

Research Article

RANK-RANKL-OPG System in COVID-19: Examining the Pathway Among Healthcare Workers in Nigeria

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Abstract

Background: Coronavirus disease 2019 (COVID-19) influences bone metabolism by altering the RANK-RANKL-OPG system. However, this has not been validated, especially among Nigerians. Consequently, the current study explored the influence of the disease on this vital skeletal pathway among Nigerian healthcare workers (HCWs). **Methods:** This was a prospective longitudinal study conducted in the Department of Chemical Pathology of the Rivers State University Teaching Hospital among HCWs in Rivers State, Southern Nigeria. Eligible HCWs (n=76) with moderate RT-PCR-confirmed COVID-19 were recruited and compared with age and sex-matched healthy controls. Demographic, anthropometric, clinical, and laboratory data were obtained at baseline upon COVID-19 onset and followed up on days four and seven. Statistical analysis was done using descriptive/inferential statistics at a p-value <0.05. **Results:** The HCWs with moderate COVID-19 had higher serum levels of pro-inflammatory markers (IL-1 β , IL-6, TNF- α) and receptor activator of nuclear factor kappa beta ligand (RANKL) but lower serum levels of osteoprotegerin (OPG) at COVID-19 diagnosis compared to the healthy controls (p<0.05). Among the HCWs with positive COVID-19 status, an increasing trend of these inflammatory markers and RANKL was observed from day one to day four and day seven, but a decreasing trend of OPG levels was observed (p<0.05). On day seven following COVID-19 diagnosis among the HCWs, a positive relationship was established between serum RANKL and all the pro-inflammatory markers (p<0.001) while an inverse relationship was only observed between OPG and IL-1 β pro-inflammatory marker (p<0.05). **Conclusion:** The study findings corroborate the negative influence of COVID-19 on the RANK-RANKL-OPG system in favor of exaggerated osteoclastogenesis.

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Keywords

COVID-19, RANK, RANKL, Osteoprotegerin

1. Introduction

The coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is a devastating viral-induced disease [1]. The pandemic induced by the disease is acclaimed to be among the most serious to have affected mankind [2]. The disease was initially reported to affect only the respiratory system and its organs [2]. However, due to the ubiquity of the viral cognate biologic receptor, recognized as the angiotensin-converting enzyme 2 (ACE2), the disease has been found to influence other organ systems including the skeletal structures [2-4].

ACE2 is highly expressed within the skeletal systems, including the bone structures [5, 6]. The cellular structures within the skeletal system, including the osteoclast and osteoblast are known to express the biologic cognate receptor of the SARS-CoV-2 viral agent [5-7]. Receptor activator of nuclear kappa beta - receptor activator nuclear kappa beta ligand - Osteoprotegerin (RANK-RANKL-OPG) pathway is a vital skeletal system pathway that regulates osteoclastic-osteoblastic balance within the skeletal structures [7].

RANKL is a protein expressed by osteoblastic cells, whereas its receptor, RANK, is expressed by the osteoclastic cells. RANKL binds to the RANK receptor on osteoblasts stimulating the activity of osteoclastic cells, which are responsible for initiating bone resorption. However, the osteoblasts also produce another soluble protein, the OPG, which acts as a “decoy receptor”, preventing the binding of RANKL to RANK and, consequently, inhibiting osteoclast activation/activities [7]. Under ideal health circumstances, the RANKL/OPG ratio is balanced in bone physiology and bone resorption is counterbalanced by bone deposition [7].

COVID-19 has been documented in several studies to impact the skeletal systems via distortions in this very RANK-RANKL-OPG pathway via several pathomechanisms [7]. However, most of the previous studies have been documented in the Western population, and to date, no such study has been conducted among Nigerians. Hence, the current study explored the influence of COVID-19 on bone metabolism among healthcare workers (HCWs) in Rivers State, Southern Nigeria.

2. Materials and Methods

2.1. Study Design, Site, and Setting

The study was a prospective longitudinal study conducted

in the Department of Chemical Pathology of one of the tertiary healthcare facilities [Rivers State University Teaching Hospital (RSUTH)] in Rivers State, Southern Nigeria. The hospital has a designated unit for isolating suspected cases of COVID-19 and also a molecular laboratory where detailed molecular analysis including the reverse transcriptase polymerase chain reaction (RT-PCR) test is conducted to confirm COVID-19 among the suspected cases. The Department of Chemical Pathology of the hospital is well-equipped with several biochemical analyzers for routine/complex biochemical investigations.

