Clinical Rheumatology (2019) 38:3189–3193
https://doi.org/10.1007/s10067-019-04637-8

BRIEF REPORT

Acroosteolysis and bone metabolism parameters distinguish female patients with limited systemic sclerosis with and without calcinosis: a case control study

Marilia M. Sampaio-Barros[1] · Lorena C. M. Castelo Branco[1] · Liliam Takayama[1] · Marco Antonio G. Pontes Filho[1] · Percival D. Sampaio-Barros[1] · Rosa Maria R. Pereira[1,2]

Received: 11 January 2019 / Revised: 23 March 2019 / Accepted: 4 June 2019 / Published online: 19 June 2019

Abstract

Calcinosis usually represents a late manifestation of systemic sclerosis (SSc), inducing tissue damage and chronic calcifications. To analyze clinical and bone metabolism parameters associated with calcinosis in limited systemic sclerosis (lSSc), thirty-six female lSSc patients with calcinosis were compared with 36 female lSSc patients without calcinosis, matched by age, disease duration, and body mass index. Organ involvement, autoantibodies, bone density, and laboratory parameters were analyzed. Statistical significance was considered if $p \leq 0.05$. Calcinosis was significantly associated with acroosteolysis (69% vs. 22%, $p < 0.001$), higher modified Rodnan skin score (mRSS 4.28 ± 4.66 vs. 1.17 ± 2.50, $p < 0.001$), and higher 25-hydroxyvitamin D (25OHD) (24.46 ± 8.15 vs. 20.80 ± 6.60 ng/ml, $p = 0.040$) and phosphorus serum levels (3.81 ± 0.41 vs. 3.43 ± 0.45 mg/dl, $p < 0.001$). 25OHD levels > 30 ng/ml were also significantly more frequent in patients with calcinosis ($p = 0.041$). Regarding treatment, current use of corticosteroids was lower in patients with calcinosis compared with patients without calcinosis (8% vs. 28%, $p = 0.032$). On logistic regression analysis, acroosteolysis (OR = 12.04; 95% CI, 2.73–53.04; $p = 0.001$), mRSS (OR = 1.37; 95% CI, 1.11–1.69; $p = 0.003$), phosphorus serum levels (OR = 5.07; 95% CI, 1.06–24.23; $p = 0.042$), and lower glucocorticoid use (OR = 0.07; 95% CI, 0.007–0.66; $p = 0.021$) are independent risk factors for calcinosis. This study showed that limited SSc patients with calcinosis present a distinct clinic and biochemical profile when compared with a matched group without calcinosis, paired by disease duration, age and BMI.

Key Points

• *Calcinosis in patients with limited SSc was associated with acroosteolysis, higher mRSS and higher serum levels of phosphorus.*

Keywords Acroosteolysis · Calcinosis · Limited systemic sclerosis

Introduction

Calcinosis usually represents a late manifestation of systemic sclerosis (SSc), inducing tissue damage and chronic calcifications. Although affecting both SSc clinical variants, it is more frequent in limited cutaneous disease [1, 2]. These calcifications can cause pain, local inflammation, ulcers, and infection, leading to a significant morbidity, mainly by functional limitation [3, 4].

Bone metabolism studies have shown an association between calcinosis and acroosteolysis [5], as well as deficiency of vitamin D and secondary hyperparathyroidism [5, 6]; nevertheless, its association with osteoporosis is still controversial [4, 7]. As an effective treatment for calcinosis is not yet completely available at the moment, the identification of prognostic factors for its presence is important in the development of therapeutic strategies for SSc.

The aim of this study was therefore to compare and analyze clinical aspects and laboratory parameters, including bone metabolism variables in patients with limited systemic sclerosis (lSSc) with and without calcinosis, paired by age, disease duration, and body mass index (BMI).

