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Hypocalcemia and Hypovitaminosis D in COVID-19 Patients are Related to High Fibroblast Growth Factor 23 and Sclerostin Concentrations

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Summary

Fibroblast Growth Factor 23 and sclerostin are indicators for COVID-19 severity. They play a crucial role in COVID-19 associated hypocalcemia through inhibition of vitamin D activation by suppressing renal 1-αhydroxylase. Better prognosis can be achieved by active vitamin D supplementation.

ABSTRACT

Background

Hypocalcemia is highly prevalent among positive COVID-19 patients which can be explained by insufficient vitamin D levels detected among them. This study measures serum levels of fibroblast growth factor 23 and sclerostin, which cause suppression of renal 1-α hydroxylase enzyme that is responsible for vitamin D activation.

Methods

It is a case control study that includes 22 healthy controls (Group A), 22 mild/moderate SARS-CoV-2 patients (Group B), and 22 severe/critical patients (Group C). Serum levels of ionized calcium, calcitriol, parathyroid hormone, fibroblast growth factor 23, and sclerostin were measured using ELISA.

Results
The lowest levels of both calcium and calcitriol were detected in group (C) and the highest levels were detected in group (A) with a significant variation between these two groups. Levels of both fibroblast growth factor 23 and sclerostin were the highest in group (C) and the lowest in group (A). Differences between all groups showed significance except the difference in sclerostin levels between group (A) and (B). Both fibroblast growth factor 23 and sclerostin levels showed significant negative correlations with calcium and calcitriol levels. A significant positive correlation was detected between sclerostin levels and fibroblast growth factor 23 levels.
Conclusion
Fibroblast Growth Factor 23 and sclerostin are strong indicators for COVID-19 infection severity. As they suppress renal 1-α hydroxylase enzyme, they have a crucial role in COVID-19 associated hypocalcemia through inhibition of vitamin D activation. Thus, better prognosis can be achieved by active vitamin D supplementation rather than inactive forms.
Keywords: Fibroblast Growth Factor 23, Sclerostin, hypocalcemia, hypovitaminosis D, Calcitriol, COVID-19.

INTRODUCTION

by the end of 2019, an epidemic of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was seen in Wuhan, and then rapidly spread to the whole world **[1].** As of February 15, 2023, the total cases were 756,291,327 with 6,841,640 fatalities worldwide as declared by WHO **[2].**

Although anyone can be infected, people older than 60 years old or those who already have pre-existing chronic illnesses are more susceptible to experience severe infection **[3].** COVID-19 infection has symptoms ranging from minor complaints to life-threatening diseases. Possible symptoms include hyperthermia, cough, dyspnea, exhaustion, decreased taste and smell sensation, pharyngitis, vomiting, and diarrhea **[4].** The respiratory system is significantly impacted by SARS-CoV-2; however, it can also affect other systems and cause gastrointestinal, hepatic, cardiovascular, and neurological manifestations **[3]**. A lot of patients show symptoms and signs of renal impairment **[5].**

Hypocalcemia is a prevalent laboratory finding detected among positive SARS-CoV-2 patients **[6].** Studies showed that positive COVID-19 patients had considerably lower ionized calcium values than those who tested negative **[7,8]**. Another study compared the serum ionized calcium levels (Ca^{++}) in those with acute respiratory illness caused by and not caused by Coronavirus-2 infection during the same period and discovered a twofold risk of hypocalcemia in PCR-positive patients in comparison to PCR-negative patients although clinical features and inflammatory markers of both groups are the same and so, they suggested that hypocalcemia is a characteristic feature of infection by Coronavirus-2 virus **[9].** A systematic review and meta-analysis study included 2032 patients from 7 studies concluded that disease prognosis is inversely linked to serum calcium levels **[10].**
Several investigations revealed that PCR-positive patients have a significant frequency of hypovitaminosis D. Lower serum calcifediol levels were detected among positive SARS-CoV-

2 patients compared to controls **[11,12].** Hypovitaminosis D has also been linked with an elevated incidence of getting Coronavirus-2 infection **[13].** PCR-positive patients with adequate level of vitamin D showed significant fewer affected lung segments, a shorter hospitalization period, and a better prognosis **[14].** Furthermore, higher risk of mortality among PCR-positive patients is strongly linked with insufficient levels of vitamin D **[15,16].** A clear link was discovered between hypocalcemia observed in positive SARS-CoV-2 patients and hypovitaminosis D in these individuals implying that hypocalcemia may be caused by vitamin D deficiency **[17].**

Fibroblast Growth Factor 23 (FGF23) is a glycoprotein primarily synthesized by osteoblasts and osteocytes **[18].** The inhibitory impact on phosphate reabsorption from the kidneys is the main function of FGF23**.** Regarding its action on vitamin D, FGF23 inhibits renal generation of the active vitamin D hormone, calcitriol **[19,20].** This action is due to its suppressing effect on renal 1-α hydroxylase enzyme **[21].** Now, it is well established that conditions marked by abnormally high levels of FGF23 cause phosphate depletion and unacceptably low levels of calcitriol in those with normal kidney function **[22].**

Sclerostin is a monomeric glycoprotein produced by osteocytes **[23].** Sclerostin drives osteoclastogenesis, decreases osteoblastic bone production, and promotes osteoblast and osteocyte mortality **[24-28].** Regarding its effect on minerals, sclerostin has been known to decrease vitamin D levels both directly and indirectly. It has a direct inhibitory impact on renal 1 α-hydroxylase activity. Also, it stimulates 24-hydroxylase enzyme, which causes vitamin D catabolism. In terms of its indirect effect, sclerostin raises circulating levels of FGF 23 that in turn inhibits renal hydroxylation of vitamin D **[29].**
This study is conducted to determine serum levels of FGF 23 and sclerostin and determine if they have a role in the pathogenesis of hypocalcemia observed in SARS-CoV-2 patients through their impact on vitamin D activation.

MATERIALD AND METHODS

Study design

A case control study done in collaboration between Medical Physiology department and Internal Medicine department at Aswan University from March 2022 to March 2023.

Participants

22 healthy controls (Group A) and 44 PCR-positive COVID-19 patients were included in this study. The positive COVID-19 patients were equally divided according to *National Institutes of Health (NIH)* into 2 subgroups: mild/moderate patients (group B) and severe/ critical patients (group C). Mild infection showed the following criteria: symptoms like hyperthermia, cough, nausea, vomiting, pharyngitis, headache, malaise, muscle ache, diminished taste, and smell sensation but no abnormal chest radiology or dyspnea. Moderate infection showed the following criteria: lower respiratory manifestations like dyspnea or abnormal chest radiology but SpO2 > 94% on room air. Severe infection showed the following criteria: SpO2 < 94% on room air, PaO2/FiO2 < 300 mmHg, lung infiltration >50%, and respiratory rate >30 breath/min. Critical illness showed respiratory failure, septic shock, or multiorgan failure **[68].**

Inclusion criteria

Positive COVID-19 male patients aged 50 years or older.

