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Original Research Article

Evaluation of fibroblast growth factor-23 and alpha klotho levels in patients with sarcoidosis

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ABSTRACT

Background: Sarcoidosis is a systemic granulomatous disease. In its pathology, it is thought that 1-alpha hydroxylase production and activation as well as 1,25-hydroxy vitamin D production from active macrophages take place. FGF-23 and alpha klotho are proteins that play a key role in the bone-parathyroid-kidney axis, regulation of phosphate and calcium metabolism, and vitamin D metabolism. We aimed to demonstrate the effects of pathologic conditions on bone metabolism and the occurrence of vascular calcification since vitamin D can be activated in non-kidney patterns and hypercalcemia and hypercalciuria can be seen in these patients.
Methods: The study was designed as a prospective case-control study. The study included 42 patients with sarcoidosis and 41 healthy volunteers who were admitted to Düzce University Medical Faculty Hospital. Age, gender, comorbidity information, BMD measurement data within the last 6 months, 24-hour urine and blood samples were taken from the patient and control groups.
Results: FGF-23 and alpha klotho levels were significantly higher in the patient group. There was a positive correlation between FGF-23 and alpha klotho. A negative correlation was found between FGF-23 and urea levels while positive correlation was found between alpha klotho and L1-L4 T-score.
Conclusions: These results suggested that there may be a relationship between sarcoidosis progression and FGF-23 and alpha klotho levels. Positive correlation between alpha klotho and L1-L4 T score in all groups suggests that alpha klotho may be associated with osteoporosis.

Keywords: Alpha klotho, FGF-23, Sarcoidosis

INTRODUCTION

Sarcoidosis is a systemic disease of unknown cause, characterized by noncaseating granulomas. The most commonly affected structures are the mediastinal lymph nodes and lungs, but many organs can be affected.[1] In renal involvement of sarcoidosis, hypercalciuria is seen in approximately 40% of patients and hypercalcemia in 10-17% of patients.[2,3] The granulomas are accused of having 1-alpha hydroxylase activities that enable the conversion of 25-hydroxycholecalciferol to 1,25-dihydroxycholecalciferol [1,25(OH)2D3] in the pathogenesis of hypercalcemia.[4]

Fibroblast growth factor (FGF-23) and alpha klotho proteins are proteins that play a key role in the bone-parathyroid-kidney axis, regulate phosphate and calcium metabolism, and are also important in vitamin D metabolism. FGF-23 is a protein that can be synthesized in small amounts in other tissues, primarily in osteocytes, and has three main functions: it inhibits phosphate reabsorption from the proximal tubule, inhibits 1-alpha

hydroxylase in the kidney, and activates 24-hydroxylase, reducing 1,25(OH)2D3 and parathyroid hormone (PTH) levels.[5,6] Alpha klotho acts as a cofactor for FGF-23-mediated receptor activation, and also increases calcium reabsorption by enzymatically modifying TRPV5 sugar chains in the distal tubule. It also plays a direct regulatory role in PTH synthesis.[5,7]

In this study, we aimed to evaluate serum FGF-23 and alpha klotho levels in patients with sarcoidosis, and to reveal the effects of these pathological conditions on bone metabolism and their relationship with the emergence of vascular calcification, since vitamin D activation can also occur through extrarenal pathways in these patients and hypercalcemia and hypercalciuria can be observed.

METHODS

This prospective study was included in the study with the approval of the ethics committee, and 42 healthy volunteers who applied to the Duzce University Medical Faculty Hospital chest diseases, nephrology and internal diseases services and polyclinics between May 2017 and December 2017, and were followed up with or newly diagnosed with sarcoidosis. Exclusion criteria were determined as those under the age of 18, those with a history of serious cardiovascular, pulmonary or endocrine disease, pregnant and lactating women, those with a chronic inflammatory and/or infectious disease, those with renal failure, those with a diagnosis of malignancy, those with a history of granulomatous disease other than sarcoidosis, those who used vitamin D or phosphorus-binding agents in the last four weeks, and patients who did not give informed consent). One person from the control group was excluded from the study due to the emergence of exclusion criteria later. Patients were selected from those who volunteered to participate in the study and written informed consent forms were obtained.

Information on the participants' age, gender, additional diseases, medications they used, and blood samples were collected. FGF-23, alpha klotho, urea, creatinine, calcium, phosphorus, 1,25(OH)2D3, PTH, hemoglobin, 24-hour urine calcium, phosphorus tests, glomerular filtration rate (GFR) and BMD measurements taken within the last six months were evaluated in the patient and control groups.

