ORIGINAL ARTICLE

Fibroblast growth factor-23 in patients with systemic sclerosis: A case–control study

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KEYWORDS
Systemic sclerosis (SSc); Subtypes (dSSc and lSSc); FGF-23; Phosphorus

Abstract  Background: Fibroblast growth factor-23 (FGF-23) is actively involved in phosphate homeostasis and skeletogenesis.

Aim of the work: To assess the serum level of FGF-23 in systemic sclerosis (SSc) patients (both diffuse dSSc and limited lSSc subtypes) in order to find if it has a role in the pathogenesis of the disease and study its relation to the clinical manifestations.

Patients and methods: The study included 30 dSSc patients, 30 lSSc and 28 age and sex matched controls. In patients, clinical examination and laboratory investigations were performed and Medsger severity scale assessed. Serum FGF-23 was measured using ELISA.

Results: The age of dSSc patients was 36.94 ± 9.89 years and the lSSc 38.36 ± 10.04 years. The serum FGF-23 level was 23.44 ± 14.86 pg/ml in dSSc patients, 20.01 ± 13.92 pg/ml in lSSc patients and 23.09 ± 11.45 pg/ml in the control (p = 0.58). No significant difference in the FGF-23 level was found according to the presence of lung fibrosis (p = 0.6). There was no significant difference in FGF levels among patients according to the severity (p = 0.39). In SSc patients there was a significant correlation between FGF and serum phosphorus especially in dSSc patients (r = 0.6, p = 0.003). Serum urea significantly correlated with FGF-23 in those with dSSc (r = 0.46, p = 0.037). There was no significant difference in the FGF-23 levels (p = 0.18) between those with a normal and impaired glomerular filtration rate.

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Systemic sclerosis (SSc) is a chronic multisystem autoimmune disease that has multiple overlapping and poorly defined clinical subsets [1]. The etiology of SSc is not still well elucidated, but the dominant phenomena are immunologic mechanisms, vascular endothelial cell injury, and activation of fibroblasts [2]. It is primarily a vascular disease that is mediated by autoimmunity and results in tissue fibrosis. The pathological findings in SSc skin document the significant loss of the peripheral vascular network, [3]. Altered balance of the opposing pro- and anti-angiogenic activities in SSc leads to abnormal new vessel growth (angiogenesis) or defective repair processes with subsequent tissue ischemia and fibrosis [4]. Even though the prognosis of the disease largely depends on visceral involvement, musculoskeletal affection is more frequent than expected in SSc patients with even reports on subclinical arthritis and presents a major cause of disability [5]. Cardiovascular and pulmonary involvements occur in SSc patients and asymptomatic coronary artery abnormalities are not uncommon [6,7].

Calcinosis cutis, deposition of calcium salts (calcium phosphate, hydroxyapatite) in the skin and subcutaneous tissues are common features of connective tissue diseases such as SSc, dermatomyositis and overlap syndromes [8–10]. Relatively fewer cases have been reported in patients with systemic lupus erythematosus (SLE) [11,12]. The pathophysiology of calcinosis cutis remains unclear. It has been suggested that alkaline phosphatase tissue activity may lead to hydrolysis of extracellular pyrophosphates normally preventing calcium deposition [13]. Alternatively, phosphate bound to denatured proteins of necrotic cells, at sites of trauma or inflammation, may serve as a nidus for dystrophic calcification [14].

Fibroblast growth factor 23 (FGF-23) is a 30 kDa secreted protein, member of the FGF family, and is thought to be actively involved in phosphate homeostasis and skeletogenesis [15]. There are inactivating mutations in its gene which were shown to be associated with an autosomal recessive disorder characterized by hyperphosphatemia and ectopic calcifications due to its reduced biological activity [16]. In animal studies, its effects were documented in FGF-23 null mice, with high serum phosphate levels, possibly due to increased renal reabsorption of phosphate, and increased vitamin D activity leading to soft tissue calcifications [17,18].

