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Short communication

Serum levels of fibroblast growth factor 23 are elevated in patients with active Lupus nephritis

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ABSTRACT

Background: Fibroblast growth factor 23 (FGF23), a phosphate-regulating hormone is an established cardiovascular risk factor. Recently, FGF23 has been related to inflammation. Lupus is an inflammatory disease, and whether FGF23 is associated with Lupus nephritis (LN) activity is unknown.
Materials and methods: We studied 15 pre-menopausal patients with recent LN diagnose (⩽2 months) and compared them to 1:1 age-matched healthy control group. We measured serum levels of intact FGF23, interleukin-6 (IL-6), tumor necrosis factor α (TNFα), and urinary levels of monocyte chemotactic protein (MCP1).
Results: LN patients (29.5 ± 10 years) presented proteinuria of 4.7 ± 2.9 g/day, and estimated glomerular filtration rate of 37 (31–87) ml/min/1.73 m^2. They demonstrated higher FGF23 levels when compared to healthy controls [106.7 (80.3–179) *vs.* 33.6 (25.8–60.9) pg/ml, $p < 0.001$]. FGF23 levels correlated with urinary MCP1 ($r = 0.62$, $p < 0.001$), serum TNFα ($r = 0.58$, $p < 0.001$) and serum IL-6 ($r = 0.46$, $p = 0.01$). Only the correlation between FGF23 and MCP1 remained significant after adjustments for 25(OH) vitamin D and renal function.
Conclusion: Newly diagnosed LN patients demonstrated elevated FGF23 levels that were positively correlated to urinary MCP1, independently of vitamin D levels and kidney function. If FGF23 may predict clinical outcomes in LN warrants further evaluation.

1. Introduction

Systemic Lupus Erythematosus (SLE) is an autoimmune disease that systemically affects several organs. Inflammatory cytokines are thought to contribute to initiation and amplification of end organ damage, although the mechanisms that might influence the loss of self-tolerance are not completely known. Despite controversies on their direct pathogenic role in SLE, Interleukin 6 (IL-6) and Tumor Necrosis Factor α (TNF α), have already been described as biomarkers of SLE activity [1]. Lupus nephritis (LN) occurs in 15% of patients at diagnosis and is characterized by intense inflammation. Urinary levels of monocyte chemoattractant protein-1 (MCP-1) are recognized as a biomarker of LN activity with good sensitivity and specificity [2].

Osteocytes secrete fibroblast growth factor 23 (FGF23), which is a-phosphate-regulating hormone. In early Chronic Kidney Disease (CKD), serum levels of FGF23 increase as a physiologic response to maintain neutral phosphate balance. However, due to the inhibition of the 1α-hydroxylase, FGF23 aggravates calcitriol deficiency, contributing to the pathogenesis of secondary hyperparathyroidism [3]. Epidemiologic studies have correlated elevated levels of FGF23 with several cardiovascular disease (CVD) manifestations in both general population and CKD patients [4]. Recent studies have demonstrated that higher FGF23 levels are associated with inflammatory markers, in an independent way, raising the possibility that inflammation might influence the correlation between FGF23 and CVD [5].

Increased serum levels of FGF23 have already been described in SLE patients, in a study enrolling patients with normal and impaired renal function [6]. However, renal function, a drive for FGF23 elevation, instead of inflammation, could explain this result. Whether FGF23 would be elevated in SLE patients and associated

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with LN activity, independently of renal function, is unknown. Therefore, we have analysed a sample of SLE patients and compared them with a healthy control group to test whether FGF23 would be increased and associated with SLE activity, regardless the renal function.

2. Material and methods

2.1. Study population

This post-hoc analysis from a previously published cross-sectional study included patients with newly diagnosed SLE and LN recruited from an outpatient clinic of an academic-based Hospital. A 1:1 age- and BMI-matched healthy population was used as a control group, from December 2010 to December 2012. Results on bone histomorphometry were previously published [7]. Briefly, patients without previous kidney disease and with a recent diagnosis of SLE were submitted to a bone biopsy. We found decreased bone formation, increased bone resorption and impaired mineralization in these bone samples.

Pre-menopausal women were diagnosed with SLE and LN in accordance to the American College of Rheumatology classification criteria within the prior two months. Exclusion criteria were prior kidney or bone disease, pregnancy or thyroid disease.

All patients were undergoing treatment with glucocorticoids. Diagnose of LN was confirmed by kidney biopsies, which were classified according to the International Society of Nephrology (ISN)/Renal Pathology Society classification by an independent pathologist.

2.2. Clinical and laboratory data

Biochemical data were collected at the initial evaluation. SLE clinical activity was estimated according to the SLE Disease Activity Index (SLEDAI). We calculated glomerular filtration rate (eGFR) by the MDRD simplified formula. Parathyroid hormone (PTH) and 25-hydroxyvitamin D_3 [25(OH)D] were measured by immunochemiluminesce [RR (reference range): 16–87 pg/ml and 30–100 ng/ml, respectively]. Total calcium (RR: 8.6–10.2 mg/dL), phosphorus (RR: 2.7–4.5 mg/dL) and alkaline phosphatase (RR: 35–104 U/L) were measured by established standard methods.