Exclusion criteria

Those with a known diagnosis of chronic kidney disease, parathyroid disease, metabolic bone disease, renal phosphate wasting disorder, or had used phosphate binder therapy or calcium therapy within the previous 3 months, or supplemented with calcifediol, or calcitriol were excluded.

Sample collection

When patients were admitted, blood samples were taken. Centrifugation was used to separate the serum for 15 minutes at 3000 rpm. Separated, clear non-hemolyzed supernatant was kept at -20°C until analysis. Serum levels of ionized calcium, calcitriol, parathyroid hormone, fibroblast growth factor 23 and sclerostin have been measured using ELISA.

Statistical analysis

The data was analyzed using SPSS 23. The test of normality used was Shapiro-Wilk test. The distribution was normal. The data were expressed as mean ± standard deviation. One-Way ANOVA test and Pearson correlation test were used. The statistical significance threshold was (p value < 0.05) and for correlation (if r = 0 no correlation, 0 < r < 1 positive correlation, -1 < r < 0 negative correlation)

RESULTS

Baseline Characteristics

Baseline personal characteristics of 66 male participating individuals including age, comorbidities like diabetes mellitus, hypertension and heart failure are summarized in **Table 1**.

Biochemical and hematological parameters

Table 2 shows that the highest levels of WBCs and CRP were detected among severe/critical patients (group C), while the lowest levels were detected among controls (group A). The differences between the studied groups in WBCs and CRP levels showed statistical significance (p value ≤ 0.001). However, the variations between group (B) and (C) regarding WBCs and CRP levels showed no significance (p value = 0.443, 0.281 respectively) **(Table 2).** Although all participating patients have normal serum levels of Blood Urea Nitrogen (BUN) and creatinine, it has been noted that highest serum BUN and creatinine values were detected among severe/critical patients (group C) while the lowest serum levels of both were detected among controls (group A). The variations between the three studied groups in the serum levels of BUN showed significance (p value < 0.001). Regarding serum levels of creatinine, a significance has been detected between the controls and severe/critical patients (p value < 0.001), but no significant variation was detected between controls and mild/moderate patients (p value = 0.112). However, another significance has been detected regarding the variation in the creatinine levels between severe/critical group and mild/moderate group (p value = 0.01) **(Table 2).**

No significant variations have been detected in Hb and platelet levels between the three groups **(Table 2).**

Serum levels of ionized calcium and calcitriol

Figure 1 revealed that the lowest serum levels of Ca^{+2} were detected among severe/critical patients (group C) and the highest levels were detected among controls (group A) with significant variation only between these two groups (*p* value = 0.001), however, the differences between group (A) and (B), and between group (B) and (C) were not significant (*p* value = 0.185, 0.096 respectively).

Results showed that the serum levels of calcitriol were the lowest among severe/critical group (C) followed by mild/moderate group (B) and the highest levels were among control group (A) **(Figure 2).** Variations in the serum levels of calcitriol between the normal controls and the other two groups showed significance (*p* value < 0.001), however, significance was observed between mild/moderate patients and severe/critical patients (*p* value = 0.757). **(Figure 2).**

Serum level of PTH

Although the highest serum levels of PTH were detected among severe/critical patients (group C) and the lowest levels were detected among controls (group A), variations between the studied groups as regard serum levels of PTH showed no significance (*p*1 = 0.989, *p*2 = 0.564, *p*3 = 0.654) **(Table 3).**

Serum levels of FGF23 and sclerostin

The highest levels of FGF23 were detected in the serum of severe/critical group (C) followed by mild/moderate group (B) while the lowest levels were detected in the serum of the control group (A). All results regarding variations in the serum levels of FGF23 between the three studied groups were statistically significant (*p1* = 0.003, *p2* < 0.001, *p3* = 0.003) **(Figure 3).**

According to **Figure 4,** the highest serum levels of sclerostin have been detected among severe/critical patients (group C) followed by mild/moderate patients (group B) and finally the control group (A). Results regarding variations in the serum levels of sclerostin between the different studied groups have showed significance (*p* value < 0.001), except the difference between group (A) and (B) which didn't (*p* value = 0.099)**.**

Correlations

Figure 5 shows that the serum levels of FGF23 is negatively correlated with the serum levels of Ca^{++} (*p* value < 0.001, r = -0.39). Another statistically significant negative correlation has been also detected between the levels of FGF23 and calcitriol levels (*p* value < 0.001, r = -0.434) **(Figure 6).** No significant correlation has been found between the levels of FGF23 and the levels of PTH (*p* value = 0.389) "results are not presented by a graph"

According to **Figure 7**, serum levels of sclerostin is negatively correlated with the serum levels of Ca^{++} (*p* value < 0.001, r = -0.432). In addition, another statistically significant negative correlation was detected between the serum levels of sclerostin and the serum levels of calcitriol (*p* value < 0.001, r = -0.431) **(Figure 8).** No significant correlation has been detected between sclerostin levels and PTH levels (*p* value = 0.300) "results are not presented by a graph"

A positive correlation between sclerostin levels and FGF23 levels was detected and showed significance (*p* value < 0.001, r = 0.563) **(Figure 9).**

DISCUSSION

Since it was first discovered, COVID-19 pandemic has attracted a lot of interest because of its high number of confirmed cases and high death rate. This study supports previous studies' results that leukocytosis, and increased CRP levels were linked to the clinical severity in positive SARS-CoV-2 patients **[30]**. Although we excluded all positive SARS-CoV-2 patients with high BUN and creatinine values, it has been noted that even if all participating patients had normal serum levels of BUN and creatinine, the highest values were detected among severe/critical patients and the lowest levels were detected among normal controls. This observation come in agreement with previous studies' results which reported that acute kidney injury (AKI) is a prevalent complication of COVID-19 infection and is linked to greater disease severity and poor prognosis **[31].**

This study observed that, the lower the serum Ca^{+2} levels among positive SARS-CoV-2 patients, the more the severity of the disease. These results are supported by previous studies' findings that revealed a high prevalence of hypocalcemia among PCR-positive patients at admission **[12,15].** Hypocalcemia was detected in about 2/3 of positive patients with severe clinical symptoms **[32]** and in about 67% of those with mild to moderate symptoms suggesting that even in non-severe individuals, hypocalcemia is a frequent finding of SARS-CoV-2 infection and is considered a feature of that illness. **[33].**

Hypocalcemia was determined as an important sign of clinical severity of SARS-CoV-2 infection **[34]**. It is considered a risk factor of higher oxygen requirement **[35],** ICU admission, mechanical ventilation **[36],** prolonged hospitalization **[37,38]**, poor prognosis, multi-organ failure, septic shock **[12],** and high mortality rate **[12,36].**

