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Different development of serum sclerostin compared to other bone remodeling markers in the first year after a liver transplant

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Summary

Objetive: Our main objective was to evaluate the development of sclerostin levels in patients with liver transplantation, and to investigate their relationship with other bone remodeling markers.

Material and method: Prospective observational study of 83 patients with liver transplantation. Sclerostin, β-crosslaps, bone alkaline phosphatase, osteocalcin and C-reactive protein values were determined the week before the transplant and subsequently, at 1, 3, 6 and 12 months. The hydroxy-vitamin D and the paratohormone were determined basally. In each revision, the existence of fractures was evaluated. The development of the markers compared to the baseline value was determined by the t-Student test. A p-value less than 0.05 was considered statistically significant.

Results: 56 men and 27 women (mean age: 56.2±10.4 years). Baseline sclerostin levels (0.76±0.35 ng/ml) decreased significantly early (0.55±0.22 ng/ml in the first month, p=0.034), a trend that remained until 12 months (0.62±0.22 ng/ml, p=0.047). On the contrary, the basal levels of osteocalcin (17±10.3 ng/ml) and β-crosslaps (0.44±0.3 ng/ml) increased significantly throughout the study; in the case of osteocalcin, up to 12 months (37.27±26.84 ng/ml, p<0.01) and β-crosslaps, up to 6 months (0.62±0.34 ng/ml, p<0.01), with a subsequent decrease (0.47±0.31 ng/ml, p=0.2).

Conclusions: There is a decrease in the levels of sclerostin after liver transplantation, as opposed to the elevation of other markers of remodeling, β-crosslaps and osteocalcin. More studies are needed to determine if these changes have an impact on the occurrence of osteoporosis in patients undergoing transplantation.

Key words: sclerostin, liver transplant, bone resorption, bone formation, vitamin D deficiency.

Introduction

Solid organ transplantation is an effective alternative in the final stage of multiple chronic diseases, increasing patients' survival. However, this improvement is associated with certain complications, such as a higher incidence of osteoporosis and an increased risk of fractures[1]. Numerous studies have concluded that there is a loss of bone mass after transplantation, more marked between the first three and six months, which lasts up to a year after the same. Subsequently there is a stabilization and even recovery of bone mass in the two subsequent years[2-4].

Liver transplantation is considered an independent risk factor in the development of osteoporosis[1-3]. In the case of patients with a liver graft, the incidence of fracture is estimated at 10-43%[1], with the spine location being the most frequent[2-4]. Among the factors that contribute to the increased risk of osteoporosis and fractures in these patients are: prolonged treatment with immunosuppressants (mainly calcineurin inhibitors)[2,5-8] and glucocorticoids[9,10], vitamin D deficiency (very common due to malnutrition) and alterations in liver function found in most patients with cirrhosis[1-3].

The biochemical markers of bone remodeling offer information based on the dynamic prediction of the same, its accepted clinical application being the evaluation of the therapeutic response with antiresorptives[11,12]

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and its potential relationship with the risk of fracture. However, at present, there is no consensus regarding the determination of biochemical markers of bone remodeling in patients with liver transplantation[3]. Sclerostin (SOST) is a protein synthesized by the osteocyte that plays a central role in the regulation of bone remodeling, since it simultaneously acts as a negative regulator of bone formation and stimulates bone resorption through the RANK-ligand[13]. Its usefulness as a biochemical marker of bone remodeling, particularly in liver transplant patients, has not been established.

Thus, our study aims to assess the development of sclerostin levels in patients with liver transplantation, and investigate their relationship with other markers of bone remodeling.

Patients and methods

Study design and patient selection

This is a prospective observational study, developed from 2015 to 2017, in a single center: the University Hospital 12 de Octubre (Bone Metabolism Unit of the Endocrinology and Nutrition Service). We included 83 Caucasian patients, fulfilling the condition of being candidates for a liver transplant (regardless of the etiology of the liver disease). Patients who had received drugs that could interfere with bone remodeling prior to transplantation were excluded. The center's Ethics Committee approved the study and a signed informed consent was obtained from all the participants. In all patients, a descending steroid regimen was used up to a maintenance dose of prednisone of 20 mg over the first six months (as part of the usual center transplant protocol). The SOST, β-crosslaps (CTX), bone alkaline phosphatase (BAP), osteocalcin (OC) and C-reactive protein (CRP) values were determined the week before the transplant and subsequently, at 1, 3, 6 and 12 months. The determination of 25 hydroxy-vitamin D [25 (OH) D] and intact parathyroid hormone (PTH) was carried out basally. Likewise, in each of the reviews, the existence of fractures was evaluated.