✉ Rosa Maria R. Pereira
rosamariarp@yahoo.com

[1] Division of Rheumatology, Hospital das Clinicas HCFMUSP Faculdade de Medicina da Universidade de Sao Paulo, Sao Paulo, SP, Brazil

[2] Disciplina de Reumatologia, Faculdade Medicina da Universidade de Sao Paulo, Av. Dr. Arnaldo, 455-3° Andar, Sala 3193, Sao Paulo, SP, Brazil

Methods

Patients This is a cross-sectional study which analyzed 36 lSSc patients with calcinosis, attending the Scleroderma Outpatient Clinic of the University of Sao Paulo. The diagnosis was considered when patients had clinical complaints and radiographic findings compatible with calcinosis. All patients were classified as having SSc according to the recent EULAR/ACR criteria [8]. Inclusion criteria were female gender, adult age (≥ 18 years), lSSc according to LeRoy et al. criteria [9], and capacity to understand the study and sign the informed consent. Exclusion criteria included diffuse SSc and overlap with another connective tissue disease (CTD). Thirty-six patients without calcinosis, paired by age, disease duration, and BMI to these patients with calcinosis, were selected and accepted to participate in this study.

Clinical data Clinical and demographic data were obtained through a direct interview and a review of the electronic register database. Modified Rodnan skin score (mRSS) was used to determine the extension of the skin involvement.

Patients were questioned about their diet and the supplementation of calcium and vitamin D, as well as the use of bisphosphonates. The daily calcium intake was determined by individual interview using a detailed food frequency questionnaire and calcium calculator, available on the Osteoporosis Society Canada website (www.osteoporosis.ca). The past and current treatment modalities (immunosuppressives, vasodilators, and glucocorticoids) were also questioned.

Laboratory data Blood was collected in the morning, with a 12-h fasting. Laboratory parameters of bone metabolism were measured in all patients: calcium, phosphorus, alkaline phosphatase, and parathormone (PTH) serum levels. A radioimmunoassay technique (DiaSorin, Stillwater, MN, USA) with a lower detection limit of 5 ng/ml was used to measure 25-hydroxyvitamin D (25OHD). According to current recommendations, 25OHD concentrations ≤ 30 ng/ml were defined as insufficiency [10] whereas values ≤ 20 ng/ml were classified as deficiency [11].

The profile of autoantibodies for each patient was also obtained: antinuclear antibodies (ANA) and anticentromere by immunofluorescence (Hep-2 cell) and anti-Scl70 by immunoblotting.

Bone mineral density BMD was analyzed by dual-energy X-ray absorptiometry (DXA; Hologic; QDR 4500, Bedford, MA, USA) of the lumbar region (L1-L4), total hip, and femoral neck. Osteoporosis was defined by a *T* score ≤ − 2.5 SD. BMI was calculated by measuring the weight and height of each patient during interview.

Ethical approval All patients gave their written informed consent, and the study was approved by the Ethics Research Committee of the University of São Paulo (Research protocol 0819/10).

Statistical analysis The results are reported as mean ± standard deviation and percentage. The data were analyzed by *t* test or Mann-Whitney test to access the differences between the groups. Fisher exact test was used for categorical variables; *p* values ≤ 0.05 were considered to be significant.

Results

The clinical and laboratory parameters of patients with and without calcinosis are shown in Table 1. Esophageal hypomotility, digital ulcers, and interstitial lung disease were the most frequent clinical manifestations of lSSc, present in similar frequency in both groups. Acroosteolysis was significantly more frequent in the group with calcinosis (69% vs. 22%; $p < 0.001$), which also presented higher mRSS (4.28 ± 4.66 vs. 1.17 ± 2.50; $p < 0.001$). Other clinical manifestations of lSSc were present in < 10% of the patients. ANA was positive in 89% in both groups. Anticentromere antibody was frequent (44% and 31%), while positive anti-Scl70 was rare in both groups (Table 1).

There were no significant statistical differences between daily dietary intake and calcium and vitamin D supplementation between the two groups. Regarding the use of medications, the only statistical association was between the current use of corticosteroids that was lower in patients with calcinosis comparing with those without calcinosis (8% vs. 28%; $p = 0.032$) (Table 2).