2.2. Ethical Considerations

Approval for the study was granted by the Rivers State Health Research Ethics Committee of the Rivers State Hospital Management Board. All study populations agreed to participate and provided written/signed informed consent. The study was conducted with strict adherence to the recommended guidelines and the principles embodied and laid down in the Helsinki Declarations of 1964, and as revised in 2013.

2.3. Study Population

The study populations consist of eligible HCWs diagnosed with RT-PCR-confirmed (with nasal and pharyngeal swab specimens) moderate COVID-19 in RSUTH. The patients were recruited upon referral to the Department of Chemical Pathology for baseline biochemical investigation following diagnosis/confirmation of moderate COVID-19 in the isolation unit. For each case recruited, an age/sex-matched healthy HCW was recruited as controls.

2.4. Sample Size Determination

The calculated minimum sample size required for this study is 76. The sample size was determined using a mathematical formula for cross-sectional studies for defined characteristics in a population >10,000 using a 0.015% prevalence of COVID-19 in Nigeria as documented by Nas and colleagues [8, 9]. However, the result from the sample size calculation was 0.230, and to enhance the power of the study, we enrolled 300% of this value; that is 76 ($0.230 \times 300\% = 76$) inclusive of a projected 10% non-compliance rate.

2.5. Eligibility Criteria

Criteria for inclusion included adult (aged ≥ 18 / <40 years of age) HCW with RT-PCR-confirmed moderate COVID-19 status for at least seven days. Criteria for exclusion are age <18 / >40 years of age, mild, severe, critical COVID-19 status, having a negative RT-PCR test within 7 days of disease confirmation, previous COVID-19 vaccination, post-menopausal, hypogonadism, hypopituitarism, thyroid disorders, pregnancy, COVID-19 re-infection, past/pre-existing comorbidities (cardiovascular disease, hypertension, chronic lung disease, asthma, sickle cell disease, HIV/AIDS, diabetes, cancer, obesity, acute/chronic kidney disease, chronic liver disease, previous/current cigarette smoker, organ transplant recipient, and receiving immunosuppressive therapy) before SARS-CoV-2 infection/COVID-19 diagnosis, and on current medications known to influence sex hormones or bone metabolism such as steroids, androgens, oral contraceptive pills, bisphosphonates, glucocorticoids, calcitonin, vitamin D supplements, calcium, etc.

2.6. Data Collection

The study populations were recruited upon referral to the Department of Chemical Pathology for baseline biochemical investigation following diagnosis/confirmation of moderate COVID-19 in the isolation unit. Upon presentation and after the acquisition of informed consent, a semi-structured questionnaire was used to obtain demographic, anthropometric, and clinical data and to determine eligibility status.

Following confirmation of eligibility status, specimens were collected for baseline laboratory parameters. The laboratory parameters included blood levels of urea, creatinine, glucose, albumin, pro-calcitonin (PCT), C-reactive protein (CRP), D-dimer levels, intact PTH, vitamin D, calcium, phosphate, magnesium, interleukin 1β (IL- 1β), interleukin 6 (IL-6), tumor necrosis factor- α (TNF- α), RANKL, and OPG. However, analysis for IL- 1β , IL-6, TNF- α , RANKL, and OPG was repeated on days four and seven while still with positive RT-PCR-confirmed COVID-19 status and on management for COVID-19.

2.7. Specimen Acquisition, Processing, and Laboratory Analysis

Whole blood specimens were drawn from subjects by well-trained research assistants at baseline (day 1), day 4, and day 7 into plain and heparin specimen tubes. These were immediately centrifuged for 15 min at $2500 \times g$ to obtain serum and plasma which were stored frozen at -80°C until assay. Heparinized plasma was analyzed for plasma sodium, potassium, bicarbonate, and chloride on an ion-selective electrode chemistry analyzer (SFRI 6000, SFRI Diagnostics, France) including the analyses for urea, creatinine, albumin, calcium, phosphate, magnesium, alkaline phosphatase, and CRP was

done on an automated chemistry analyzer (BS200, Mindray, China). Serum intact parathyroid hormone (PTH), vitamin D [25(OH)D], IL- 1β , IL-6, TNF- α , RANKL, and OPG levels were determined via the enzyme-linked immunoassay (ELISA) method using standard reagents kits (Elabsience, Texas, USA).