Biochemical determinations

The patients' serum samples were obtained between 8:00 and 9:00 hours, after an overnight fast, and they were kept frozen at -70ºC. Bone metabolism markers included: OC (Cobas e602, electrochemiluminescence, normal range: 8-48 ng/ml) and BAP (IDS, Roche Diagnostics, enzyme immunoassay, normal range: 4.0-20.0 ng/ml) as parameters of bone formation, and CTX (Cobas e602, electrochemiluminescence, normal range: 0.200-0.704 ng/ml) as a resorption parameter. Likewise, SOST was determined by enzyme immunoassay (Human Sclerostin, TECO Medical Group, normal range: 0.22-1.1 ng/ml). PTH levels were determined by electrochemiluminescence (Cobas e602, normal range: 7.0-57.0 pg/ml). Serum levels of 25 (OH) D were determined by chemiluminescence (Architect 2000, Abbot Diagnostics). Although there is currently no criterion on the optimal serum levels of 25 (OH) D, most authors define a deficiency of values below 20 ng/ml as deficiency. Serum levels between 21 and 29 ng/ml can be considered as relative insufficiency, and higher than 30 ng/ml indicate sufficiency of the same[14]. PCR was determined by immunoturbidimetry (C-Reactive Protein Gen.3, Roche Diagnostics, normal range less than 0.1-0.5 mg/dL).

Fractures

In each of the revisions made to the patients, the existence of fractures was evaluated by means of clinical anamnesis made to the patient and re-evaluation of risk factors for them. In the case of suspicion of asymptomatic or paucisymptomatic osteoporotic fractures (such as vertebral crushing), a thoracolumbar radiography was carried out[15].

Statistic analysis

The statistical analysis was carried out using the Statistical Package for the Social Sciences, SPSS (version 21.0, IBM, Armonk, New York, USA). The normal distribution was confirmed by the Shapiro Wilks test. The evolution of the markers with respect to the baseline value was determined by the t-Student test. All results were expressed as mean ± standard deviation (SD). A p-value of less than 0.05 was considered statistically significant.

Results

A total of 83 patients receiving a liver transplant (56 males and 27 females) were included in the study. The average age of the patients was 56.2±10.4 years. The group's average weight was 72.1±18.7 kg and its BMI was 27.7±7.5 kg/m^2.

Evolution of markers of bone remodeling

The basal levels of SOST were 0.76±0.35 ng/ml and decreased significantly early (0.55±0.22 ng/ml in the first month, p=0.034), a trend that was maintained until the end of the study. of the study (0.62±0.22 ng/ml, p=0.047) (Table 1). There were no significant differences between both sexes in the evolution of the aforementioned marker. The SOST levels did not correlate with the development of fractures. On the contrary, OC levels (17±10.35 ng/ml) showed a progressive and significant increase from the 3rd month after transplantation (31.85±26 ng/ml, p<0.01), which it was maintained until the end of the follow-up period (37.27±26.84 ng/ml, p<0.01). In both sexes, the evolution of OC levels was similar.

In relation to CTX, the levels prior to transplantation were 0.44±0.35 ng/ml. One month after transplantation, a significant increase was observed (0.81±0.47 ng/ml, p<0.01) that persisted until 6 months (0.62±0.34 ng/ml, p<0.01) compared to basal level. At 12 months, there was a marked decrease in CTX towards the value before transplantation (0.47±0.31 ng/ml, p=0.2). There were no differences regarding CTX development between both sexes but, since the third month, the group of women had significantly higher levels than men (0.94±0.62 ng/ml and 0.61±0,34 ng/ml, respectively, p<0.01). The levels of PA presented discrete variations throughout the study, without showing significant changes in any of the determinations (Table 1), nor differences between sexes.

There were no statistically significant correlations between the different markers of bone metabolism in the study.

Vitamin D and PTH

At the time of transplantation, 25 (OH) D levels were in the deficiency range: 10.4±6.5 ng/ml. 82.1% of the patients had deficiency (levels of 25 (OH) D <20 ng/ml) and 17.9% levels of relative insufficiency. As for the PTH, the initial mean value was slightly above the high limit

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Table 1. Development of sclerostin and the rest of the markers of bone remodeling throughout the study (mean ± standard deviation)

	Basal	1st month	3rd month	6th month	12th month
OC (ng/ml)	17 ± 10.35	17.95 ± 12.40	31.85 ± 26	35.75 ± 32.63	37.27 ± 26.84
AP (ng/ml)	34.87 ± 17.8	30.16 ± 13.77	27.97 ± 11.93	42.07 ± 21.23	31.05 ± 11.41
CTX (ng/ml)	0.44 ± 0.35	0.81 ± 0.47	0.72 ± 0.48	0.62 ± 0.34	0.47 ± 0.31
SOST (ng/ml)	0.76 ± 0.35	0.55 ± 0.22	0.63 ± 0.23	0.63 ± 0.30	0.62 ± 0.22

OC: osteocalcin; AP: alkaline phosphatase; CTX: β-crosslaps; SOST: sclerostin.

of the normal range (78.8±52 pg/ml). No correlations of interest were found between the serum values of PTH and 25(OH)D and the markers of bone remodeling included in the study.

Inflammation

C-reactive protein (CRP) levels were elevated before transplantation (4.77±3.8 mg/dL). After the intervention, there was a progressive and significant decrease during the first 3 months of the study, up to a figure of 1.3±3.5 mg/dL ($p<0.005$).

Fractures

Throughout the year of follow-up, 3 fractures were observed: a vertebral crush, and 2 Colles fractures (one of them after a trauma in an accident). There was no statistically significant correlation between the development of fracture after transplantation and the different markers of bone metabolism.

