THE CLINICAL IMPORTANCE OF BIOCHEMICAL BONE MARKERS IN PATIENTS WITH ALCOHOLIC AND VIRAL LIVER CIRRHOSIS

KLINIČKI ZNAČAJ BIOHEMIJSKIH KOŠTANIH MARKERA KOD PACIJENATA SA ALKOHOLNOM I VIRUSNOM CIROZOM JETRE

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Summary

Background: Metabolic bone disease in patients with chronic liver disease is called hepatic osteodystrophy and is primarily a sequel to osteopenia/osteoporosis, and rarely secondary to osteomalacia. The aim of this work was to define the influence of vitamin D_3 and parathyroid hormone (PTH) in the pathogenesis of hepatic osteodystrophy, as well as the predictive significance of biochemical bone markers.

Methods: This prospective study included 58 male patients with alcoholic (49) and viral (9) cirrhosis. The concentrations of serum vitamin D_3, PTH, osteocalcin and β-carboxy-terminal cross-linked telopeptide of type I collagen (β-CTX) were determined. Bone density was measured by dual energy X-ray absorptiometry in the L1-L4 spinal segment and the femoral neck.

Results: Lower bone mineral density (BMD) was measured in 41 patients (70.7%). There was no significant correlation between PTH and vitamin D_3 values and T score in the femoral neck (p=0.51; p=0.063) and lumbar spine (p=0.49; 0.064). Also, no significant correlation was found between the osteocalcin values in lumbar spine BMD (p=0.944) and femoral neck (p=0.161), or with β-CTX values and BMD in the lumbar spine (p=0.347) and femoral neck (p=0.73). Statistically significant difference was confirmed between the stage A osteocalcin (p=0.000) and β-CTX (p=0.008) values in relation to advanced stages B and C.

Kratak sadržaj

Uvod: Metabolička bolest kostiju kod pacijenata sa hroničnim oboljenjem jetre naziva se hepatička osteodistrofija i primarno je posledica osteopenije ili osteoporoze a ređe osteomalacije. Cilj rada je bio da se determiniše uloga paratireoidnog hormona (PTH) i vitamina D_3 u patogenezi hepatičke osteodistrofije, kao i prediktivni značaj biohemijskih koštanih markera.

Metode: U prospektivnoj studiji, obuhvaćeno je 58 pacijenata muškog pola sa alkoholnom (49) i virusnom (9) cirozom jetre. Određivane su serumske koncentracije vitamina D_3, PTH, osteokalcina i β-C-terminalnog telopeptida tip I kolagena (β-CTX). Koštana gustina je merena dvostrukom X-zračnom apsorpciometrijom na L1-L4 segmentu kičme i vratu butne kosti.

Rezultati: Smanjena mineralna koštana gustina (BMD) izmerena je kod 41 (70,7%) pacijenta. Nije bilo značajne korelacije vrednosti PTH i vitamina D_3 sa T skorom na vratu butne kosti (p=0,51; p=0,063) i lumbalnoj kičmi (p=0,49; p=0,064). Statistička značajnost nije uočena ni prilikom uporeðivanja vrednosti osteokalcina i BMD lumbalne kičme (p=0,944) i vrata butne kosti (p=0,161), kao ni vrednosti β-CTX i BMD lumbalne kičme (p=0,347) i vrata butne kosti (p=0,73). Međutim, statistički značajna razlika je potvrđena korelisanjem vrednosti osteokalcina (p=0,000) i β-CTX (p=0,008) u stadijumu A u odnosu na odmakle stadijume bolesti B i C.

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Conclusions: PTH and vitamin D3 do not influence the development of hepatic osteodystrophy. In patients with cirrhosis, osteocalcin and β-CTX are not valid indicators of decreased BMD, but their values correlate with the degree of liver insufficiency.

Keywords: liver cirrhosis, osteoporosis, osteocalcin, β-CTX

Zaključak: PTH i vitamin D_3 ne utiču na razvoj hepatičke osteodistrofije. Kod pacijenata sa cirozom jetre osteokalcin i β-CTX nisu validni pokazatelji smanjenja mineralne koštane gustine, ali direktno korelišu sa stepenom insuficijencije jetre.

