

Serum Sclerostin in Hepatitis C Virus Infected Patients

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Background: Sclerostin inhibits osteoblast functions, differentiations, and survival rates. As an endogenous inhibitor of the Wnt/ β -catenin pathway, the sclerostin should be related to decreased bone masses, although several studies indicate opposite results. In addition, it may be related to insulin resistances and carbohydrate metabolisms, a relation shared with other markers of bone metabolisms, such as osteocalcin. Hepatitis C virus (HCV) infected patients may present osteoporosis, and frequently show liver steatosis, which is a consequence of insulin resistance. The behaviour of sclerostin in these patients is yet unknown. The aim of this work is to analyse the relationships between serum sclerostin and osteocalcin levels and bone mineral density (BMD), liver functions, the intensity of liver steatosis and biochemical markers of bone homeostasis and insulin resistance in HCV-infected patients. **Methods:** Forty HCV patients with 20 years of age and gender-matching controls were included in this study and underwent bone densitometry. Serum sclerostin, osteocalcin, collagen telopeptide, adiponectin, leptin, insulin, resistin, tumor necrosis factor (TNF)- α , and interleukin (IL)-6 were determined. Liver fat was histomorphometrically assessed. **Results:** Sclerostin levels were slightly higher in patients than in controls, and were directly related to BMD at different parts of the skeleton, also to the serum telopeptide, and to the liver steatosis and TNF- α . On the contrary, osteocalcin showed a significant direct relationship with serum adiponectin, and an inverse one with IL-6. **Conclusions:** Serum sclerostin levels were within the normal range in HCV patients, and correlated directly with BMD and serum telopeptide. In addition, the relationships of sclerostin and osteocalcin with variables associated with insulin resistance suggested the role of bones for intermediary metabolisms.

Key Words: Fatty liver, Hepacivirus, Insulin resistance, Osteocalcin

INTRODUCTION

Sclerostin is secreted by osteocytes.[1] It is a key signalling protein involved in the mechanosensing function of these cells, being an endogenous inhibitor of the Wnt/ β -catenin pathway.[2] By binding to low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6), it antagonizes Wnt/ β -catenin signaling[3] and decreases bone turn-over[4,5] and bone mass.[6,7] However, several reports have failed to find these expected results. Sheng et al.[8] found low sclerostin levels in osteoporotic women; Faje et al.[9] found no changes in sclerostin levels in girls affected by anorexia nervosa; García-Martín et al.[10] also report lower sclerostin lev-

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els in type 2 diabetic patients with osteoporosis. In a recent report we showed that sclerostin levels, although higher among alcoholics, were related to liver function derangement instead to bone mineral density (BMD), although a relationship was observed with decreased markers of bone synthesis and increased markers of bone breakdown.[11] In addition, Cejka et al.[12] and Polyzos et al.[13] also report a positive correlation between sclerostin levels and BMD in renal patients and osteoporotic postmenopausal women, respectively. In diseases characterized by increased bone turnover, such as Paget disease or metastatic prostate cancer, serum sclerostin levels were significantly increased.[14] Therefore, although it seems clear that sclerostin levels are related to bone turn-over markers, relationships with BMD are, in most cases, either absent or even opposite to what expected.

In some studies, correlations were reported between sclerostin and parameters apparently unrelated to bone mass, such as liver function,[11] glycated haemoglobin,[10] or atheromatosis.[15] High levels of serum sclerostin have been reported for patients affected by type 2 diabetes.[16] The possibility exists that sclerostin is related to insulin resistance, adding a new link in the recently described connection between bone-secreted proteins, such as osteocalcin or sclerostin, beta cell function, adipokines, and insulin sensitivity.[17]