Discussion

In recent years it has been proposed that SOST, glycoprotein of 213 amino acids secreted by the osteocyte, and that produces an inhibition of bone formation by suppressing the Wnt/β-catenin intracellular signaling pathway, could be a biomarker of central importance in the evaluation of bone remodeling[16]. However, there is little information about SOST levels after a solid organ transplant, although patients undergoing this procedure suffer osteoporosis very often and, in particular, vertebral fractures. Thus, in a study carried out on bone biopsies of patients undergoing different types of transplantation (kidney, liver, heart), an increase in SOST expression evaluated by immunohistochemistry has been described[17].

In the present observational prospective study, the evolution of the levels of sclerostin (SOST) and other markers of bone remodeling during 12 months after a liver transplant was evaluated. Our results show a significant decrease in the serum levels of SOST, as opposed to an increase in the rest of remodeling markers (OC and CTX).

These results are similar to those described in patients with kidney transplantation, in whom a marked decrease (30-60%) in serum levels of SOST after transplantation is observed[18,19], especially in the first 2 months after the intervention. In our study the most marked decrease also occurs in the first month. Until now, it has not been possible to establish a relationship between the levels of SOST in patients with kidney transplants and the risk of fractures or cardiovascular events, although it does occur with the presence of vascular calcifications[20].

In renal transplantation, one of the main factors that justify the initial reduction of SOST could be an increased loss of urine due to tubular dysfunction due to overload, typical of the initial period after transplantation[21]. In the case of the liver, previous studies have shown that in certain diseases that may require transplant, such as primary biliary cirrhosis, patients had increased sclerostin levels[22]. Among the possible factors that would justify the initial decrease in SOST could be the imporved pro-inflammatory situation after surgery and immunosuppression.

Regarding inflammation, in our study CRP levels were basally elevated and decreased significantly throughout the year (although there was no correlation between the value of SOST and that of CRP). However, in the literature there are examples in which the possible relationship between SOST and inflammation has not been confirmed. Thus, in a previous study in patients with rheumatoid arthritis treated with TNF inhibitors (tumor necrosis factor) no effect of anti-inflammatory treatment on the levels of SOST was evidenced[23].

The decrease in SOST observed in the patients of our study could be one of the causes that justifies the improvement in mineral metabolism after the intervention, just as it has been considered in patients undergoing a kidney transplant18,19. One of the proposed hypotheses is that the changes in the SOST would reflect the optimization of osteocyte function after transplantation[24].

In a study previously carried out in kidney transplant patients, men had a similar SOST level than women before surgery[17]. Similarly, in our study we did not observe any difference between SOST serum concentrations in both sexes, neither at the beginning of the study nor during the follow-up.

Regarding the rest of the bone remodeling markers, in our study we observed an increase in markers of bone formation (OC), as well as those of bone resorption (CTX). These results are consistent with those already presented in the literature, after liver transplantation25. Recently, an increase in CTX and N-terminal propeptide of type I procollagen (P1NP) was observed 6 months after liver transplantation[25]. These results, similar to those of our group, seem to confirm the existence of a high bone remodeling in patients undergoing a transplant. In this context, it is worth highlighting the influence of steroids (as part of the treatment after transplantation), favoring resorption and suppressing bone formation, especially during the first six months after surgery[9,10].

In addition to the determination of bone remodeling markers, in our study a high 25 (OH) D deficiency rate prior to transplantation (82.1%) was also confirmed. After adequate supplementation, a significant improvement in 25 (OH) D levels was observed, until the mean value was placed in the insufficiency range. In parallel, slightly elevated levels of PTH were observed (in a probable context of hyperparathyroidism secondary to vitamin D deficiency) before surgery, with normalization at 12 months, after improvement in the 25 (OH) D figures. There are multiple factors that influence the insufficient levels of 25 (OH) D prior to transplantation: proinflammatory state, higher prevalence of malnutrition, loss of liver contribution by hydroxylation of its precursor, etc.3 Several previous studies have reported high rates of 25 (OH) D deficiency, although not as high as those shown in our cohort. Thus, in a group of patients undergoing liver transplantation, a deficiency rate of 25 (OH)D of 37% was observed, improved at 6 months, with a deficiency rate of 17%[25].

Our study presents several strengths. First, its optimal sample size (n=83) and the fact that this is a longitudinal and prospective study. Finally, it is the first study that includes SOST determination after liver transplantation. Despite this, the study has certain limitations. Bone mineral density was not evaluated, nor the etiology of the liver disease that motivated the transplant. On the other hand, the low number of fractures observed does not allow us to draw conclusions about the real impact of these changes on the risk of post-transplant osteoporosis[26].

In summary, our results show a decrease in SOST levels after liver transplantation, which goes in the opposite direction to the variations observed in other remodeling markers such as CTX and OC.

Deficiency of 25 (OH) vitamin D pre-transplant is high and improves after supplementation. More studies are needed to determine if these changes have a significant impact on the occurrence of osteoporosis or long-term cardiovascular disease in patients undergoing transplantation.

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Conflict of interests: The authors declare no conflict of interest.

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