A high viral load among COVID-19 patients can explain the disruption of the calcium homeostasis as every stage of the viral life cycle requires calcium **[6].** Other important risk factors include hypovitaminosis D which was found to be highly prevalent among those patients **[6,17].** Malnutrition, weight loss and cachexia can also explain hypocalcemia, particularly during hospitalization **[6,39-42].**

This study is among the first studies that determine serum levels of active form of vitamin D, calcitriol, among SARs-CoV-2 infected patients. The levels of calcitriol were the lowest in the serum of severe/critical patients while the serum of normal controls showed the highest levels with significant variations between the control group and the other two groups. These results can be explained by low serum levels of calcifediol observed in positive SARS-CoV-2 patients **[11,15,43].** This deficiency may be due to either malnourishment or lack of sun exposure that may lead to chronic hypovitaminosis D **[44].** Another explanation of diminished calcitriol levels with progression of COVID-19 infection is inhibition of the renal 1-α hydroxylation of provitamin D either due to SARS-CoV-2-associated kidney impairment **[31]** or due to elevated serum levels of FGF23 and sclerostin.

According to previous studies, a lack of vitamin D increases one's vulnerability to SARS-CoV-2 infection **[6,45,46]** and linked with greater risk of respiratory failure, mechanical ventilation, and high mortality rate **[16,47].**

Regarding the efficacy of cholecalciferol, calcifediol and calcitriol supplementation in decreasing the incidence of Coronavirus-2 infection and improving outcomes, a randomized clinical trial study reported that a single large dosage of cholecalciferol had no beneficial effect on reduction of the duration of hospitalization **[48].** This may be due to decreasing levels of vitamin D binding protein (VDBP) in the serum during acute diseases, which could reduce the therapeutic efficacy of cholecalciferol **[49]** or may be due to elevated serum levels of both FGF23 and sclerostin leading finally to diminished activation of provitamin D forms either stored in the body or supplemented during hospitalization.

A randomized pilot study revealed increased oxygenation and decreased need for ICU admission, risk of readmission, and rate of mortality among COVID-19 patients receiving calcitriol compared to those not receiving it **[50].** Another Spanish cohort study found that supplementing with calcifediol did not lower the risk of SARS-CoV2 infection or its associated death rate in the entire cohort, whereas supplementing with cholecalciferol was only slightly associated with protection against COVID-19 infection **[51].** A population-based study indicated that calcitriol administration may be beneficial for those with advanced chronic kidney disease (CKD) during the COVID-19 infection **[52].** Although calcifediol supplementation in CKD individuals during COVID-19 pandemic has also decreased the risk of COVID-19 infection and mortality, the outcomes were less dramatic than what was seen in calcitriol-treated individuals **[51,52].** In those with genetic VDBP deficiency, calcitriol rather than cholecalciferol can restore calcium balance **[53]**, this can explain why calcitriol is more effective than cholecalciferol in improving COVID-19 clinical outcomes **[48].**

Although some studies revealed that cholecalciferol and calcifediol supplementation is substantially linked to decreased disease severity, ICU admission, and death rate, their results were not compared with the results of calcitriol supplementation **[54-57].**

Although serum levels of PTH were the highest in severe/critical cases and the lowest among controls, the differences were statistically insignificant. An earlier study also reported insignificant higher serum PTH levels in COVID-19 patients with hypovitaminosis D **[58].** Another study also observed no significant variations in the serum levels of PTH between PCR-positive individuals with vitamin D insufficiency and those with sufficient levels, or between those with hypocalcemia and those with normocalcemia **[17].** In contrast, low serum PTH levels has been detected in patients with moderate/severe hypocalcemia **[59].** Decreased PTH secretion and hypoalbuminemia were reported to play important roles in the development of hypocalcemia while receiving inpatient treatment for COVID-19 infection **[60].**

This study detected that the highest serum levels of both FGF23 and sclerostin were detected among severe/critical cases and the lowest levels were detected among normal controls. The variations in the serum levels of FGF23 between the three studied groups were considered statistically significant. Regarding the serum levels of sclerostin, differences between groups were considered statistically significant except the difference between the control group and the mild/moderate group which was not. Serum levels of FGF23 and sclerostin showed significant inverse relationship with both serum Ca^{++} level and serum calcitriol level. Sclerostin serum level showed significant direct relationship with the serum level of FGF23.

These findings may be a result of an inflammatory response that has a crucial role in FGF23 and sclerostin regulation causing an increase in their production. In a human study, positive correlations were detected between inflammatory markers and serum levels of FGF23 **[61]**. Another experimental study demonstrated that inflammation increased FGF23 values in both normal and uremic rats **[62].** Other studies have reported that almost all inflammatory conditions have stimulatory effects on osteocytes leading to increased generation of both FGF23 and sclerostin **[63,64].** Serum levels of FGF23 also increases due to elimination of kidney function which is proved by a previous experimental study **[65].** This is another explanation why the more the severity of SARS-CoV-2 infection, the higher the serum levels of FGF23.

FGF23 inhibits 1-α hydroxylase whose function is to convert calcifediol to active calcitriol **[21].** It has also a role in exaggerating vitamin D catabolism by stimulating 24-hydroxylase enzyme whose function is to break down calcitriol **[66].** Therefore, high levels of FGF23 detected in positive SARS-CoV-2 patients may be the cause of further lowering of serum levels of active calcitriol and responsible for significant hypocalcemia observed in these patients. It may be also the cause of reduced efficacy of cholecalciferol and calcifediol supplementation compared to calcitriol.

Sclerostin was found to have a negative impact on 1,25 dihydroxycholecalciferol production by directly inhibiting renal 1- α-hydroxylase enzyme, thus decreasing the circulating levels of calcitriol and subsequently leading to hypocalcemia. In addition, its effect on FGF23 is another factor determining calcitriol levels. This can be explained by its inhibitory effect on PHEX, an enzyme whose action is to inhibit FGF23 catabolism, so sclerostin indirectly increases circulating levels of FGF23 **[67].** This can also explain the observed high FGF23 serum levels among SARS-CoV-2 patients. This explanation has been proved by an experimental study which detected elevated serum calcitriol level, decreased 24,25-dihydroxyvitamin D level, and decreased FGF23 level in SOST knockout mice **[29].**

CONCLUSION

In conclusion, serum levels of FGF23 and sclerostin are considered strong indicators of COVID-19 severity, progression, and outcomes. As they suppress renal 1-α hydroxylase enzyme, they have a crucial role in hypocalcemia observed in these patients through inhibition of vitamin D activation. Our findings agree with the theory that hypovitaminosis D and hypocalcemia are potential risk factors for disease severity and progression in COVID-19 patients.