Liver steatosis constitutes one of the outstanding manifestations of insulin resistance in hepatitis C virus (HCV) infection. HCV by itself -especially genotype 3- may lead to liver steatosis, but obesity and concomitant alcohol abuse are the main factors involved.[18] The "two hit theory" sustains that cytokine activation and increased lipid peroxidation contribute to the progression of simple steatosis to steatohepatitis.[19] In this scenario, fat tissue is not only the source of fatty acids, but also produces a number of proinflammatory cytokines which are of paramount importance in the progression of liver disease. Most of them are involved in insulin resistance, but some others, such as adiponectin, exert a protective role. Interestingly, it has been recently described that osteocalcin stimulates secretion of adiponectin from osteocytes.[20] On the other hand, adiponectin may stimulate proliferation, differentiation and mineralization of osteoblasts.[17]

Several studies report osteopenia among patients with HCV infection,[21-25] although possibly osteopenia is rath-

er related to liver function derangement and/or altered nutritional status than to virus infection. In fact, some studies including relatively large series fail to detect significant bone alterations in HCV patients.[26,27] Moreover, osteosclerosis has been described in several cases of chronic HCV infection.[28-30]

Based on the aforementioned statements, it is important to explore which is the role of serum sclerostin on bone alterations observed in HCV patients, and which is the relation of sclerostin and osteocalcin with fat-derived cytokines involved in insulin resistance and with one of the consequences of insulin resistance, such as liver fat accumulation.

METHODS

We included 40 treatment-naïve, HCV-infected patients, who were admitted to the hospital for programmed liver biopsy previous to antiviral treatment with interferon and ribavirin, 22 men and 18 women, aged 41.18 ± 9 years. Eleven of them also drank ethanol in significant amounts (>80 g/day during at least 5 years), and 14 were co-infected by human immunodeficiency virus (HIV).

Patients were compared with 20 controls, 4 women and 16 men ($\chi^2=2.60$; $P=0.11$), aged 43.65 ± 9.7 years ($t=0.98$; $P=0.33$), drinkers of less than 10 g ethanol/day. Diagnosis of HCV infection was assessed both by the presence of anti-HCV antibodies and HCV RNA by reverse transcriptase polymerase chain reaction (PCR). According to PCR, 27 belonged to genotype 1, 7 to genotype 3, and 4 to genotype 4. In two cases, genotype was unknown.

1. Bone densitometry

After informed consent, patients underwent densitometric evaluation with a Lunar Prodigy Advance device (General Electric, Piscataway, NJ, USA), recording BMD, fat, and lean mass at different parts of the body, such as upper limbs, ribs, pelvis, lower limbs, spine, and total body. Body mass index (BMI; as weight [in kg]/height² [in m]) was also recorded.

2. Biochemical assessment

Blood samples were taken at 8.00 am in fasting conditions, and immediately frozen at -20° . In addition to routine laboratory evaluation, the following parameters were determined :

Serum sclerostin, by one step enzyme-linked immunosorbent assay (ELISA) (Biomedica Gruppe, Vienna, Austria; interassay variation coefficient 4-6%; intra-assay variation coefficient 5%); tumour necrosis factor (TNF)- α by immunometric chemiluminiscent assay; (intra-assay variation coefficient ranging 4-6.5%, interassay variation coefficient ranging 2.6-3.6%, recovery 92-112%, Diagnostic Products Corporation [DPC], Los Angeles, CA, USA; interleukin (IL)-6, by chemiluminiscent assay; (interassay variation coefficient ranging 5.3-7.5%, recovery=85-104%, DPC, Los Angeles, CA, USA); serum insulin, by immunoanalysis (Chemiflex); interobserver variation coefficient=1.9-5.2%; intraobserver variation coefficient=1.7-4.2%; sensitivity=1 μ U/mL; recovery=91.1-101.6% (Architect system; Abbott, Wiesbaden Germany); serum resistin, by ELISA (sensitivity=0.033 ng/mL; intra-assay variation coefficient=2.8-3.4%; interassay variation coefficient ranging 5.1-6.9%, recovery=85.2-99.2%, [Biovendor, Heidelberg, Germany]); serum leptin, by ELISA (sensitivity=0.2 ng/mL; intra-assay variation coefficient=4.2-7.6%; interassay variation coefficient ranging 4.4-6.7%, recovery=85.7-98.0%, [Biovendor, Heidelberg, Germany]); serum adiponectin by ELISA (sensitivity=26 ng/mL; intra-assay variation coefficient=3.9-5.9%; interassay variation coefficient ranging 6.3-7%, recovery=92.4-102.9%, [Biovendor, Heidelberg, Germany]); insulin resistance was estimated by the homeostatic model assessment (HOMA). We also determined serum osteocalcin, by immunometric chemiluminiscent assay (recovery=97-121%; variation coefficients of assays ranging from 3.5-7.1%; DPC [Los Angeles, CA, USA]), as a marker of bone synthesis, and C-terminal telopeptide of type I collagen (CrossLaps), by one step ELISA, with a recovery ranging from 94-107% and an intra- and interassay variation coefficient ranging 4.7-4.9% and 5.4-8.1%, respectively (Osteometer Bio Tech A/S, Herlev, Denmark), as a marker of bone breakdown. As shown in table 1, not all the variables were determined to all the patients and controls.