We measured serum intact FGF23 by ELISA (Kainos Laboratories, Tokyo, Japan, RR = 8.2–54.3 pg/ml), as well as urinary MCP1 (Human MCP1, R&D Systems), also standardized to urine creatinine (Cr) using the same spot urine and expressed as pg/mg Cr. Serum levels of IL-6 and TNFα were also measured using ELISA assays from R&D Systems, USA (RR: 0.7–3.12 pg/ml and 0.5–15.6 pg/ml; respectively).

The local ethics committee of the Clinics Hospital of the Sao Paulo University Medical School approved the study protocol (CAPPesq 0373/10), and patients provided written informed consent.

2.3. Statistical analysis

Results are expressed as mean ± standard deviation, median (25–75 percentile) or number and percentage. For comparisons between patients and control groups, we used Mann–Whitney test. Correlations between variables were determined using the Spearman correlation coefficient. Skewed variables were log transformed and linear regression models were built using Ln urinary MCP1 as dependent variable, with TNFα, IL-6, 25(OH)D and GFR as independent variables. Analyses were performed using the SPSS v 20.0 software. Tests were all two-sided we considered significant p value < 0.05.

3. Results and discussion

Women (8 white/7 non-white) with lupus nephritis (N = 15) aged 21 ± 6 years and were on prednisone treatment for 34 ± 12 days. Proliferative LN was observed in 86.6% of cases, with a mean proteinuria of 4.7 ± 2.9 g/day and an eGFR of 37 (31–87) ml/min/1.73 m^2. All SLE patients presented low levels of vitamin D (range 4–20), possibly contributing to increased PTH levels.

When compared to age-matched healthy controls, LN patients had higher FGF23 levels, lower eGFR and 25(OH)D levels, higher levels of serum IL-6, serum TNFα, and urinary MCP1 (Table 1).

FGF23 correlated negatively with estimated GFR (eGFR; r = −0.68, p < 0.001) and 25(OH)D (r = −0.73, p < 0.001), and positively with urinary MCP1 (r = 0.62, p < 0.001), serum TNFα (r = 0.58, p < 0.001) and serum IL-6 (r = 0.46, p = 0.01), as shown in Fig. 1. No correlation was found between FGF23 and SLEDAI. Linear regression analysis showed that the correlation between urinary MCP1 and FGF23 remained significant after adjustment for 25(OH)D and eGFR (adjusted R^2 = 0.39; beta = 0.353; p = 0.042). The correlations between urinary MCP1 and both TNFα and IL-6 were lost after adjustment for 25(OH)D and eGFR.

Our study showed that newly diagnosed LN patients present elevated levels of FGF23 that are not entirely explained by the reduction of renal function. Inflammation, instead, should explain this, as FGF23 correlates with urinary MCP1. Knowing that MCP1 is a biomarker of LN activity, FGF23 also arises as a potential new marker of this renal disease.

Epidemiologic data indicate that SLE patients present at least 2- to 3-fold increased risk of CVD manifestations and mortality when compared to the general population. Traditional risk factors do not fully explain this increased risk of CVD, suggesting that SLE-related factors may contribute to accelerated atherosclerosis. Beyond the possible adverse effects of glucocorticoid treatment, a growing number of studies suggest that altered immune system function may act as a primary contributor to the initiation and progression of atherosclerosis [8].

Although FGF23 levels are elevated in CKD [3], it is important to note that, in our patients, reduced GFR was clearly the result of glomerular acute inflammation and not a CKD condition, which we have proved during the follow-up, in which there was a recovery of renal function [7]. Indeed, recently it was demonstrated that even acute kidney injury is associated with high levels of FGF23 [9].

Elevated levels of FGF23 have already been associated with inflammatory markers in CKD patients [10]. Although the exact

Table 1
Clinical and biochemical characteristics of lupus nephritis (LN) patients and healthy controls.

	Lupus nephritis (n = 15)	Healthy controls (n = 15)	P
Age (years)	29.5 ± 10	31.7 ± 6.4	0.25
Body Mass Index (BMI) (kg/m^2)	24 ± 3	24 ± 4	0.74
eGFR (ml/min/1.73 m^2)	37 (31–87)	90 (73–100)	**0.009**
25(OH)D (ng/ml)	9.9 ± 4.4	24.3 ± 6.2	**<0.001**
FGF23 (pg/ml)	106.7 (80.3–179)	33.6 (25.8–60.9)	**<0.001**
Serum IL-6 (pg/ml)	5.8 (1.9–11.3)	1.2 (1.1–1.4)	**0.002**
Serum TNFα (pg/ml)	9.1 (8.2–19.9)	5.6 (5.2–6.7)	**<0.001**
Urinary MCP1 (pg/mg Cr)	1594 (595–2447)	177 (113–267)	**<0.001**

Results are expressed as mean ± stand deviation or median (25–75). eGFR (glomerular filtration rate estimated by the MDRD); 25(OH)D (25-hydroxyvitamin D_3); FGF 23 (Fibroblast growth factor 23); IL-6 (Interleukin 6); TNFα (tumor necrosis factor α); MCP1 (monocyte chemotactic protein 1).
Except for FGF23, data were already presented in a previous publication, cited at Ref. [7].