PERSPECTIVES AND SIGNIFICANCE

This study suggests that supplementation of an active form of vitamin D for COVID-19 patients may show better prognosis compared to inactive forms of vitamin D due to suppression of 1-α-hydroxylase enzyme. However, further clinical trial studies should be conducted to support our findings.

LIMITATIONS

There are several limitations on the current investigation. First off, the study is a single-center investigation with a modest sample size, which might have an impact on our findings. Furthermore, we did not assess serum levels of calcifediol or VDBP. Therefore, we think that bigger multicenter studies should be conducted to further explore the results of our research.

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STATEMENTS AND DECLARATIONS

Data Availability Statement

The corresponding author can provide the data which supports the results of this study upon request.

Acknowledgement

Not applicable. This study was done without any financial assistance.

Author contribution

MR, AA and NA designed research. NA and MA collected samples. NA carried out biochemical analysis. NA and AA conducted statistical analysis and wrote the whole manuscript. MR, AA and MA revised the results and the manuscript. This work was reviewed and authorized by all authors.

Ethics approval

Institutional Review Board (IRB) permission number: 603/03/22 was given to this study by the ethics committee of Aswan University's Faculty of Medicine.

Ethics, consent, and permissions

The background, methods of the study, and the risks of blood sampling such as dizziness, fainting, or hematoma at the site of blood collection were explained to all participants. Written informed consents were taken from the participants. They have the option to leave the study at any moment and without providing any reason.

Consent to publish

All authors give the consent to publish this research article with all data identified in it. As every participant was coded, it was possible to analyze the data without revealing the names of the participants, therefore all participants agree to publish this research article. All participants were informed by the findings of this study.

Clinical trials registration

This research has clinical trials registration under the name “Fibroblast Growth Factor 23 and Sclerostin in Relation to Calcium in COVID-19 Patients.”
Date of registry: 11 March 2022
Identifier number: NCT05275491
URL: https://classic.clinicaltrials.gov/ct2/show/NCT05275491
Date of enrollment of the first participant to the trial: 6 January, 2022

Competing interests

The authors declare that they have no competing interests.

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List of abbreviations

AKI: Acute kidney injury

BUN: Blood urea nitrogen

Ca^{+2}: Ionized calcium

CKD: Chronic kidney disease

CRP: C-reactive protein

ELISA: Enzyme-Linked Immunoassay

FGF23: Fibroblast Growth Factor 23

PTH: Parathyroid hormone

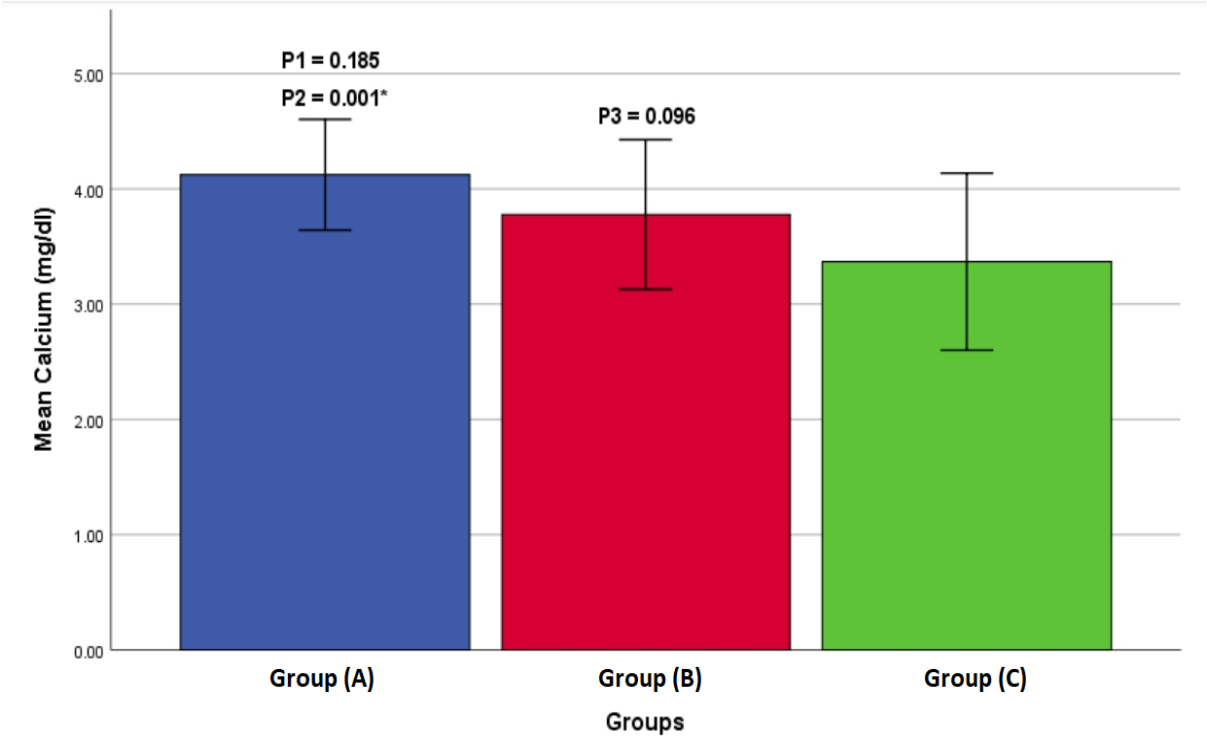
SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2