Ključne reči: ciroza jetre, osteoporoza, osteokalcin, β-CTX

Introduction

Impaired bone metabolism in liver cirrhosis brings about pathological fractures, lower quality of life and a higher mortality rate. Metabolic bone disease in patients with chronic liver disease is called hepatic osteodystrophy and is primarily the consequence of osteopenia or osteoporosis and considerably more rare osteomalacia. Histopathological changes seen in hepatic osteodystrophy are similar to findings of postmenopausal osteoporosis where the loss of trabecular bone is more intensive than the loss of cortical bone (1, 2).

The genesis of hepatic osteodystrophy is multifactorial and, according to the different causes of liver diseases, it may be caused by: a lower body mass index (<19 kg/m^2), reduced muscular mass, physical inactivity, alcoholism, hyperbilirubinemia, lower insulin-like growth factor 1 and osteoprotegerin levels, a calcium deficit, a lowered vitamin D_3 value, the vitamin D receptor genotype, hypogonadism and other hormonal disorders, increased tissue iron depositing, long-term cholestasis, the use of antiviral medication such as ribavirin, corticosteroids (prednisolone, 5 mg/day over 3 months) and immunosuppressive therapy as well as liver insufficiency itself (3–5).

It has been suggested that bone strength may be assessed, independently of the bone mineral density (BMD) level, by measuring bone turnover using specific serum and urinary markers of bone formation and resorption (6).

The aim of the study was to define the influence of vitamin D_3 and parathyroid hormone (PTH) in the pathogenesis of hepatic osteodystrophy, as well as the predictive significance of the biochemical bone markers.

Patients and Methods

Patients

A prospective study, conducted in the period 2010–2011, included 58 patients with liver cirrhosis who were examined and treated at the Gastroenterology and Hepatology Clinic, Clinical Center of Serbia, Belgrade. The concentrations of parathormone, bone density markers and vitamin D_3 were measured at the Center for Biochemistry, Clinical Center of Serbia. Osteodensitometry was carried out at the Railway Health Care Institute.

Hepatologic examinations were focused on the detection of the etiology of disease and differentiation of the liver insufficiency extent. With this in mind, the following diagnostic procedures were carried out: history and clinical examination, laboratory analysis, ultrasonographic and histopathologic diagnostics.

Diagnosis of B viral cirrhosis was based on serology (HBsAg, HBeAg, anti-HBe and anti-HBc IgG) and determination of polymerase chain reaction hepatitis B virus deoxyribonucleic acid (PCR HBV DNA); C viral cirrhosis was verified by the presence of anti-HCV antibodies and detection and determination of viral load per milliliter of blood (PCR HCV RNA). Alcoholic cirrhosis was diagnosed on the basis of: medical history data (consumption of pure alcohol more than 50 g/day over a five-year period), clinical and biochemical parameters and/or liver biopsy. The study included only patients with at least a 6-month abstinence period.

The liver insufficiency degree, as determined by the generally accepted Child-Pugh classification, was divided into three stages: A, B and C (score: A ≤ 6; 7 < B < 9; C ≥ 10). The Child-Pugh score includes five parameters: total bilirubin, serum albumins, INR, ascites and hepatic encephalopathy (*Table I*) (7).

Females and patients with cholestatic diseases, liver malignancy, portal venous system thrombosis, earlier immobilization, renal failure, diabetes, hyper and hypothyroidism, as well as patients treated by corticosteroids, ribavirin, interferon and bisphosphonates were excluded from the study.

Table I Child-Pugh classification.