Osteoporosis (lumbar spine, total hip, and/or femoral neck) was more frequent in the group with calcinosis (31% vs. 17%), although not statistically significant. There were no significant differences between BMD and *T* score in patients with and without calcinosis (Table 3). Regarding laboratory data, mean serum levels of 25OHD (24.46 ± 8.15 vs. 20.80 ± 6.60 ng/ml; $p = 0.040$) and phosphorus (3.81 ± 0.41 vs. 3.43 ± 0.45 mg/dl; $p < 0.001$) were significantly higher in patients with calcinosis. 25OHD levels > 30 ng/ml were also significantly more frequent in patients with calcinosis ($p = 0.041$). Serum levels of calcium, alkaline phosphatase, and PTH were similar in both groups (Table 3). Logistic regression analysis showed that acroosteolysis (OR = 12.04; 95% CI, 2.73–53.04; $p = 0.001$), mRSS (OR = 1.37; 95% CI, 1.11–1.69; $p = 0.003$), phosphorus serum levels (OR = 5.07; 95% CI, 1.06–24.23; $p = 0.042$), and lower glucocorticoid use (OR = 0.07; 95% CI, 0.007–0.66; $p = 0.021$) are independent risk factors for calcinosis.

Discussion

The originality of the present study, analyzing only female patients with limited SSc excluding overlap with other CTD,

Table 1 Anthropometric and clinical parameters in limited SSc patients with and without calcinosis

	With calcinosis $n = 36$	Without calcinosis $n = 36$	p value
Anthropometric			
Age (years)	58.31 (7.92)	54.53 (10.33)	0.086
Weight (kg)	64.86 (14.97)	65.83 (11.86)	0.764
Height (m)	1.55 (0.05)	1.56 (0.07)	0.496
BMI (kg/m^2)	26.70 (5.89)	26.61 (3.74)	0.937
Disease duration (years)	19.61 (9.59)	15.56 (9.48)	0.075
Clinical			
RP duration (years)	18.72 (11.23)	15.94 (9.95)	0.270
mRSS	4.28 (4.66)	1.17 (2.50)	**< 0.001**
Digital ulcers, *n* (%)	27 (75)	25 (69)	0.605
Interstitial lung disease, *n* (%)	17 (47)	12 (33)	0.235
Esophageal hypomotility, *n* (%)	25 (69)	26 (72)	0.798
Acroosteolysis, *n* (%)	25 (69)	8 (22)	**< 0.001**
Autoantibodies			
ANA, *n* (%)	32 (89)	32 (89)	1.000
Anti-Scl70, *n* (%)	6 (17)	10 (28)	0.263
Anticentromere, *n* (%)	16 (44)	11 (31)	0.229

bold: p value < 0.05

BMI, body mass index; *mRSS*, modified Rodnan skin score; *RP*, Raynaud's phenomenon

Data are expressed in mean (SD) or *n* (percentage)

resides in the comparison with a group of patients without calcinosis, paired by age, disease duration, and BMI. As calcinosis is commonly a late manifestation of SSc, this process of pairing by age and disease duration was rather difficult.

Many variables are associated with calcinosis in the literature, especially acroosteolysis [12, 13] and digital ulcers [2]. In the present study, while acroosteolysis was significantly associated with the presence of calcinosis, digital ulcers were frequent and affected both groups. Although acroosteolysis and digital ulcers are associated with decreased blood flow, other vascular manifestations of SSc, as pulmonary hypertension and scleroderma renal crisis, were not associated with calcinosis in the literature.

Although not yet completely understood, a few studies have shown that both hypoxia and vascular impairment seem to be implicated in the etiopathogenesis of calcinosis [4, 14] and acroosteolysis [5, 15]. The main mechanism could be associated with the role of the hypoxia-inducible factor α/vascular endothelial growth factor (HIF-1α/VEGF) signaling pathway in regulating osteoclastic bone-resorption and angiogenesis. This hypothesis provides evidence that increased osteoclastogenesis and higher VEGF levels may contribute to acroosteolysis in patients with SSc [15].

