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Serum fibroblastic growth factor 23 in acute sarcoidosis and normal kidney function

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Abstract. *Background:* Serum fibroblastic growth factor (FGF) 23 has recently been established as a major physiological regulator of phosphate homeostasis and may have a causal role in adverse cardiovascular and bone outcomes. However its role in states of disordered phosphate homeostasis and normal kidney function is as yet under characterised. *Aims:* To investigate whether this biomarker of vascular calcification and adverse bone outcomes is detectable in patients with sarcoidosis. *Design:* We conducted a cross sectional study on a convenience sample of patients presenting with acute sarcoidosis to a respiratory tertiary referral unit. *Methods:* We set out to systematically examine the characteristics and determinants of serum FGF-23 in patients presenting with acute sarcoidosis. *Results:* We studied 39 patients, 26 were male. Mean (SD) age was 33 (9.6) years. 15.4% of patients had a serum level of FGF-23 ≥ 9.9 pg/mL. The remaining 84.6% of patients had a serum FGF-23 < 9.9 pg/mL. Those with a detectable serum FGF-23 had a significantly higher serum calcium (P = 0.007), and lower serum iPTH (P<0.001). Serum phosphate and 25-hydroxyvitamin D were not statistically significantly different between groups (P=0.25 and P=0.83). The proportion of patients with stage II disease on CXR was higher in those with a detectable FGF-23 (P<0.001). *Conclusions:* Serum FGF-23 was below the level of detection in the majority of this cohort of patients presenting with acute sarcoidosis. A detectable serum FGF-23 was associated with a higher serum calcium and lower serum iPTH. *(Sarcoidosis Vasc Diffuse Lung Dis 2016; 33: 139-142)*

Key words: FGF-23, sarcoidosis, calcium

Introduction

Serum fibroblastic growth factor (FGF) 23 has recently been established as a major physiological regulator of phosphate homeostasis in hyperphosphataemic states, and particularly in chronic kidney disease (CKD) (1). It is predominately produced by osteocytes and osteoblasts to down regulate renal proximal tubular phosphate co-transporters to reduce phosphate reabsorption (1, 2). Moreover, FGF-23 simultaneously reduces the expression of proximal tubular 1α hydroxylase to suppress renal production of 1,25-dihydroxyvitamin D, and increases the production of 24α hydroxylase thus enhancing the degradation of 1,25-dihydroxyvitamin D (3).

FGF-23 is associated with vascular calcification independent of kidney function (10) and has a possible causal role in the development of left ventricular hypertrophy (11, 12), and adverse cardiovascular events in the presence of normal kidney function (13). Since FGF-23 is a small molecule (30 kDa) (1, 2). it is not entirely clear whether the elevation of FGF-23 in CKD is merely a consequence of reduced renal clearance or an independent function of elevat-

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ed serum phosphate levels (1). The investigation of FGF-23 in states of altered phosphate homeostasis and normal renal function is therefore essential to further characterise our understanding of this molecule.

The determinants of serum FGF-23 in sarcoidosis is thus of considerable interest since this disorder is a model of extra-renal vitamin D activation (5-7), with resultant hypercalciuria and hypercalcaemia in a proportion of patients.(8) We set out to characterize the prevalence of detectable serum FGF-23 and determinants of serum levels in consecutive patients presenting with acute sarcoidosis.

Methods

We conducted a cross sectional study on a convenience sample of 39 patients presenting with acute sarcoidosis to our tertiary referral respiratory unit. Clinical and radiological features as well as serum and urine biochemical parameters were collected at the time of presentation. Diagnostic tissue biopsies were also taken as part of the clinical evaluation of these patients including transbronchial and thoracoscopic lung biopsies and skin biopsies. All patients gave written informed consent and local ethical committee approval was obtained prior to study commencement.

FGF-23 was measured using a human FGF-23 ELISA (Merck Millipore Corporation, MA, USA), performed according to manufacturers instructions. The FGF-23 in each serum sample was quantified using a standard curve (9.9 pg/mL to 2400 pg/mL); therefore the lower level of detection was 9.9 pg/mL. Estimated glomerular filtration rate (eGFR) was measured using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) serum creatinine based equation.

