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ORIGINAL ARTICLE

Sclerostin serum levels in patients with systemic autoimmune diseases

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Systemic autoimmune diseases (SADs) are associated with lower bone mass and an increased risk of fractures. Sclerostin has a pivotal role in bone metabolism. Available data on circulating sclerostin levels in healthy subjects are limited, whereas those in SAD patients are absent. Our objective was to determine circulating sclerostin concentrations in systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and Crohn's disease (CD) patients, and to analyze the factors associated with sclerostin concentrations. In this cross-sectional case–control study, serum sclerostin levels were measured in 38 SLE patients, 20 CD patients, 8 SSc patients and 20 healthy controls using a sclerostin ELISA. The mean values of the sclerostin (95% confidence interval) were 35.36 pmol l^{-1} (12–101) in patients and 33.92 pmol l^{-1} (2.31–100) in control subjects. The mean sclerostin value was 36.4 pmol l^{-1} (22.1–48.5) in SLE patients, 26.7 pmol l^{-1} (17.3–36.3) in CD patients and 51.8 pmol l^{-1} (26.5–77.1) in SSc patients ($P = 0.001$). Serum sclerostin levels were positively correlated with age ($P < 0.001$), body mass index (BMI) ($P = 0.01$) and lumbar spine Z-score ($P = 0.001$) and negatively with creatinine clearance ($P = 0.001$). Glucocorticoid treatment did not affect sclerostin levels. Sclerostin levels seem to have a heterogeneous pattern in different autoimmune diseases. SLE and SSc patients did not differ from healthy controls regarding sclerostin levels. The CD group had significantly lower values compared with SSc patients. Factors associated with sclerostin levels in autoimmune diseases seem to be the same than in the general population.

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Introduction

Sclerostin was discovered in 2001 from the genetic and molecular study of two rare sclerosing dysplasias, sclerosteosis and van Buchem disease, in which there is absence or decreased gene expression of this protein.[1,2] Sclerostin is a 190 amino-acid glycoprotein secreted mainly, but not exclusively, by mature osteocytes, although it is also produced by chondrocytes and cementocytes.[3] In addition, it is also produced by the liver, the vascular wall and the kidneys.[4] Sclerostin inhibits the functions, differentiation and survival rates of osteoblasts, as it promotes the apoptosis of these cells.[5] By binding to low-density lipoprotein receptor-related protein 5/6 receptors (LRP-5/6), this protein blocks the Wnt signaling pathway in osteoblasts.[6] In addition, sclerostin may upregulate the expression of RANK-L;[7] binding of RANKL to its receptor RANK constitutes the key step that drives the development of osteoclasts from hematopoietic progenitor cells, as well as the activation of mature osteoclasts.[8] Therefore, sclerostin has a pivotal role in bone biology and turnover.[6] This protein is secreted into circulation, and consequently its levels can be determined in serum samples. In keeping with this, a strong correlation between the levels of sclerostin in bone and its serum levels has been observed.[9] In spite of this, the exact function of sclerostin is intriguing. Unlike patients with sclerosteosis, in whom serum sclerostin levels are undetectable,[10] individuals heterozygous for inactivating

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Table 1 Demographic information of the patients with autoimmune disease and controls included in the study

	Patients, n = *66 (76.7%)*	*Control group,* n = *20 (23.3%)*	P-*value*
Age (years, mean ± s.d.)	44.24 ± 14.19	44.40 ± 10.82	0.964
Range	[21–79]	[26–62]	
Female gender	57 (86.4%)	17 (85%)	1
Postmenopausal	21 (36.8%)	5 (29.4%)	0.784
Creatinine clearance (CKD-EPI)[a] (ml min^{-1} 1.73 m^{-2}), mean ± s.d.	94.74 ± 24.38	97.57 ± 10.69	0.747
Sclerostin (pmol l^{-1}), mean ± s.d.	35.36 ± 15.40	33.92 ± 18.48	0.729

[a]CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration formula.

sclerostin mutations have serum levels of nearly half of those observed in matched controls. However, bone formation rates are significantly increased compared with control subjects.[11] Although it seems clear that sclerostin levels are related to markers of bone turnover, associations with bone mineral density (BMD) are, in most cases, either absent or even opposite to what is expected, and in numerous studies sclerostin levels have been correlated with higher bone mass.[12,13] There is no doubt about the key role of sclerostin in bone biology, and, in fact, the treatment with monoclonal antibodies against this protein is considered as a potential therapeutic strategy for osteoporosis. However, it is still unknown whether the measurement of circulating levels of sclerostin will be useful at the clinical practice.[14]

Systemic autoimmune diseases (SADs) are characterized by a trend to lose bone mass, and by a higher incidence of fractures, associated not only with characteristics intrinsic to the patients (age, sex, hormones, body mass index (BMI) and genetic characteristics) but also with treatments (glucocorticoids and immunosuppressive drugs) and disease characteristics (disease activity, sedentariness, hormonal disbalance or malnutrition). The fact that sclerostin is involved in bone diseases and in bone remodeling, as well as the previously described association between inflammation and increased sclerostin levels,[15] makes it plausible to think that alterations in its concentration could also promote a dysfunctional osteoblastogenesis process in SAD patients.