VDBP: Vitamin D binding protein

WBCs: White blood calls

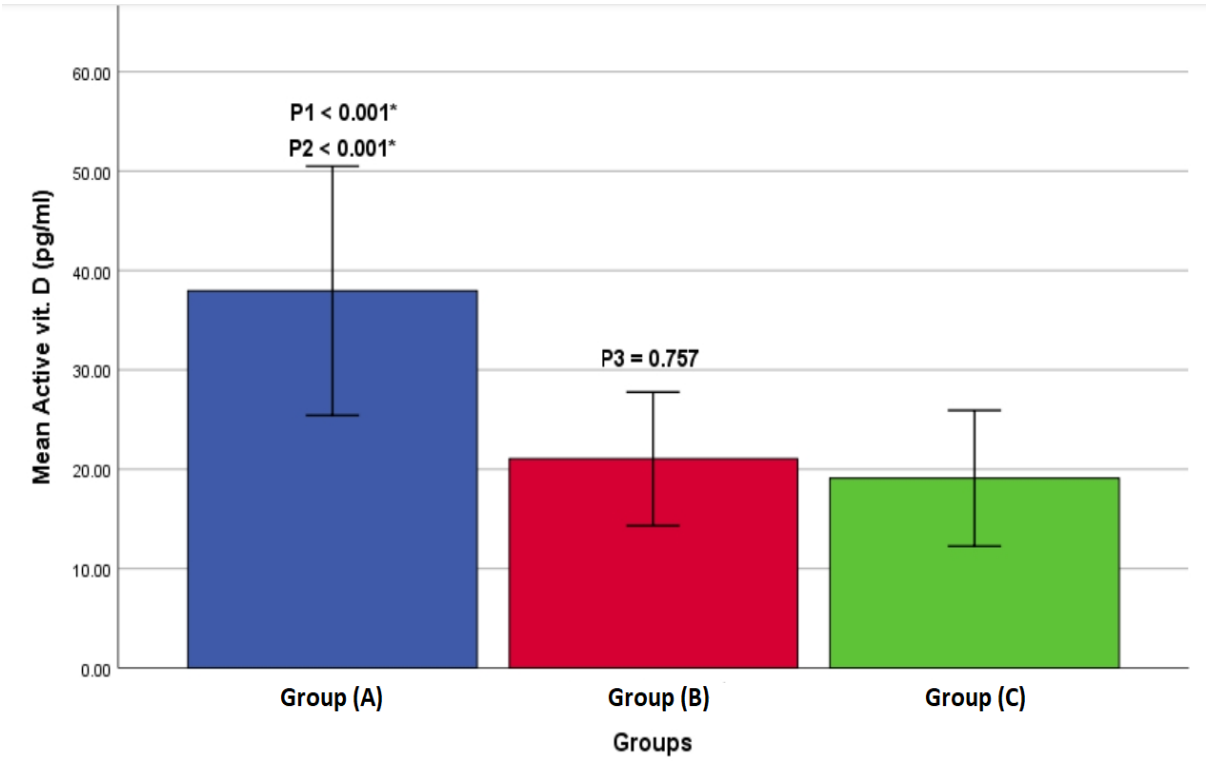
Figures

Figure (1): Serum levels of ionized calcium between the studied groups



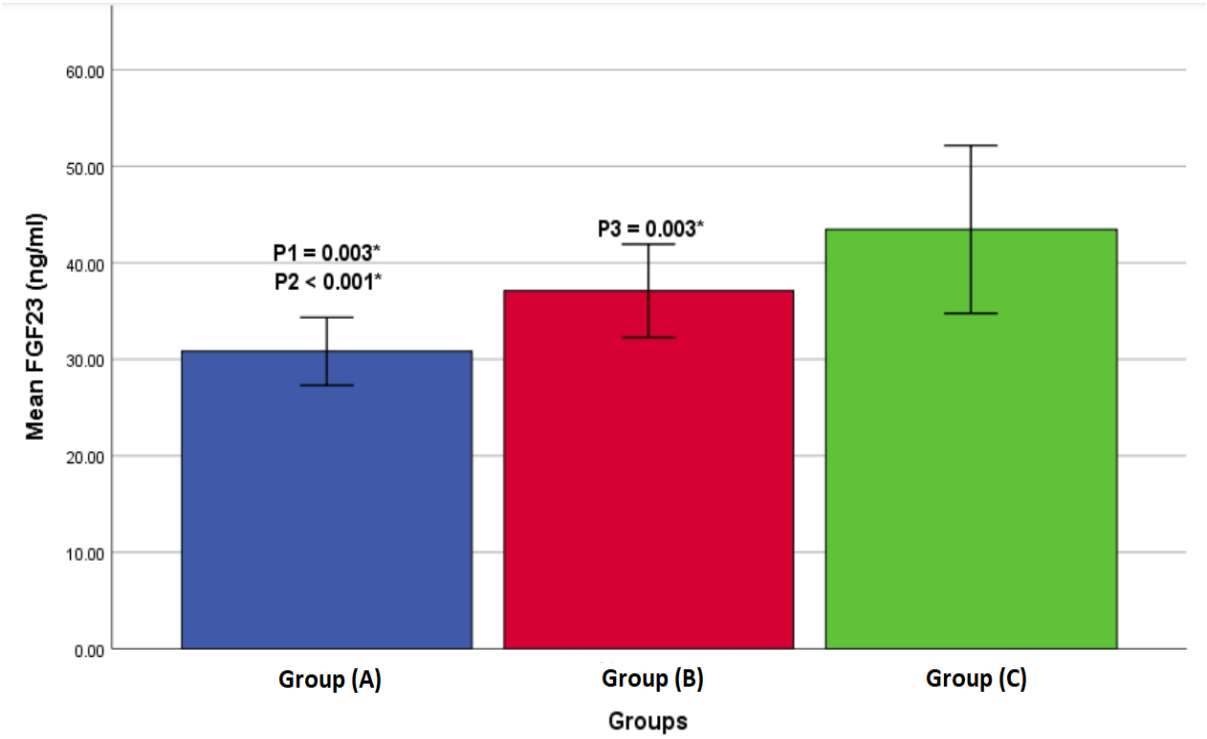
Legend : Data is expressed as **mean ± standard deviation, Group (A):** controls**, Group (B):** mild/moderate patients**, Group (C):** severe/critical patients, **p1**: probability value for difference between Groups (A & B)**, p2**: probability value for difference between Groups (A & C)**, p3**: probability value for difference between Groups (B & C)**, *:** statistically significant at **p ≤ 0.05,** One-Way ANOVA test was used.

Figure (2): Serum levels of Active vit. D (Calcitriol) between the studied groups



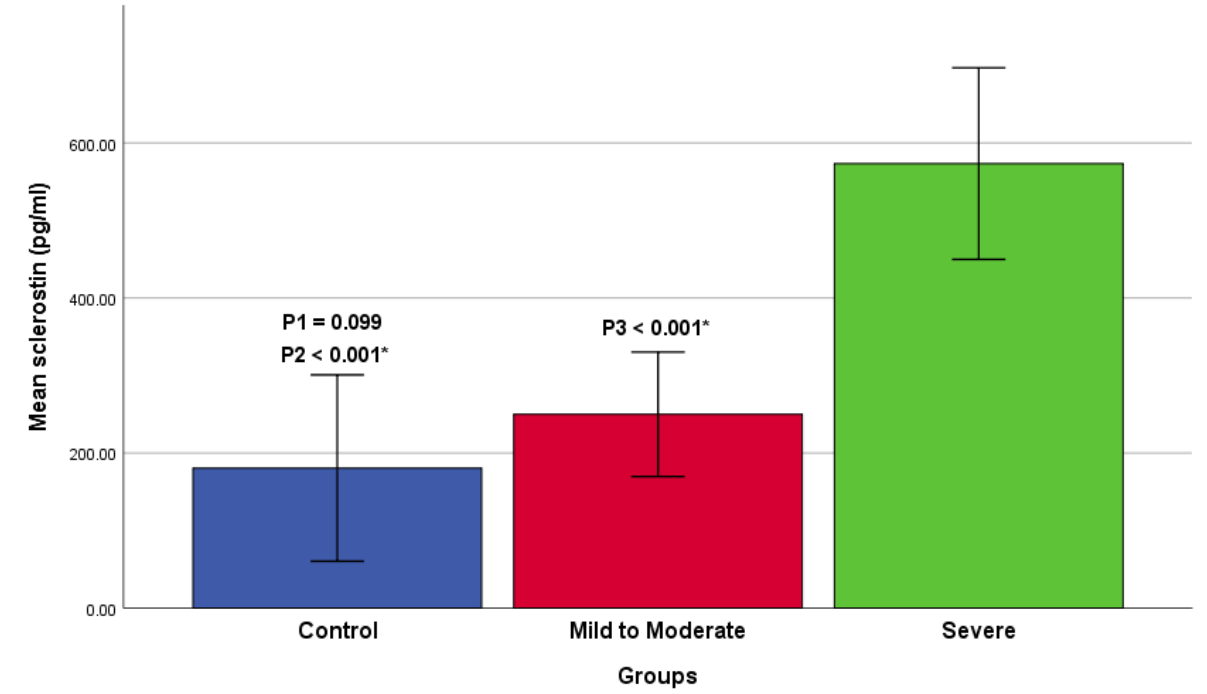
Legend: Data is expressed as **mean ± standard deviation, Group (A):** controls**, Group (B):** mild/moderate patients**, Group (C):** severe/critical patients, **p1**: probability value for difference between Groups (A & B)**, p2**: probability value for difference between Groups (A & C)**, p3**: probability value for difference between Groups (B & C)**, *:** statistically significant at **p ≤ 0.05,** One-Way ANOVA test was used.

