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Growth hormone/insulin-like growth factor-1 axis, calciotropic hormones and bone mineral density in young patients with chronic viral hepatitis

Czynność osi GH/IGF-I, stężenie hormonów kalciotropowych we krwi oraz gęstość mineralna kości u młodych osób z przewlekłym wirusowym zapaleniem wątroby

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Abstract

Introduction: Chronic liver disease caused by HBV and HCV infections, due to its great prevalence and serious medical consequences, is at the present time a significant clinical problem. An impaired liver function can provoke severe disturbances in calcium and phosphorus homeostasis, and consequently in the bone metabolism resulting in hepatic osteodystrophy. The aim of this study was to determine whether there are significant differences in bone mineral density (BMD) and/or circadian levels of hormones connected with bone metabolism and bone turnover markers in patients with chronic viral hepatitis.

Material and methods: Circadian levels (AUC, area under the curve) of GH, IGF-I, IGFBP-3, osteocalcin (BGLAP), C-terminal telopeptide of type I collagen (ICTP), PTH, 25(OH)D, total calcium and total phosporus were measured in the blood of members of the study group (n = 80). BMD was assessed using the dual-energy X-ray absorptiometry method of the L2-L4 lumbar spine. Data was compared to that of healthy individuals (n = 40).

Results: BMD (1.05 g/cm^3 *vs.* 1.20 g/cm^3), total calcium concentration (2.20 mmol/L *vs.* 2.45 mmol/L), total phosphorus concentration (1.06 mmol/L *vs.* 1.33 mmol/L), IGF-I (AUC 3,982.32 ng/mL *vs.* 5,167.61 ng/mL), IGFBP-3 (AUC 725.09 ng/L *vs.* 944.35 ng/L), 25(OH)D (AUC 356.35 ng/mL *vs.* 767.53 ng/mL) and BGLAP (AUC 161.39 ng/L *vs.* 298 ng/L) were lower in the study group. GH (AUC 88.3 ng/mL *vs.* 48.04 ng/mL), iPTH (AUC 1,201.94 pg/mL *vs.* 711.73 pg/mL) and ICTP (AUC 104.30 μg/L *vs.* 54.49 μg/L) were higher in patients with hepatitis. Positive correlations were noted between bone mineral density and IGF-I, IGFBP-3, and BGLAP levels.

Conclusions: Chronic viral hepatitis causes a decrease in bone mineral density. Impaired liver function disrupts homeostasis of the calcium–vitamin D–parathyroid hormone axis and provokes secondary hyperparathyroidism. Chronic viral hepatitis induces a decrease in the synthesis of IGF-I and IGFBP-3 and an increase in GH secretion. Hepatic osteodystrophy is probably caused by both changes in calciotropic hormones as well as in the somatotropin hormone axis. **(Endokrynol Pol 2015; 66 (1): 22–29)**

Key words: *GH; IGF-I; PTH; osteocalcin; vitamin D; BMD; chronic hepatitis; bone; liver*

Streszczenie

Wstęp: Przewlekłe zakażenia HBV i HCV są obecnie znaczącym problemem klinicznym. W wyniku zaburzeń czynności wątroby może dochodzić do zaburzeń w homeostazie wapnia i fosforu oraz w metabolizmie kostnym prowadzących do osteodystrofii wątrobowej. Celem badania była ocena gęstości mineralnej kości (BMD), okołodobowych stężeń hormonów związanych z metabolizmem kości oraz markerów obrotu kostnego u chorych na przewlekłe wirusowe zapalenie wątroby.

Materiał i metody: W grupie badanej (n = 80) oznaczano we krwi okołodobowe stężenia (AUC, area under the curve [pole pod krzywą]) GH, IGF-I, IGFBP-3, osteokalcyny (BGLAP), C-terminalnego telopeptydu kolagenu typu I (ICTP), PTH, 25(OH)D, całkowitego wapnia oraz fosforu. BMD (L2-L4) oceniono z użyciem DEXA. Dane porównano ze zdrową grupą kontrolną (n = 40).

