FGF23-Klotho axis in patients with rheumatoid arthritis

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Abstract

Objective

We aimed to compare serum Klotho and fibroblast growth factor-23 (FGF-23) levels between rheumatoid arthritis (RA) patients and healthy controls. Possible association between FGF-23 and soluble Klotho with different characteristic of the disease as well as their potential role as surrogate markers of cardiovascular disease (CVD) were studied.

Methods

Sixty-three patients with RA recruited at Vega-Baja Hospital, Orihuela (Spain) from November 2016 to May 2018 and sixty-five age- and sex-matched healthy controls were included in this study. Serum Klotho and FGF-23 were analysed using ELISA.

Results

Patients had higher serum levels of Klotho than healthy controls ($p<0.0001$). They were positively associated with the presence of anticitrullinated peptide antibody and rheumatic factor ($p<0.05$). Klotho serum levels were higher in RA patients treated with biologic agents than in those undergoing conventional therapy ($p=0.008$). However, no association with carotid intima media thickness was found. Although no significant differences in serum FGF-23 levels between patients and controls were found ($p=0.43$), FGF-23 levels were positively associated with low-density lipoprotein (LDL-c) level ($p<0.05$) and smoking ($p=0.008$) in patients with RA.

Conclusion

The increased serum Klotho levels in RA patients, especially in those undergoing biologic therapy, may indicate a potential implication in the pathogenesis of the disease. Although levels of FGF-23 were related to LDL-c levels, the FGF-23-Klotho axis does not seem to be related to subclinical arteriosclerosis in RA.

Key words

FGF-23, Klotho, rheumatoid arthritis, cardiovascular disease

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic and disabling disease affecting 0.5–1% of the general population. It is characterised by the presence of autoantibodies including rheumatoid factor (RF) and anti-citrullinated peptide antibodies (ACPAs) (1). RA patients have an increased risk of morbidity and mortality from cardiovascular (CV) events as a result of accelerated atherosclerosis (2, 3).

Interestingly, RA and atherosclerosis are both chronic inflammatory diseases sharing inflammatory biomarkers as well as similar pattern of cellular activation consistent with chronic inflammation. A main role toward the development of CV disease in RA has been attributed to inflammation and autoimmunity. Considering the high incidence of CV events in RA patients, an important step might be the identification of high-risk individuals that may benefit from treatment in order to prevent overt CV disease. In this regard, different non-invasive surrogate markers have demonstrated the presence of subclinical atherosclerosis in asymptomatic RA patients (4, 5). Information on serological biomarkers of CVD in patients with RA is limited (6).

The bone-derived fibroblast growth factor-23 (FGF-23) is a novel marker of chronic kidney disease (CKD)-associated mineral bone disorder, which increases progressively with declining renal function (7-12) and is markedly increased in end-stage renal disease (ESRD) (7-9). FGF-23 regulates phosphate and vitamin D balance by inducing urinary phosphate excretion in the proximal tubule, and it also inhibits the conversion of 25-hydroxy vitamin D to its active form. The actions of FGF-23 are mediated through FGF-receptors (FGFRs) with the aid of its co-receptor α-Klotho (Klotho) (7-9). Klotho is a membrane-bound protein predominantly expressed in the kidney, parathyroid gland and choroid plexus. It is also shed from the cell surface by α-secretases, acting in its soluble form independent of FGF-23 (13, 14).

In recent years, a growing body of evidence points towards FGF-23 as a novel predictor of mortality in adult CKD (15-19). The association between FGF-23 and mortality is mainly attributed to the increased risk of cardiovascular events, but also due to progression of CKD in itself. Specifically, FGF-23 is associated with left ventricular hypertrophy (LVH) (19-21), impaired left ventricular function (19), endothelial dysfunction (22, 23), heart failure (24) and progression of renal failure (15-19) in adult CKD. Recent experimental data suggest that FGF-23 can induce LVH directly by activating fibroblast growth factor receptor 4 (FGFR4) in cardiomyocytes (25). Further, soluble Klotho has been shown to have protective effects on the cardiovascular system by preventing endothelial dysfunction, vascular calcifications (26), cardiac fibrosis and cardiac hypertrophy (27).

