J Musculoskelet Neuronal Interact 2014; 14(2):189-194

Original Article

Hylonome

Increased bone mineral density at the hypoxia prone site of the juxta-articular metacarpal bone in patients with limited systemic sclerosis: a cross-sectional study

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Abstract

Objectives: Low levels of oxygen has been shown to be involved in the induction of osteogenesis, particularly in bone repair. It is unknown whether hypoxia leads to osteogenesis at the hypoxia prone skeletal sites in limited systemic sclerosis. This study determined the total and trabecular volumetric bone mineral density (vBMD) at the hypoxia prone site of the juxta-articular metacarpal bone. **Methods:** In this cross-sectional study, female patients with limited systemic sclerosis were included and compared to healthy controls. Peripheral quantitative computed tomography was used to measure cross-sectional area, total vBMD, and trabecular vBMD at the radius, the tibia and the third metacarpal bone. Disease severity was assessed by the modified Rodnan Skin Score. **Results:** Twenty consecutive patients were included in the sclerosis group and 20 in the control group. Mean age was 60 years (range 52-68 years), and mean disease duration was 45 months (range 4-156 months). Age, height, and weight were comparable between the groups. The mean modified Rodnan Skin Score was 1.78 (range 0 to 8). The sclerosis group showed both higher total and trabecular vBMD at the distal metacarpal bone (p=0.05 and 0.04, respectively). vBMD of the tibia and radius did not differ in both groups. **Conclusions:** vBMD at the juxta-articular metacarpal bone in patients with limited systemic sclerosis is increased, possibly due to an alteration in local bone metabolism and hypoxia induced local osteogenesis.

Keywords: Limited Systemic Sclerosis, Scleroderma, peripheral Quantitative Computed Tomography, Bone Mineral Density, Metacarpal bone, Hypoxia, Bone Formation

Introduction

Systemic sclerosis (SSc; Scleroderma) is a connective tissue disorder with a wide variety of clinical manifestations. It mainly affects the skin and internal organs such as lung, heart and gastrointestinal tract[1]. More women than men are affected with a ratio of 3-5:1. Estimates of its incidence ranges from 9-19 cases per million per year, prevalence rate estimates range from 28-250 cases per million[1]. From a clinical point of view, SSc can be categorized in two main subtypes[2-5]: The limited form usually shows an skin thickening of the extremities distal to elbows and/or knees, and the diffuse form with a skin thickening proximal to the elbow and with a rapidly progressive involvement of internal organs. Nearly all SSc patients are positive for ANA. Limited disease shows a centromere pattern, whereas the diffuse form presents anti-topoisomerase antibodies, also known as anti-Scl-70[6].

The etiology of SSc is unknown. A genetic predisposition is likely to exist, and a positive familial history is currently considered the strongest risk factor[7]. The disorder is charac-

The authors have no conflict of interest.

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Edited by: J. Rittweger
Accepted 26 March 2014

Abbreviations

vBMD	volumetric bone mineral density
PQCT	peripheral quantitative computed tomography
SSc	Systemic sclerosis, Scleroderma
CSA	cross-section area
TGF-β	transforming growth factor β
VEGF	vascular endothelial growth factor
HIF-1α	hypoxia-inducible factor-1

terized by a triad of activation of humoral and cellular immune autoimmunity, an obliterative vasculopathy of small arteries and arterioles, combined with vascular and interstitial fibrosis of target organs.

