relationship between CTX-I and other tests, and Fig. 3 shows the relationship between CTX-I and CRP

Discussion

To the best of our knowledge, this is the first study reporting on serum CTX-I levels in patients with COVID-19. The findings demonstrated that serum CTX-I levels were statistically higher in the COVID-19 patient group than in the healthy controls. However, there were no differences between COVID-19 patients divided into subgroups according to disease severity (mild, moderate and severe).

Many studies on diverse viruses have sought to characterize their roles in bone metabolism,[10] with those on bone metabolism in SARS-CoV-2 indicating that much more research is required on the risk of fracture in patients recovering from SARS-CoV-2 infection.[10] Although not all features of COVID-19 are fully understood, pathological studies on the lungs, liver and heart have shown the potential negative effects of COVID-19 on certain tissues.[11] In this regard, bone-forming capacity is prone to worsen due to the progression of underlying diseases resulting from SARS-CoV-2 infection.[12]

Serum CTX-I levels increase following proteolysis of type I collagen, the main component of the bone extracellular organic matrix, during mature bone resorption. Therefore, CTX-I levels decrease in patients with suppressed bone resorption.[9,13] Most research on viruses

and CTX-I shows that virus infection causes increased CTX-I levels. Huang et al.[14] showed that the dengue virus caused a temporary inflammatory reaction in bone tissue and increased osteolytic activity to release CTX-I. Research on human immunodeficiency virus (HIV) and hepatitis C virus (HCV) infections showed that HIV was related to increased bone resorption and formation, and increases in CTX-I, whereas HCV infection was not associated with CTX-I.[15] Increased bone resorption has been shown in the pathogenesis of bone loss, which was evaluated by measuring CTX-I in hemophiliacs and correlations with HIV infection.[16]

A study on biochemical markers of bone metabolism in SARS conducted in Hong Kong showed significant bone resorption, as indicated by a significant increase in serum CTX-I from certain days after fever onset.[17] Patients with chronic hepatitis C (CHC) and osteoporosis or osteopenia had higher serum CTX-I levels, which may mean that bone loss in the early stages of CHC is not due to reduced bone synthesis but secondary to increased bone resorption.[18] Furthermore, animal studies on SARS-CoV-2 demonstrate a significant reduction in several bone parameters.[19]

Tao et al. hypothesized that angiotensin-converting enzyme 2 (ACE2) deficiency caused by SARS-CoV2 invasion may result in bone matrix reduction and that COVID-19 can cause anaphase bone loss and earlier muscle disorder by targeting ACE2 in the bone marrow microenvironment.[12] The current study supports this hypothesis by showing higher CTX-I levels in patients with COVID-19 than in the healthy controls.

Magnesium has many biological functions, especially in bone, with 60% of the total body content found in bone.[20] A recent meta-analysis showed an association between unstable Mg homeostasis and COVID-19, and suggested incorporating Mg into the ionogram and supplementing it externally.[21] In parallel, a lower Mg level was observed in the COVID-19 group in our study, but this decrease was not significant. In addition, moderate and severe patients had lower Mg than non-complicated patients, though this difference was not significant. A retrospective study by Pal et al.[22] found widespread hypocalcemia in mildly severe COVID-19 patients and attributed it to the disease, though they did not clarify its pathophysiological mechanism.

In a study of 420 COVID-19 patients and 165 healthy controls, serum Ca was lower in the COVID-19 patients. Although the current study found a slight discrepancy in Ca levels, it was not considered significant.

Alkaline phosphatase is a bone-building enzyme with several isoforms. Although studies on bone-specific ALP (B-ALP) in COVID-19 are limited to only a few case reports,[23,24] a study showing elevated total ALP in COVID-19 patients suggested it was primarily due to liver damage. In the current study, serum ALP values were similar in both groups and although it increased with COVID-19 severity, this difference was not significant.

Since CTX-I excretion takes place in the kidneys, the CTX-I level may be high in the plasma due to impaired renal function. However, kidney function tests of serum creatinine and BUN showed no differences between the healthy group and COVID-19 patients, indicating that the high CTX-I level was of COVID-19 origin. The CRP serum level, which is a positive acute phase reactant, increases during infection,[25] with many reports showing increased CRP in COVID-19 patients.[26,27] Our findings showed an increase in CRP levels in COVID-19 patients, which is compatible with current research results. In addition, a positive weak relationship was found between CTX-I and CRP.

