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Coronavirus Pandemic

Role of serum fibroblast growth factor-23 and Klotho level in COVID-19 infection-related mortality: a prospective study

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Abstract

Introduction: This study investigated the role of fibroblast growth factor 23 (FGF23)/Klotho in the mortality of patients hospitalized with coronavirus disease 2019 (COVID-19), excluding those with chronic kidney disease (CKD).

Methodology: A prospective cross-sectional study was conducted from April 2021 to May 2022. Patients who tested positive for COVID-19 via polymerase chain reaction and were hospitalized, were classified into two groups (survivors and non-survivors) at the end of their hospital follow-up. Demographic data, clinical status, and prognosis were analyzed.

Results: A total of 66 patients (age 58.8 ± 17.0 years, 60.6% male) were included. The mean age of non-survivors (67.2 ± 1 years) was significantly higher than the survivors (49.2 ± 1 years; $p < 0.0001$). FGF23 was significantly elevated in non-survivors (301 ± 20 pg/mL), compared to survivors (160 ± 36 pg/mL; $p < 0.0001$). Factors with significant differences ($p < 0.001$) between the two groups were investigated as independent mortality predictors using logistic regression analysis. FGF23 ($p = 0.01$), age ($p = 0.045$), and oxygen saturation at admission ($p = 0.02$) were independent predictors of mortality. High serum FGF23 levels were associated with COVID-19-related mortality; Klotho levels were lower ($p = 0.028$). Vitamin D was not significantly different between the groups.

Conclusions: Elevated serum FGF23 and parathyroid hormone (PTH), and lower Klotho levels, were associated with COVID-19-related mortality in patients without CKD. There was no association with serum vitamin D levels. Further studies are required to establish the relationship between mortality and FGF23/Klotho, PTH, and vitamin D levels.

Key words: fibroblast growth factor-23; klotho; COVID-19; mortality; vitamin D.

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Introduction

Fibroblast growth factor 23 (FGF23) is a hormone involved in the regulation of vitamin D and phosphate metabolism, particularly in patients with end stage renal disease (ESRD) [1]. Recent evidence suggests that besides its role in bone-mineral homeostasis, systemic FGF23 may have effects beyond the kidneys and parathyroid glands, potentially impacting tissues not previously identified as targets of FGF23. FGF23 acts as an immunomodulatory hormone and can influence the immune response due to its indirect effects on non-immune tissues and direct effects on immune cells, including macrophages and polymorphonuclear neutrophils (PMN). Elevated FGF23 levels are associated with increased inflammation and mortality in patients with chronic kidney disease (CKD) [2]. Experimental mouse models and in vitro human PMN cells have shown that FGF23 can lead to immune dysfunction [3]. Furthermore, clinical studies in hemodialysis (HD) patients [4] and in patients with moderate to severe CKD not undergoing HD [5], have demonstrated that high plasma levels of FGF23 are associated with an increased rate of infections. Epidemiological findings in both the general population and in individuals with kidney disease are consistent and indicate a relationship between FGF-23 and mortality, cardiovascular disease, and inflammation [6].

The role of vitamin D in the immune response to coronavirus disease 2019 (COVID-19) can be two-fold. Firstly, vitamin D supports the production of antimicrobial peptides in the respiratory epithelium, thereby reducing the likelihood of viral infection and the development of COVID-19 symptoms. Secondly, vitamin D may reduce the inflammatory response to

severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This response, particularly the deregulation of the renin-angiotensin system, is characteristic of COVID-19 and its excessive activation is associated with a worse prognosis. It is known that vitamin D interacts with angiotensin converting enzyme 2 (ACE2), which is used by SARS-CoV-2 as an entry receptor. SARS-CoV-2 down-regulates the expression of *ACE2*, and vitamin D supports the expression of this gene [7]. FGF23 and Klotho levels, which play important roles in vitamin D regulation, may be underlying mechanisms for the severity and mortality of COVID-19 patients [8].

The aim of this study was to investigate the role of FGF23/Klotho in mortality among patients who did not have CKD and are hospitalized with COVID-19 infection.

Methodology

This prospective cross-sectional study was conducted from April 2021 to May 2022. A total of 66 patients who tested positive for COVID-19 via polymerase chain reaction (PCR) and had an indication for hospitalization – based on the World Health Organization (WHO) COVID-19 clinical management living guidance, 25 January 2021 – were included in the study [9]. Patients with known oncological diseases, severe heart disease, diabetes mellitus, and kidney disease were excluded. Those who were under 18 years of age, pregnant women, and patients with signs of shock or multiple organ failure were also excluded. The decision for hospitalization was made by emergency department doctors and consultant physicians according to clinical criteria, without intervention from the research team. Basic clinical, laboratory data, and blood samples were collected on the day of hospital admission.