3. Histological assessment

The amounts of liver steatosis and fibrosis were histomorphometrically determined, using a specific software (LEICA-QW, version 3.0, Wetzlar, Germany) at 40x, using samples stained with haematoxylin-eosin and Masson trichromic. Both parameters were measured as proportion of fat area in relation with the total area, and proportion of fibrous tis-

sue in relation with total area. Knodell index and Metavir score were also recorded

The study protocol was approved by the local ethics committee of our Hospital and was carried out in accordance with the ethical guidelines of the 1975 Declaration of Helsinki.

4. Statistics

The Kolmogorov-Smirnov test was used to test for normal distribution. Student's t test and Mann-Whitney's U test (for variables with non-parametric distribution) were used to compare mean values between two different groups. Spearman's correlation and Pearson's correlation were used to compare quantitative variables, and χ^2 test was used to compare qualitative variables.

All these analyses were performed using SPSS software (SPSS Inc., Chicago, IL, USA).

RESULTS

They are shown in Table 1. All patients had normal serum creatinine values (range=0.50-1.10 mg/dL; median [interquartile range]=0.70 [0.62-0.86] mg/dL). Patients had higher BMD values at spine, arms, and total BMD ($P<0.05$ in all the cases) than controls (Table 1).

Sclerostin levels were slightly, non-significantly, higher in patients (17.78 ± 6.37 pmol/L) than controls (15.06 ± 3.93 pmol/L, $t=1.09$; $P=0.28$). A direct correlation was observed between sclerostin levels and BMD at arms ($r=0.33$), ribs ($r=0.34$), pelvis ($r=0.38$), legs ($r=0.45$) and total BMD ($r=0.39$; $P<0.05$ in all the cases). BMD was negatively correlated with age, especially at legs ($r=-0.51$), pelvis ($r=-0.46$) and total BMD ($r=-0.47$, $P<0.005$ in all the cases), but no relation was observed between sclerostin and age. No differences in sclerostin levels were observed between alcoholic and non-alcoholic patients, or between those co-infected by HIV and those not co-infected by HIV.

1. Relationship with biochemical markers of bone homeostasis and hormones

HCV patients showed lower osteocalcin, and higher telopeptide levels than controls (Table 1), suggesting an increased bone breakdown than controls. Sclerostin showed a direct correlation with serum telopeptide ($r=0.44$; $P=0.05$), but not with osteocalcin. It also showed a direct cor-