Figure (3): Serum levels of FGF 23 between the studied groups



Legend: Data is expressed as **mean ± standard deviation, FGF23:** fibroblast growth factor 23**, Group (A):** controls**, Group (B):** mild/moderate patients**, Group (C):** severe/critical patients, **p1**: probability value for difference between Groups (A & B)**, p2**: probability value for difference between Groups (A & C)**, p3**: probability value for difference between Groups (B & C)**, *:** statistically significant at **$p \leq 0.05$,** One-Way ANOVA test was used.

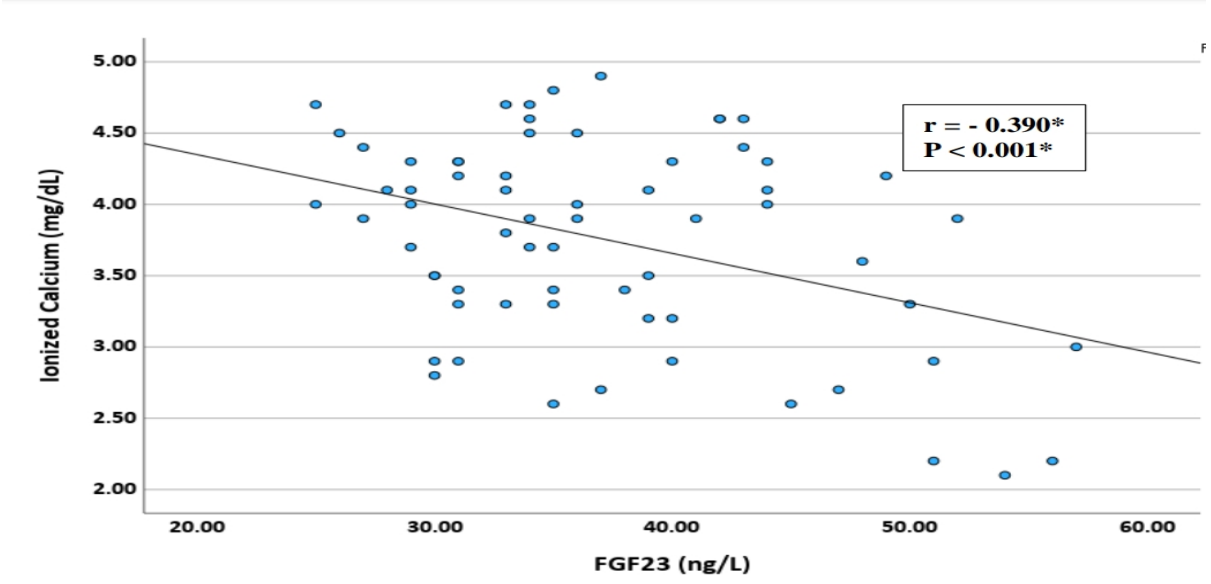
Figure (4): Serum levels of sclerostin between the studied groups



Legend: Data is expressed as **mean ± standard deviation, Group (A):** controls**, Group (B):** mild/moderate patients**, Group (C):** severe/critical patients, **p1**: probability value for

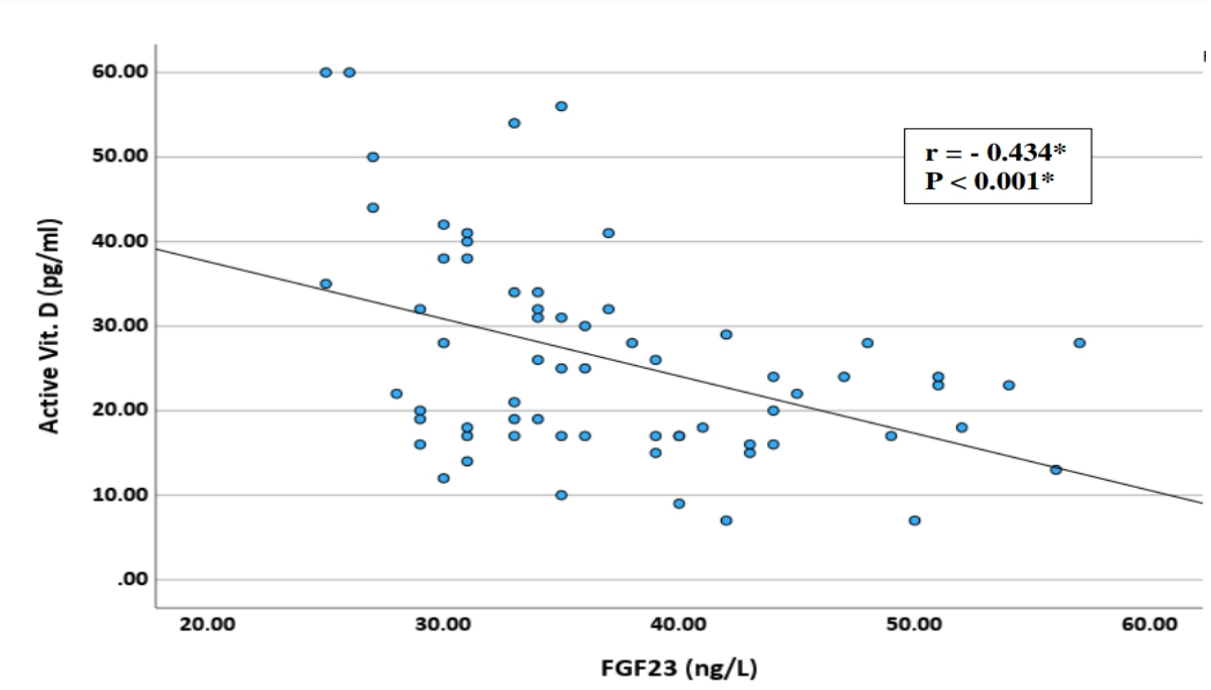
difference between Groups (A & B)**, p2**: probability value for difference between Groups (A & C)**, p3**: probability value for difference between Groups (B & C)**, *:** statistically significant at **$p \leq 0.05$,** One-Way ANOVA test was used.