Statistical analysis

Descriptive statistics were reported using median (25^{th}-75^{th} centiles) and mean (SD) where appropriate. Wilcoxon rank sum test was used to compare independent groups comprised of those with and without detectable serum FGF-23 levels. The Fisher's exact test was used to compare proportions between these groups, and Spearman's rank correlation was used to assess for bivariate correlations between variables. SPSS version 20 was used for data analysis.

Results

We studied 39 consecutive patients, 26 were male. Mean (SD) age was 33 (9.6) years. All patients included in this study were White, and none of the patients included were on oral or systemic corticosteroids prior to or at the time of FGF-23 measurement. 26% (10/39) of this cohort were current smokers, and none had a history of diabetes mellitus. 28% (11/39) of patients presented with Lofgren's syndrome. All patients in this study had a tissue biopsy as part of their clinical evaluation, and 13 patients had negative biopsies, all of whom were diagnosed with sarcoidosis on clinical grounds.

In the overall cohort the median (25^{th}-75^{th} centile) 24-hour urinary calcium excretion was 4.8 (2.8-7.5) mmol/L. The mean (SD) iPTH was 25.35 (12) ng/dL, serum calcium was 2.32 (0.16) mmol/L, serum phosphate was 1.07 (0.21) mmol/L, serum 25-hydroxyvitamin D was 38 (18.5) nmol/L and eGFR was 117 (4) ml/min/1.73m^2.

84.6% (33/39) of patients had undetectable serum FGF-23 levels (i.e. < 9.9 pg/mL by the study assay). Hence 15.4% (6/39) of patients had a detectable serum level of FGF-23 at ≥ 9.9 pg/mL; 3 were female, all but one of these patients also had granulomatosis on initial diagnostic tissue biopsy. Three of the six patients with a detectable serum FGF-23 were current smokers.

In those without a detectible serum FGF-23 (n=33), median (25-75^{th} centile) serum iPTH was 24.8 (8.62-31.03) ng/dL, serum calcium was 2.29 (2.23-2.36) mmol/L, serum phosphate was 1.08 (0.87-1.19) mmol/L, and 24-hour urinary calcium was 4.97 (2.9-8.7) mmol/L. Median (25-75^{th} centiles) 25-hydroxyvitamin D was 34.7 (24.4-51.45) nmol/L.

In those with a detectable serum FGF-23 (n=6), median (25-75^{th} centile) level was 45.76 (15.7-108.84) pg/ml, serum iPTH was 7.0 (6-10.8) ng/dL, serum calcium was 2.62 (2.31-2.72) mmol/L, serum phosphate was 1.26 (0.87-1.36) mmol/L, and 24-hour urinary calcium was 6.69 (3.88-12.63) mmol/L. Median (25-75^{th} centiles) 25-hydroxyvitamin D was 40.9 (15.5-63.6) nmol/L.

When comparing those with and without a detectable serum FGF-23, serum iPTH was statistically significantly lower with a mean difference (95% CI) of -20 (-11, -30) ng/dL (P<0.001) and serum calcium was statistically significantly higher 0.26 (0.15, 0.38) mmol/L in those patients with a detectable serum FGF-23 level (P<0.001). Although serum phosphate and 24-hour urinary calcium tended to be higher in those with a detectable FGF-23, there was no statistically significant difference (P = 0.25 and P = 0.15). When comparing serum 25-hydroxyvitamin D levels between these groups, there was no statistically significant difference with a mean difference (95% CI) of -2.0 (-20.7, 16.7) nmol/L (P = 0.83). Lastly, there was also no difference between groups in the proportion of patients with granulomatosis on initial diagnostic biopsy (P = 0.37), or in smoking status at presentation (P = 0.14).

Serum FGF-23 levels were correlated inversely with serum iPTH (r = -0.7, P < 0.001), and positively with serum calcium (r = 0.44 P = 0.004), but were not correlated with serum phosphate (r = 0.19 P = 0.23), 24-hour urinary calcium excretion (r = 0.23, P = 0.13), or 25-hydroxyvitamin D levels (r = 0.08, P = 0.67). iPTH was inversely associated with serum calcium (r = -0.53, P = 0.006), and 24-hour urinary calcium excretion was inversely correlated with serum iPTH (r = -0.5, P = 0.02) while serum calcium was positively correlated with 24-hour urinary calcium excretion (r = 0.37, P = 0.02). The presence of granulomatosis was moderately inversely correlated with serum iPTH (r = -0.5, P = 0.006).