Limitations

Study limitations include the absence of B-ALP and radiological images to support bone resorption. While this study includes critical clinical laboratory parameter results, the small sample size could be a limitation, particularly within the COVID-19 subgroups. Another limitation is the lack of parathyroid hormone (PTH) and calcitriol measurement since they play a role in bone metabolism.

Conclusions

The study showed that COVID-19 patients had increased serum CTX-I levels, a reference marker for bone resorption, though there were no differences based on disease severity. Our data may be foundational to clinical studies investigating bone metabolism in COVID-19.

Supplementary data

The Supplementary materials are available at https://doi.org/10.5281/zenodo.10719329. The package includes the following files:

Supplementary Table 1. Verification of parametric test assumption for control.

Supplementary Table 2. Verification of parametric test assumption for COVID-19 patient group.

Supplementary Table 3. Verification of parametric test assumption for NCG COVID-19 subgroup.

Supplementary Table 4. Verification of parametric test assumption for MG COVID-19 subgroup.

Supplementary Table 5. Verification of parametric test assumption for SG COVID-19 subgroup.

Supplementary Table 6. Tests of homogenity.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

ORCID IDs

Mehmet Ali Gul ⓘ https://orcid.org/0000-0002-5849-0116
Nezahat Kurt ⓘ https://orcid.org/0000-0002-1685-5332

