Role of fibroblast growth factor-23 in calcinosis in women with systemic sclerosis

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ABSTRACT

Objective: Systemic sclerosis (SSc) is a complex disorder of unknown etiology. The purpose of this study was to evaluate fibroblast growth factor-23 (FGF-23) serum levels in women with SSc compared with healthy controls and to examine a possible association between FGF-23 serum levels with the presence of calcinosis in SSc patients.

Methods: This cross-sectional study was performed in San Cecilio Hospital, Granada (Spain) from November 2017 to May 2019. Sixty-two women with SSc and 62 age and sex matched healthy controls were included in this study. FGF-23 serum concentration was evaluated by indirect enzyme-linked immunosorbent assay (ELISA).

Results: There was no significant difference in FGF-23 levels between SSc patients and healthy controls (78.2 ± 60.5 vs. 80.3 ± 56.3 pg/mL, p= 0.662). Regarding the characteristics of the disease, we found a relationship between the values of FGF-23 and the presence of calcinosis. The levels of FGF-23 are higher in patients suffering from calcinosis (p= 0.028).

Conclusion: We observed the presence of higher levels of serum FGF-23 in SSc female patients with calcinosis. Therefore, FGF-23 could be a possible therapeutic target for future treatments.

Keywords: Fibroblast growth factor-23; Systemic sclerosis; calcinosis.

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INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease of unknown etiology, characterized by skin fibrosis, vascular walls and some internal organs, such as the digestive tract, lung, heart or kidney. This fibrosis appears as a result of a process not fully understood, which includes microvascular damage, immunological disorder and hyperproduction of collagen and extracellular matrix proteins. Clinically, it is very heterogeneous, so it is usually divided into two large groups: SSc with diffuse cutaneous involvement (dcSSc), with extensive dermal hardening and frequent organic involvement and SSc limited cutaneous (lcSSc), with more limited hardening and less organic involvement[1].

Calcinosis cutis, is a characteristic clinical manifestation of SSc, and the reason why calcium salts (calcium phosphate, hydroxyapatite) are deposited in the skin and subcutaneous tissues remains unclear[2].

Fibroblast growth factor-23 (FGF-23), is a protein of 251 amino acids synthesized and secreted by bone cells, mainly the osteocyte[3]. This full-length protein is recognized as a biologically active hormone that plays a key role in the complex network between the bones and other organs[4]. FGF-23 has been described to have detrimental effects on the heart, promoting left ventricular hypertrophy; the liver, leading to production of inflammatory cytokines; the bones, inhibiting mineralization; and the bone marrow, by reducing the production of erythropoietin[5-8]. Included in the group of hormones known as phosphatonins[9], it has been identified as the main regulatory factor of phosphate metabolism[10]. The increase in serum phosphate levels stimulates production of FGF-23 by bone and viceversa[11]. Alterations in both FGF-23 and phosphate levels can lead to the appearance of extraosseous calcifications[12], caused by the dangerous association of hypercalcemia and hyperphosphatemia. Given the broad list of effects associated with the high levels of FGF-23, it

would be reasonable to think of FGF-23 as a clinical target.

There is a lack of evidence regarding the potential role of FGF-23 in the pathogenesis of SSc, so we aimed to evaluate FGF-23 serum levels in women with SSc compared with healthy controls and to examine a possible association between FGF-23 serum levels with the presence of calcinosis in SSc patients.

MATERIALS AND METHODS

STUDY SUBJECTS

This cross-sectional study was performed in San Cecilio Hospital, Granada (Spain) from November 2017 to May 2019. We prospectively enrolled 62 consecutive female patients affected by SSc ≥ 18 years old attending our Systemic Autoimmune Diseases Unit. In addition, a control group of 62 healthy women age-matched, recruited mainly among nonmedical staff of our hospital that attended their annual medical health examination and who were invited to participate were included. All patients included in this study had normal serum creatinine (Cr) levels, and met the 2013 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for SSc[13]. The clinical diagnosis of SSc was per-formed by the rheumatologists.

We selected only women for the study because the distribution of FGF-23 concentration differs by sex[14]. This can be influenced by different hormones, among which are estrogens (estradiol)[15]. To exclude these problems, we selected female subjects in the current study.

At the clinic visit, participants completed questionnaires about their lifestyle characteristics, medical history, previous and concomitant treatment, and demographic data were taken. Informed consent was obtained for all subjects, and the study was approved by the Research Ethics Committee of Hospital Clinico Universitario San Cecilio in Granada, Spain, and conducted in accordance with the guidelines in the Declaration of Helsinki.

LABORATORY MEASUREMENTS

In all the cases, a fasting blood sample was taken in the morning, and was stored at -70°C until the assays were performed.

The sera were tested for creatinine, calcium, phosphorus, alkaline phosphatase, 25-Hydroxy vitamin D, parathyroid hormone, and FGF-23.