All patients with a detectable serum FGF-23 level had stage II disease on chest x-ray (CXR) at presentation. When comparing those with and without a detectable serum FGF-23 level there was a statistically significant difference (P < 0.001) in the proportion of patients exhibiting stage II disease on CXR at presentation in comparison to those who had an undetectable FGF level who predominately (79%) had stage I disease at presentation.

In the cohort overall, median (25-75th centiles) FEV1 was 3.89 (3.18-4.6) L, FVC was 5.16 (2.97-6) L and FEV/FVC ratio was 79.5 (72.3-84.5) %. Median (25-75th centiles) DLCO was 86% (67-96). There was no statistically significant difference in any of these lung function parameters between those with and without a detectible serum FGF-23 (all P > 0.05).

Discussion

Serum FGF-23 was detectable only in a minority of patients in this cohort, however our findings suggest that there appears to be substantial differences in terms of a number of clinical characteristics between those with and without a detectable level.

Typically patients with a detectable serum FGF-23 had a value for serum calcium in the hypercalcaemic range and a suppressed serum iPTH in contrast to those without a detectable FGF-23 level. These patients were also significantly more likely to have stage II disease on CXR at presentation. One might speculate that these observations relate to greater activated vitamin D levels in these patients, although we cannot confirm this without serum levels of 1,25-dihydroxyvitamin D, which is a limitation of our study.

Our a priori hypothesis was that sarcoidosis might be associated with higher serum FGF-23 levels due to vitamin D induced gastrointestinal phosphate absorption, however there are no published data on the relationship between serum FGF-23 and sarcoidosis.

With higher serum phosphate levels, one would expect an elevated serum iPTH, however we found the opposite in those with a detectable FGF-23, perhaps the enhanced renal phosphate excretion induced by FGF-23 offset the rise in iPTH, or perhaps the concomitant hypercalcaemia suppressed serum iPTH. This model is a relatively unique physiological scenario where both calcium and phosphate are absorbed simultaneously, and so while one would expect serum iPTH to increase urinary phosphate excretion, it is simultaneous being suppressed by enhanced calcium absorption (14). We did not find a relationship between serum phosphate and FGF-23, however serum phosphate levels were typically higher in those with a detectable FGF-23, and perhaps our sample size lead to a type II error in this regard.

Recent data implicates serum FGF-23 in a causal relationship with adverse cardiovascular events such as left ventricular hypertrophy (LVH) (15). Mirza et al. found that serum FGF-23 levels were associated with increased left ventricular mass index and the presence of left ventricular hypertrophy, even when within the normal range (15). The relevance of this observation to patients with sarcoidosis is unknown. FGF-23 is also thought to directly activate osteo-

clastic activity and may contribute to the development of osteoporosis (16). Recent evidence suggests that baseline levels of serum FGF-23 are directly correlated with overall fracture risk independent of renal function, particularly at levels above 55.7 pg/mL. (17) Whether serum FGF-23 monitoring has any utility in identifying patients at high risk of osteoporosis is not known but may also warrant further investigation given the prevalence of osteoporosis in sarcoidosis (19).

Manufactures of FGF-23 measurement kits do not generally provide reference values, due to the fact that these assays are mainly for research purposes only at present, however reference values have been proposed in a number of studies (18). Using these reference values, 50% of our cohort with detectable levels were well above the upper limit of normal, and why the majority of our sarcoidosis cohort had levels below the reference range is also entirely unclear.

This study has a number of limitations including the lack of serum 1,25-dihydroxyvitamin D levels and measures of urinary phosphate excretion. Unfortunately were we unable to measure these parameters of interest due to the fact that only small amounts of serum were collected and stored at acute presentation, which limited the number of analysis we could perform. In addition, while the lack of serum FGF-23 measurements in control subjects is suboptimal, FGF-23 has been studied in normal controls previously and our study was designed as a pilot study to test the potential relevance of this biomarker in sarcoidosis patients.

In this study of serum FGF-23 measurement in acute sarcoidosis, FGF-23 was detectable only in a minority of patients, who tended to have higher serum calcium levels and stage II disease on CXR. The significance of this observation is unclear but with mounting evidence of a causal role for FGF-23 in adverse cardiovascular and bone outcomes, perhaps this biomarker may be of interest in a subset of patients with sarcoidosis. Despite its limitations our study provides some useful information, which may help to inform further investigation on the role of serum FGF-23 in sarcoidosis.

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