Table 1. Main variables utilized in this study in patients and controls

	Patients		Controls		
	N	X ± SD Median (IQ range)	N	X ± SD Median (IQ range)	
Insulin (µU/mL)	37	13.25 ± 16.71 9.41 (5.45-14.57)	10	8.34 ± 4.34 7.15 (5.08-10.63)	Z=0.54; NS
Resistin (ng/mL)	37	4.66 ± 1.41	10	4.28 ± 1.42	T=0.75; NS
Adiponectin (ng/mL)	37	11.41 ± 9.42	16	24.92 ± 21.84	T=2.38; P=0.04
Leptin (ng/mL)	37	13.93 ± 16.07 7.19 (1.86-22.28)	10	18.41 ± 16.03 12.89 (4.65-34.42)	Z=1.32; NS
Tumor necrosis factor-α (pg/mL)	40	13.18 ± 9.06 10.90 (6.98-13.08)	16	5.86 ± 2.00 5.05 (4.10-8.15)	Z=4.65; P<0.001
Interleukin- 6 (pg/mL)	37	5.60 ± 4.91 3.22 (2.0-7.89)	16	5.94 ± 1.75 5.0 (5.0-6.55)	Z=4.78; P<0.001
Body mass index (kg/m ²)	40	24.80 ± 3.58	20	26.05 ± 3.66	T=1.26; NS
Total BMD (g/cm ²)	38	1.166 ± 0.112	20	1.094 ± 0.101	T=2.40; P=0.02
Lower limbs BMD(g/cm ²)	38	1.267 ± 0.158	20	1.319 ± 0.098	T=1.33; NS
Upper limbs BMD(g/cm ²)	38	0.929 ± 0.155	20	0.850 ± 0.051	T=3.02; P=0.004
Ribs BMD (g/cm ²)	38	0.679 ± 0.072	20	0.683 ± 0.070	T=0.20; NS
Pelvis BMD (g/cm ²)	38	1.132 ± 0.127	20	1.142 ± 0.113	T=0.28; NS
Spine BMD (g/cm ²)	38	0.924 ± 0.155	20	0.798 ± 0.080	T=4.10; P<0.001
Femoral neck BMD	36	0.979 ± 0.140	20	0.947 ± 0.083	T=1.06; NS
Total femur BMD	26	1.012 ± 0.144	20	1.051 ± 0.101	T=1.02; NS
Osteocalcin (ng/mL)	39	4.62 ± 2.36	20	7.37 ± 2.56	T=4.11; P<0.001
Serum telopeptide (ng/mL)	20	0.371 ± 0.221	19	0.210 ± 0.101	T=2.95; P=0.007
Sclerostin (pmol/L)	40	17.78 ± 6.37	7	15.06 ± 3.93	T=1.09; NS
Serum calcium (mg/dL)	39	9.26 ± 0.45	-	-	-
Serum alkaline phosphatase (U/mL)	39	79.10 ± 23.20	-	-	-
Fat area (in % of total biopsy area)	36	4.51 ± 7.43 1.21 (0.07-5.66)	-	-	-
Amount of fibrosis (%)	36	8.51 ± 8.35 5.30 (3.30-9.25)	-	-	-
Prothrombin activity (%)	40	95.60 ± 6.32	-	-	-
Serum albumin (g/dL)	40	4.39 ± 0.34	-	-	-
Serum bilirubin (mg/dL)	40	0.78 ± 0.61	-	-	-

We provide means ± standard deviations (SD), in addition to median and interquartile (IQ) ranges for those variables with a non-parametric distribution. In these cases, Mann-Whitney's U test was used to compare patients and controls. Otherwise, Student's t test was applied. $P > 0.10$. BMD, bone mineral density; NS, non significant.

relation with serum calcium levels ($r=0.40$; $P=0.012$) and with total alkaline phosphatase ($r=0.35$; $P=0.028$).

2. Relationship with liver function, cytokines, and liver histology

Sclerostin was significantly, directly correlated with serum bilirubin ($r=0.32$; $P=0.046$), but no relationships at all were observed with albumin, prothrombin activity, age, Knodell index or metavir activity and metavir fibrosis score.

Liver fat could be properly analysed in 36 out of the 40 patients. We found a direct significant relationship be-

tween sclerostin levels and liver fat amount ($r=0.44$; $P=0.007$) and serum TNF-α levels ($r=0.39$; $P=0.014$), but not with adiponectin, leptin, resistin, insulin, or HOMA. Osteocalcin was inversely related with IL-6 ($r=-0.36$; $P=0.033$), and directly with adiponectin ($r=0.51$; $P=0.001$), but not with liver fat, Knodell index or metavir scores.