Wyniki: BMD (1,05 g/cm^3 *vs.* 1,20 g/cm^3), stężenia wapnia (2,20 mmol/l *vs.* 2,45 mmol/l) i fosforu (1,06 mmol/l *vs.* 1,33 mmol/l), IGF-I (AUC 3982,32 ng/ml *vs.* 5167,61 ng/ml), IGFBP-3 (AUC 725,09 ng/l *vs.* 944,35 ng/l), 25(OH)D (AUC 356,35 ng/ml *vs.* 767,53 ng/ml), BGLAP (AUC 161,39 ng/l *vs.* 298 ng/l) okazały się niższe w grupie badanej niż w grupie kontrolnej, zaś stężenia GH (AUC 88,3 ng/ml *vs.* 48,04 ng/ml),

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PTH (AUC 1201,94 pg/ml *vs.* 711,73 pg/ml) i ICTP (AUC 104,30 μg/l *vs.* 54,49 μg/l) były większe u osób z zapaleniem wątroby. Stwierdzono dodatnią korelację między BMD a stężeniami IGF-I, IGFBP-3 oraz BGLAP.
Wnioski: Przewlekłe wirusowe zapalenie wątroby prowadzi do zmniejszenia gęstości mineralnej kości. Upośledzona funkcja wątroby zakłóca homeostazę wapnia, witaminy D, PTH, prowadzi do wtórnej nadczynności przytarczyc. Dochodzi do zmniejszenia syntezy IGF-I i IGFBP-3 oraz do zwiększenia wydzielania GH. Osteodystrofia wątrobowa jest prawdopodobnie spowodowana zarówno poprzez zmiany stężenia hormonów kalciotropowych, jak i zaburzenia funkcjonowania osi somatotropinowej. **(Endokrynol Pol 2015; 66 (1): 23–29)**

Słowa kluczowe: *GH; IGF-I; osteokalcyna; witamina D; gęstość mineralna kości; przewlekłe zapalenie wątroby*

Introduction

Chronic liver disease caused by HBV and HCV infections is at the present time a significant clinical problem, given the numerous complications of liver dysfunction as a consequence of a chronic inflammatory state. Moreover, the prevalence of HBV and HCV infections is estimated at respectively 0.1-7% and 0.4-20% of the general population in some European regions [1].

The liver, due to its complex metabolic and secretory functions, has long been classified as a functional part of the endocrine system [2–5]. Among the many aspects of the interaction with the endocrine system, an interesting one is the effect on bone metabolism. This influence is manifested by the interaction with the somatotropic axis, the regulation of the calciotropic hormones — PTH and vitamin D, or even bone turnover markers such as osteocalcin, or C-terminal telopeptide of type I collagen [6–10].

According to previous studies, osteopenia, osteoporosis or osteomalacia may affect as many as 40–50% of patients suffering from impaired liver function. These changes appear in the literature under the name of hepatic osteodystrophy [11–14]. Primary osteoporosis is characterised by specific features: it applies to younger patients, requires a primary disease, and appears as the result of a combination of different pathogenetic factors [15–17]. To date, the pathogenesis and mechanism of hepatic osteodystrophy has not been well explored. It is still not possible to consistently and unambiguously present the factors contributing to this phenomenon [5, 11–13, 18–21].

The aim of this study was to evaluate some potential pathogenetic factors influencing bone status in patients with chronic active viral hepatitis, and to determine their importance in hepatic osteodystrophy development. For this purpose, we decided to assess the bone mineral density (BMD) and hormones and markers associated with bone metabolism. In evaluating these parameters we took into account the existence of diurnal chronobiological cycles, by making multiple measurements throughout the day. Among the chosen parameters were: somatotropic axis hormones — growth hormone (GH), insulin-like growth factor-I (IGF-I), insulin-like growth factor binding protein 3 (IGFBP-3), and calciotropic hormones — parathyroid hormone (PTH) and calcidiol [25(OH)D] reflecting the level of vitamin D. Of bone turnover markers, we chose osteocalcin (BGLAP; bone gamma-carboxyglutamate protein) as an indicator of osteogenesis, and C-terminal telopeptide of type I collagen (ICTP) as an indicator of bone loss.