Pathologic vascular alterations that can be detected in an early disease state using nailfold capillary microscopy seem to play a key role in the pathogenesis of the disease[8]. They are leading to a liberation of different cytokines such as transforming growth factor (TGF)-β, initiating and promoting the fibrotic response, and endothelin-1, a potent vasoconstrictor inducing proliferation of smooth muscle cells and activation of fibroblasts[9,10]. Vascular remodeling with hypertrophy of intima and media and microangiopathy are observed[10,11]. The resulting hypoxia triggers the expression of vascular endothelial growth factor (VEGF)[12]. Excessive production of extracellular matrix by activated fibroblasts further reduces the blood supply of the tissue by increasing the diffusion distance between capillaries and tissue cells[12]. When the oxygen saturation drops below 5%, there is an exponential increase in hypoxia-inducible factor (HIF)-1α[13-16]. HIF-1α is a ubiquitous transcription factor and one of the most important regulators of cellular adaption under hypoxic conditions[17,18]. Its accumulation favors an increased expression of various angiogenic mediators, namely VEGF[19]. In hypoxic tissue, VEGF stimulates angiogenesis by activating endothelial cells and by mobilizing and recruiting endothelial precursor cells[20]. Recently, it has been postulated that HIF-1α and VEGF are not only involved in the development of new vessels, but also play a role in local bone metabolism[21]. In fractures for example, the generation of new bone is not only positively influenced by mechanical stimuli and inflammatory mediators, but also by a local hypoxia because of intermediate interruption of perfusion and nutrient supply[22]. Therefore, the hypoxia-related accumulation of HIF-1α may support localized osteogenesis in patients with SSc. According to our knowledge, no study has examined this hypothesis yet.

Methods

We conducted a monocentric cross-sectional study to investigate the influence of limited SSc on volumetric bone mineral density (vBMD) and bone geometry at the distal epiphysis and shaft of radius, tibia and metacarpal bone by peripheral quantitative computed tomography (pQCT) in patients with limited SSc. The results were compared to a healthy control group comparable in terms of age, height, and weight. The cantonal ethics committee of Bern approved the study protocol.

Subjects

Patients with limited SSc[4] were recruited at the Department of Rheumatology and Clinical Immunology/Allergology of the University Hospital Bern, Switzerland. Inclusion criteria were a confirmed diagnosis of limited SSc (Raynaud's phenomenon, with two major criteria or one major and one additional criteria), age 18 years or older, and female gender. Diseases duration was calculated as months since diagnosis. Exclusion criteria were pregnancy, lactation, eating disorder, malignancies, endocrinopathies such as thyroid and parathyroid disorders, prior retinoid therapy, prior or ongoing treatment with bisphosphonates, rheumatoid arthritis, and spondylarthropathy. The data for the healthy control group was collected from control samples of healthy female volunteers participating in a prior study, matched for age, body weight, and body height. All patients and healthy controls gave written informed consent to participate in the study.

Assessment of skin alterations

To assess skin alterations, we used the modified Rodnan Skin Score[23].

Bone measurements

We measured metacarpal, radial, and tibial vBMD and geometry once in all study participants using pQCT. The characteristics of the scanner used (Stratec XCT 3000, Stratec Medical, Pforzheim, Germany) are described elsewhere. Scans were performed according to a published standardized protocol[24]. Measurements were taken for the third metacarpal bone and the peripheral radius of the non-dominant upper extremity and for the tibia of the contralateral side. The length measurement of the third metacarpal bone was performed by palpation of the distal articular surface and measurement with a measuring tape (accuracy in 5 mm). After a scout view of the metacarpal bone, we manually set a reference line at the distal end of the bone and collected two measurements (at 4% and 50% of total bone length measured from the distal bone end). The CT scanning speed for the third metacarpal bone was 15 mm/s. The slice thickness was 2 mm and the voxel size was set at 0.3 mm. Tibia length was determined from the medial knee joint cleft to the end of the medial malleolus using a measuring tape (accuracy in 5 mm). Radius bone length was set equal to ulnar length that was measured with a measuring tape by palpation from the olecranon to the ulnar styloid (accuracy in 5 mm). A scout view of the distal end of the tibia/radius was performed. The automated detection algorithm provided by the manufacturer was used to place the reference line at the distal bone end. Scans were performed at 4% and 66% of the bone's total length measured from the reference line. The scanning speed for all tibia/radius CT scans was 20 mm/s with a slice thickness of 2 mm and a voxel size set at 0.5 mm.