The aim of this project was to determine serum levels of sclerostin in patients affected by different SADs, in particular systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and Crohn's disease (CD). Furthermore, we aimed to analyze the factors associated with sclerostin concentrations. To the best of our knowledge, no studies regarding circulating sclerostin levels in patients with these diseases have been published to date.

Results

Demographic information about patients with autoimmune disease and control group is shown in **Table 1**.

The mean values of sclerostin were 35.36 pmol l^{-1} in patients and 33.92 pmol l^{-1} in controls. When patients were stratified by disease, the mean sclerostin values were 26.78 pmol l^{-1} in CD, 36.40 pmol l^{-1} in SLE and 51.83 pmol l^{-1} in SSc. We found statistically significant differences when sclerostin levels were compared between healthy controls and SAD patients (Kruskal–Wallis test, $P = 0.001$). By pairs, it was found that the CD group had significantly lower values compared with SLE and SSc ($P = 0.002$ and $P = 0.001$, respectively). When our cohort was stratified by sex, differences in sclerostin serum concentrations were found, with significantly higher levels in men.

When we assessed the potential association between sclerostin levels and factors related to them, a positive correlation with age ($r = 0.552$; $P < 0.001$) and BMI ($r = 0.341$; $P = 0.01$) was found. We also observed a negative association with creatinine clearance, as assessed by the Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI), ($r = -0.408$; $P = 0.001$).

Twenty-five patients were under chronic treatment with glucocorticosteroids, receiving a dose equivalent to 6.15 ± 3.05 mg per day of prednisone. No difference in sclerostin levels was observed when patients were stratified according to whether they were under treatment with glucocorticosteroids or not (33.94 ± 12.32 pmol l^{-1} vs 35 ± 148.28 pmol l^{-1}, $P = 0.7$).

No differences in sclerostin levels were noticed when patients who displayed pathologic densitometry (osteopenia and osteoporosis) were compared with those who had normal bone mass (32.63 ± 11.37 pmol l^{-1} vs 36.24 ± 13.53 pmol l^{-1}, $P = 0.3$).

Sclerostin concentration also showed a statistically significant correlation (Rho Spearman) with the densitometric parameters assessed: hip *Z*-score ($r = 0.482$, $P = 0.001$), spine *Z*-score ($r = 0.443$; $P = 0.001$), hip *T*-score ($r = 0.443$; $P = 0.001$) and spine T score ($r = 0.294$; $P = 0.035$). In addition, sclerostin levels were also associated with lumbar spine BMD ($r = 0.371$; $P = 0.031$) and were marginally associated with femoral neck BMD ($P = 0.055$).

Thirty-five out of the sixty-six SAD patients were taking calcium and vitamin D supplements (40.7%). No differences were disclosed in sclerostin levels between patients who were taking such supplements and those who were not (36.77 ± 15.2 pmol l^{-1} vs 36.86 ± 18.93 pmol l^{-1}).

Discussion

The results obtained in our study suggest that sclerostin levels are altered in some SAD such as SSc and CD when compared with those observed in healthy controls. However, the behavior of different diseases was heterogeneous. Patients with CD had lower levels when compared with those observed in healthy controls. These results remained significant even after adjustment for age, as CD patients were younger. We also disclosed that female SSc patients showed higher levels of sclerostin, even if it did not reach statistical significance after adjusting the results for postmenopausal status and age (as it has been reported that sclerostin levels increase with these factors). Taking into account the low number of female

SSc patients included in this study ($n = 8$), we believe that statistical significance could be reached by increasing the sample size.

Some studies have reported an association between inflammation and higher sclerostin levels,[15] which might lead us to expect higher levels in SAD patients. However, previous data on this issue are controversial. In a recent study, rheumatoid arthritis patients showed similar sclerostin levels to controls.[16] On the other hand, although some groups have reported higher sclerostin levels in ankylosing spondylitis (mainly in patients with active disease[17,18]), others have reported lower levels and that these low serum sclerostin levels were significantly associated with the formation of new syndesmophytes.[18] Finally, in untreated patients diagnosed with juvenile idiopathic arthritis (JIA),[4] serum sclerostin concentrations were significantly higher than in controls and dropped significantly after treatment with anti-TNF. In the present study, we found increased sclerostin levels in patients with SSc, but such results could be explained by the fact that SSc patients were older. It is well known that the expression of each of the proteins of the Wnt signaling pathway in the osteoblasts is individually regulated by age.[19] In a study performed in 1235 premenopausal women and 568 postmenopausal women from 20 to 79 years of age, the potential changes in serum levels of sclerostin in association with age were assessed. In this study, sclerostin levels were found to be stable between 35 and 45 years of age and that they increased from the age of 45.[20] Another study measured sclerostin levels in postmenopausal women as a control group, by using the same technique than in our study.[13] They reported that mean sclerostin values ($37.2 \pm 18.6\ \text{pmol l}^{-1}$, mean age 57.7 ± 7.8 years) were also lower than the mean concentration observed in our SSc patients. In addition, half of the patients in our study suffered from calcinosis. Nevertheless, it did not show any correlation with the changes observed in the mean sclerostin values.