Creatinine was determined by Jaffe method (Siemens Healthcare Diagnostic Inc. NY, USA). Calcium and phosphorus were determined colorimetrically using commercial reagents in an automated chemical analyzer (Siemmens Healthcare Diagnostic Inc. NY, USA). Alkaline phosphatase was determined with standard automated equipment. The 25-Hydroxy vitamin D and parathyroid hormone were measured by chemiluminescent assays (Dia Sorin.Saluggia, Italy). Antinuclear antibodies were assessed using ELISA kits produced by Generic Assay Dahlewitz Germany.

Serum FGF-23 (Elabscience, USA) was measured by ELISA according to the manufacturer's recommendations. All other routine serum biochemistries were measured at the Department of Clinical Chemistry, San Cecilio Hospital.

STATISTICAL ANALYSIS

The results were analyzed using SPSS (version 21). Continuous variables were reported as means ± standard deviations and median (Q1–Q3) as suitable, while categorical variables were expressed as frequency and percentage. Comparisons of data between groups were made by Mann-Whitney U test and Wilcoxon test where appropriate. P values of less than 0.05 were considered statistically significant. Assuming an alpha risk of 0.05%, with a power of 80% and with a maximum error of 15 units, a minimum sample size of 58 patients was calculated. The subgroup analyses were pre-specified.

RESULTS

CHARACTERISTICS OF THE STUDY SUBJECTS

The main features of the 62 women with SSc and 62 controls included in this study are shown in Table I. The mean age (SD) of the patients was 53 ± 10 years. The majority were Caucasian (90.5%). The mean disease duration was 8.8 ± 6.9 years. Forty-four (70.9%) patients had a limited form of the disease and 18 (29.1%) had a diffuse form. Twelve (19%) patients had calcinosis and all patients had Raynaud Phenomenon.

The mean age (SD) of the controls was 52 ± 9 years. Most of them were also Caucasian (98.3%).

The main difference found between the two groups was the presence of a greater number of smokers in healthy controls.

LABORATORY RESULTS

Laboratory tests of the patients and healthy controls

TABLE I. CHARACTERISTICS OF WOMEN WITH SYSTEMIC SCLEROSIS AND HEALTHY CONTROLS

	SSc	HC	
	mean ± SD	mean ± SD	p-value
Age, years	53.2 ± 10.1	52.7 ± 9.7	0.71
Height, cm	159.5 ± 5.6	160.9 ± 7.1	0.23
Body weight, kg	66.8 ± 12	67.2 ± 12	0.90
Body mass index, kg/m2	26.3 ± 4.9	25.9 ± 4.3	0.90
Waist circumference	83.5 ± 11	83.1 ± 13.4	0.64
Smoking, n (%)	11 (17.7)	15 (24.2)	0.04
Hypertension, n (%)	8 (12.9)	11 (17.7)	0.87
Diabetes mellitus, n (%)	2 (3.2)	2 (3.2)	0.62
Dyslipidemia, n (%)	21 (33.8)	14 (22.5)	0.17
Disease duration, year	8.8 ± 6.9	-	-
lcSSc, n (%)	44 (70.9)	-	-
dcSSc, n (%)	18 (29.1)	-	-
Calcinosis, n (%)	12 (19.3)	-	-
ANAs, n (%)	54 (87.1)	-	-
Anti-centromere, n (%)	34 (54.8)	-	-
Anti-Scl70, n (%)	6 (9.6)	-	-

SD: standard deviation; SSc: systemic sclerosis; HC: healthy control; lcSSc: limited cutaneous SSc; dcSSc: diffuse cutaneous SSc; ANA: antinuclear antibodies.

TABLE II. SERUM FGF-23 AND SERUM BIOCHEMISTRIES OF THE PATIENTS AND HEALTHY CONTROLS

	SSc	HC	
	mean ± SD	mean ± SD	p-value
FGF-23, pg/ml	78.2 ± 60.5	80.3 ± 56.3	0.662
CRP, mg/dl	0.4 ± 0.4	0.2 ± 0.1	0.007
ESR, mm/h	21.1 ± 16	11.3 ± 10.2	0.001
Serum phosphate, mg/dl	3.6 ± 0.5	3.4 ± 0.5	0.04
Serum calcium, mg/dl	9.5 ± 0.3	9.4 ± 0.4	0.05
Alkaline phosphatase, IU/L	104 ± 130.5	72.9 ± 19.5	0.07
25-(OH)D, ng/ml	26 ± 12	23.1 ± 7.3	0.31
Intact PTH, pg/ml	50.7 ± 27.3	54.9 ± 19.6	0.02
Serum creatinine, mg/dl	0.7 ± 0.8	0.7 ± 0.2	0.12
eGFR, ml/min	93 ± 17.2	97 ± 13.2	0.19