Bone parameters measured by pQCT

Both scan processing and calculation of bone parameters was performed using the software provided by the manufacturer (version 5.5D).

Epiphyseal scans (at 4%): After separating bone from soft tissue using a contour detection algorithm, total mineralized vBMD and bone cross-section area (CSA) were calculated. To determine the trabecular vBMD, concentric pixel layers were peeled off from the bone's perimeter until a central area covering 45% (radius and tibia) and 50% (metacarpal bone) of the total bone was remaining.

	Systemic sclerosis (n=20)		Healthy controls (n=20)		p value
	Mean	SD	Mean	SD	
Age [yrs]	60.85	8.96	59.50	8.14	0.46
Height [cm]	161.25	5.91	161.45	5.05	0.89
Weight [kg]	61.75	9.56	61.70	8.30	0.89
Age at menopause [yrs]	48.71	4.58	50.63	3.54	0.21

Table 1. Baseline characteristics of the systemic sclerosis group and the healthy control group (SD: standard deviation; p values for two-sided Mann-Whitney test).

Diaphyseal scans (at 66% for radius/tibia; at 50% for metacarpal bone): The proximal cortical bone fractions were determined using a standardized threshold of 710 mg/cm^3.

Additionally, we assessed the following parameters: cortical volumetric vBMD (mg/cm^3), total CSA (mm^2; including bone marrow), cortical CSA (mm^2; excluding bone marrow) and cortical wall thickness (mm; difference between outer and inner radius of a cylindric model).

Additional assessments

All participants provided additional information such as calcium and vitamin D intake, comorbidities and daily habits using a standardized questionnaire.

Soft tissue assessment

Of the diaphyseal scans at 66% of the forearm and lower leg, subcutaneous fat CSA was determined by selecting the area with thresholds of 240 to +40 mg/cm^3 HA density after smoothing the image. Muscle CSA was determined by subtracting the total bone CSA and subcutaneous fat CSA from the total limb CSA. The fat CSA/muscle CSA ratio was also calculated.

Statistical analysis

We compared the subject characteristics as well as bone and soft-tissue parameters between the two groups by non-parametric two-sided Mann-Whitney tests. In the SSc group, we used the Spearman rank correlation coefficient to assess the correlation between the metacarpal total vBMD as well as the trabecular vBMD and the modified Rodnan Skin score, the disease duration, positivity for anti-centromere antibody, the age of onset of menopause, and the radial muscle area. Statistical analyses were performed with IBM SPSS Statistics 21 (SPSS Inc., Zurich, Switzerland), Bonferroni's Multiple Comparison Test performed with GraphPad Prism 5 was used for statistical correction. Statistical significance was set at an alpha of 0.05.

Results

Subjects

Out of 33 contacted consecutive patients with limited SSc, ten had to be excluded for comorbidities. A total of 20 patients were finally measured. Of the 100 control subjects recruited for a related study, 20 consecutive subjects were selected on the basis of their age, height and weight in order to form a control group that were comparable with regard to age, height and weight. In two controls no tibia was measured. In the SSc group, the mean disease duration was 45.71 months (range 4-156 months). In four patients, disease duration was unknown. Twelve patients were positive for indirect immunofluorescence staining for anti-centromere antibody. The mean modified Rodnan Skin Score was 1.78 (range 0 to 8). Regarding age (p=0.46), body weight (p=0.89), and body height (p=0.89), there was no significant difference between the SSc group and the control group. The same applied to the age of onset of menopause (p=0.21) (Table 1). However, age of onset of menopause was known in only eight individuals of the control group, while it was known for 19 SSc patients.