Circulating FGF-23 levels change by dietary phosphorus, serum phosphorus, serum calcium, and 1,25-dihydroxyvitamin D level [19–21]. In patients with chronic renal failure, an increase in FGF-23 levels has been shown to antedate the development of secondary hyperparathyroidism and may thus be beneficial in predicting which patients will develop this disorder [22]. Therefore, FGF-23 has emerged as an essential regulator of normal phosphate and 1,25(OH)2D3 homeostasis. Phosphate and 1,25(OH)2D3 increase FGF-23 levels; FGF 23 then acts on the renal proximal tubule to suppress the synthesis of 1,25(OH)2D3 and decrease the reabsorption of phosphate. The interrelation between FGF-23, vitamin D and soft tissue calcification is not yet clear. It was suggested that the lack of FGF-23, through a negatively regulated circuit of 1,25(OH)2D3 synthesis, was responsible for abnormal tissue calcification [23,24]. In another study, FGF-23 levels have been found to be independently associated with peripheral vascular calcifications in adult patients on hemodialysis [25], and also with total body atherosclerosis/future heart failure in adult chronic kidney disease patients [26,27]. It was seen that familial tumoral calcinosis, characterized by periarticular calcified masses often localized in the hip, elbow or shoulder [28] can be caused by mutation in the FGF-23 gene [16].

The level of FGF-23 was found to be significantly increased in patients with high atherosclerosis scores [29]. Data are consistent with the findings that higher FGF-23 associates with endothelium dysfunction [30,31]. Another point of view is that vascular injury and activation are likely to be the initiating events in systemic sclerosis. Evidence of vascular involvement is early and widespread, and is associated with significant clinical sequelae. The initial vascular insult is apparently endothelial cell injury, possibly triggered by unidentified serum cytotoxic factors or some other causes [26].

To the best of our knowledge, there was no research about the effect of FGF-23 in pathogenesis of calcinosis or endothelial cell injury in patients with scleroderma, so we designed this research to evaluate FGF-23 serum level in patients with limited and diffuse scleroderma compared to normal subjects.

2. Patients and methods

This study was conducted on 60 SSc patients; 30 with diffuse cutaneous scleroderma (dSSc), 30 with limited cutaneous scleroderma (lSSc) and 28 normal subjects selected by convenient and sequential method. The patients were recruited from the rheumatology clinic of the Hafiz hospital of Shiraz affiliated to Shiraz University of Medical Sciences from September 2009 to September 2010 based on the Leroy criteria for the diagnosis of scleroderma [32]. The normal participants of the study were chosen from blood donors referring to Blood Transfusion Organization in Shiraz. All procedures of the study were in accordance with the ethical standards of the Ethics Committee of the Shiraz University of Medical Sciences (Accepted proposal No. 90-5608) on human experimentation and with the 1975 Helsinki Declaration. A written informed consent was signed by the patients before inclusion in the study.

Screening, demographic measurements, and clinical assessment were performed by the rheumatologists involved in this research protocol. The Medsger severity scale [33] was considered for the patients. The study groups were scleroderma patients with the age of more than 16 years old and less than

Conclusion: The mean serum level of FGF-23 in this study showed no significant difference between systemic sclerosis patients and their subtypes with the normal subjects. It seems to have no role in the clinical manifestations of the disease.

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60 years old. Patients with overlap syndrome, impaired renal function (GFR < 60 ml/min/1.73 m²), diabetes mellitus, and abnormal parathyroid function were excluded from the study.

In all cases, a blood sample was withdrawn in the morning after an overnight fasting and stored at −70 °C until assayed. Patients and control subjects were matched for age and sex. The sera were tested for serum creatinine, urea, albumin, calcium, phosphorus, glucose, alkaline phosphatase (ALP), parathyroid hormone, and FGF-23. Calcium, phosphorus, creatinine, ALP and albumin were determined with standard automated equipment. Total calcium was corrected for variance in serum albumin by the formula: corrected total Ca (mg/dl) = measured Ca (mg/dl) + 0.8 x [4 − Alb (g/dl)]. The intact PTH was measured using immunoradiometric assay (Beckman Coulter, Immunotech, Czech Republic; normal range, 9–65 pg/ml). The Estimated creatinine clearance rate (eCCr) using Cockcroft–Gault formula was checked to find the GFR of patients. Biologically active and intact FGF-23 in using Cockcroft–Gault formula was checked to find the GFR of patients. Biologically active and intact FGF-23 in patients with systemic sclerosis (SSc) was measured by FGF-23 ELISA (Kinos, Japan) according to the manufacturer's recommendations.