Patients who met the inclusion criteria and signed an "informed consent form" were included in the study. Serum obtained from blood samples taken for routine biochemical and hemogram tests were used. Blood samples, collected in a two mL biochemical tube, were centrifuged at 3500 rpm for 20 minutes at room temperature within 15 minutes of collection, and the obtained sera were stored at -80 °C until the day of analysis. All patients were monitored after hospitalization. At the end of the follow-up, they were divided into two groups: those who died in the hospital and those who were discharged.

Measurement of serum FGF23 and Klotho levels

Serum FGF23 (E0059Hu, BT Lab, Shanghai, China) and Klotho (E2781Hu, BT Lab, Shanghai, China) levels were measured using enzyme-linked immunosorbent assay (ELISA) kits according to the provided test protocols.

PCR analysis

Reverse transcriptase polymerase chain reaction (RT-PCR; Rotor Gene, Qiagen, USA) using Biospeedy SARS CoV-2 Double Gene RT-qPCR Kit (Lot: 2B01114NF25OG100-TK20, Bioeksen, İstanbul, Turkey) was used to test for COVID-19 in the samples taken from nasopharyngeal and oropharyngeal regions.

Statistical analyses

Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as numbers and percentages. Student's t-test was used for comparing independent group differences when parametric test assumptions were met; Mann-Whitney U test was used for nonparametric data. Differences between categorical variables were examined using Chi-square analysis. $p < 0.05$ was considered statistically significant. Independent factors affecting mortality were evaluated by binary logistic regression analysis. The data were analyzed using SPSS 25.0 (IBM SPSS Statistics 25 software, IBM Corp, Armonk, NY, USA).

Results

A total of 66 patients with COVID-19 who met the inclusion criteria were included in the study. The baseline characteristics of these patients are shown in Table 1. The mean age of the patients was 58.8 ± 17.0 years, and 60.6% were male. More than half of the patients (50.8%) presented to the hospital within the first 5 days after symptom onset. Dyspnea and cough

Table 1. Clinical parameters of patients according to COVID-19 infection-related mortality status (n = 66).

Parameters	Survivors of COVID-19 (n = 31)	COVID-19-related death (n = 35)	*p* value
Age (years)	49.2 ± 1	67.2 ± 1	$p < 0.0001$
BMI (kg/m²)	28.7 ± 0.8	28.3 ± 0.9	NS
Blood Pressure (mmHg)	119 ± 15	120 ± 18	NS
Heart rate (min)	92 ± 1	101 ± 1	0.014
Respiratory rate (min)	24.5 ± 5	27.6 ± 4	0.014
Oxygen saturation (%)	87 ± 7	81 ± 6	$p < 0.0001$

BMI: body mass ındex; COVID-19: coronavirus disease 2019; NS: not significant.

Table 2. Comparison between survivors of COVID-19 and COVID-19-related deaths (n = 66).

Parameters	Survivors of COVID-19 (N = 31)	COVID-19-related death (N = 35)	*p* value
FGF23 (pg/mL)	160 ± 36	301 ± 20	$p < 0.0001$
Klotho (pg/mL)	161 ± 153	103 ± 74	0.028
PTH (ug/L)	61 ± 39	122 ± 141	0.012
Vitamin D (ug/L)	24.3 ± 13	21.5 ± 1	NS
Calcium (mg/dL)	8.1 ± 5	8.1 ± 4	NS
Phosphorus (mg/dL)	3.2 ± 7	3.2 ± 8	NS
Albumin (g/L)	32.3 ± 7	32.0 ± 7	NS
HbA1c (%)	6.1 ± 1	6.1 ± 1	NS
FBG (mg/dL)	133 ± 44	149 ± 36	NS
Creatinine (mg/dL)	0.83 ± 0.3	1.1 ± 0. 4	0.001
CRP (mg/L)	81.8984 ± 63	131.1200 ± 78	0.007
D-dimer(mg\L)	1540 ± 2497	1782 ± 1560	NS
Troponin (ng/L)	14 ± 26	125 ± 329	$p < 0.0001$

COVID-19: coronavirus disease 2019; CRP: C-reactive protein; FBG: fasting blood glucose; FGF23: fibroblast growth factor 23; NS: not significant; PTH: parathyroid hormone.

were the presenting symptoms in 77.3% of the cases. The vaccination rate was 4.5%. After hospitalization, 35 patients died from COVID-19 infection-related causes, while 31 patients were discharged.

In the COVID-19-related death group, the average age, respiratory rate, and heart rate were significantly higher, while their oxygen saturation was significantly lower, than the survivor group.