With the growing evidence on the role of FGF-23 and soluble Klotho in the development of cardiovascular disease and the significant increase in cardiovascular morbidity and mortality in RA patients, the objectives of this study were to: analyse FGF-23 and soluble Klotho in a cohort of RA patients, examine possible associations between FGF-23 and soluble Klotho with different characteristic of the disease and investigate possible associations between FGF-23 and soluble Klotho as surrogate measures of CVD.

Materials and methods

Patients and controls

This cross-sectional study was performed in Vega-Baja Hospital, Orihuela (Spain) from November 2016 to May 2018. We prospectively enrolled 63 consecutive women patients affected by RA and followed at the Vega-Baja Hospital (Orihuela, Spain) and 65 matched healthy women controls. All patients included in this study had normal serum creatinine (Cr) levels and met the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) criteria for RA (28).

At the clinic visit, participants completed questionnaires about their medical history, current medication used, and lifestyle characteristics. Informed consent was obtained for all subjects, and the study was approved by the lo-

cal ethics committees and conducted in accordance with the guidelines in the Declaration of Helsinki.

Cardiovascular assessment

Disease severity was scored through the disease activity score of 28 joints and joint damage was evaluated based on the Steinbrocker radiographic criteria (I-IV). Current smokers were defined as those who reported having smoked ≥1 cigarette per day regularly during the year preceding the examination. Waist circumference, height, and weight were measured and body mass index (BMI) was calculated as weight (kg)/height (m^2). Blood pressure (BP) was measured twice in the left arm of the seated subject with a mercury column sphygmomanometer. The average of the 2 readings was used as the examination BP, and hypertension was defined as systolic BP ≥140 mm Hg, or a diastolic BP ≥90 mm Hg, or self-reported antihypertensive medication use. Type 2 diabetes mellitus (T2DM) was defined by a fasting glucose level ≥126 mg/dL, or self-reported use of insulin, or oral hypoglycemic medications. Kidney function was assessed using the estimated glomerular filtration rate (eGFR) calculated by the CKD-Epi study equation (29).

The CV risk was assessed using the Modified Systemic Coronary Risk Evaluation (mSCORE). The mSCORE was calculated using validated risk tables for both high and low risk populations. For this study, the low risk table was used since Spain has been classified as a low risk country for cardiovascular disease. Carotid intima-media thickness (c-IMT) was measured by performing carotid ultrasound examination in the Common Carotid artery and the detection of focal plaques in the extracranial carotid tree by manual technique using a commercially available scanner equipped with 7–12 MHz linear transducer as the patient was lying in the supine position with the neck rotated to the opposite side of examination as previously reported (30). Carotid plaques were counted in each territory and defined as no plaque, unilateral plaque or bilateral plaques (30). Values of cIMT greater than 0.9 mm were considered abnormal (cIMT thickening) and plaques were defined if the cIMT was greater than 1.2 mm (31). In our study, ankle-arm index were evaluated using a BIDOP model ES-100V3 vascular screening system (Hadeco, Inc, Kawasaki, Japan).

Table I. Characteristics of patients with rheumatoid arthritis and healthy controls.

	RA mean ± SD	HC mean ± SD
Age, years	53.1 ± 8.3	52.7 ± 9.7
Height, cm	160.8 ± 6.2	169.9 ± 7.1
Body weight, kg	68.7 ± 14.5	67.2 ± 12
Body mass index, kg/m^2	26.5 ± 5.6	25.9 ± 4.3
Waist circumference	103.8 ± 13.1	83.1 ± 13.4
Ankle-arm index	1.1 ± 0.1	1.2 ± 0.2
cIMT	0.7 ± 0.1	0.6 ± 0.2
mSCORE	2 ± 2.3	1.8 ± 2.5
Duration of RA, years	8.5 ± 5.8	-
DAS28-ESR	3 ± 1.3	-
HAQ	0.75 ± 0.67	-
Steinbrocker's stage	2.75 ± 1.17	-
Steinbrocker's class	1.87 ± 0.69	-
Smoking, n (%)	14 (22.2)	14 (21.5)
Hypertension, n (%)	10 (15.8)	11 (16.9)
Diabetes mellitus, n (%)	3 (4.7)	2 (3)
Dyslipidaemia, n (%)	13 (20.6)	14 (21.5)
Prednisone, mg/day	6.5 ± 3.5	-
Methotrexate, mg/week	11.5 ± 4.8	-
Biological agent use, n (%)	32 (50.7)	-
RF positive, n (%)	46 (73)	-
ACPAs positive, n (%)	45 (71.4	-
CRP, mg/dL	0.6 ± 0.8	0.2 ± 0.1
ESR, mm/h	23.9 ± 15.8	11.3 ± 10.2
Serum creatinine, mg/dL	0.58 ± 0.11	0.7 ± 0.2
eGFR, mL/min	108.7 ± 28.7	99 ± 13.2