SD: standard deviation; SSc: systemic sclerosis; HC: healthy control; FGF-23: fibroblast growth factor-23; 25-(OH)D: 25-hydroxy vitamin D; PTH: parathyroid hormone; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; GFR: estimated glomerular filtration rate

included in the present study are shown in Table II. As expected, laboratory markers of inflammation found at the time of the study were higher in women with SSc than in controls. In this regard, the mean CRP in SSc patients was 0.4 ± 0.4 mg/dl versus 0.2 ± 0.1 mg/dl in controls (p =0.007). Likewise, the mean ESR in the group of SSc patients was 21.1 ± 16 mm/1st hour versus 11.3 ± 10.2 mm/1st hour in controls (p <0.001).

Antinuclear antibodies (ANAs) were detected in 54 patients (87.1%), anti-centromere antibodies in 34 patients (54.8%) and anti-Scl-70 antibodies in 6 patients (9.6%).

There were no significant differences in FGF-23 levels between the patients and controls [78.2 ± 60.5 *vs.* 80.3 ± 56.3, pg/ml; p=0.662].

TABLE III. CORRELATIONS BETWEEN FGF-23 AND ANALYTICAL PARAMETERS IN SSC PATIENTS.

	Correlation coefficient	p
Serum phosphate	0.05	0.704
Serum calcium	-0.25	0.051
25-(OH)D	0.21	0.106
Intact PTH	0.05	0.728
Alkaline phosphatase	-0.14	0.276

FGF-23: fibroblast growth factor-23, SSc: systemic sclerosis, 25-(OH)D: 25-hydroxy vitamin D, PTH: parathyroid hormone

FGF-23 LEVELS IN SSC PATIENTS WITH DIFFERENT CLINICAL CHARACTERISTICS

In the sub-group analysis, based on diffused and limited forms of the disease, no significant differences were found in serum FGF-23 levels [92.2 ± 93.3 *vs.* 95.4 ± 78.7 pg/ml; *p*=0.786]. No significant differences were found also regarding the levels of FGF23 in terms of the presence of anti-centromere antibody [91.9 ± 74.6 *vs.* 87.4 ± 91.5, pg/ml; *p*=0.837], anti-Scl-70 antibody [74.6 ± 37.2 *vs.*90.7 ± 84, pg/ml; *p*=0.745] and ANAs antibodies [85.7 ± 87 *vs.* 104.2 ± 63.3, pg/ml; *p*=0.480]. No significant differences were found in serum FGF-23 levels according to the presence of cardiac [91.7 ± 80 *vs.* 84.6 ± 73.2, pg/ml; *p*=0.729] or lung involvement [94.2 ± 73.3 *vs.* 87.5 ± 82, pg/ml; *p*=0.592].

When patients were subdivided according to the presence of calcinosis, a significant difference was observed in serum FGF-23 concentration. The levels of FGF-23 were higher in patients with calcinosis [156.7 ± 126.1 *vs.* 73.3 ± 59.6, pg/ml; *p*=0.028] (Figure 1).

CORRELATION BETWEEN FGF-23 LEVELS AND ANALYTICAL PARAMETERS

Table III shows the correlation coefficients between FGF-23 and analytical parameters in SSc patients. FGF-23 levels did not show correlation with 25-