2.8. Infection Prevention and Control Measures

Adequate infection prevention and control measures, as recommended by the Nigeria Center for Disease Control, were strictly adhered to during specimen collection and laboratory analysis [10].

2.9. Variable Definitions/Stratifications

The clinical spectrum of COVID-19 disease was categorized as follows [11]:

Pre-symptomatic/asymptomatic COVID-19 infection: Individuals who test positive for SARS-CoV-2 using an RT-PCR from a nasopharyngeal swab but who have no symptoms/signs consistent with COVID-19.

Mild illness: Individuals who have any of the various signs and symptoms of COVID-19 (e.g., fever, cough, sore throat, malaise, headache, muscle pain, nausea, vomiting, diarrhea, loss of taste and smell) but who do not have shortness of breath and dyspnea.

Moderate illness: Individuals who show evidence of lower respiratory disease during clinical assessment or imaging and who have an oxygen saturation (SpO_2) $\geq 94\%$ on room air at sea level. Severe illness: Individuals who have $\text{SpO}_2 < 94\%$ on room air at sea level, a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) < 300 mm Hg, a respiratory rate > 30 breaths/min, or lung infiltrates $> 50\%$.

Critical illness: Individuals who have respiratory failure, septic shock, and/or multiple organ dysfunction.

2.10. Data Management/Statistical Analyses

Data management and statistical analyses were done using a statistical package for social sciences software for Windows (version 25; IBM Co., Armonk, NY, USA). The continuous data were initially evaluated for conformity to a normal distribution pattern using the Shapiro-Wilk tests. Continuous data violating the normal distribution patterns were log-transformed before analysis, expressed using means \pm standard deviations, and compared by independent student t-test or analysis of variance, as applicable. Categorical data were reported as counts/percentages and compared with the Chi-square or Fisher's tests, as appropriate. Linear logistic regression was used to evaluate associations between continuous variables. A p-value < 0.05 was deemed statistically significant.

3. Results

During the studied period (2020-2023), 76 eligible HCWs with moderate RT-PCR-confirmed COVID-19 presented in the department through the COVID-19 isolation unit of RSUTH. For each recruited, age and sex-matched healthy control participants were recruited.

As depicted in Table 1, the HCWs with moderate COVID-19 patients had lower oxygen saturation (SpO₂) status, at diagnosis than the healthy controls (p=0.004).

At diagnosis as shown in Table 2, the HCWs with moderate COVID-19 had higher serum levels of inflammatory markers (IL-1 β , IL-6, TNF- α) and RANKL but lower serum levels of OPG compared to healthy controls (p<0.05).

As shown in Table 3, there was an increasing trend/worsening of the pro-inflammatory markers (IL-1 β , IL-6, TNF- α) and serum RANKL levels from day 1 through day 4 and day 7 but a decreasing trend of serum OPG levels following COVID-19 diagnosis among the HCWs (p<0.05).

As shown in Table 4, a positive relationship was established between RANKL and all the pro-inflammatory markers in both crude and adjusted linear regression analysis (p<0.001) on day 7 post-COVID-19 diagnosis. However, while inverse relationships were established between OPG and all the pro-inflammatory markers on day 7 post-COVID-19 diagnosis, a statistically significant threshold was only achieved between the serum OPG levels and IL-1 β pro-inflammatory marker serum levels (p<0.001).

Table 1. Distribution of demographic/clinical parameters of studied population.

Variables	COVID-19 patients n = 76	Healthy Controls n = 76	p-value
	Mean \pm SD/n	Mean \pm SD/n	
Age, mean, years	31.33 \pm 3.45	32.09 \pm 3.36	0.243
Body mass index, kg/m ²	28.66 \pm 3.03	29.04 \pm 2.89	0.171
Gender: males versus females	38/38	38/38	NA
Oxygen saturation (SpO ₂), %	96.41 \pm 4.32	98.61 \pm 5.65	0.004*
Vaccination status (Yes/No)	44/32	40/36	0.304

*Statistically significant; SD: Standard deviation

Table 2. Distribution of baseline laboratory parameters of studied population.