FGF-23 and alpha klotho were studied using ELISA (MyBioSource, San Diego, CA, USA).

Statistical analysis

In the statistical evaluation of the data, the distribution of continuous variables was examined with the Shapiro-Wilks test and in the comparison of groups, the independent samples t-test was used for two-group comparisons depending on the distribution of the data. In the analysis of categorical data, the Pearson Chi-Square test was used depending on the expected value rule. In the examination of correlations between continuous variables, Pearson and Spearman correlation analysis were used depending on the distribution of the variables. Statistical analyses were performed with the SPSS v.22 package program and the significance level was considered as 0.05 and $p \leq 0.05$ was considered statistically significant.

RESULTS

A total of 83 participants were included in the study. 50.6% (n=42) of the participants were patients diagnosed with sarcoidosis and 49.4% (n=41) were healthy controls. 52% (n=30) of the patient group were female and 48% (n=12) were male, and 48% (n=28) of the control group were female and 52% (n=13) were male. The distribution of patients with and without sarcoidosis was statistically similar in terms of gender (p=0.756).

The mean age of sarcoidosis patients was 50.9±11.5 years (29-72) and the mean age of the control group was 50.9±12.7 years (25-82). No statistically significant difference was found between the groups in terms of mean age (p=0.985).

In the patient group, serum calcium (p=0.027), serum phosphorus (p=0.017) and 24-hour urine calcium levels (p=0.016) were found to be statistically significantly higher than in the control group, while PTH levels were significantly lower (p=0.027). No statistically significant difference was found between the two groups in terms of hemoglobin, urea, creatinine, 1,25(OH)2D3, 24-hour urine phosphorus levels and GFR (Table 1).

In the patient group, the mean T score of L1-L4 lumbar vertebrae was -0.45±1.32, and the mean T score of femoral neck was -0.52±1.04. In the control group, the mean T score was -0.04±1.25 in L1-L4 vertebrae and -0.45±1.03 in femur. No statistically significant difference was found between the bone mineral density of the patient and control groups.

Serum FGF-23 levels were found to be 47.39±130.46 pg/ml (0-816.3) on average in sarcoidosis patients. In the control group, it was 8.68±23.74 pg/ml (0-111.6). Mean serum alpha klotho levels were 2162±11181 pg/ml (0-4906) in sarcoidosis patients and 1350±1224 pg/ml (0-3673) in the control group. FGF-23 (p=0.015) and alpha klotho (p=0.019) levels were found to be statistically significantly higher in the patient group compared to the control group (Table 2).

When the relationship between FGF-23 and alpha klotho was evaluated according to Spearman's correlation analysis, FGF-23 was found to be positively correlated with alpha klotho (r:0.533, p<0.001).

According to Spearman's correlation analysis, when the relationship between FGF-23 and alpha klotho values and

all other parameters was examined, a negative correlation was found between FGF-23 and urea levels (r: -0.243, p=0.027), and a positive correlation was found between alpha klotho and L1-L4 T-score (r: 0.221, p=0.045). No statistically significant difference was found between other variables.

Table 1: Biochemical parameters of patient and control groups.

	Patient group	Control group	P value
Urea	30.19±11.5 (11.8-74)	30.75±30.75 (11.2-62)	0.709
Creatinine	0.73±0.17 (0.5-1.3)	0.7±0.23 (0.4-1.3)	0.181
Calcium	9.57±0.52 (8.6-10.9)	9.33±0.45 (8.09-10.3)	0.027
Phosphorus	3.65±1.07 (2.5-9.8)	3.24±0.59 (1.9-4.9)	0.017
1,25(OH)2D3	27.86±5.46 (20-46)	25.66±4.25 (14-34)	0.129
PTH	45.69±35.76 (6-193)	59.85±38.2 (13.9-198)	0.027
24-hour urine calcium excretion	214.6±133.94 (15-633)	150.63±96.96 (23-490)	0.016
24-hour urine phosphorus excretion	67.08±254.14 (285-1248)	604.02±245.5 (66-1095)	0.254
GFR	104.18±27.39 (55-179)	114.01±32.69 (65-191)	0.202
Haemoglobin	13.36±2.45 (0.9-17.1)	12.71±2.09 (8.3-17.6)	0.084

Table 2: FGF-23 and alpha klotho levels in patient and control groups.

	Patient group	Control group	P value
FGF-23	47.39±130.46 (0-816.3)	8.68±23.74 (0-111.6)	0.015
Alpha klotho	2162.88±1181.4 (0-4906)	1350.31±1224.53 (0-3673)	0.019

FGF-23 and alpha klotho levels were compared separately in male and female genders among sarcoidosis patients, control group and all participants, and no significant difference was found between FGF-23 and alpha klotho levels according to gender in any group.