Recent studies have shown an association between calcinosis and osteoporosis [2, 4, 16]. The higher frequency of osteoporosis in SSc patients with calcinosis commonly

Table 2 Dietary calcium, vitamin D, calcium supplementation, and treatment in limited SSc patients with and without calcinosis

Variables	With calcinosis $n = 36$	Without calcinosis $n = 36$	p value
Dietary calcium intake (mg)	547 (311)	496 (317)	0.489
Calcium supplementation, *n* (%)	15 (42)	13 (36)	0.152
Vitamin D supplementation, *n* (%)	23 (64)	21 (58)	0.252
Medications			
Current immunosuppressant, *n* (%)	16 (44)	24 (67)	0.059
Current glucocorticoid, *n* (%)	03 (8)	10 (28)	**0.032**
Vasodilator, *n* (%)	31 (86)	35 (97)	0.090
Bisphosphonates, *n* (%)	07 (19)	2 (6)	0.076
Anticonvulsant, *n* (%)	2 (6)	1 (3)	0.562

Data are expressed in mean (SD) or *n* (percentage)

Table 3 Laboratory parameters, bone mineral density (BMD), *T* score, and frequency of osteoporosis in limited SSc patients, with and without calcinosis

Variables	With calcinosis $n = 36$	Without calcinosis $n = 36$	*p* value
Total calcium (mg/dl)	9.44 (0.41)	9.36 (0.54)	0.477
Phosphorus (mg/dl)	3.81 (0.41)	3.43 (0.45)	**< 0.001**
Alkaline phosphatase (UI/l)	80.88 (39.49)	80.77 (26.01)	0.988
25 OHD (ng/ml)	24.46 (8.15)	20.80 (6.60)	**0.040**
PTH (pg/ml)	54.61 (31.27)	51.94 (21.85)	0.676
25OHD > 30 ng/ml, *n* (%)	8 (22)	2 (6)	**0.041**
25OHD > 20 ng/ml, *n* (%)	18 (50)	19 (53)	0.816
PTH > 65 (pg/ml), *n* (%)	10 (28)	6 (17)	0.263
C-reactive protein (mg/ml)	7.02 (8.04)	5.03 (4.64)	0.203
ESR (mm) 1st hour	19.13 (23.29)	19.50 (15.83)	0.938
Albumin (g/dl)	4.45 (0.27)	4.44 (0.28)	0.965
L1–L4 BMD (g/cm^2)	0.930 (0.15)	0.926 (0.12)	0.892
T score L1–L4	− 1.28 (1.47)	− 1.34 (1.32)	0.846
Total hip BMD (g/cm^2)	0.817 (0.16)	0.839 (0.12)	0.510
T score total hip	− 1.10 (1.28)	− 0.88 (0.92)	0.406
Femoral neck BMD (g/cm^2)	0.716 (0.13)	0.742 (0.11)	0.377
T score femoral neck	− 1.33 (1.16)	− 1.07 (0.98)	0.317
Osteoporosis, *n* (%)	11 (31)	6 (17)	0.169

ESR, erythrocyte sedimentation rate; *25OHD*, 25-hydroxyvitamin D; *BMD*, bone mineral density. Data are expressed in mean (SD) or *n* (percentage)

presents a negative association with disease duration [7, 16]. The fact that patients were paired by age, BMI, and disease duration should have significantly contributed to the finding of no differences regarding BMD values in patients with and without calcinosis in our study.