VALUE	1 POINT	2 POINTS	3 POINTS	UNITS
Total bilirubin	<34 (<2)	34–50 (2–3)	>50 (>3)	mmol/L (mg/dL)
Serum albumins	>35	28–35	< 28	g/L
INR	<1.7	1.71–2.20	>2.20	No
Ascites	No	Responsive to diuretics	Refractory	No
Hepatic encephalopathy	No	Grade I–II	Grade III–IV	No

Biochemistry

Vitamin D status was evaluated by measuring the 25-OH vitamin D3 after morning blood collection. 25-OH vitamin D3 was first extracted from 500 µL of serum by means of the original fluid-fluid extraction procedure, which is an integral part of the »Bio-Rad« HPLC reagent kit for vitamin D measurement. Fifty µL was injected in the HPLC line consisting of: an isocratic HPLC pump »Waters 515«, an auto-sampler »Waters 27-7«, a column heater, and a UV/VIS detector »Waters 2489«. HPLC separation of vitamins D3 and D2 was carried out by the original mobile phase and column manufactured by the »Bio-Rad« company. Column temperature was 40 °C, and wavelength 265 nm. All concentrations were calculated by the manufacturer's internal standard. Chromatographic data were processed by the original »Empower 2-Waters« chromatographic program. Normal vitamin D values were > 32 ng/L.

The intact PTH (N=15–65 pg/mL), N-MID osteocalcin (N=14–46 µg/L), β-CTX (N=0–704 pg/mL) were determined by Elecsys®/Cobas eTM reagents (Roche Diagnostics) on the Elecsys® 2010/cobas™ 601 immunoanalyzer (Roche Diagnostics) in compliance with the manufacturer's instructions. Detection was based on the electro-chemiluminescent immunoassay (ECLIA), using the tris (bipyridyl)-ruthenium (II) complex (8).

Bone densitometry

All patients underwent dual energy X-ray absorptiometry (DEXA) by means of a Hologyc Discovery (S/N 83200) apparatus. The imaging of the femoral neck and lumbar spine (L1–L4) was carried out. According to the World Health Organisation recommendations, normal bone mass was defined as T score >-1 SD, osteopenia as T score ranging from -1 to -2.5 SD, osteoporosis as T score ≤-2.5 SD, and severe osteoporosis as T score ≤2.5 along with bone fractures. According to the same recommendations, T score is a number of SD in relation to mean value of BMD at the time of maximal bone mass (9).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS®, version 17.0). Basic descriptive statistics included mean, median, standard deviations, ranges and percentages. Differences between groups were compared with the parametric t-test or nonparametric Kruskal-Wallis test. Correlation analysis was processed via the Spearman method. Values at the $p \leq 0.05$ level were considered statistically significant.

Results

The study included 58 male patients with liver cirrhosis; mean age 54.1±9.8 years. The youngest and the oldest patient were 26 and 75 years old, respectively. Alcoholic cirrhosis was shown in 49 (84.4%) and viral C and B cirrhosis was diagnosed in 9 (15.6%) patients. Stages A, B and C were recorded in 22 (37.9%), 17 (29.3%) and 19 (32.8%) patients, respectively.

Lower bone density was measured by osteodensitometry in 41 patients (70.7%), of which osteopenia was found in 31 (53.4%) and osteoporosis in 10 (17.3%) patients. T score <-1.0 was verified in 14 (63.6%) patients with the stage A, in 12 (70.6%) with the stage B and 15 (78.9%) patients with the stage C. No significant difference of T score and stage of disease was found (p=0.562). Mean BMD value of the femoral neck in cirrhosis stage A was 0.806±0.13 g/cm², and it was 0.780±0.14 g/cm² in patients with B + C stages. In stage A, mean BMD value of the lumbar spine was 0.996±0.14 g/cm², and in stages B+C it was 1.015±0.16 g/cm². There was no significant difference between the femoral neck BMD (p= 0.305) and lumbar spine (p=0.420), and stage of the liver disease (*Table II*).

Normal PTH values were found in 48 (82.7%) patients and increased PTH values were found in 10 patients (17.3%). The average PTH values were in stage A 81.46±67.12 pg/mL and in stages B+C 40.05±17.82 pg/mL. There was neither a statistically

Table II Mineral bone density, PTH, vitamin D3 and biochemical bone markers according to the Child-Pugh score.