Material and methods

Eighty patients with chronic viral hepatitis (B or/and C) were included in the study. The study group consisted of 45 men with a mean age of 37 years (SD = 6.3 years) and 35 women with a mean age of 34 years (SD = 5.2 years). The inclusion criterion was a diagnosis of chronic B or C hepatitis. This diagnosis had been based on the clinical picture, laboratory tests results — ALT, AST, GGTP, alkaline phosphatase, total bilirubin, INR, proteinogram, serological tests results — HBsAg, HBcAg, HBeAg, anti-HBs, anti-HBc, anti-HBe, molecular tests results — in B-type hepatitis the presence of HBV DNA and DNA-polymerase activity, in C-type hepatitis the presence of HCV RNA and liver biopsy. Hepatitis group candidates were excluded if they suffered from chronic diseases of the endocrine system, cardiovascular system, respiratory system, or excretory system. Also candidates who used calcium supplements, vitamin D, corticosteroids or other drugs influencing bone metabolism were excluded. Additional exclusion criteria were a history of alcohol abuse or a family history of osteoporosis. The control group consisted of 40 healthy volunteers. This group included 20 men and 20 women with a mean age of 36 years (SD = 6.7 years) who did not meet exclusion criteria. All participants underwent a full clinical examination including calculation of BMI and routine biochemical blood tests. Permission to conduct the study was obtained from the Local Ethics Committee for Scientific Research of the Medical University of Silesia.

Blood sample analyses

All participants had a peripheral venous catheter established into their antecubital vein one hour before the start of the study. Blood samples, in the amount of 8 ml each, were collected through this catheter at 08.00, 12.00, 16.00, 20.00, 24.00 and 04.00. The samples were centrifuged to obtain blood serum, which was frozen

Table I. *Characteristics of patients with chronic viral hepatitis and control group*

Tabela I. *Charakterystyka chorych z przewlekłym wirusowym zapaleniem wątroby oraz grupy kontrolnej*

	Chronic hepatitis (N = 80)	Control group (N = 40)	p value
Age [years]	34 (SD ± 0.82)	36 (SD ± 1.50)	NS
BMI [kg/m^2]	22.3 (SD ± 0.50)	22.8 (SD ± 0.89)	NS
ALT [IU/L]	120.3 (SD ± 12.74)	19.2 (SD ± 1.65)	P < 0.001
ALP [IU/L]	180.43 (SD ± 7.51)	182.30 (SD ± 7.02)	NS
Total bilirubin [μmol/L]	19.4 (SD ± 2.57)	13.2 (SD ± 1.58)	NS
Creatinine [μmol/L]	89.6 (SD ± 3.38)	84.7 (SD ± 3.89)	NS

BMI — body mass index; ALT — alanine transaminase; ALP — alkaline phosphatase; NS — statistically non-significant

Table II. *Bone mineral density, total calcium and inorganic phosphorus parameters of patients with chronic hepatitis and control group*

Tabela II. *Gęstość mineralna kości, stężenie wapnia oraz fosforu we krwi u chorych z przewlekłym wirusowym zapaleniem wątroby oraz w grupie kontrolnej*

	Chronic hepatitis	Control s	p value
BMD [g/cm^3]	1.05 (SD ± 0.029)	1.20 (SD ± 0.032)	p = 0.0011
BMD Z-score	–0.36	0.48	p < 0.05
BMD T-score	–0.52	0.18	p < 0.05
Total calcium [mmol/L]	2.20 (SD ± 0.031)	2.45 (SD ± 0.054)	p < 0.001
Inorganic phosphorus [mmol/L]	1.06 (SD ± 0.029)	1.33 (SD ± 0.036)	p < 0.001

BMD — bone mineral density

at –70°C for subsequent analysis. In every serum sample, the radioimmunological assay method was used to assess the concentration of selected parameters: Growth hormone (GH-RIA kit, OPiD POLATOM, Poland), insulin-like growth factor-I (IGF-I RIA-kit, Biosource, Belgium), insulin-like growth factor binding protein-3 (IGFBP-3 IRMA-kit, Diagnostic Systems Laboratories, TX, USA), osteocalcin (BGLAP IRMA-kit, Biosource, Belgium), C-terminal telopeptide of type I collagen (ICTP RIA-kit, Orion Diagnostica, Finland), parathyroid hormone (iPTH IRMA-kit, Biosource, Belgium), and calcidiol (25(OH)D3 IRMA-kit, Biosource, Belgium).Total calcium serum concentration was assessed by the colorimetric method (Calcium Liquicolor, Humen, Germany), as was total inorganic phosphorus concentration (Phosphorous Liquirapid, Humen, Germany).