Correlations between hypovitaminosis D and SSc clinical features were observed in the literature, although their results were extremely variable according to the different studies. In particular, lower 25OHD levels were occasionally observed in patients with longer disease duration, calcinosis, and severity of skin and/or pulmonary involvement, while the inverse correlations with PTH levels or bone mass density were reported in only a few studies [6, 17, 18]. In our study, an interesting finding was the significantly higher serum levels of 25OHD in patients with calcinosis, although both groups had mean levels of 25OHD < 30 ng/ml. Some speculations could explain these findings in our SSc patients. First is a genetic predisposition to calcinosis, not analyzed in this study. Second is the analysis of paired patients according to gender, age, disease duration, and BMI, not previously described in the literature. Third, since the supplementation of vitamin D was similar in both groups, higher 25OHD serum levels could be associated with an increase of calcium absorption leading to deposition of subcutaneous calcinosis.

The finding of higher levels of phosphate (phosphorus) in patients with calcinosis in this study shed light to the possible role of phosphorus in the pathogenesis of calcinosis in SSc. Phosphate concentration is characterized by a high physiological variation, depending on age, gender, physiological state (e.g., pregnancy), and even season (due to the seasonal variation of vitamin D which is directly involved in the regulation of phosphate concentration) [19]. Recent findings of the association of high serum levels of phosphate with cardiovascular and soft tissue calcification in patients with normal kidney function [20] suggest its possible role in the process of calcinosis in SSc. Further studies focusing phosphorus in SSc are necessary to better explain these findings.

Regarding the use of medications, the only distinct result was the lower frequency of current corticosteroids use in the calcinosis group. It is known that glucocorticoids have an action in the inhibition of calcium absorption, by opposing 25OHD action. Thus, in the calcinosis group that received less corticosteroids, there could be a greater absorption of calcium and 25OHD, leading to a predisposition to calcification [7]. It is important to emphasize that acroosteolysis (OR = 12.04), higher Rodnan skin score (OR = 1.37), phosphorus serum levels (OR = 5.07), and lower glucocorticoid use (OR = 0.07) were demonstrated on the logistic regression analysis as independent risk factors for calcinosis.

In summary, the present study provides evidence that the presence of calcinosis in female patients with limited SSc is associated with clinical (acroosteolysis, higher Rodnan skin score), laboratory (higher phosphorus serum levels) and therapeutic (lower glucocorticoid use) variables, when compared with patients without calcinosis paired by age, disease duration, and BMI. Multicenter studies will be necessary to better analyze the bone metabolism alterations associated with calcinosis in SSc.

Support and grants This work was supported by grants from Programa Nacional de Pós-Doutorado da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (PNPD/ CAPES) (no. 1412229 to M.M.S.B.) and Federico Foundation (to P.D.S.B. and R.M.R.P.).

Compliance with ethical standards

All patients gave their written informed consent, and the study was approved by the Ethics Research Committee of the University of São Paulo (Research protocol 0819/10).

Disclosures None.