SD: standard deviation; RA: rheumatoid arthritis; HC: healthy control; DAS: disease activity score; ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; eGFR: estimated glomerular filtration rate; RF: Rheumatoid Factor; ACPAs: Anti-citrullinated protein antibodies; HAQ: Health Assessment Questionnaire; cIMT: carotid intima-media thickness; mSCORE: Modified Systemic Coronary Risk Evaluation.

Laboratory measurements

In all the cases, a blood sample was taken in the morning after overnight fasting and stored at -70°C until the assays. Sera were tested for creatinine, urea, calcium, phosphorus, intact parathyroid hormone (iPTH), 25-hydroxy vitamin D (25-(OH)D), 1,25-dihydroxy vitamin D3 (1,25 (OH2)D3), FGF-23, and Klotho. Calcium and phosphorus, were determined colorimetrically using commercial reagents in an automated chemical analyser (Siemmens Healthcare Diagnostic Inc. NY, USA). Creatinine levels were determined by Jaffe method (Siemmens Healthcare Diagnostic Inc. NY, USA). The iPTH, 25-(OH)D and 1,25 (OH2)D3 were measured by chemiluminescent assays (Dia Sorin. Saluggia, Italy). Serum Klotho (Elabscience, USA) and FGF-23 (Elabscience, USA) were measured by ELISA according to the manufacturer's recommendations. ACPAs were detected using a second-generation ELISA (ACPAs) kit (ORGENTEC Diagnostika GmbH. Mainz, Germany) while IgM RF was determined as part of routine analysis by turbidimetric assay (Siemmens Healthcare Diagnostic Inc. NY, USA) according to the manufacturers' instructions. Fasting plasma glucose was measured in fresh specimens with a hexokinase reagent kit (Siemmens Healthcare Diagnostic Inc. NY, USA). Total cholesterol and triglyceride levels were determined by fully enzymatic techniques. High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein B (apoB)–containing lipoproteins with magnesium sulfate and dextran sulfate. Low-density lipoprotein (LDL) was calculated using the Friedewald formula. All other

routine serum biochemistries were measured at the Department of Clinical Chemistry, Vega-Baja Hospital.

Statistical analysis

Data were analysed by statistical software SPSS 18 (Chicago, IL, USA) and with the programme R v. Rx64.3.5.0 (Vienna, Austria), using independent samples *t*-test, Mann-Whitney U-test, and Chi-square test where appropriate. Spearman's coefficient and Pearson's correlation were calculated as suitable to determine the correlation between the bio-chemical parameters. Biserial-Puntual correlation was calculated when it was indicated. *p*-values less than 0.05 were considered statistically significant. The quantitative data were shown as mean ± standard deviation (SD) and median (Q1–Q3) as suitable.

Results

Characteristics of the study subjects

The characteristics of the study subjects are shown in Table I. A total of 63 female patients were included in our study, with a mean (SD) age of 53±8 years. The majority were Caucasians (90.5%). The mean duration of RA was 8.5±5.8 years. The mean disease activity scores in 28 joints (DAS28) according to the erythrocyte sedimentation rate (ESR) indicated low disease activity 3.0±1.3. The mean health assessment questionnaire (HAQ) was 0.75±0.67. The mean Steinbrocker´s stage was 2.75±1.17 and the mean Steinbrocker's class was 1.87±0.69. At the time of the study 32 patients were receiving biological agent (9 with etanercept, 9 with certolizumab pegol, 7 with tocilizumab, 6 with adalimumab and 1 with rituximab). Twenty-eight patients were treated with methotrexate, with a median weekly dose of 11.5±4.8 mg and sixteen patients were treated with prednisone with a median daily dose of 6.5±3.5 mg.