DISCUSSION

This study shows that sclerostin levels were only slightly raised in HCV patients compared with controls. This result

could be explained, at least in part, by the fact that BMD in the included patients was similar, or even higher, than that of the controls, an already reported finding.[27] However, a paradoxical positive correlation was observed between BMD and sclerostin. As commented earlier, sclerostin antagonizes Wnt/ β -catenin signaling,[3] and decreases bone turn-over[4,5] and bone mass,[6,7] but several authors report results similar to those obtained in this study: a positive correlation between sclerostin and BMD, instead of a negative one. This is the case of Čejka et al.[12] in renal patients, Polyzos et al.[13] and Sheng et al.[8] in postmenopausal women, or García-Martín et al.[10] in diabetic patients, among other authors. The mechanisms underlying these paradoxical results are unknown. It has been hypothesized that by slowing bone turn-over, with lower than normal bone synthesis but even a more decreased bone breakdown, high sclerostin levels could be related to increased bone mass,[12] but we also found a direct correlation between sclerostin levels and bone breakdown, in accordance with the observations of Yavropoulou et al.[14] who report increased sclerostin levels in diseases characterized by increased bone turnover, such as Paget disease or metastatic prostate cancer, and those of Gaudio et al.[31] who found a direct correlation between sclerostin and crosslaps in long-term immobilized patients. The significant correlations with serum calcium and total alkaline phosphatase levels reported in this study could be also related with increased bone breakdown, an effect theoretically opposite to the mechanisms of action of sclerostin. Others have proposed that increased sclerostin levels observed in these situations is due to inflammation.[17] The positive correlation between TNF- α and sclerostin reported in the present study is in accordance with that hypothesis.

We found a significant correlation between the intensity of liver steatosis and sclerostin levels. This result is apparently unexpected. However, in recent times, there are data which support a role of bone on intermediary metabolism. For instance, osteocalcin may promote insulin secretion and exert insulin-sensitizing effects;[32,33] and, as commented, a significant relationship between sclerostin levels and glycated haemoglobin was reported.[10] In this sense, adiponectin may be also viewed as a "protective" cytokine, whereas leptin, resistin, TNF- α and IL-6 may be associated to insulin resistance.[34] In accordance with these functions, we found an inverse correlation between osteo-

calcin and IL-6 levels, but a direct one with adiponectin. In very recent reports, inverse correlations have been found between arteriosclerosis and osteocalcin,[33,35] fully in accordance with the results of this study.

In HCV-infected patients, liver steatosis is a consequence of insulin resistance, which may be due both to a direct effect of genotype 3 viral particles and to disordered adipokine and insulin metabolism. One of the adipokines with potent contrainsular effects is TNF- α . In this study, remarkably, TNF- α was also related to serum sclerostin, a result consistent with the finding of a correlation with liver fat accumulation. However, besides the relationship with bilirubin, sclerostin was unrelated to any other parameter of liver function, in contrast with the results observed among alcoholic patients,[11] in whom sclerostin was clearly related with liver function impairment. The relative preservation of a good liver functional reserve among the patients included in this study may explain the lack of correlation obtained.

On the contrary, sclerostin and osteocalcin did show some relationships which suggest a role of bone on intermediary metabolism and glucose homeostasis, not only in our study, but in others performed elsewhere. In this sense, García-Martín et al.[10] found a significant correlation between both glycated haemoglobin and the duration of type 2 diabetes mellitus and sclerostin, which was directly -not inversely- related with BMD, as in this study. Further analysis led to the discovery of a relationship between sclerostin and arteriosclerosis,[15] a condition also related to altered lipid metabolism. The precise mechanisms involved in the relation of osteocalcin and sclerostin with carbohydrate and lipid metabolism are only partially known, but in this study we also report findings which are in accordance with the hypothesis that, in fact, bone may exert regulatory functions on glucose and fat metabolism. In this context we can explain the direct correlation between osteocalcin and adiponectin, the relationships between sclerostin and liver steatosis, and the relationships of both bone proteins with TNF- α . and IL-6, cytokines strongly linked to insulin resistance.