FGF-23 refers to a control sample from 28 healthy individuals, receiving no treatment whatsoever, with similar age and sex distributions to the treatment sample, with a mean value of 23.09 ± 11.45 ng/l (5.08–61.06).

2.1. Statistical analysis

The results were analyzed using SPSS (version 13.0). Comparisons of data between groups were made by analysis of variances (ANOVA). Pearson correlation was used for detection of the relation between two variables. P value less than 0.05 was considered as statistically significant.

3. Results

Initially, 96 subjects were selected for the study, 32 cases in each group: group 1: diffuse systemic sclerosis (dSSc), group 2: limited systemic sclerosis (ISSc) and group 3: normal subjects). During the course of the study, 8 subjects were excluded from the study for not showing up for tests. The final population in the study consisted of 88 subjects (6 males and 82 females). In groups 1 and 2 there were 28 females and 2 males (F:M 14:1) and in the control there were 26 females and 2 males (F:M 13:1). The mean age of the patients in group 1 was 36.94 ± 9.89 years (18–55 year), group 2 was 38.36 ± 10.04 years (16–58 year) and for the control group was 36.94 ± 9.89 years (18–55 year), group 2 was 38.36 ± 10.04 years (16–58 year) and for the control group was 37.13 ± 9.65 years (18–56 year). There was no significant difference among patients with different severities (p = 0.6). According to the Medsger severity scale there was no significant difference in FGF levels among patients with different severities (p = 0.39).

The subjects were also tested for serum calcium, phosphorus, albumin, alkaline phosphatase (ALP), glucose, creatinine, urea, and PTH (parathyroid hormone) and the glomerular filtration rate (GFR) was estimated. There was no significant correlation between FGF-23 and the previously mentioned parameters with the exception of phosphorus (r = 0.28, p = 0.02) (Table 1). On comparing the studied parameters, there was a significantly different level of serum albumin and ALP between the patients and control (p = 0.007 and p = 0.03 respectively), otherwise the other laboratory investigations were comparable. In SS patients there was a significant correlation between FGF levels and serum phosphorus (r = 0.32, p = 0.02); this significant correlation is especially present in the dSSc patients (r = 0.6, p = 0.003) and not in ISSc or controls. Serum urea significantly correlated with FGF-23 only in group 1 (r = 0.46, p = 0.037) but not in groups 2 and 3. The GFR was checked in 44 patients with a mean of 78.2 ± 15.9 ml/min (38.2–109.3 ml/min). There were significant differences in the mean level of FGF-23 among the three groups (p < 0.5).

The range of FGF-23 in group 1 was 5.08–76.7 pg/ml, (mean 23.44 ± 14.86 pg/ml; median 46.2); in group 2 the range was 1.08–63.97 pg/ml (mean of 20.01 ± 13.92 pg/ml; median 39.5) and in the control it was 5.08–61.06 pg/ml (mean of 23.09 ± 11.45 pg/ml; median 47.6). There was no significant difference in the mean level of FGF-23 among the three groups (p = 0.58); the differences between dSSc and ISSc (p = 0.29), ISSc and control (p = 0.25) or between the dSSc and control (p = 0.79) were insignificant.

There was no cardiac involvement in our scleroderma patients. The FGF-23 level was not different between patients with and without pulmonary manifestations including dyspnea, basilar rales, abnormal chest X-ray, high resolution CT (HRCT) chest or pulmonary function tests (p = 0.54). Unfortunately, we do not have data on pulmonary hypertension because our patients did not have pulmonary hypertension.

The HRCT chest of 20 patients showed lung fibrosis (mean FGF-23 18.1 ± 12.1 pg/ml) and the HRCT in another 2 did not detect lung fibrosis (mean FGF-23 12.5 ± 8.5 pg/ml). The rest of the patients did not have an available HRCT chest.

Comparing the FGF-23 level among these groups showed no significant difference (p = 0.6). According to the Medsger severity scale there was no significant difference in FGF levels among patients with different severities (p = 0.39).

The results were analyzed using SPSS (version 13.0). Comparisons of data between groups were made by analysis of variances (ANOVA). Pearson correlation was used for detection of the relation between two variables. P value less than 0.05 was considered as statistically significant.