A total of 65 healthy women controls were included in our study, with a mean (SD) age of 52±9 years. The majority were Caucasian (98.3%).

Laboratory results

Serum biochemistries of the patients and healthy controls are presented in Table II. The mean serum total ACPAs and RF in RA patients were 571.22±1040.89 U/ml, 173.73±751.8 U/ml, respectively. Mean serum total cholesterol, HDL-C, LDL-C, and triglycerides in RA patients were 212.74±41 mg/dL, 69.92±19.45 mg/dL, 120.18±29.24 mg/dL and 112.93±55.67 mg/dL, respectively. Mean serum total cholesterol, HDL-C, LDL-C, and triglycerides in healthy controls were 211.8±37.3 mg/dL, 63.3±13.1 mg/dL, 129.7±30.7 mg/dL, 107.7±53.9 mg/dL, respectively.

Serum Klotho concentrations were significantly higher in the RA patients than those in the control group: [4.68 (1.4–7.8) *vs*. 3.5 (0.6–6.1), ng/mL; $p<0.0001$] (Fig. 1). There was no significant differences in FGF-23 levels between the patients and controls [85.7 (5.2–275.4) *vs*. 81.2 (2.6–269.9), pg/ml; $p=0.4316$] (Fig. 2).

Cardiovascular disease risk factors

Patients had a mean BMI of 26.58±5.61 kg/m^2, waist circumference of 103.8±13.19 cm, ankle-arm index of 1.14±0.1, cIMT of 0.71±0.13, mSCORE 2±2.34. Fourteen (22%) of them had a smoking history.

Healthy controls had a mean BMI of

Table II. Serum fibroblast growth factor 23, Klotho and serum biochemistries of the patients and healthy controls.

	RA mean ± SD	HC mean ± SD
FGF-23, pg/mL	85.6 ± 56.5	81.2 ± 61.4
Klotho, ng/ml	4.6 ± 1.1	3.5 ± 1.2
Serum phosphate, mg/dL	3.5 ± 0.5	3.4 ± 0.5
Serum calcium, mg/dL	9.4 ± 0.3	9.4 ± 0.4
25(OH)D, ng/mL	24.2 ± 11.4	23.1 ± 7.3
1,25(OH2)D3, ng/ml	45.8 ± 15.5	44.5 ± 12.6
Intact PTH, pg/mL	58.5 ± 22.9	54.9 ± 19.6
Cholesterol, mg/dl	212.7 ± 41	211.8 ± 37.3
LDL-C, mg/dl	120.1 ± 29.2	129.7 ± 30.7
HDL-C, mg/dl	69.9 ± 19.4	63.3 ± 13.1
Triglycerides, mg/dl	112.9 ± 55.6	107.7 ± 53.9
Uric acid, mg/dl	3.9 ± 1.3	4.6 ± 1.3
NT-proBNP, pg/ml	79.8 ± 54.8	59.7 ± 38.4
Fe, mg/dl	77.7 ± 28.9	85.6 ± 32.3

SD: standard deviation; RA: rheumatoid arthritis; HC: healthy control; FGF-23: fibroblast growth factor-23; 25(OH)D: 25-hydroxy vitamin D; 1,25(OH2)D3: 1,25-dihydroxy vitamin D; PTH: parathyroid hormone; LDL: low density lipoprotein; HDL: high density lipoprotein; Fe: iron; NT-proBNP: prohormone brain natriuretic peptide.