Therefore, we conclude that sclerostin levels are only slightly, non-significantly raised in treatment-naïve HCV patients with preserved liver function and normal BMD. However, sclerostin levels show a positive correlation with BMD and increased bone breakdown. Moreover, sclerostin

levels are related to the intensity of liver steatosis, and to TNF- α levels. On the other hand, osteocalcin is closely related to adiponectin, and, inversely, to IL-6. All these data suggest that osteocalcin and sclerostin may play a role on intermediary metabolism in HCV patients, this last being also related with increased bone mass and bone breakdown. These results are paradoxical, but are in accordance with those reported by other researchers in other pathological conditions, and support the existence of a link between bone cells-derived proteins, insulin resistance, and inflammation.

REFERENCES

1. van Bezooijen RL, Papapoulos SE, Löwik CW. Bone morphogenetic proteins and their antagonists: the sclerostin paradigm. *J Endocrinol Invest* 2005;28 Suppl:15-7.
2. Williams BO, Insogna KL. Where Wnts went: the exploding field of Lrp5 and Lrp6 signaling in bone. *J Bone Miner Res* 2009;24:171-8.
3. Li X, Zhang Y, Kang H, et al. Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. *J Biol Chem* 2005; 280:19883-7.
4. Gaudio A, Privitera F, Battaglia K, et al. Sclerostin levels associated with inhibition of the Wnt/beta-catenin signaling and reduced bone turnover in type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012;97:3744-50.
5. Ardawi MS, Rouzi AA, Qari MH. Physical activity in relation to serum sclerostin, insulin-like growth factor-1, and bone turnover markers in healthy premenopausal women: a cross-sectional and a longitudinal study. *J Clin Endocrinol Metab* 2012;97:3691-9.
6. van Bezooijen RL, Roelen BA, Visser A, et al. Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. *J Exp Med* 2004; 199:805-14.
7. Poole KE, van Bezooijen RL, Loveridge N, et al. Sclerostin is a delayed secreted product of osteocytes that inhibits bone formation. *FASEB J* 2005;19:1842-4.
8. Sheng Z, Tong D, Ou Y, et al. Serum sclerostin levels were positively correlated with fat mass and bone mineral density in central south Chinese postmenopausal women. *Clin Endocrinol (Oxf)* 2012;76:797-801.
9. Faje AT, Fazeli PK, Katzman DK, et al. Sclerostin levels and bone turnover markers in adolescents with anorexia nervosa and healthy adolescent girls. *Bone* 2012;51:474-9.
10. García-Martín A, Rozas-Moreno P, Reyes-García R, et al. Circulating levels of sclerostin are increased in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 2012; 97:234-41.
11. González-Reimers E, Martín-González C, de la Vega-Prieto MJ, et al. Serum sclerostin in alcoholics: a pilot study. *Alcohol Alcohol* 2013;48:278-82.
12. Cejka D, Jäger-Lansky A, Kieweg H, et al. Sclerostin serum levels correlate positively with bone mineral density and microarchitecture in haemodialysis patients. *Nephrol Dial Transplant* 2012;27:226-30.
13. Polyzos SA, Anastasilakis AD, Bratengeier C, et al. Serum sclerostin levels positively correlate with lumbar spinal bone mineral density in postmenopausal women--the six-month effect of risedronate and teriparatide. *Osteoporos Int* 2012;23:1171-6.
14. Yavropoulou MP, van Lierop AH, Hamdy NA, et al. Serum sclerostin levels in Paget's disease and prostate cancer with bone metastases with a wide range of bone turnover. *Bone* 2012;51:153-7.
15. Morales-Santana S, García-Fontana B, García-Martín A, et al. Atherosclerotic disease in type 2 diabetes is associated with an increase in sclerostin levels. *Diabetes Care* 2013; 36:1667-74.
16. Gennari L, Merlotti D, Valenti R, et al. Circulating sclerostin levels and bone turnover in type 1 and type 2 diabetes. *J Clin Endocrinol Metab* 2012;97:1737-44.
17. Starup-Linde J. Diabetes, biochemical markers of bone turnover, diabetes control, and bone. *Front Endocrinol (Lausanne)* 2013;4:21.
18. Woreta TA, Sutcliffe CG, Mehta SH, et al. Incidence and risk factors for steatosis progression in adults coinfecting with HIV and hepatitis C virus. *Gastroenterology* 2011;140:809-17.
19. James OF, Day CP. Non-alcoholic steatohepatitis (NASH): a disease of emerging identity and importance. *J Hepatol* 1998;29:495-501.
20. Motyl KJ, McCabe LR, Schwartz AV. Bone and glucose metabolism: a two-way street. *Arch Biochem Biophys* 2010; 503:2-10.
21. Gallego-Rojo FJ, Gonzalez-Calvin JL, Muñoz-Torres M, et al. Bone mineral density, serum insulin-like growth factor I, and bone turnover markers in viral cirrhosis. *Hepatology* 1998;28:695-9.