Bone characteristics in SSc patients

Trabecular vBMD at the distal epiphysis of the third metacarpal bone was 8% higher in the SSc group compared to the control group (p=0.04, Table 2). Total vBMD was 7% higher at the distal metacarpal bone in the SSc group vs. healthy control (p=0.05, Table 2). Cortical thickness, vBMD, and total CSA were comparable for the metacarpal bone, tibia and radius between the groups. The slopes between total vBMD at the metacarpal bone and total vBMD at the tibial epiphysis were the same for SSc patients and healthy controls (R^2=0.218 [p=0.05] for controls, and R^2=0.235 [p=0.03]) with a difference of intercept of the two regression lines of 32 (211.01 for controls, and 243.41 for patients with SSc). The slopes between trabecular vBMD of the metacarpal bone and trabecular vBMD of the tibial epiphysis were the same for SSc patients and healthy controls (R^2=0.196 [p=0.07] for controls, and R^2=0.154 [p=0.09] for patients with SSc) with a difference of intercept of the two regression lines of 33.38 (175.66 for controls, and 209.49 for patients with SSc).

Muscle and fat CSA of the forearm and the lower leg were comparable between the groups, with a tendency of higher muscle CSA at the lower leg in the control group (Table 3). There was no correlation between total vBMD as well as trabecular vBMD and the modified Rodnan Skin Score, the disease duration, and the positivity for anti-centromere antibody.

		Systemic Sclerosis			Healthy controls			
		N	Mean	SD	N	Mean	SD	p value
Metacarpal bone 4%	BMC [g/cm]	20	44.675	4.755	19	42.336	6.200	0.20
	total CSA [mm²]	20	120.190	10.979	20	121.252	11.590	0.87
	total vBMD [mg/cm³]	20	372.735	34.756	20	346.715	41.009	**0.05**
	trab vBMD [mg/cm³]	20	324.235	38.790	20	298.925	44.385	**0.04**
Metacarpal bone 50%	BMC [g/cm]	20	47.992	7.722	19	45.396	6.411	0.21
	total CSA [mm²]	20	57.618	6.295	19	56.231	5.929	0.54
	cort CSA [mm²]	20	35.523	5.855	19	34.560	4.024	0.49
	cort vBMD [mg/cm³]	20	1178.705	43.272	19	1175.900	45.405	0.89
Radius 4%	BMC [g/cm]	20	1.078	0.179	20	1.014	0.201	0.12
	total CSA [mm²]	20	327.86	38.156	20	330.70	50.040	0.95
	total vBMD [mg/cm³]	20	331.304	58.372	20	309.314	51.857	0.20
	trab vBMD [mg/cm³]	20	194.350	42.023	20	174.687	39.392	0.18
Radius 66%	BMC [g/cm]	20	0.9240	0.143	20	0.871	0.119	0.24
	total CSA [mm²]	20	135.487	27.464	20	127.960	20.614	0.43
	cort CSA [mm²]	20	68.062	10.984	20	63.789	9.790	0.12
	cort vBMD [mg/cm³]	20	1115.961	48.493	20	1124.31	54.770	0,66
Tibia 4%	BMC anti[g/cm]	20	2.929	0.405	18	2.903	0.387	0.54
	total CSA [mm²]	20	1096.112	103.791	18	1064.958	110.753	0.46
	total vBMD [mg/cm³]	20	268.043	34.911	18	274.484	39.626	0.58
	trab vBMD [mg/cm³]	20	214.024	28.404	18	219.970	36.176	0.79
Tibia 66%	BMC [g/cm]	20	3.612	0.394	18	3.512	0.328	0.19
	total CSA [mm²]	20	533.425	58.558	18	541.069	75.776	0.86
	cort CSA [mm²]	20	278.725	32.183	18	266.597	28.439	0.09
	cort vBMD [mg/cm³]	20	1080.989	185.972	18	1122.569	38.655	0.68

Table 2. Bone variables of systemic sclerosis and healthy control group (SD: standard deviation; CSA: cross section area; BMC: bone mineral content; vBMD: volumetric bone mineral density; p-values for two-sided Mann-Whitney test).