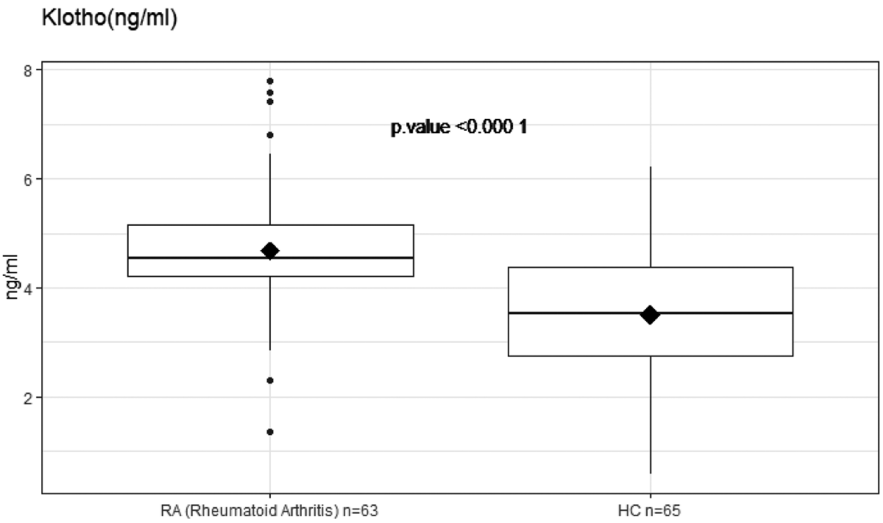

Fig. 1. Median serum concentration of Klotho in RA patients and healthy controls. Two-tailed Mann Whitney U-test for unpaired sample. HC: healthy controls.

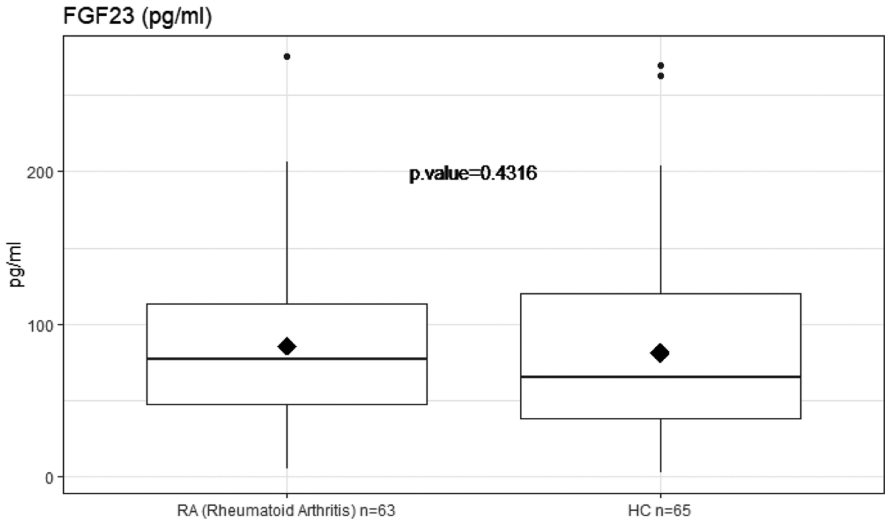

Fig. 2. Median serum concentration of FGF-23 in RA patients and healthy controls. Two-tailed Mann Whitney U-test for unpaired sample. HC: healthy controls.

Table III. Correlations between Klotho and study parameters in rheumatoid arthritis patients.

	Correlation coefficient	*p*-value
Age	-0.12	0.335
Height	0.01	0.881
Body weight	-0.001	0.988
Body mass index	-0.02	0.820
Waist circumference	-0.06	0.635
Smoking	0.04	0.780
Hypertension	-0.08	0.518
cIMT	-0.11	0.380
mSCORE	0.03	0.796
Disease duration of RA	0.08	0.487
DAS28-ESR	-0.22	0.071
HAQ	-0.17	0.174
Methotrexate dose	0.38	0.041
Prednisone dose	-0.11	0.682
Biological agent use	**0.34**	**0.007**
RF	**0.28**	**0.022**
ACPAs	**0.25**	**0.039**
C-reactive protein	0.0	0.874
Erythrocyte sedimentation rate	-0.10	0.393
Fibrinogen	-0.20	0.102
Serum creatinine	0.07	0.583
Serum urea	**-0.27**	**0.027**
eGFR	-0.15	0.241
Serum phosphate	0.005	0.963
Serum calcium	0.02	0.849
25(OH)D	0.19	0.130
1,25(OH2)D3	0.09	0.443
Intact PTH	-0.06	0.608
Cholesterol, mg/dl	0.01	0.924
LDL-C, mg/dl	0.06	0.635
HDL-C, mg/dl	-0.11	0.374
Triglycerides, mg/dl	0.08	0.530
Uric acid, mg/dl	-0.11	0.363
NT-proBNP, pg/ml	-0.01	0.910
Fe, mg/dl	0.08	0.491