Figure (5). Correlation between serum levels of FGF 23 and serum ionized calcium levels



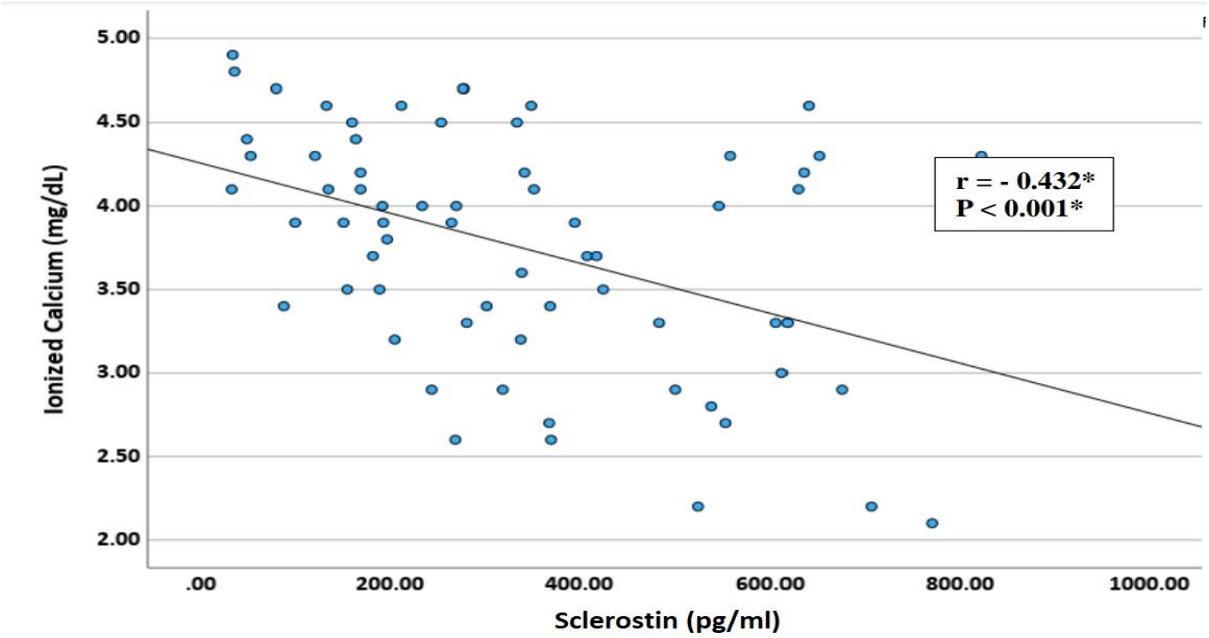
Legend: **FGF23:** fibroblast growth factor 23, ***:** statistically significant at **$p \leq 0.05$**, **r =** Pearson correlation coefficient, Pearson correlation test was used.

Figure (6). Correlation between serum levels of FGF23 and serum levels of Active Vit. D (Calcitriol)



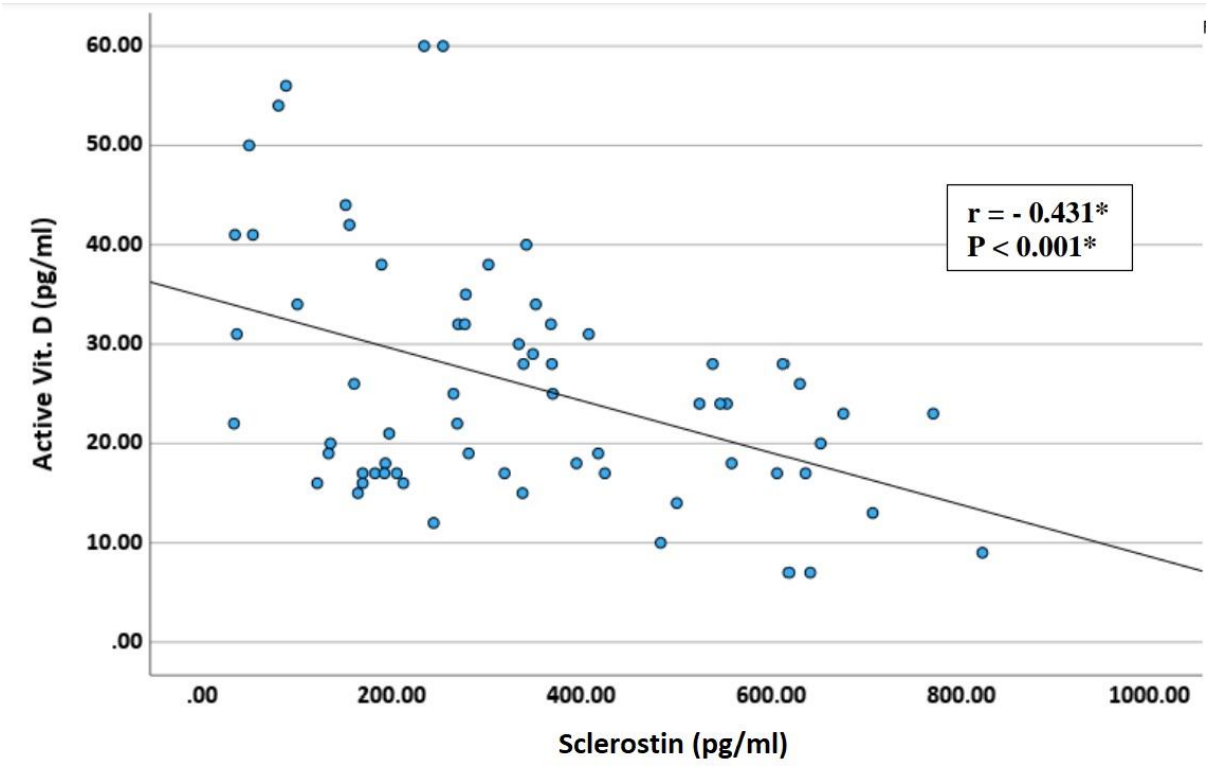
Legend: **FGF23:** fibroblast growth factor 23, ***:** statistically significant at **$p \leq 0.05$**, **r =** Pearson correlation coefficient, Pearson correlation test was used