Variables	COVID-19 Patients (Day 1) n = 76	Healthy Controls n = 76	p-value
	Mean \pm SD/n	Mean \pm SD/n	
Urea, mmol/L	4.04 \pm 1.19	3.89 \pm 1.03	0.118
Creatinine, μ mol/L	86.36 \pm 5.31	84.11 \pm 5.10	0.078
Adjusted total calcium, mmol/L	2.30 \pm 0.47	2.27 \pm 0.42	0.231
Phosphate, mmol/L	1.14 \pm 0.28	1.12 \pm 0.27	0.144
Magnesium, mmol/L	0.84 \pm 0.12	0.83 \pm 0.14	0.325
Intact parathyroid hormone, ng/L	41.77 \pm 3.33	40.99 \pm 4.17	0.108
Vitamin D, nmol/L	40.11 \pm 5.41	42.03 \pm 5.37	0.085
Albumin, g/L	32.74 \pm 4.44	33.67 \pm 4.14	0.081
Interleukin-1 β , pg/mL	217.19 \pm 12.77	10.41 \pm 1.66	<0.001*
Interleukin-6, pg/mL	44.41 \pm 6.71	4.03 \pm 1.05	<0.001*
Tumor necrosis factor- α , pg/mL	198 \pm 11.66	17.51 \pm 2.22	<0.001*
RANKL, pg/mL	445.77 \pm 17.61	226.43 \pm 10.67	<0.001*

Variables	COVID-19 Patients (Day 1) n = 76	Healthy Controls n = 76	p-value
	Mean \pm SD/n	Mean \pm SD/n	
OPG, ng/ml	3.05 \pm 1.23	5.39 \pm 1.34	<0.001*

*Statistically significant; SD: Standard deviation; RANKL: Receptor activator of nuclear factor kappa β ; OPG: Osteoprotegerin

Table 3. Dynamics of relevant laboratory parameters among COVID-19 patients.

Parameters	Day 1	Day 4	Day 7	p-value
	Mean \pm SD/n	Mean \pm SD/n	Mean \pm SD/n	
Interleukin-1 β , pg/mL	217.19 \pm 12.77	258.43 \pm 20.46	304.66 \pm 20.87	<0.001*
Interleukin-6, pg/mL	44.41 \pm 6.71	61.51 \pm 6.41	74.21 \pm 6.67	<0.001*
Tumor necrosis factor- α , pg/mL	198 \pm 11.66	251 \pm 12.74	298 \pm 12.90	<0.001*
RANKL, pg/mL	445.77 \pm 17.61	566.65 \pm 17.41	617.73 \pm 17.08	<0.001*
OPG, ng/ml	3.05 \pm 1.23	2.55 \pm 1.20	1.73 \pm 1.04	<0.001*

*Statistically significant; SD: Standard deviation; RANKL: Receptor activator of nuclear factor kappa β ; OPG: Osteoprotegerin

Table 4. Relationship between RANKL/OPG and pro-inflammatory markers on day 7 following COVID-19 diagnosis.

	RANKL, pg/mL	OPG, pg/ml
Panel A	Crude Linear Regression Parameters	
Pro-inflammatory Markers	β ; p-value	β ; p-value
Interleukin-1 β , pg/mL	0.649; <0.001*	-0.373; <0.001*
Interleukin-6, pg/mL	0.581; <0.001*	-0.201; 0.161
Tumor necrosis factor- α , pg/mL	0.467; <0.001*	-0.144; 0.087
Panel B	Adjusted Linear Regression Parameters**	
Pro-inflammatory Markers	β ; p-value	β ; p-value
Interleukin-1 β , pg/mL	0.602; <0.001*	-0.334; <0.001*
Interleukin-6, pg/ml	0.511; <0.001*	-0.198; 0.203
Tumor necrosis factor- α , pg/mL	0.433; <0.001*	-0.139; 0.117

*Statistically significant; SD: Standard deviation; RANKL: Receptor activator of nuclear factor kappa β ; OPG: Osteoprotegerin; **adjusted for age, sex, vaccination status, and oxygen saturation

4. Discussion

4.1. Major Findings

The studied HCWs with moderate COVID-19 had higher

serum levels of pro-inflammatory markers (IL-1 β , IL-6, TNF- α) and RANKL but lower serum levels of OPG at COVID-19 diagnosis compared to the healthy controls. Among the HCWs with positive COVID-19 status, an increasing trend of these inflammatory markers and RANKL was observed from day one to day four and day seven, but a decreasing trend of OPG levels was observed. On day seven following COVID-19 diagnosis

among the studied HCWs, a positive relationship was established between serum RANKL and all the pro-inflammatory markers while an inverse relationship was only observed between OPG and IL-1 β pro-inflammatory marker.