Bone densitometry

All participants underwent assessment of bone mineral density (BMD) with the use of the dual-energy X-ray absorptiometry method (DEXA) of the L2-L4 lumbar spine. A Lunar DPX densitometer (General Electric, USA) was used to make measurements. Measurement results were presented as either absolute density values [g/cm^3] or T-score and Z-score values.

Statistics

All acquired data was implemented to create a database using Microsoft Excel forming a part of Microsoft Office 2000 (Microsoft, USA). For each quantitative parameter, basic statistical characteristics were made — mean, median, standard deviation (SD), and standard error of the mean (SEM). Area under curve (AUC) values of the parameters was determined by the trapezoidal method. Shapiro-Wilk test was performed for the evaluation of a normal distribution parameter. Student's t-test was used for the comparison of unpaired groups of parameters that follow normal distribution. In the use of unrelated variables, Fisher's exact test was used to check the homogeneity of variance. Pearson's correlation coefficient was used to investigate multivariate data correlation. A p value < 0.05 was accepted as statistically significant.

Results

We found a significant difference in the ALT serum concentration. It was greatly elevated (120.3 IU/L *vs.* 19.2 IU/L, p < 0.001) in patients compared to controls (Table I and II).

We observed a difference in BMD in the L2-L4 vertebrae densitometry (DEXA) between study participants and the control group, taking into account both the absolute BMD values (1.05 g/cm^3 *vs.* 1.20 g/cm^3, p = 0.0011) and the indications of Z-score (–0.36 *vs.* 0.48, p < 0.05) and T-score (–0.52 *vs.* 0.18, p < 0.05). We noted, however, that despite much lower values compared to the controls, the results of the study group members were within the reference values. In the group of patients with chronic hepatitis, we found in blood lower total calcium levels (2.20 mmol/L *vs.* 2.45 mmol/L, p < 0.001) and inorganic phosphorus levels (1.06 mmol/L *vs.* 1.33 mmol/L, p < 0.001) (Table III).

We observed significantly increased total daily release of GH in the study group compared to controls (88.3 ng/mL *vs.* 48.04 ng/mL, p < 0.001). However, we

Table III. *Diurnal oscillations of GH, IGF-I and IGFIBP-3 concentrations in blood of patients with chronic viral hepatitis and control group*

Tabela III. *Okołodobowe stężenia GH, IGF-I, IGFBP-3 we krwi chorych z przewlekłym wirusowym zapaleniem wątroby oraz w grupie kontrolnej*