Figure (7). Correlation between serum levels of sclerostin and serum ionized calcium levels



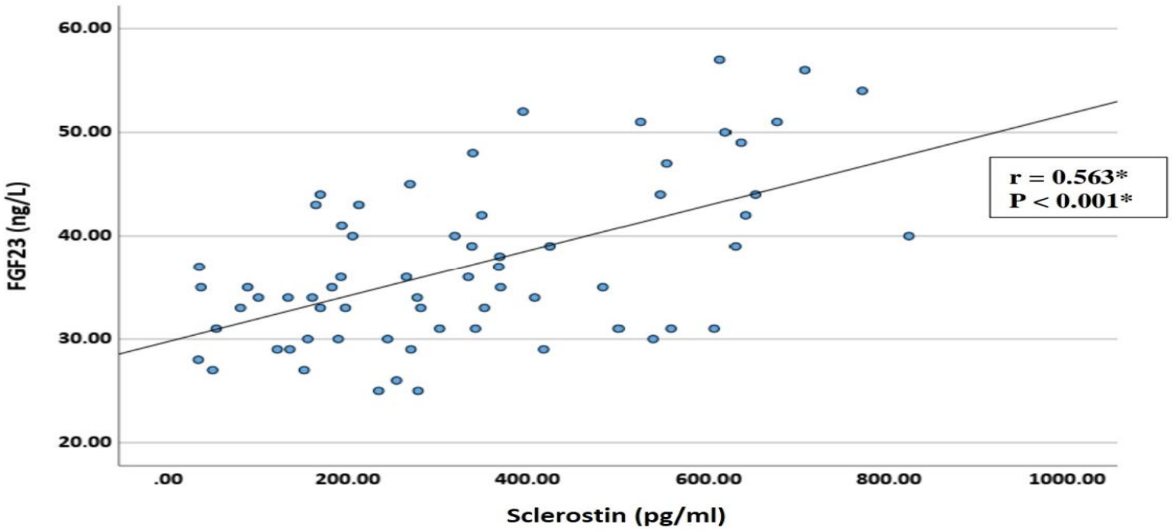
Legend: ***:** Statistically significant **at $p \leq 0.05$, r =** Pearson correlation coefficient**,** Pearson correlation test was used.

Figure (8). Correlation between serum levels of sclerostin and serum levels of Active Vit. D (Calcitriol)



Legend: ***:** statistically significant at **$p \leq 0.05$, r** = Pearson correlation coefficient**,** Pearson correlation test was used.

Figure (9). Correlation between serum levels of sclerostin and serum levels of FGF23



Legend: **FGF23:** fibroblast growth factor 23, ***:** statistically significant at **$p \leq 0.05$**, **r** = Pearson correlation coefficient, Pearson correlation test was used.

Tables

Table 1: Baseline Characteristics of the studied groups

	Group A (n = 22)	Group B (n = 22)	Group C (n = 22)
Age			
Mean ± SD	57.77 ± 4.14	58.55 ± 5.5	59.41 ± 4.36
Significance	*p1* = 0.850, *p2* = 0.486, *p3* = 0.816		
Comorbidities			
Diabetes (n, %)	8 (36.36)	12 (54.54)	14 (63.64)
Hypertension (n, %)	7 (31.82)	10 (45.45)	13 (59.09)
Heart failure (n, %)	2 (9.09)	3 (13.64)	4 (18.18)
Tobacco smoking (n, %)	16 (72.73)	19 (86.36)	17 (77.27)

Age is expressed as **mean ± standard deviation, n:** number of persons, **Group (A):** controls**,** **Group (B):** mild/moderate patients**, Group (C):** severe/critical patients, ***p*1**: probability value for difference between Groups (A & B)**, *p*2**: probability value for difference between Groups

(A & C)**, *p*3**: probability value for difference between Groups (B & C), **P** is significant if it is **≤ 0.05,** One-Way ANOVA test was used.

Table (2): Serum levels of Hb, WBCs, Platelets, CRP, BUN and creatinine in the studied groups

	Group A (n = 22)	Group B (n = 22)	Group C (n = 22)
Hb (g/dL)			
Mean ± SD	11.4 ± 1.01	11.44 ± 1.28	11.15 ± 1.12
Significance	*p1* = 0.991, *p2* = 0.766, *p3* = 0.687		
WBCs (x 10^9/L)			
Mean ± SD	3.88 ± 1.79	6.67 ± 1.63	7.32 ± 1.88
Significance	*p1* < 0.001*, *p2* < 0.001*, *p3* = 0.443		
PLTs (x 10^9/L)			
Mean ± SD	173.59 ± 33.65	184.05 ± 36.84	180.27 ± 28.77
Significance	*p1* = 0.553, *p2* = 0.784, *p3* = 0.925		
CRP (mg/L)			
Mean ± SD	3.0045 ± 1.64	31.53 ± 24.60	43.35 ± 36.68
Significance	*p1* = 0.001*, *p2* < 0.001* , *p3* = 0.281		
BUN (mg/dL)			
Mean ± SD	11.55 ± 2.11	15 ± 2.18	19.64 ± 1.65
Significance	*p1* < 0.001* , *p2* < 0.001* , *p3* < 0.001*		
Creatinine (mg/dL)			
Mean ± SD	0.94 ± 0.16	1.03 ± 0.16	1.17 ± 0.15
Significance	*p1 = 0.112* , *p2* < 0.001* , *p3* = 0.01*		

All data is expressed as **mean ± standard deviation, n:** number of persons, **HB:** Hemoglobin**,** **WBCs:** white blood cells**, PLTs:** platelets**, CRP:** C-reactive protein**, BUN:** blood urea nitrogen**, Group (A):** controls**, Group (B):** mild/moderate patients**, Group (C):** severe/critical patients. **, *p*1**: probability value for difference between Groups (A & B)**, *p*2**: probability value

for difference between Groups (A & C)**, *p*3**: probability value for difference between Groups (B & C)**, *:** statistically significant at $p \leq 0.05$**,** One-Way ANOVA test was used.

Table (3): Serum levels of PTH in the studied groups

	Group A (n = 22)	**Group B (n = 22)**	**Group C (n = 22)**
PTH (pg/mL)			
Mean ± SD	38.95 ± 11.53	39.79 ± 21.48	44.95 ± 23.18
Significance	*p1* = 0.989, *p2* = 0.564, *p3*= 0.654		

Data is expressed as **mean ± standard deviation, n:** number of persons, **PTH:** parathyroid hormone**, Group (A):** controls**, Group (B):** mild/moderate patients**, Group (C):** severe/critical patients, ***p*1**: probability value for difference between Groups (A & B)**, *p*2**: probability value for difference between Groups (A & C)**, *p*3**: probability value for difference between Groups (B & C)**, P** is significant if it is **≤ 0.05,** One-Way ANOVA test was used.