4.2. Relationship with Previous Studies

Although few studies have highlighted the impact of COVID-19 on bone metabolism since the advent of the disease [12, 13], a handful of these studies have examined the relationship between the disease and the RANKL-OPG system [14-16]. In one of these studies which examined the RANKL-OPG system among Brazilian COVID-19 patients, the authors reported that COVID-19 patients also presented with increased serum RANKL but reduced OPG compared to healthy controls [14]. In another study examining the relationship between periodontitis and COVID-19 among another subset of Brazilian COVID-19 patients, the authors indicated an increase in the levels of salivary RANKL compared to controls [15]. Our findings are in tandem with the conclusions of these two previous [14, 15] and tend to indicate a link between COVID-19 and the RANKL-OPG system. However, another similar study conducted among the Iraqi patients indicated that bone mineral density was lower in patients with SARS-CoV-2 infection but OPG levels were higher among patients infected with SARSCoV-2. The serum level of RANKL was not measured in that Iraqi study but the authors suggested that the higher OPG levels may be a reaction to an increased osteoclast activity to maintain bone homeostasis [16].

4.3. Cellular/Molecular Mechanistic Insight

RANKL is a transmembrane protein mainly expressed by osteoblasts, whereas its cognate biologic receptor, the RANK, is expressed by osteoclasts. Upon binding to RANK, RANKL stimulates the formation and activity of osteoclasts, which are responsible for bone resorption. However, osteoblasts produce an additional soluble protein, OPG, which acts as a “decoy receptor”, preventing the binding of RANKL to RANK and, consequently, osteoclast activation. Under normal health status, the ratio of RANKL to OPG is balanced, and bone resorption is counterbalanced by bone deposition [17]. However, COVID-19 has been shown to induce the release of several pro-inflammatory cytokines, especially IL-1, IL-6, and TNF- α , which stimulate osteoclast activity, enhancing bone resorption through alteration of the normal balanced RANKL-OPG system [12, 13]. This was corroborated in this study where a positive relationship was established between RANKL and the inflammatory markers with an inverse relationship observed between OPG and the inflammatory markers.

4.4. Relevance to Clinicians and Future Studies

The findings here highlight the need to consider the impact

of COVID-19 on bone physiology during the management of the disease. In subsequent studies, it is imperative to decipher the exact pathophysiologic basis of COVID-19-associated negative impacts on bone physiology.

4.5. Strength and Limitations

The study was strongly strengthened by the recruitment/analysis of only those COVID-19 patients with confirmed positive RT-PCR tests. However, the study was limited by some factors which are potential areas for improvement in future studies. The study was a single-center study with predominantly black populations, so, its findings may not represent of the larger population within the studied region. The smaller sample size was another limitation that should also be improved upon in future studies.

5. Conclusion

HCWs with moderate COVID-19 had higher serum levels of pro-inflammatory markers and RANKL but lower serum levels of OPG at COVID-19 diagnosis compared to the healthy controls. An increasing trend of these pro-inflammatory markers and RANKL was also observed from day one to day four and day seven, but a decreasing trend of OPG levels was observed. On day seven following COVID-19 diagnosis among the studied HCWs, a positive relationship was established between serum RANKL and all the pro-inflammatory markers, while an inverse relationship was only observed between OPG and IL-1 β pro-inflammatory marker. These findings corroborate the negative influence of COVID-019 on the RANK-RANKL-OPG system in favor of exaggerated osteoclastogenesis.

Abbreviations

COVID-19	Coronavirus Disease 2019
HCWs	Healthcare Workers
Sars-Cov-2	Severe Acute Respiratory Syndrome Coronavirus-2
RANK	Receptor Activator of Nuclear Factor Kappa Beta
RANKL	Receptor Activator of Nuclear Factor Kappa Beta
ACE2	Angiotensin Converting Enzyme 2
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
RSUTH	Rivers State University Teaching Hospital
PCT	Pro-calcitonin
CRP	C-reactive Protein
IL-1 β	Interleukin 1 β
IL-6	Interleukin 6
TNF α	Tumour Necrosis Factor α
ELISA	Enzyme-Linked Immunosorbent Assay

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Data Availability Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author (CA) upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

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