Sampling time	GH [ng/mL]			IGF-I [ng/mL]			IGFBP-3 [ng/L]		
	Study group	Control group	p value	Study group	Control group	p value	Study group	Control group	p value
08.00	2.70 (SD ± 0.073)	1.21 (SD ± 0.034)	p < 0.001	183.93 (SD ± 8.29)	263.03 (SD ± 9.25)	p < 0.001	39.97 (SD ± 1.27)	50.16 (SD ± 2.37)	p < 0.001
12.00	3.51 (SD ± 0.071)	1.23 (SD ± 0.034)	p < 0.001	181.24 (SD ± 6.45)	251.07 (SD ± 9.59)	p < 0.001	38.62 (SD ± 1.19)	48.39 (SD ± 1.87)	p < 0.001
16.00	3.92 (SD ± 0.092)	2.52 (SD ± 0.123)	p < 0.001	206.55 (SD ± 7.72)	252.94 (SD ± 8.62)	p < 0.001	38.34 (SD ± 1.44)	46.70 (SD ± 2.41)	p = 0.0036
20.00	5.93 (SD ± 0.193)	4.56 (SD ± 0.119)	p < 0.001	217.49 (SD ± 7.82)	274.87 (SD ± 9.07)	p < 0.001	35.84 (SD ± 1.37)	48.79 (SD ± 2.20)	p < 0.001
24.00	6.01 (SD ± 0.116)	3.56 (SD ± 0.106)	p < 0.001	233.76 (SD ± 8.03)	282.19 (SD ± 9.29)	p < 0.001	34.13 (SD ± 1.17)	48.29 (SD ± 2.22)	p < 0.001
04.00	4.37 (SD ± 0.205)	1.32 (SD ± 0.141)	p < 0.001	181.45 (SD ± 7,06)	240.39 (SD ± 8.37)	p < 0.001	31.43 (SD ± 1,32)	42.98 (SD ± 2.39)	p < 0.001
AUC	88.30 (SD ± 1.824)	48.04 (SD ± 1.294)	p < 0.001	3,982.32 (SD ± 133.012)	5,167.61 (SD ± 162.95)	p < 0.001	725.09 (SD ± 21.83)	944.35 (SD ± 37.67)	p < 0.001

GH — growth hormone concentration; IGF-I — insulin-like growth factor 1; IGFBP-3 — insulin-like growth factor binding protein 3; AUC — area under curve

did not notice significant changes in the daily oscillation profile of GH ejection. The study group was characterised by lower daily concentrations of IGF-I (3,982.32 ng/mL *vs.* 5,167.61 ng/mL, p < 0.001) compared to controls. IGFBP-3 showed similar changes (725.09 ng/L *vs.* 944.35 ng/L, p < 0.001). We did not observe changes in the daily oscillations of these parameters (Table IV).

Daily secretion of calcidiol was lower in patients with chronic viral hepatitis compared to control group (356.35 ng/mL *vs.* 767.53 ng/mL, p < 0.001). Moreover, we noted a flattening of the profile of daily 25(OH)D oscillation in patients with hepatitis, manifested as the disappearance of the peak of the highest concentration which occurred about 12.00 in the healthy control group. An inverse relationship concerned the diurnal concentration of intact parathyroid hormone (iPTH), which was higher in the study group (1,201.94 pg/mL *vs.* 711.73 pg/mL, p < 0.001) (Table V).

We observed that the daily concentration of osteocalcin was lower in patients with hepatitis than in the control group (161.39 ng/L *vs.* 298.00 ng/L, p < 0.001). In the case of ICTP, we noticed an increase in its release among participants of the study group (104.30 μg/L *vs.* 54.49 μg/L, p < 0.001).

Our results revealed a statistically significant positive correlation between BMD values and diurnal serum concentrations of IGF-I, IGFBP-3 and BGLAP in blood. In addition, we found a positive link between the concentrations of IGFBP-3 and BGLAP. We also observed a significant negative bond between BMD values and the daily GH secretion. Additionally, we found a negative correlation linking the daily levels of IGF-I and ICTP or between IGF-I and iPTH.

Discussion

The material analysed in this paper is highly homogeneous. Among the participants of the study group, we did not identify the features of cirrhosis of the liver, severe cholestasis, or other conditions that may have an impact on the calcium-phosphate metabolism. The general characteristics differentiating feature of research subjects for the members of the control group was a significantly elevated level of alanine aminotransferase (ALT; 120.3 IU/L *vs.* 19.2 IU/L, p < 0.001), reflecting active inflammation of the liver. Moreover, individuals included in our study were characterised by a relatively low age (mean age 34 years, SD = 5.2 years), which enabled us to exclude changes in bone metabolism associated with senility [3, 14, 21–26]. In our study, we implemented repeated measurements over the course of 24 hours, allowing us to reflect diurnal level variations with respect to chronobiological cycles [3, 4, 8, 27–29].