cIMT: carotid intimal medial thickness; mSCORE: modified systematic coronary risk evaluation; DAS: disease activity score; HAQ: Health Assessment Questionnaire; RF: rheumatoid factor; ACPAs: Anti-citrullinated protein antibodies; eGFR: estimated glomerular filtration rate; 25(OH)D: 25-hydroxy vitamin D; 1,25(OH2)D3: 1,25-dihydroxy vitamin D; PTH: parathyroid hormone; LDL: low density lipoprotein; HDL: high density lipoprotein; Fe: iron; NT-proBNP: prohormone brain natriuretic peptide.

25.9±4.3 kg/m^2, waist circumference of 83.1±13.4 cm, ankle-arm index of 1.2±0.2, cIMT of 0.6±0.2, mSCORE 1.8±2.5. Fourteen (21.5%) of them had a smoking history.

Relationship between Klotho levels and characteristic of the disease in patients with RA

Table III shows the correlation coefficients between Klotho and other markers in RA patients. Higher plasma Klotho concentrations were significantly associated with higher ACPAs, RF, and DAS-28-ESR. The level of Klotho was higher in RA patients undergoing treatment with biological agent. Klotho was not correlated with age, height, body weight, HAQ, prednisone dose, duration of RA or methotrexate dose.

Relationship between FGF-23 levels and characteristic of the disease in patients with RA

Table IV shows the correlation coefficients between FGF-23 and other markers in RA patients. FGF-23 was not correlated with age, height, body weight, prednisone dose, disease duration, ACPAs, RF, DAS-28-ESR, HAQ, methotrexate dose or biological agent use.

Relationship between Klotho levels and cardiovascular disease risk factors in patients with RA

Klotho was not correlated with smoking, hypertension, waist circumference, cIMT, BMI, mSCORE, glucose levels or dyslipidaemia.

Relationship between FGF-23 levels and cardiovascular disease risk factors in patients with RA

Higher plasma FGF-23 concentrations were significantly associated with higher cholesterol levels**,** LDL-c concentrations and with smoking. FGF-23 was not correlated with hypertension, waist circumference, ankle-arm index, cIMT, BMI, mSCORE or glucose levels.

Relationship between characteristic of the RA and cardiovascular disease risk factors

Table V shows the correlation between disease characteristics and cardiovascular disease risk factors. An inverse

correlation between moderate-high activity in DAS-28-ESR and ankle-arm index was observed.

Discussion

The bone-derived hormone, FGF-23, and its co-receptor, Klotho, depict a novel endocrine axis regulating mineral metabolism in health and disease states. Binding of FGF-23 to FGFRs on target cells and its subsequent action require the presence of its co-receptor, Klotho (32, 33).

Recent evidence suggests that FGF-23 and Klotho may play important roles in the development of cardiovascular morbidity, both in the general population and in patients with CKD (20, 21, 25, 34). Both clinical (21) and experimental studies (20, 25, 34) have causally linked FGF-23 to cardiac hypertrophy, cardiac dysfunction and congestive heart failure. A direct role of the calcineurin-NFAT (nuclear factor of activated T-cells) signaling pathway, triggered by FGFR-4 activation in cardiac myocytes, has been reported in this context (20).