DISCUSSION

FGF-23 is a phosphatonin that plays an important role in the calcium-phosphorus-PTH axis. It binds to its receptor by forming a complex with alpha klotho. It reduces phosphate absorption from the kidney, inhibits the production of 1,25(OH)2D3 and increases its destruction.[5] In this study, we aimed to determine the levels of FGF-23 and alpha klotho in patients with sarcoidosis and to determine their possible relationships with these pathologies, since sarcoidosis causes 1,25(OH)2D3 activation through extrarenal pathways and changes in calcium metabolism, and pathologies such as hypercalcemia, hypercalciuria and related kidney damage, bone metabolism disorders, and vascular calcifications can be observed in its course.

Serum calcium levels were significantly higher in the patient group compared to the control group. Other studies have reported that hypercalcemia is common in patients with sarcoidosis, with an incidence ranging from 2% to 63% In a study by McCort et al, the incidence of hypercalcemia was reported as 63%.[8] In a study conducted by Mayock et al on 509 patients with sarcoidosis, they reported that hypercalcemia was seen in 17% of the patients.[9] The largest study on this subject was conducted by James et al. 3676 sarcoidosis patients worldwide were included in the study and the incidence of hypercalcemia was reported as 11%.[10] The development of hypercalcemia in sarcoidosis may be caused by the 1-alpha hydroxylase enzyme released from granulomas, which was first reported by Adams et al in 1985.[11] In these studies, it has been suggested that hypercalcemia develops due to tissue and systemic increases in 1,25(OH)2D3. In a study conducted by Zeimer et al, PTHrP levels were found to be high in two hypercalcemic patients, and PTHrP expression was shown in granulomas in 11 out of 19 sarcoidosis patients. It was thought that PTHrP has a possible role in the development of hypercalcemia in sarcoidosis patients and that 1,25(OH)2D3 is not the only factor in the development of hypercalcemia.[12] In our study, similar to other studies, serum calcium levels in the patient group were statistically significantly higher than in the control group. Serum 1,25(OH)2D3 levels in the patient group were higher than in the control group, but not statistically significantly. Some publications have reported cases of hypercalcemic sarcoidosis with normal serum 1,25(OH)2D3 and PTHrP levels, and it has been stated that further studies are needed to elucidate the pathology of hypercalcemia in sarcoidosis.[13,14] In the case reports by Berlin and colleagues, it was suggested that measured serum vitamin D levels may not reflect true serum vitamin D levels, and that cellular vitamin D levels at the tissue level may be high while serum vitamin D levels are low.[15] In light of this information in the literature, we thought that serum 1,25(OH)2D3 activity may have increased in our patients, but it may not have been measured in the serum because there was an increase at tissue levels. In addition, since serum PTHrP levels were

not measured in our study, it could not be determined whether PTHrP played a role in the increased calcium in the patient group.

Lebacq et al examined 152 sarcoidosis cases and found that 11% of patients had hypercalcemia, 62% had hypercalciuria, and 13.8% had nephrolithiasis.[16] In a study conducted by Şen and her colleagues in our country, they examined 55 patients diagnosed with sarcoidosis and found that 24-hour urinary calcium excretion was above normal values in 40.8% of them.[17] Researchers believed that hypercalciuria was caused by increased 1,25(OH)2D3 activity and hypercalcemia suppressing PTH, and that hypercalciuria developed in these patients due to suppression of PTH synthesis and increased renal calcium load.[2,4,16] In our study, 24-hour urine calcium levels were compared between the patient group and the control group. Similar to other studies, 24-hour urine calcium levels were found to be statistically significantly higher in the sarcoidosis patient group.

Sarcoidosis-associated hypoparathyroidism is mentioned in many case reports.[2,15,18,19] Vucinic et al reported that PTH secretion in sarcoidosis is suppressed by hypercalcemia and high 1,25(OH)2D3 levels.[19] In the hypercalcemia sarcoidosis case series published by Berlin and colleagues, patients had low or normal vitamin D levels and normal and suppressed PTH levels. The researchers suggested that unknown factors other than vitamin D may also cause hypercalcemia and that high calcium levels suppress PTH.[15] This view is also supported by other case reports and studies. In our study, when PTH levels were compared between the two groups, they were found to be significantly lower in sarcoidosis patients. It was thought that the reason for the lower PTH levels in the sarcoidosis patient group may be due to high calcium levels and an increase in 1,25(OH)2D3.