Child-Pugh score			
	A	B + C	p value
BMD femoral neck (g/cm²)	0.806±0.13	0.780±0.14	p=0.305
BMD lumbar spine (g/cm²)	0.996±0.14	1.015±0.16	p=0.420
PTH (pg/mL)	81.46±67.12	40.05±17.82	p=0.77
Vitamin D (ng/mL)	38.23±23.93	29.12±16.73	p=0.27
Osteocalcin (µg/L)	15.48±8.4	8.51±4.6	p=0.000
β-CTX (pg/mL)	450.71±366.1	739.20±381.2	P=0.008

BMD (bone mineral density), PTH (parathyroid hormone), β-CTX (β-carboxy-terminal cross-linked telopeptide of type I collagen)

significant difference between the groups (p=0.77) nor a significant correlation between PTH values and T score in the femoral neck (p=0.51) and lumbar spine (p=0.49) (*Table II*).

Lower vitamin D_3 values were measured in 31 patients (53.5%). The average vitamin D_3 values were in stage A 38.23±23.93 ng/L and in stages B+C 29.12±16.73 ng/L. Although there was no statistically significant difference between the groups (p=0.27), patients with advanced liver disease had lower values of the vitamin D_3. There was no signifi-

cant correlation between vitamin D_3 values and T score in the femoral neck (p=0.063) and lumbar spine (p=0.064) (*Table II*).

Osteocalcin values were lowered in 46 (79.3%) patients. In stage A, reduced osteocalcin values were measured in 14 (63.6%) patients, in stage B in 15 (88.2%), and in stage C in 17 (89.5%) patients (*Figure 1a*). A significant difference between stage A 15.48±8.4 μg/L osteocalcin level and advanced stages B+C 8.51±4.6 μg/L was found (p=0.000) (*Table II*). Increased β-CTX values were obtained in
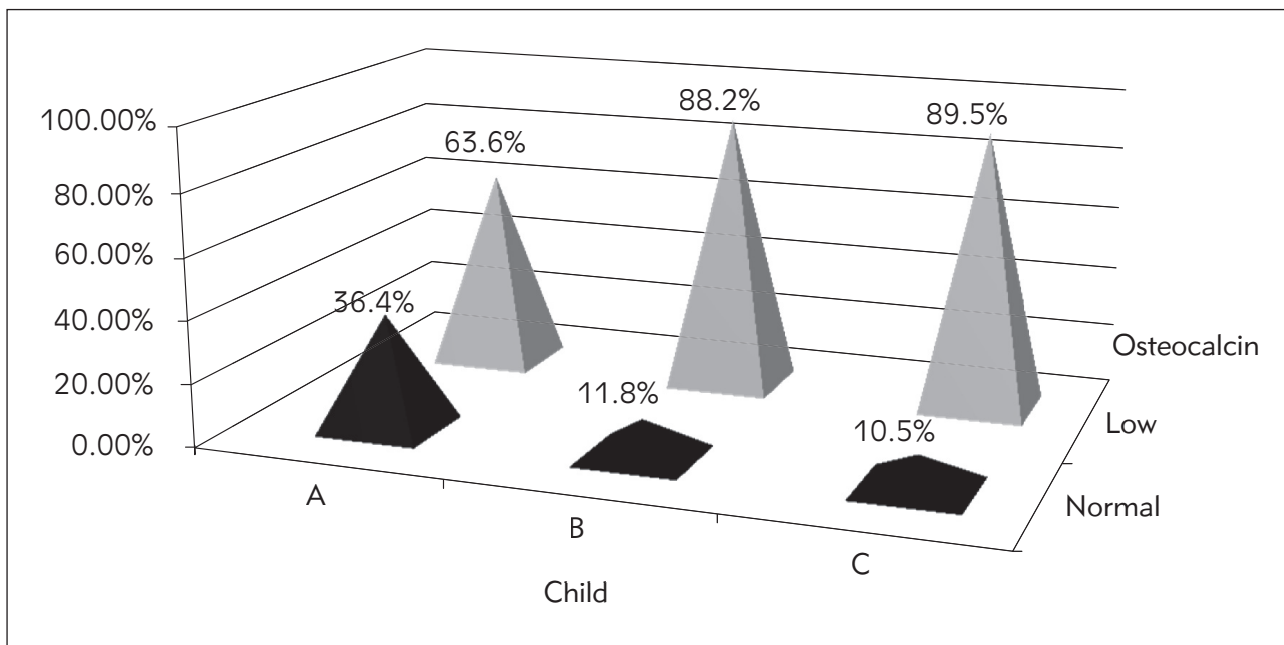