In our study, we found a statistically significant difference in BMD expressed either as absolute values, or as a T-score and Z-score occurring between patients with hepatitis and the controls (BMD *vs.* 1.05 g/cm^3. 1.20 g/cm^3; p = 0.0011, T-score –0.52 *vs.* 0.18, p < 0.05, Z-score –0.36 *vs.* 0.48, p < 0.05). The results, despite

Table IV. *Diurnal oscillations of 25(OH)D and iPTH concentrations in blood of patients with chronic viral hepatitis and in control group*

Tabela IV. *Okołodobowe stężenia 25(OH)D i iPTH we krwi chorych z przewlekłym wirusowym zapaleniem wątroby oraz w grupie kontrolnej*

Sampling time	25(OH)D [ng/mL]			iPTH [pg/mL]		
	Study group	Control group	P value	Study group	Control group	p value
08.00	18.35 (SD ± 1.35)	34.90 (SD ± 3.82)	$p < 0.001$	56.46 (SD ± 8.97)	35.72 (SD ± 6.53)	$p = 0.0384$
12.00	18.20 (SD ± 1.43)	48.10 (SD ± 6.32)	$p < 0.001$	53.11 (SD ± 5.36)	33.25 (SD ± 4.78)	$p = 0.0061$
16.00	17.49 (SD ± 1.69)	37.12 (SD ± 4.37)	$p < 0.001$	64.98 (SD ± 10.71)	38.33 (SD ± 8.09)	$p = 0.0107$
20.00	17,72 (SD ± 1,60)	38.90 (SD ± 7.13)	$p = 0.0062$	55.21 (SD ± 6.68)	33.04 (SD ± 5.06)	$p = 0.0056$
24.00	17.25 (SD ± 1.39)	35.84 (SD ± 3.86)	$p < 0.001$	57.05 (SD ± 6.50)	32.12 (SD ± 4.43)	$p = 0.0013$
04.00	17.85 (SD ± 1.82)	32.38 (SD ± 4.23)	$p = 0.0055$	70.44 (SD ± 10.95)	39.38 (SD ± 9.51)	$p = 0.0033$
AUC	356.35 (SD ± 28.74)	767.53 (SD ± 356.71)	$p < 0.001$	1,201.94 (SD ± 114.62)	711.73 (SD ± 121.68)	$p < 0.001$

25(OH)D — calcidiol; iPTH — intact parathyroid hormone; AUC — area under curve

Table V. *Diurnal oscillations of BGLAP and ICTP concentrations in blood of patients with chronic viral hepatitis and in control group*

Tabela V. *Okołodobowe stężenia BGLAP i ICTP we krwi chorych z przewlekłym wirusowym zapaleniem wątroby oraz w grupie kontrolnej*

Sampling time	BGLAP [ng/L]			ICTP [μg/L]		
	Study group	Control group	p value	Study group	Control group	p value
08.00	8.26 (SD ± 0.71)	13.36 (SD ± 1.04)	$p < 0.001$	5.72 (SD ± 0.46)	2.77 (SD ± 0.29)	$p < 0.001$
12.00	7.58 (SD ± 0.52)	14.61 (SD ± 1.28)	$p < 0.001$	4.87 (SD ± 0.36)	2.69 (SD ± 0.35)	$p < 0.001$
16.00	8.32 (SD ± 0.91)	13.44 (SD ± 1.09)	$p = 0.0014$	4.77 (SD ± 0.40)	2.46 (SD ± 0.37)	$p < 0.001$
20.00	7.72 (SD ± 0.69)	14.52 (SD ± 1.22)	$p < 0.001$	4.70 (SD ± 0.34)	2.52 (SD ± 0.26)	$p < 0.001$
24.00	7.96 (SD ± 0.84)	15.30 (SD ± 1.27)	$p < 0.001$	5.40 (SD ± 0.384)	2.67 (SD ± 0.24)	$p < 0.001$
04.00	8.66 (SD ± 1.08)	17.60 (SD ± 1.59)	$p < 0.001$	6.18 (SD ± 0.49)	3.23 (SD ± 0.26)	$p < 0.001$
AUC	161.39 (SD ± 13.40)	298.0 (SD ± 21.44)	$p < 0.001$	104.30 (SD ± 6.90)	54.49 (SD ± 4.84)	$p < 0.001$