References

1. Ren LL, Wang YM, Wu ZQ, et al. Identification of a novel coronavirus causing severe pneumonia in human: A descriptive study. *Chin Med J (Engl)*. 2020;133(9):1015–1024. doi:10.1097/CM9.0000000000000722
2. Khan IH, Zahra SA, Zaim S, Harky A. At the heart of COVID-19. *J Card Surg*. 2020;35(6):1287–1294. doi:10.1111/jocs.14596
3. Le Bert N, Tan AT, Kunasegaran K, et al. SARS-CoV-2-specific T cell immunity in cases of COVID-19 and SARS, and uninfected controls. *Nature*. 2020;584(7821):457–462. doi:10.1038/s41586-020-2550-z
4. Kermali M, Khalsa RK, Pillai K, Ismail Z, Harky A. The role of biomarkers in diagnosis of COVID-19 A systematic review. *Life Sci*. 2020; 254:117788. doi:10.1016/j.lfs.2020.117788
5. Marx RE, Cillo JE, Ulloa JJ. Oral bisphosphonate-induced osteonecrosis: Risk factors, prediction of risk using serum CTX testing, prevention, and treatment. *J Oral Maxillofac Surg*. 2007;65(12):2397–2410. doi:10.1016/j.joms.2007.08.003
6. Lazarovici TS, Mesilaty-Gross S, Vered I, et al. Serologic bone markers for predicting development of osteonecrosis of the jaw in patients receiving bisphosphonates. *J Oral Maxillofac Surg*. 2010;68(9):2241–2247. doi:10.1016/j.joms.2010.05.043
7. Seibel MJ. Biochemical markers of bone turnover. Part I: Biochemistry and variability. *Clin Biochem Rev*. 2005;26(4):97–122. PMID:16648882. PMCID:PMC1320175.
8. Chavassieux P, Portero-Muzy N, Roux JP, Garnero P, Chapurlat R. Are biochemical markers of bone turnover representative of bone histomorphometry in 370 postmenopausal women? *J Clin Endocrinol Metab*. 2015;100(12):4662–4668. doi:10.1210/jc.2015-2957
9. Chubb SAP. Measurement of C-terminal telopeptide of type I collagen (CTX) in serum. *Clin Biochem*. 2012;45(12):928–935. doi:10.1016/j.clinbiochem.2012.03.035
10. Salvio G, Gianfelice C, Firmani F, Lunetti S, Balercia G, Giacchetti G. Bone metabolism in SARS-CoV-2 disease: Possible osteoimmunology and gender implications. *Clinic Rev Bone Miner Metab*. 2020;18(4):51–57. doi:10.1007/s12018-020-09274-3
11. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. *Lancer Respir Med*. 2020;8(4):420–422. doi:10.1016/S2213-2600(20)30076-X
12. Tao H, Bai J, Zhang W, et al. Bone biology and COVID-19 infection: Is ACE2 a potential influence factor? *Med Hypotheses*. 2020;144:110178. doi:10.1016/j.mehy.2020.110178
13. Lee CYS, Suzuki JB. CTX biochemical marker of bone metabolism: Is it a reliable predictor of bisphosphonate-associated osteonecrosis of the jaws after surgery? Part I: Biological concepts with a review of the literature. *Implant Dent*. 2009;18(6):492–500. doi:10.1097/ID.0b013e3181c6837c
14. Huang YL, Chen ST, Liu RS, et al. CLEC5A is critical for dengue virus-induced osteoclast activation and bone homeostasis. *J Mol Med*. 2016;94(9):1025–1037. doi:10.1007/s00109-016-1409-0
15. Bedimo R, Cutrell J, Zhang S, et al. Mechanisms of bone disease in HIV and hepatitis C virus: Impact of bone turnover, tenofovir exposure, sex steroids and severity of liver disease. *AIDS*. 2016;30(4):601–608. doi:10.1097/QAD.0000000000000952
16. Katsarou O, Terpos E, Chatzismalis P, et al. Increased bone resorption is implicated in the pathogenesis of bone loss in hemophiliacs: Correlations with hemophilic arthropathy and HIV infection. *Ann Hematol*. 2010;89(1):67–74. doi:10.1007/s00277-009-0759-x
17. Chan MHM, Chan PKS, Griffith JF, et al. Steroid-induced osteonecrosis in severe acute respiratory syndrome: A retrospective analysis of biochemical markers of bone metabolism and corticosteroid therapy. *Pathology*. 2006;38(3):229–235. doi:10.1080/00313020600696231
18. Lin JC, Hsieh TY, Wu CC, et al. Association between chronic hepatitis C virus infection and bone mineral density. *Calcif Tissue Int*. 2012;91(6):423–429. doi:10.1007/s00223-012-9653-y
19. Awosanya OD, Dalloul CE, Blosser RJ, et al. Osteoclast-mediated bone loss observed in a COVID-19 mouse model. *Bone*. 2022;154:116227. doi:10.1016/j.bone.2021.116227
20. De Baaij JHF, Hoenderop JGJ, Bindels RJM. Magnesium in man: Implications for health and disease. *Phys Rev*. 2015;95(1):1–46. doi:10.1152/physrev.00012.2014
21. Trapani V, Rosanoff A, Baniasadi S, et al. The relevance of magnesium homeostasis in COVID-19. *Eur J Nutr*. 2022;61(2):625–636. doi:10.1007/s00394-021-02704-y
22. Pal R, Ram S, Zohmangaihi D, et al. High prevalence of hypocalcemia in non-severe COVID-19 patients: A retrospective case-control study. *Front Med (Lausanne)*. 2021;7:590805. doi:10.3389/fmed.2020.590805
23. Erat T, Atar M, Kontbay T. Transient benign hyperphosphatasemia due to COVID-19: The first case report. *J Pediatr Endocrinol Metab*. 2021;34(3):385–387. doi:10.1515/jpem-2020-0503
24. Ploegmakers DJM, Zielman-Blokhuis AM, van Duijnhoven HJR, de Rooy JWJ, Geurts ACH, Nonnekes J. Heterotopic ossifications after COVID-19 pneumonia [in Dutch]. *Ned Tijdschr Geneeskd*. 2020; 164:D5357. PMID:33331722.
25. Pepys MB, Hirschfield GM. C-reactive protein: A critical update. *J Clin Invest*. 2003;111(12):1805–1812. doi:10.1172/JCI200318921
26. Liu F, Li L, Xu M, et al. Prognostic value of interleukin-6, C-reactive protein, and procalcitonin in patients with COVID-19. *J Clin Virol*. 2020;127:104370. doi:10.1016/j.jcv.2020.104370
27. Lagunas-Rangel FA. Neutrophil-to-lymphocyte ratio and lymphocyte-to-C-reactive protein ratio in patients with severe coronavirus disease 2019 (COVID-19): A meta-analysis. *J Med Virol*. 2020;92(10): 1733–1734. doi:10.1002/jmv.25819