Serum phosphorus levels were compared in the patient group and the control group, and serum phosphorus levels were found to be significantly higher in the patient group. In a study conducted by Grekin et al serum phosphorus levels were found to be high in 5 of 11 sarcoidosis patients, but most of them had impaired renal function tests.[20] In contrast to our study, phosphorus levels were found to be within the normal range in patients with sarcoidosis in a study conducted by Harrel et al.[21] Although there are some case reports in the literature where phosphorus levels were detected to be high, most of them were observed to have impaired renal function. In our study, we thought that the high serum phosphorus levels might be due to an increase in 1,25(OH)2D3 activity and suppression of PTH.

In our study, FGF-23 and alpha klotho levels were compared between the two groups, considering that FGF-23 levels may be high due to increased 1,25(OH)2D3 activity in sarcoidosis. Serum FGF-23 and alpha klotho levels were found to be statistically significantly high in sarcoidosis patients. There is only one study in the literature investigating FGF-23 levels in sarcoidosis patients. In this study conducted by Sexton et al., FGF-23 levels were measured in 39 patients with acute sarcoidosis who had normal renal function, and serum FGF-23 levels were found to be high in 15.4% of the patients. The researchers stated that the high FGF-23 level may be due to increased gastrointestinal phosphorus absorption mediated by vitamin D.[22] However, in this study, only FGF-23 levels were compared between acute sarcoidosis patients with normal renal function, and there was no control group. We also thought that the high FGF-23 levels in the sarcoidosis patient group in our study could be due to increased 1,25(OH)2D3 activity and high serum phosphorus levels. However, there are not enough studies in the literature evaluating FGF-23 and alpha klotho levels in sarcoidosis patients, and additional studies on this subject with larger samples are needed.

In our study, we found a positive relationship between FGF-23 and alpha klotho. Similar to our findings, in the study conducted by Silva et al to determine the relationship between serum klotho levels and insulin resistance albumin-creatinine ratios in type 2 diabetic patients, serum FGF-23 and alpha klotho levels were found to be correlated with each other.[23] Since FGF-23 requires alpha klotho to show activity, it is an expected finding that alpha klotho levels increase in patients with high FGF-23. It is also known that 1,25(OH)2D3 increases alpha klotho expression in the kidney.[7] The fact that the increase in 1,25(OH)2D3 is a factor that can increase alpha klotho levels in patients with sarcoidosis may be an explanatory reason for the high alpha klotho levels we found in our study.

Many studies in the literature have shown that FGF-23 is associated with the progression of kidney damage. In a study conducted by Sarmento-Dias et al to reveal the relationship between FGF-23 and uremic vasculopathy in 48 peritoneal dialysis patients, a correlation was found between FGF-23 and urea and creatinine values.[24] Raafat and colleagues also observed a positive correlation between FGF-23 and urea in a study they conducted in 2015 to investigate the clinical importance of FGF-23 in chronic kidney disease patients.[25] In our study, a negative correlation was found between FGF-23 and urea. In our study, we evaluated patients with normal renal function. Therefore, the negative correlation between FGF-23 and BFT in these patients without abnormal urea increase suggests that FGF-23 production is triggered by impaired renal function, but its production is suppressed when renal function is normal. On the other hand, it is also possible that FGF-23 has an accumulative effect in the body when renal clearance decreases.

When the relationship between alpha klotho levels and other parameters was examined, a positive correlation was found between alpha klotho and L1-L4 T score. In a study conducted by Kuro-o et al, it was reported that mice lacking the klotho gene developed osteoporosis, and

they concluded that the absence of klotho protein both reduced the number of osteoblasts and affected the function of osteoblasts, thus affecting bone mineralization.[26] In a study conducted by Zheng et al, the relationship between BMD measurements and serum FGF-23 and klotho levels in hemodialysis patients was examined, and a positive correlation was found between serum klotho protein levels and femoral neck and lumbar vertebral T scores.[27] These findings also support our study.

The limitation of our study was that it was a single-center experience and therefore the number of patients was small.

CONCLUSION

In conclusion, FGF-23 and alpha klotho are proteins that have been shown to be associated with cardiovascular diseases, progression in CKD, vascular calcification, osteoporosis, skin atrophy, infertility and life span. In our study, we found that the levels of these proteins were higher in patients with sarcoidosis. When evaluated from this perspective, the question of whether these proteins can be used as markers in predicting cardiovascular diseases, osteoporosis and renal damage comes to mind. It will be possible to reveal this relationship with studies conducted with larger patient groups.

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