BGLAP — bone gamma-carboxyglutamate protein, osteocalcin; ICTP — carboxy-terminal telopeptide of type I collagen; AUC — area under curve

a decrease in BMD values, do not meet the criteria for the diagnosis of osteopenia or osteoporosis [15, 16]. Our results comply with those of previous reports [30-35], but the loss of bone mass among our subjects was significantly lower than has been found by some other authors. We believe that this is due to the decreased severity of hepatic impairment presented by our patients. Pelazas-Gonzalez et al. [36] observed that chronic HCV infection in well-nourished patients with preserved liver function does not cause osteoporosis. The selection of our study group, excluding cholestasis, helped us to eliminate the influence of abnormal intestinal absorption of calcium caused by bile obstruction [37] and cholestasis itself, which is considered to be an independent risk factor for osteoporosis [38].

Among patients with hepatitis, we noticed a statistically significant reduction in total calcium concentration in blood (2.20 mmol/L *vs.* 2.45 mmol/L, $p < 0.001$) as well as the level of inorganic phosphorus (1.06 mmol/L *vs.* 1.33 mmol/L, $p < 0.001$) compared to the value achieved by the control group. We believe that our results are derived from abnormalities in the metabolism of vitamin D and PTH [11, 15].

The common complication described in chronic liver diseases, and cirrhosis in particular, is hypogonadism [4, 26, 39], which also stands as an important risk factor for osteoporosis [10, 15, 40, 41]. This disorder manifests in laboratory tests as hyperoestrogenism [4, 9, 42–44], and a decrease in free testosterone serum level [43, 44]. It is true that our study did not have marked levels of sex hormones, but by carefully collecting medical history and physical examination we were most probably able to exclude the influence of gonadal dysfunction among study participants. None of the study participants showed any signs of alcohol abuse. The consumption of excessive amounts of alcohol is a risk factor for osteoporosis, independent of hypogonadism and liver dysfunction [26, 45].

In the course of our study, we observed that in patients with chronic hepatitis daily IGF-I level (3,982.32 ng/mL *vs.* 5,167.61 ng/mL, $p < 0.001$) and IGFBP-3 level (725.09 ng/mL *vs.* 944.35 ng/mL, $p < 0.001$) were reduced compared to the control group. Moreover, these phenomena were accompanied by increased daily secretion of GH in the study group (88.3 ng/mL *vs.* 48.04 ng/mL, $p < 0.001$). This trend has been observed by different authors [33, 46, 47]. We noted a positive correlation linking BMD and IGF-I and IGFBP-3.

These observations confirm the importance of growth hormone axis function and, consequently, the concentrations of IGF-I and IGFBP-3, for bone metabolism [48–50]. Another feature is the appearance of a positive relationship between the values of IGF-I and BGLAP and negative between IGF-I and ICTP and PTH. These relationships indicate a direct effect of liver function on bone anabolism and catabolism respectively.

In our opinion, the cause of the increase in the daily GH secretion in patients with chronic hepatitis disorder feedback was due to impaired production of IGF-I in the liver [8, 51]. However, there are reports which undermine the aforementioned hypothesis. In animal studies, the elimination of hepatic IGF-I synthesis caused its level to decrease by 75%, although that action did not have any significant influence on the BMD [52, 53]. Furthermore, some scientists deny the existence of a link between IGF-I and BMD in men [54–56]. Papers dealing with osteoporosis in liver disease suggest the existence of a link between reduced BMI and decreased IGF-I and BMD [57-61]. However, our observation did not confirm such a relationship.