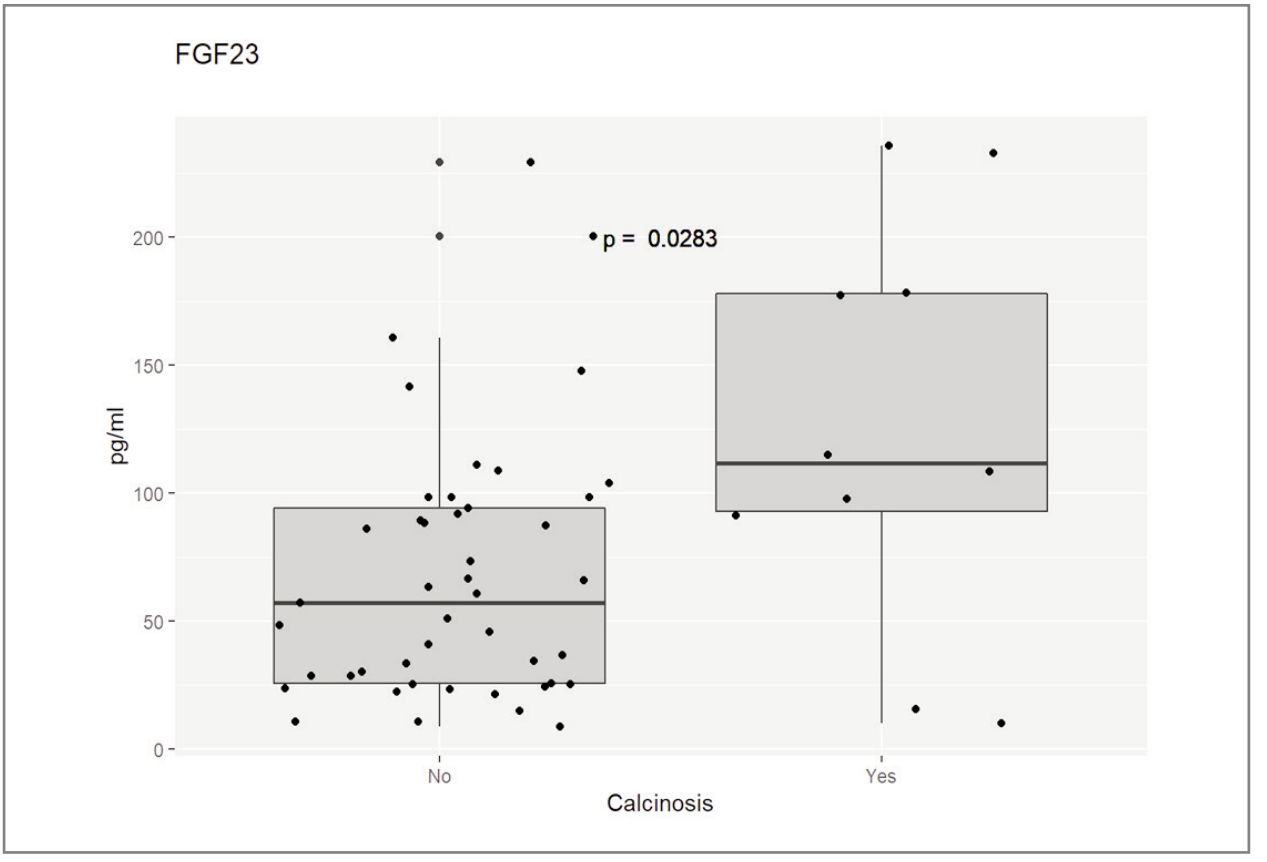

FIGURE 1. Median serum concentration of fibroblast growth factor-23 in Systemic sclerosis patients with calcinosis (n=12) and Systemic sclerosis patients without calcinosis (n=50). Wilcoxon test for paired sample.

(OH)D, iPTH, alkaline phosphatase, total calcium and phosphate levels.

DISCUSSION

In the present study, there was no significant difference in the FGF-23 levels between SSc female patients and healthy controls; however, the levels of FGF-23 were higher in patients suffering from calcinosis.

SSc is a multisystemic disease characterized by vascular, immunological alterations and excessive accumulation of connective tissue components that cause cutaneous sclerosis and fibrosis of different organs[16].

FGF-23 is an osteocyte-derived hormone that participates in vitamin D and phosphate metabolism. Changes in the serum levels of FGF-23 are particularly involved in the calcification of peripheral vasculature, the coronary artery and soft tissue[17,18].

We have not found a significant difference in FGF-23 levels between SSc patients and healthy controls in our study, which was in agreement with the study of Ahmadi[19]. Shenavandeh et al.[20], in a study in Iran, they also found no difference between FGF-23 levels of their patients with SSc and healthy controls, and when they analyzed the existence of differences in serum levels of FGF-23 among patients with diffuse or localized disease, they also found no significant differences. In relation to this data, we have not observed differences in FGF-23 levels between patients with dcSSc and patients with lcSSc.

In SSc, we can find the presence of calcinosis in both subtypes of the disease (diffuse and limited form)[2]. We found significantly elevated FGF-23 serum levels in SSc patients with calcinosis compared with SSc patients without calcinosis. FGF-23, specifically binds FGF-receptor-1-expressing kidney cells, leading to the decreased synthesis of active hormone of vitamin D, the $1,25(OH)_2D_3$, by causing repression of the gene for α-1 hydroxylase, thereby regulating calcium homeostasis. Ordinarily, FGF-23 also inhibits renal tubular reabsorption of phosphate by decreasing the gene expression of Na^+-dependent phosphate transporters (Na/Pi-2a and Na/pi-2c) in the proximal convoluted tubules. Another function of FGF-23 is to decreases mRNA transcription of parathyroid hormone, and inhibit its secretion[21]. Since FGF-23 is a known regulator of all three factors, it is plausible to that FGF-23 could be involved in the mechanism of vascular and soft tissue calcification.

There are some limitations in our study that should be considered: this study focused only on SSc female patients; therefore, the findings of this study cannot be generalized to men with SSc. This study was a cross-sectional analysis that reflected the status of a population in a particular period, and the small sample size, with only 12 patients with calcinosis.

CONCLUSION

We observed the presence of higher levels of serum FGF-23 in SSc female patients with calcinosis. Therefore, FGF-23 could be a possible therapeutic target for future treatments.

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