Figure 1a Osteocalcin values according to the Child-Pugh score.
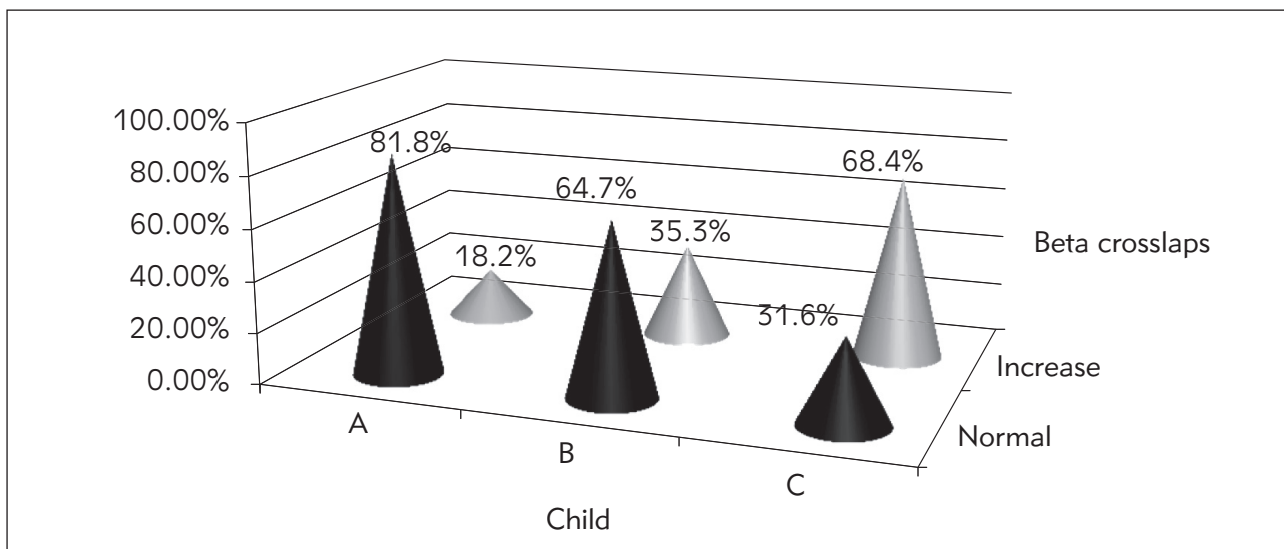

Figure 1b β-CTX values according to the Child-Pugh score.

23 (39.7%) patients. In the liver cirrhosis stage A, higher β-CTX values were measured in 4 (18.2%) patients, in stage B in 6 (35.3%) and in 13 (68.4%) patients with the stage C (*Figure 1b*). A highly significant difference between stage A 450.71±366.1 pg/mL β-CTX and stages B+C 739.2±381.2 pg/mL was established (p=0.008) (*Table II*). There was no significant correlation between the osteocalcin values in the lumbar spine (p=0.944) and femoral neck (p=0.161), nor with β-CTX values and T score in the lumbar spine (p=0.347) and femoral neck (p=0.73).

Discussion

The majority of authors agree that the prevalence of hepatic osteodystrophy in cholestatic diseases is 15%–30% (primary sclerosing cholangitis 15%, primary biliary cirrhosis 20%–30%) (2). In contrast, regarding the parenchymal diseases, there is no unique standpoint on the prevalence of hepatic osteodystrophy (10).

In the study of Cijevschi et al. (11) impaired bone metabolism was confirmed in 32.5% of patients with viral and in 47.3% of patients with alcoholic cirrhosis. A similar opinion was presented by Javad et al. (12) who reported that, in patients with viral B or C cirrhosis, osteopenia and osteoporosis were recorded in 42% and 26% of patients, respectively. High frequency of reduced bone density was reported by George et al. (13) who described 72 patients with alcoholic and viral cirrhosis out of whom 68% had lower bone mineral density.