Our results show a significant reduction in blood levels of calcidiol in study subjects compared to the control group (712.71 ng/mL *vs.* 1,535 ng/mL, $p < 0.001$). Vitamin D deficiency may lead to hypocalcaemia, the development of secondary hyperparathyroidism, and hypophosphatemia [62]. Our research revealed a similar tendency, but it should be mentioned that, despite significant differences in the concentrations of calcium and phosphorus in relation to controls, they stayed within the range of reference values for a healthy population. Results demonstrating reduced levels of (25(OH)D) in the serum of patients with impaired liver function, including cirrhosis, have appeared in the past [5, 30, 63, 64]. However, some reports remain contradictory. Diamond et al. [65] and Tsunoka et al. [14] observed that the levels of (25(OH)D) and (1,25(OH)2D) show significant differences between the groups of patients with liver cirrhosis and chronic hepatitis. They point to the lower values of these parameters in patients with cirrhosis, suggesting a relationship between both calcidiol or calcitriol levels and liver efficiency. But there are also other opinions: Duarte et al. [66] did not show any differences in this area between people with liver cirrhosis and a healthy control group. It is worth noting that we found a change in the circadian oscillation profile of serum (25(OH)D); in the patient group, it showed a much narrower range of diurnal changes in addition to an overall lower level compared to healthy subjects. We believe this is the premise suggesting liver dysfunction in the regulation of vitamin D, far in advance of the development of cirrhosis.

Our study showed significantly elevated levels of PTH in the hepatitis group members compared to the control group (1,201.94 pg/mL *vs.* 711.73 pg/mL, $p < 0.001$). An increase in PTH levels in patients with chronic hepatitis or cirrhosis is usually the result of secondary hyperparathyroidism [32, 66, 67]. As previously mentioned, many authors have suggested that the reason for hyperparathyroidism in such patients is the decrease of vitamin D level [67, 68]. Previous studies have shown that the lowest normal calcidiol levels, at which PTH secretion is minimal, is on average 40 ng/mL [62, 69]. Among participants in our study, in all the samples taken during the day, the concentrations of (25(OH)D) levels were below the designated threshold. This seems to confirm the hypothesis of hyperparathyroidism being dependent on vitamin D levels in patients with chronic active hepatitis. The effect of PTH on bone metabolism has been the subject of numerous considerations. In the course of hyperparathyroidism, the intensification of bone resorption with an increase in the number of osteoclasts, severe alteration of bone structure and a reduction of BMD has been demonstrated [25, 70], which is consistent with our results.

However, there have also been reports that short-pulse increases in PTH have anabolic effects on bone mass and increases their mechanical strength [24, 71]. There is a suggestion that this effect results from the extension of the life of osteoblasts by PTH-dependent inhibition of apoptosis [72]. In patients with chronic active hepatitis with secondary hyperparathyroidism, one would expect an increased bone turnover; however, this occurs only in about 50% of patients [30, 54, 73], and in the remainder a dynamic bone disease has been described [73].

Our study showed significantly lower serum BGLAP concentration in patients with hepatitis (161.39 ng/L *vs.* 298 ng/L, $p < 0.001$), and significantly higher levels of ICTP (104.30 μg/L *vs.* 54.49 μg/L, $p < 0.001$) than in the control group. In addition, we noted a statistically significant positive correlation joining BMD with BGLAP concentration. Our findings are similar to those from other authors [74, 75], while Gallego-Rojo et al. [30] showed a reduction in the concentration of BGLAP only in patients with end-stage liver cirrhosis. Previous studies have also shown increased activity of bone resorption including ICTP [30, 76]. Most research leads us to the hypothesis that the severity of the resorption process is the predominant cause of hepatic osteodystrophy.

Conclusions

In our study, we have shown that the impairment of liver function in chronic hepatitis due to HBV or HCV infection leads in young patients to significant disorders of bone metabolism. These changes manifest themselves as a decrease in bone mineral density which may reflect in an increased risk of pathological bone fractures.

In our research we observed that chronic liver inflammation leads to impaired function of the calcium-vitamin D-parathyroid hormone axis, causes secondary hyperparathyroidism and, consequently, an imbalance in bone turnover processes. Furthermore, we have shown that in chronic active hepatitis, somatotropin axis adjustment is disturbed; this manifests as a decrease in the synthesis of IGF-I and IBFBP-3 and, coupled with these, an increase in growth hormone secretion.

These observations allow us to conclude that an important pathogenetic role underlying hepatic osteodystrophy is played by both changes in calciotropic hormones and the hormone axis. We believe that there may be some significance in a change in the metabolism of bone turnover markers — BGLAP and ICTP.

We believe that the issue we have raised in this study requires further research.

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