Our results show a significant difference in the serum concentration of Klotho in RA patients compared to healthy controls; moreover, the level of Klotho was associated to disease activity, according to DAS-28-ESR and with the use of biological agent, the patients who were in treatment with biological agent had higher levels of Klotho. Moreno *et al*. (35) showed that TNF-α downregulate Klotho expression through an NFκB-dependent mechanism, anti-TNF neutralising antibodies prevented TNF-induced Klotho mRNA downregulation. In addition, we found a significant association between high Klotho levels and the positivity for ACPAs and RF. ACPAs and RF represent a hallmark for RA, and RA patients with high ACPAs and RF titres often have a worse prognostic. This result represented a paradox; we initially hypothesised that Klotho levels would decrease in RA patients since serum Klotho has been reported to act as an anti-inflammatory molecule (36, 37). The positive correlation found in our cohort suggests that the increase in serum levels of Klotho may be a compensatory response to inflammation. In this regard, serum levels of Klotho were found remarkably high in subjects with inflammation-related stress, such as in smokers, in subjects with sleep deprivation and high psychological stress. These situations yield an elevation of inflammatory markers (38-40). These observations are in line with our results and support the hypothesis that an increase in Klotho is a compensatory response to stress and that Klotho functions as an anti-inflammatory molecule. The use of soluble Klotho as a biomarker has been investigated in sys-

Table IV. Correlations between fibroblast growth factor 23 and study parameters in rheumatoid arthritis patients.

	Correlation coefficient	*p*-value
Age	0.03	0.806
Height	-0.05	0.722
Body weight	0.13	0.316
Body mass index	0.15	0.246
Waist circumference	0.17	0.181
Smoking	**0.34**	**0.007**
Hypertension	0.11	0.399
Ankle-arm index	-0.13	0.293
cIMT	-0.13	0.320
mSCORE	0.22	0.086
Disease duration of RA	0.17	0.175
DAS28-ESR	0.08	0.516
HAQ	-0.03	0.791
Methotrexate dose	0.11	0.570
Prednisone dose	0.36	0.166
Biological agent use	-0.02	0.884
RF	0.01	0.944
ACPAs	0.19	0.137
C-reactive protein	0.03	0.818
Erythrocyte sedimentation rate	0.05	0.690
Fibrinogen	0.14	0.273
Serum creatinine	0.06	0.644
Serum urea	-0.03	0.790
eGFR	-0.07	0.588
Serum phosphate	0.08	0.543
Serum calcium	-0.04	0.772
25(OH)D	-0.12	0.366
1,25(OH2)D3	0.04	0.761
Intact PTH	-0.07	0.562
Cholesterol, mg/dl	**0.31**	**0.012**
LDL-C, mg/dl	**0.35**	**0.005**
HDL-C, mg/dl	0.04	0.730
Triglycerides, mg/dl	0.18	0.159
Uric acid, mg/dl	0.14	0.279
NT-proBNP, pg/ml	-0.01	0.911
Fe, mg/dl	0.19	0.135

cIMT: Carotid intimal medial thickness; m-SCORE: modified systematic coronary risk evaluation; DAS: disease activity score; HAQ: Health Assessment Questionnaire; RF: rheumatoid factor; ACPAs: Anti-citrullinated protein antibodies; eGFR: estimated glomerular filtration rate; 25(OH)D: 25-hydroxy vitamin D; 1,25(OH2)D3: 1,25-dihydroxy vitamin D; PTH: parathyroid hormone; LDL: low density lipoprotein; HDL: high density lipoprotein; Fe: iron; NT-proBNP: prohormone brain natriuretic peptide.

Table V. Correlation between characteristic of the rheumatoid arthritis and cardiovascular disease risk factors.

	DAS28-ESR		RF		ACPAs	
	r	*p*	r	*p*	r	*p*
Ankle-arm index	**-0.255**	**0.04**	0.208	0.10	0.218	0.08
cIMT	0.004	0.97	-0.161	0.20	-0.157	0.21
mSCORE	0.074	0.56	0.230	0.06	0.012	0.92

cIMT: carotid intimal medial thickness; mSCORE: modified systematic coronary risk evaluation; DAS: disease activity score; RF: rheumatoid factor; ACPAs: anti-citrullinated protein antibodies.

temic lupus erythematosus (SLE) (41), and more recently, its proangiogenic effect has been tested in a wound healing assay using human scleroderma-derived microvascular endothelial cells (42). In our study we did not find any correlation between Klotho and CRP, ESR, disease duration of RA or HAQ.