22. Corazza GR, Trevisani F, Di Stefano M, et al. Early increase of bone resorption in patients with liver cirrhosis secondary to viral hepatitis. *Dig Dis Sci* 2000;45:1392-9.
23. Carey EJ, Balan V, Kremers WK, et al. Osteopenia and osteoporosis in patients with end-stage liver disease caused by hepatitis C and alcoholic liver disease: not just a cholestatic problem. *Liver Transpl* 2003;9:1166-73.
24. Schiefke I, Fach A, Wiedmann M, et al. Reduced bone mineral density and altered bone turnover markers in patients with non-cirrhotic chronic hepatitis B or C infection. *World J Gastroenterol* 2005;11:1843-7.
25. Hofmann WP, Kronenberger B, Bojunga J, et al. Prospective study of bone mineral density and metabolism in patients with chronic hepatitis C during pegylated interferon alpha and ribavirin therapy. *J Viral Hepat* 2008;15:790-6.
26. Nanda KS, Ryan EJ, Murray BF, et al. Effect of chronic hepatitis C virus infection on bone disease in postmenopausal women. *Clin Gastroenterol Hepatol* 2009;7:894-9.
27. Pelazas-González R, González-Reimers E, Alemán-Valls MR, et al. Bone alterations in hepatitis C virus infected patients. *Eur J Intern Med* 2013;24:92-6.
28. Manganelli P, Giuliani N, Fietta P, et al. OPG/RANKL system imbalance in a case of hepatitis C-associated osteosclerosis: the pathogenetic key? *Clin Rheumatol* 2005;24:296-300.
29. Fiore CE, Riccobene S, Mangiafico R, et al. Hepatitis C-associated osteosclerosis (HCAO): report of a new case with involvement of the OPG/RANKL system. *Osteoporos Int* 2005;16:2180-4.
30. Shaker JL, Reinus WR, Whyte MP. Hepatitis C-associated osteosclerosis: late onset after blood transfusion in an elderly woman. *J Clin Endocrinol Metab* 1998;83:93-8.
31. Gaudio A, Pennisi P, Bratengeier C, et al. Increased sclerostin serum levels associated with bone formation and resorption markers in patients with immobilization-induced bone loss. *J Clin Endocrinol Metab* 2010;95:2248-53.
32. Wieczorek-Baranowska A, Nowak A, Pilaczyńska-Szcześniak Ł. Osteocalcin and glucose metabolism in postmenopausal women subjected to aerobic training program for 8 weeks. *Metabolism* 2012;61:542-5.
33. Confavreux CB, Szulc P, Casey R, et al. Higher serum osteocalcin is associated with lower abdominal aortic calcification progression and longer 10-year survival in elderly men of the MINOS cohort. *J Clin Endocrinol Metab* 2013;98:1084-92.
34. Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999;257:79-83.
35. Ogawa-Furuya N, Yamaguchi T, Yamamoto M, et al. Serum osteocalcin levels are inversely associated with abdominal aortic calcification in men with type 2 diabetes mellitus. *Osteoporos Int* 2013;24:2223-30.