The mean sclerostin values found in our SLE patients were similar to those reported for the healthy population, as indicated by Durosier *et al*,[21] and to the values observed in the healthy controls matched by age and sex that were included in our study.

It has previously been described that sclerostin levels in patients treated with glucocorticoids depend on the dose and on the duration of treatment. Acute administration of glucocorticoids reduces serum levels of sclerostin during a 96-h administration; this reduction may reflect glucocorticoid-induced rapid apoptosis of osteocytes.[22] In a prospective study with 25 patients who were started on glucocorticoids with a dose of 7.5 mg per day or greater, and 60 healthy controls,[23] a significant decrease in bone turnover markers with similar serum sclerostin levels was found over a short period of time. A progressive increase in serum sclerostin levels was observed after 12 months, compared with controls. Sclerostin levels increased and correlated with the glucocorticoid dose. In our study, we did not find any difference in this regard. This might be due to the fact that our patients had been under treatment with glucocorticoids with lower doses and for a shorter period of time.

We have observed a positive correlation between sclerostin levels and *T*-score and *Z*-score values. Similar findings have been reported in type 2 diabetes mellitus patients[13] and also in the general population.[21,24,25] However, paradoxically, increased sclerostin levels have been associated with a higher incidence of fractures, especially if accompanied by lower BMD.[26]

Higher sclerostin serum concentrations levels were found in men, which had previously been reported.[19]

In addition, a trend toward higher sclerostin levels in patients with lower glomerular filtration rate could also be observed. Higher sclerostin levels have also been described in patients with chronic renal failure, who did not suffer from any autoimmune disease.[27] The exact cause of that increase is still unknown.

Recently, it has been shown that sclerostin levels are increased in type 2 diabetes mellitus patients who present vascular damage[28] when compared with those patients who do not have vascular damage. This could be particularly important in patients with different autoimmune diseases, in whom vascular risk is increased. This issue has not been assessed in our present study.

In conclusion, patients with different SADs appear to have different levels of sclerostin. Furthermore, factors such as age or renal function, which are usually associated with its levels, do not seem to have a different influence, in comparison with the general population. The treatment with low doses of glucocorticoids, calcium or vitamin D supplements do not modify sclerostin levels. The association between sclerostin levels and a higher bone mass would not support, a priori, the hypothesis of a potential benefit of the use of anti-sclerostin antibodies for the treatment of osteoporosis in patients with autoimmune diseases. Serum sclerostin values cannot be used to identify patients with autoimmune disorders who have low BMD. However, as this was a cross-sectional study, we feel that further prospective studies are needed to fully elucidate the implication of sclerostin in SADs.

Materials and Methods

We have performed a descriptive cross-sectional case–control study, which included 66 patients who had been diagnosed with SLE ($n = 38$), SSc ($n = 8$) or CD ($n = 20$) and 20 healthy controls from the Andalusian Public Health's System's Biobank. A subject's written consent was obtained from all the patients and controls. Patients with diagnosis of overlap syndrome were excluded. The healthy controls included in this study were matched by age and sex. Clinical and analytical data (sex, age, BMI, diagnostic, altered renal function, glomerular filtration rate, osteopenia, osteoporosis, femoral neck and lumbar spine BMD, previous treatment with glucocorticoids and vitamin D) were recovered from clinical records.

Subjects' mean age was 44 years. Regarding sex distribution, 57 patients were female (87%). In the healthy control group, 17 of them were female (85%).

Sclerostin serum levels were determined by a commercial enzyme-linked immunoassay (Sclerostin ELISA Kit, BI-20492, BIOMEDICA, Vienna, Austria. Intra- and inter-assay coefficient of variation: 6 and 6.5%, respectively. Detection limit: $2.6\ \text{pmol l}^{-1}$), following the manufacturer's instructions. Hundred microliters of serum was employed for each measurement. The ELISA was performed at the Epidemiology, Genetics and Atherosclerosis Research Group on Systemic Inflammatory Diseases (Santander, Spain), led by Dr Miguel Angel González-Gay.

A descriptive statistical analysis was performed. Central tendency and dispersion measures were calculated for numeric variables, whereas absolute and relative frequencies were calculated for the qualitative ones. Median values were compared by Student's *t*-test for independent samples and ANOVA, if they followed a normal distribution,

or Mann–Whitney and Kruskal–Wallis test, if they did not. Potential bivariate correlations were also assessed by Spearman's Rho (ρ). Statistical significance was defined as a P-value of $\leqslant 0.05$, and all analyses were performed using the IBM SPSS Statistics 19 statistical software (IBM, Armonk, NY, USA).

Conflict of Interest

The authors declare no conflict of interest.

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