### Table 1: Correlation of the serum FGF-23 level and the studied laboratory investigations in the patients and control (n = 88).

<table>
<thead>
<tr>
<th>Investigation</th>
<th>FGF-23 (pg/ml)</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.14</td>
<td>0.28</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>0.09</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Calcium</td>
<td>0.20</td>
<td>0.10</td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.02</td>
<td>0.87</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>0.03</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.28</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>0.10</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>PTH</td>
<td>0.04</td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

ALP: Alkaline phosphatase; PTH: Parathormone.

### Table 2: Comparison of the laboratory investigation levels between those systemic sclerosis (SSc) patients with normal and high levels of FGF-23.

<table>
<thead>
<tr>
<th>Investigation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>0.007</td>
</tr>
<tr>
<td>ALP</td>
<td>0.03</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.57</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.74</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.53</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>1</td>
</tr>
<tr>
<td>Urea</td>
<td>0.61</td>
</tr>
<tr>
<td>PTH</td>
<td>0.69</td>
</tr>
</tbody>
</table>

ALP: Alkaline phosphatase; PTH: Parathormone.
39 patients with GFR > 60 ml/min (81.5 ± 13.4 ml/min) and 5 with GFR < 60 (52.3 ± 8.3 ml/min). There was no significant difference in the FGF-23 levels (p = 0.18) between these two groups (GFR < 60: 19.58 ± 12.55 pg/ml and GFR > 60: 24.82 ± 5.05 pg/ml).

When patients were classified into patients with normal and high FGF levels (FGF levels higher than control’s mean ± 2SD), there were no differences in the laboratory findings between patients with normal and high levels of FGF (Table 2). There was also no difference in the presence or absence of lung abnormality between patients with high or normal levels of FGF (p = 0.77 and p = 0.86, respectively).

4. Discussion

In systemic sclerosis, both subtypes of the disease (diffuse and limited) showed susceptibility to calcinosis [8,9,34]. Vascular injury and activation are also likely to be the initiating events in systemic sclerosis [26]. The FGF-23 has emerged as an essential regulator of normal phosphate and 1,25(OH)2D3 homeostasis and also a factor contributing to endothelial cell dysfunction and atherosclerosis [16,29]. In the current study, the serum level of FGF-23 is evaluated in 60 SSc patients (30 dSSc and 30 lSSc) and compared with control subjects for the first time.

In the present study, the serum FGF-23 levels showed a wide range in all patients and controls but with no significant differences. There was no significant difference in the FGF-23 levels between the dSSc and ISSc subtypes or with the controls. There were no correlation between FGF-23 level and clinical manifestations in the SSc patients. So this study did not show that FGF-23 level could be a key factor in the processes of SSc or have a role in the diffuse or limited subtypes. Future investigations are required to find any role in the disease especially in patients with obvious calcinosis or those with special manifestations like digital gangrene.

The results did not show a significant correlation between the serum FGF-23 level and any of the measured laboratory investigations including calcium, albumin, alkaline phosphatase, creatinine, urea, glucose, and PTH in the SSc patients. Only the serum phosphorus level significantly correlated with FGF-23 (r = 0.28, p = 0.02) especially in those with dSSc. Additionally, a significant correlation was shown between the FGF-23 and the urea in dSSc patients (r = 0.46, p = 0.037). It should be considered that these two factors were in the normal range in all three groups. It is likely that increasing the number of patients may verify results in future studies.

In one study conducted by Kojima et al. on chronic hemodialysis patients, it was shown that the FGF-23 significantly correlated with serum creatinine (r = 0.42, p < 0.0001), albumin (r = 0.23, p = 0.03), calcium (r = 0.39, p = 0.0001) and phosphorous (r = 0.74, p < 0.0001). There was a negative correlation between FGF-23 and glucose (r = -0.23, p = 0.029) [35]. Results were different in our patients who did not have any kidney problem, renal insufficiency or metabolic disease; there was no correlation between the serum FGF-23 level and any of the estimated investigations.

In conclusion, the mean serum level of FGF-23 in this study showed no significant difference between systemic sclerosis patients and their subtypes with the normal subjects. It seems to have no role in the clinical manifestations of the disease.

Conflict of interest

None.

Acknowledgments

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