		Systemic Sclerosis			Healthy controls			
		N	Mean	SD	N	Mean	SD	p value
Forearm	Muscle CSA [mm²]	20	2463.412	325.084	20	2361.925	265.101	0.29
	Fat CSA [mm²]	20	1432.337	659.697	20	1414.925	449.133	0.92
	Fat CSA/Muscle CSA [%]	20	59.482	27.427	20	60.352	21.322	0.91
Lower leg	Muscle CSA [mm²]	20	6360.175	885.207	18	5852.527	791.128	0.07
	Fat CSA [mm²]	20	2959.38	1141.352	18	2887.110	842.469	0.84
	Fat CSA/Muscle CSA [%]	20	46.985	16.821	18	50.199	18.689	0.58

Table 3. Soft tissue composition of the forearm and lower leg of Scleroderma and healthy control group (SD: standard deviation; CSA: cross section area; p-values for two-sided Mann-Whitney test).

Discussion

In this cross-sectional study, we compared the vBMD of the third metacarpal bone, the tibia and the radius between 20 female patients with limited SSc and 20 healthy controls using pQCT measurements. We were able to demonstrate that at juxta-articular metacarpal bone – possibly also the hypoxia and connective tissue fibrosis prone site – the vBMD is higher compared to the tibial and radial vBMD in patients with limited SSc. Furthermore, the vBMD of the metacarpal bone was higher than the vBMD of the metacarpal bone in healthy controls. Muscle and soft tissue at the forearm and the lower leg were comparable between female SSc patients and healthy controls. According to our knowledge, others have not performed similar investigations. Therefore, the described observations are the first of its kind.

Several studies described SSc as a potential risk factor for osteoporosis[25-29]. However, there are conflicting results regard-

ing BMD alterations in SSc. A systematic review[30] of case control studies showed a decreased bone mineralization in SSc patients in three studies[27,31,32], one study did not demonstrate a difference as compared to a healthy control group[29], and one study revealed normal to high BMD values in SSc patients[33]. A recent study of Yacoub et al. showed a decreased BMD and an increased incidence of osteoporosis in 60 female patients with SSc as compared to a healthy control group[34]. As it was the case in our analysis, this was a cross-sectional study. Radiologic investigations were performed at the lumbar spine and at the femur. A significant relationship between BMD and duration of disease, joint involvement, malabsorption, and antibody status was demonstrated in Moroccan female patients with SSc. These study results contrast our findings. However, we specially focused on vBMD in peripheral bones (metacarpal bone, radius, and tibia). We found a higher total and trabecular vBMD of the third metacarpal bone. This may suggest an influence of limited SSc with predominantly peripheral manifestation on local bone metabolism.

It should be noted that the small sample size is likely to be linked to a sampling bias. Despite a comprehensive interview of all patients by means of a questionnaire, we cannot exclude an imbalanced distribution of risk factors for osteoporosis between the two groups (accidental bias). Comorbidities with a potential influence on bone metabolism were largely excluded. We assessed other relevant factors such as familial history, physical activity, nutritional habits, and smoking. However, these factors were not considered in the statistical analysis.

Conclusions

We conclude that limited SSc might be associated with bone formation at the connective tissue fibrosis prone site of the juxta-articular metacarpal bone due to an alteration in local bone metabolism and local hypoxia. This results are in agreement with the hypothesis that low levels of oxygen may induce osteogenesis. Further studies should address the impact of hypoxia on SSc on systemic and local bone metabolism.

Acknowledgements

The study was financially supported by a personal grant from the Böni Stiftung to D.A. We would like to thank all participating patients and volunteers. We thank Prisca Eser and Jolanda Widmer for technical help.

Authors' Contributions

VH carried out pQCT-measurements, performed the statistical analysis and drafted the manuscript. IGL helped to perform the statistical analysis, PV provided intellectual and administrative support, DA conceived the study, helped to draft the manuscript and is responsible for the overall content. All authors read and approved the final manuscript.

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