The serum level of FGF23 was significantly elevated in the cohort of patients who succumbed to COVID-19 (301 ± 20 pg/mL) compared to those who survived (160 ± 36 pg/mL) ($p < 0.0001$). Similarly, significant differences were observed in serum levels of Klotho and parathyroid hormone (PTH) between the two groups ($p = 0.028$ and $p = 0.012$, respectively; Table 2).

The factors that were significantly ($p < 0.001$) different between the groups of patients – those who died of COVID-19-related causes and the suvivors – were investigated to identify independent predictors of mortality using logistic regression analysis. FGF23 ($p = 0.01$), age ($p = 0.045$), and oxygen saturation at admission ($p = 0.02$) were identified as independent predictors of mortality (Table 3).

Discussion

In this prospective study, levels of FGF23/Klotho and PTH were found to be independent predictors of COVID-19-related mortality. Furthermore, high serum FGF23 and PTH, and low Klotho levels, were independent of other variables associated with increased infections such as CKD and diabetes. This study is one of the first clinical prospective studies evaluating the relationship between high serum FGF23 and PTH, and low Klotho levels, and COVID-19 infection in patients without underlying diseases such as CKD or diabetes.

Previous studies, such as those by Toro *et al*., demonstrated that high serum FGF23 levels in patients with end-stage kidney disease undergoing chronic HD were associated with higher hospitalization rates and severe COVID-19 outcomes, including death [10]. Numerous studies have associated high FGF23 levels with all-cause mortality rates in both the general population and individuals with CKD [6]. While most studies focused on cardiovascular mortality, infection-related deaths are also significant. Epidemiological studies involving HD patients in the HEMO study have shown an independent relationship between FGF23 and infection-related mortality [4]. Elevated FGF23 levels

Table 3. Regression analysis parameters for COVID-19 related mortality.

Variables in the Equation									
								95% CI for EXP(B)	
		B	SE	Wald	df	Sig.	Exp(B)	Lower	Upper
Step 1	FGF23 (pg/mL)	0.111	0.043	6.585	1	0.010	1.117	1.026	1.215
	Age (years)	0.070	0.035	4.030	1	0.045	1.072	1.002	1.147
	O_2 sat (%)	– 0.277	0.119	5.423	1	0.020	0.758	0.600	0.967
	Creatinine (mg/dL)	– 1.081	1.968	0.302	1	0.583	0.339	0.007	16.055
	Troponin I	0.035	0.0,21	2.777	1	0.096	1.036	0.994	1.080
	CRP	– 0.004	0.010	0.198	1	0.996	0.996	0.976	1.015
	Constant	– 0.960	6.617	0.021	1	0.383	0.383		

CI: confidence interval; COVID-19: coronavirus disease 2019; CRP: C-reactive protein; df: degrees of freedom; FGF23: fibroblast growth factor 23; O_2 sat: oxygen saturation; SE: standard error: Sig: significance level.

may be associated with increased inflammation in patients. However, evidence regarding the potential mechanisms linking high FGF23 levels with increased risk of severe infection remains uncertain. Myrou *et al*. stated that while FGF23 levels showed significant differences across age groups in symptomatic COVID-19 infection, the relationship with the severity or stage of infection and/or other comorbidities remained unclear [11].

The effects of vitamin D on immunity represent a new and exciting research area. While some retrospective studies have shown a correlation between vitamin D status and the severity and mortality rate of COVID-19, other studies failed to find this correlation when confounding variables were adjusted. In this study, no protective effect or mortality correlation was found when examining changes in vitamin D levels.

FGF23 levels (p = 0.01), age (p = 0.045), and oxygen saturation at admission (p = 0.02) were identified as independent predictors of mortality between the groups of patients who died from COVID-19 and those who survived.

Limitations of the study include a small patient group and, deficiencies in the study design, which may limit the generalizability of the findings.

Conclusions

High serum FGF23 and, PTH, and low Klotho levels, independent of CKD, were associated with mortality in COVID-19 patients. This association was not observed with serum vitamin D levels. Further studies with larger patient cohorts are required to establish a reliable correlation between FGF23/Klotho and other laboratory parameters.

Authors' contributions

DA, BBA: patient recruitment, clinical follow-up, and data analysis; DA, BBA, NE, MA: patient enrollment for the study and clinical data collection; GG, OKE: ELISA measurements. DA, SO: clinical study design and manuscript draft preparation. All authors reviewed and approved the final version of the manuscript.

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Institutional review board statement

The study was conducted following the Helsinki Declaration and approved by the Pamukkale University Non-Interventional Clinical Research Ethics Board (approval no: E-60116787-020-37820) (16.03.2021 and numbered 06).

Informed consent statement

Informed consent was obtained from all patients participating in the study.

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