References

1. Vayssairat M, Hidouche D, Abdoucheli-Baudot N, Gaitz JP (1998) Clinical significance of subcutaneous calcinosis in patients with systemic sclerosis. Does diltiazem induce its regression? Ann Rheum Dis 57:252–254
2. Valenzuela A, Baron M, Canadian Scleroderma Research Group, Herrick AL, Proudman S, Stevens W et al (2016) Calcinosis is associated with digital ulcers and osteoporosis in patients with systemic sclerosis: a Scleroderma Clinical Trials Consortium study. Semin Arthritis Rheum 46:344–349
3. Boulman N, Slobodin G, Rozenbaum M, Rosner I (2005) Calcinosis in rheumatic diseases. Semin Arthritis Rheum 34:805–812
4. Valenzuela A, Song P, Chung L (2018) Calcinosis in scleroderma. Curr Opin Rheumatol 30:554–561
5. Braun-Moscovici Y, Furst DE, Markovits D, Rozin A, Clements PJ, Nahir AM et al (2008) Vitamin D, parathyroid hormone, and acroosteolysis in systemic sclerosis. J Rheumatol 35:2201–2205
6. Serup J, Hagdrup HK (1984) Parathyroid hormone and calcium metabolism in generalized scleroderma. Increased PTH level and secondary hyperparathyroidism in patients with aberrant calcifications. Prophylactic treatment of calcinosis. Arch Dermatol Res 276:91–95
7. Di Munno O, Mazzantini M, Massei P, Ferdeghini M, Pitaro N, Latorraca A et al (1995) Reduced bone mass and normal calcium metabolism in systemic sclerosis with and without calcinosis. Clin Rheumatol 14:407–412
8. van den Hoogen F, Khanna D, Fransen J, Johnson SR, Baron M, Tyndall A, Matucci-Cerinic M, Naden RP, Medsger TA Jr, Carreira PE, Riemekasten G, Clements PJ, Denton CP, Distler O, Allanore Y, Furst DE, Gabrielli A, Mayes MD, van Laar JM, Seibold JR, Czirjak L, Steen VD, Inanc M, Kowal-Bielecka O, Müller-Ladner U, Valentini G, Veale DJ, Vonk MC, Walker UA, Chung L, Collier DH, Csuka ME, Fessler BJ, Guiducci S, Herrick A, Hsu VM, Jimenez S, Kahaleh B, Merkel PA, Sierakowski S, Silver RM, Simms RW, Varga J, Pope JE (2013) 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/ European League Against Rheumatism collaborative initiative. Arthritis Rheum 65:2737–2747
9. LeRoy EC, Black C, Fleischmajer R, Jablonska S, Krieg T, Medsger TA Jr et al (1988) Scleroderma (systemic sclerosis): classification, subsets and pathogenesis. J Rheumatol 15:202–205
10. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R (2005) Estimates of optimal vitamin D status. Osteoporos Int 16:713–716
11. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357:266–281
12. Koutaissoff S, Vanthuyne M, Smith V, De Langhe E, Depresseux G, Westhoven R et al (2011) Hand radiological damage in systemic sclerosis: comparison with a control group and clinical and functional correlations. Semin Arthritis Rheum 40:455–460
13. Johnstone EM, Hutchinson CE, Vail A, Chevance A, Herrick AL (2012) Acro-osteolysis in systemic sclerosis is associated with digital ischaemia and severe calcinosis. Rheumatology (Oxford) 51:2234–2238
14. Pai S, Hsu V (2018) Are there risk factors for scleroderma-related calcinosis? Mod Rheumatol 28:518–522
15. Park JK, Fava A, Carrino J, Del Grande F, Rosen A, Boin F (2016) Association of acroosteolysis with enhanced osteoclastogenesis and higher blood levels of vascular endothelial growth factor in systemic sclerosis. Arthritis Rheumatol 68:201–209
16. Rios-Fernández R, Callejas-Rubio JL, Fernández-Roldán C, Simeón-Aznar CP, García-Hernández F, Castillo-García MJ, Fonollosa Pla V, Barnosi Marín AC, González-Gay MÁ, Ortego-Centeno N (2012) Bone mass and vitamin D in patients with systemic sclerosis from two Spanish regions. Clin Exp Rheumatol 30:905–911
17. Atteritano M, Sorbara S, Bagnato G, Miceli G, Sangari D, Morgante S, Visalli E, Bagnato G (2013) Bone mineral density, bone turnover markers and fractures in patients with systemic sclerosis: a case control study. PLoS One 8:e66991
18. Quarles LD (2012) Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. Exp Cell Res 318:1040–1048
19. Sheridan K, Logomarsino JV (2017) Effects of serum phosphorus on vascular calcification in a healthy, adult population: a systematic review. J Vasc Nurs 35:157–169
20. Baron M, Pope J, Robinson D, Jones N, Khalidi N, Docherty P, Kaminska E, Masetto A, Sutton E, Mathieu JP, Ligier S, Grodzicky T, LeClercq S, Thorne C, Gyger G, Smith D, Fortin PR, Larché M, Abu-Hakima M, Rodriguez-Reyna TS, Cabral-Castaneda AR, Fritzler MJ, Wang M, Hudson M (2016) Calcinosis is associated with digital ischaemia in systemic sclerosis-a longitudinal study. Rheumatology (Oxford) 55:2148–2155