Sato *et al*. (43) reported an association between higher FGF-23 concentrations and disease activity and inflammation in patients with RA, which was independent of the renal function. In our patients, there was no significant difference in FGF-23 levels between the RA patients and healthy controls and we did not find correlation between FGF-23 and ACPAs, RF, CRP, ESR, disease duration of RA, DAS-28-ESR, HAQ or with the treatment with a biological agent. The mechanism linking inflammation and serum FGF-23 has not yet been elucidated. The altered iron metabolism associated with chronic inflammation may be the link between FGF-23 and inflammation. Under inflammatory conditions, elevated IL-6 increases the hepatic production of hepcidin, which inhibits iron absorption from the gut blocking iron release from macrophages and hepatocytes (44). Iron deficiency anaemia is associated with high levels of FGF-23, which decreases with the administration of intravenous elemental iron (44). Thus, lower circulating iron levels and/or higher ferritin levels resulting from chronic inflammation could increase serum FGF-23. In our study, we found normal levels of iron and we did not observe a correlation between FGF-23 and iron levels. We think it may be the explanation for the lack of association between FGF-23 and parameters of inflammation observed in our study.

Regarding cardiovascular risk factors, we did not find correlation between Klotho levels and surrogate measures of CVD. It was also the case for mSCORE, cIMT or traditional risk factors of markers of metabolic syndrome such as smoking, hypertension, waist circumference, BMI, glucose levels or dyslipidaemia. In contrast, we disclosed associations between serum FGF-23 levels and elevated total-cholesterol, LDL cholesterol and with smoking. Previous studies have linked FGF-23 to traditional CVD risk factors, including smoking, adiposity, dyslipidaemia and metabolic syndrome components (45-48). There is currently a paucity of evidence to support a biochemical mechanism by which FGF-23 might control lipid regulation. An attractive theory is that FGF-23 can signal through multiple FGFRs previously thought limited to other FGF-19 subfamily members. In fact, FGF-23 is closely related in structural homology to both FGF-15/19 and FGF-21. FGF-15/19 signaling is primarily implicated in bile acid metabolism and gallbladder filling, while experimental data supports a role for FGF-21 in regulation of lipolysis (49). Possible explanations for the association between smoking and FGF-23 are that smoking reduces FGF-23 sensitivity, thus necessitating an increased production to maintain phosphate excretion (45).

RA severity markers such as autoantibody production (RF, ACPAs), markers of systemic inflammation (ESR, CRP), number of inflamed joints, early functional decline and the presence of extraarticular features have all been reported to be strongly associated with adverse CV outcomes in RA (50-53). Yoo (54) found that female RA patients had significantly higher levels of LDL-C and LP(a) and lower level of TC than female controls and positive correlations between CRP and LDLC/HDL-C ($r=0.30$, $p<0.05$).

In our study we did not find any correlation between FGF-23 and ankle-arm index, cIMT or mSCORE. A possible explanation to these results could be that we did not find excess FGF-23 levels with declining Klotho levels in our study what is necessary for hyperphosphataemia and abnormal vitamin D metabolism, which are also important factors in vascular calcification (55) and coronary artery calcification is a strong predictor of cardiac events (56).

There are some limitations in our study that should be considered: this study was a cross-sectional analysis that reflected the status of a population in a particular period and our study evaluated the soluble fraction of Klotho, exploring its hormonal functions; but we did not perform immunohistochemical tests on RA tissues, so the real amount of Klotho, either as soluble or transmembrane, produced in situ and presiding a paracrine control, was not assessed. We selected only women for the study because the distribution of FGF-23 concentration differed by sex and female sex were associated with higher FGF-23 concentration at baseline (57), to exclude these problems, we selected women subjects in the current study.

We can conclude that RA patients have elevated levels of Klotho that correlate with disease activity. In addition, we report an independent association between higher FGF-23 concentrations with lipid disturbances and with smoking, in RA patients, suggesting that its possible participation as a cardiovascular risk factor is, at least in part, mediated by traditional cardiovascular risk factors.

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