In accord with the results of George et al, our study also confirmed high frequency (70.7%) of reduced BMD values.

The frequency and severity of osteoporosis increase as the liver insufficiency progresses. However, the study by Cijevschi et al. (11) reported that no significant correlation between the BMD, severity and etiology of the liver disease was found.

Similar to previous reports, our study failed to confirm that the stage of liver cirrhosis interfered with the T score. It was noted, however, that the frequency of osteopenia or osteoporosis, in the femoral neck, increased with the higher degree of liver insufficiency.

The role of the calcium-PTH-vitamin D axis in hepatic osteodystrophy is controversial. Some authors reported that PTH was increased in liver insufficiency, while others underscored that PTH values were normal or even reduced in this condition. The prevailing opinion is that the changes in PTH concentrations have no significant effect on the development of osteoporosis in cirrhotic patients (10, 14, 15). Although vitamin D inadequacy is very common with these patients, secondary hyperparathyroidism is rather rare (16).

Our results also suggested that PTH values had no significant impact on hepatic osteodystrophy.

The latest studies have demonstrated very high prevalence of vitamin D deficiency and insufficiency in patients with chronic liver disease and cirrhosis (17). Recently, it has been recognised that vitamin D also regulates cell proliferation and differentiation, and has immunomodulatory, antiinflammatory and antifibrotic properties. The role of vitamin D in the activation and regulation of both innate and adaptive immune systems may explain its importance in the liver diseases (18).

However, in spite of the decreased value of vitamin D particularly in advanced cirrhosis patients, the influence of the lower 25-hydroxy vitamin D_3 values on the BMD was not determined in the study of Gonzales-Calvin et al. (15). Based on regression analysis, the authors concluded that, in cirrhotic patients, 25-hydroxy vitamin D_3 was not a valid predictor of osteoporosis.

Furthermore, in the pathogenesis of hepatic osteodystrophy, reduced tissue sensitivity to circulating vitamin due to a modified vitamin D receptor genotype may have a role. In healthy people and patients with postmenopausal osteoporosis, polymorphism of the vitamin D receptor alleles designated as: B/b, a/a and T/t alleles correlates with the bone mineral density (3).

Similar to previous studies, in our analyses, patients with advanced disease had a lower vitamin D_3 level, but there was no statistical confirmation of the effect of vitamin D_3 levels on BMD values.

The rates of bone production and destruction can be evaluated either by measuring predominantly osteoblastic or osteoclastic enzyme activities or by assaying bone matrix components released into the bloodstream and excreted in the urine. The most sensitive markers for bone formation are serum total osteocalcin, bone alkaline phosphatase and the procollagen type I N-terminal propeptide. For the evaluation of bone resorption, most assays are based on the detection in serum or urine of type I collagen fragments. These include free pyridinoline and deoxypyridinoline, pyridinoline cross-linking telopeptides (β-CTX and N-terminal cross-linked telopeptide type I collagen) (19–21).

In cases of increased values of the bone ALP, osteocalcin and CTX, screening should be done for osteoporosis, in particular associated to high bone remodeling diseases such as hyperparathyroidism or endogenous hypercorticisolism, or unknown vertebral fracture (22).

George et al. (13) while analyzing a group of 72 patients with viral liver cirrhosis confirmed lower osteocalcin values in 68% of subjects and increased

free deoxypyridinoline in 79% of patients. Subsequently, Goral et al. (10) reported that lower values of osteocalcin as a marker of bone formation were found in patients with virus B and C liver cirrhosis.

Decreased osteocalcin values and increased values of β-CTX were also confirmed in our study. There was no significant correlation between the bone metabolism markers values and bone mineral density in the lumbar spine and femoral neck. However, a reduction in serum osteocalcin and increase in β-CTX can be explained by impaired osteoblast activity with reduced synthesis of collagen matrix and increased osteoclastic activity with bone resorption.

Conclusion

PTH and vitamin D_3 do not influence the development of hepatic osteodystrophy. In the patients with cirrhosis, osteocalcin and β-CTX are not valid indicators of decreased bone mineral density, but their values correlate well with the degree of liver insufficiency.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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