



## Clinical benefits of biochemical markers of bone turnover in Egyptian children with chronic liver diseases

Karam A Mahdy, Hanaa H Ahmed, Fathia Mannaa, Azza Abdel-Shaheed

Karam A Mahdy, Medical Biochemistry Department, National Research Centre, Egypt

Hanaa H Ahmed, Hormones Department, National Research Centre, Egypt

Fathia Mannaa, Medical Physiology Department, National Research Centre, Egypt

Azza Abdel-Shaheed, Child Health Department, National Research Centre, Egypt

Supported by the National Research Centre during the research plan from 2001-2004 by projects 5/4/5 and 5/5/5 in collaboration with National Liver Institute of Menoufyia University

Correspondence to: Karam A Mahdy, Medical Biochemistry Department, National Research Centre, El Bohouth Street, Dokki 12311, Cairo, Egypt. karammahdy@yahoo.com

Telephone: +2-2-3371499 Fax: +2-2-3370931

Received: 2006-08-23 Accepted: 2006-12-20

### Abstract

**AIM:** To investigate the association between serum insulin-like growth factor 1 (IGF-1), osteocalcin, and parathyroid hormone (PTH) levels with the etiology and clinical condition of patients with chronic liver disease.

**METHODS:** Eighty children with hepatocellular damage were divided into 3 groups according to the etiology of disease infection: bilharziasis (9 patients), hepatitis B virus (HBV, 12 patients) and hepatitis C virus (HCV, 29 patients). The Child score index was found as A in 24 patients, B in 22 patients, C in 4 patients. Thirty healthy children served as control group. HBsAg, HbAbIgM, HbAbIgG, and anti-HCV were detected using ELISA technique. HCV-RNA was measured by reverse transcription polymerase chain reaction (RT-PCR). Anti-bilharzial antibodies were detected by indirect haem-agglutination test. Liver function tests were performed using autoanalyser. Serum IGF-1, osteocalcin and PTH levels were measured by ELISA technique. Abdominal ultrasonography was also conducted.

**RESULTS:** Serum IGF-1 level was significantly lower in all patient groups with liver diseases, while serum osteocalcin and PTH levels were significantly elevated in patients with HBV and HCV infections compared with the control group. Serum osteocalcin and PTH concentrations were measured with the severity of liver disease from Child A to C. Child A patients unexpectedly showed significantly reduced IGF-1 levels in comparison to patients staged as Child B or C. Serum osteocalcin level was negatively correlated with albumin ( $14.7 \pm 0.54$  vs  $3.6 \pm 0.10$ ,  $P < 0.05$ ), while that for PTH was positively

correlated with total protein ( $70.1 \pm 2.17$  vs  $6.7 \pm 0.10$ ,  $P < 0.05$ ) in patients with HCV infection.

**CONCLUSION:** Low serum IGF-1 level seems to play a critical role in the bone loss in patients with chronic liver disease. Elevated biochemical markers of bone remodeling suggest high-turnover in patients with viral infection and reflect severity of the clinical stage.

© 2007 The WJG Press. All rights reserved.

**Key words:** Liver disease; Bone turnover; Insulin-like growth factor-1; Osteocalcin; Parathyroid hormone

Mahdy KA, Ahmed HH, Mannaa F, Abdel-Shaheed A. Clinical benefits of biochemical markers of bone turnover in Egyptian children with chronic liver diseases. *World J Gastroenterol* 2007; 13(5): 785-790

<http://www.wjgnet.com/1007-9327/13/785.asp>

### INTRODUCTION

Bone tissues are metabolically active and undergo continuous remodeling throughout their life. Skeletal remodeling is achieved by two counteracting processes: bone formation which is accomplished through osteoblasts and bone resorption that is attributed to osteoclast activity<sup>[1]</sup>. Bone manifestations are well-known as extrahepatic complications of chronic liver disease<sup>[2]</sup>. Patients with chronic liver disease are at increased risk of developing hepatic osteodystrophy manifested as osteomalacia or osteoporosis<sup>[3]</sup>. Osteoporosis can be described as a disturbance in the bone remodeling process. Bone loss is a result of an imbalance of the bone remodeling process, where bone resorption exceeds bone formation<sup>[4]</sup>.

The prevalence of osteoporosis in patients with chronic liver diseases ranges from 10% to 60%<sup>[5,6]</sup>. In general, secondary factors such as malabsorption and nutritional deficiencies may cause bone changes in chronic liver disease<sup>[7]</sup>. Insulin like growth factor (IGF) family is considered as important anabolic hormones, which play a role in anabolic metabolism and stimulating DNA synthesis, cell proliferation and meiotic division throughout life<sup>[8]</sup>. Since most circulating IGFs are synthesized by hepatocytes, lower levels of these parameters could be found in patients with liver diseases<sup>[8]</sup>.

Regulation of bone metabolism is achieved by various factors such as mechanical motion, minerals, and hormones, all influencing bone turnover<sup>[4]</sup>. Osteocalcin is a protein produced by osteoblasts. Its level is reduced in the presence of osteodystrophy and can be recommended as a very sensitive and specific marker for bone formation/turnover<sup>[9]</sup>.

Parathyroid hormone has a dual effect on bone cells. It can stimulate osteoblast activity and lead to substantial increase in bone density. In contrast, when secreted continuously at relatively high rates, as in hyperparathyroidism patients it can stimulate osteoclast-mediated bone resorption and suppress osteoblast activity<sup>[11]</sup>. The anabolic effect of parathyroid hormone (PTH) on osteoblasts is probably both direct and indirect via growth factors such as insulin like growth factor-1 (IGF-1) and transforming growth factor- $\beta$  (TGF- $\beta$ )<sup>[11]</sup>.

However, the role of hepatocellular dysfunction in hepatic osteodystrophy is not clear. Therefore, this study aimed to clarify the influence of hepatocellular dysfunction on bone loss, and to outline the clinical benefits of controlling serum IGF-1, osteocalcin and parathyroid hormone levels in children with chronic liver diseases.

## MATERIALS AND METHODS

Eighty children were enrolled in this study, from Liver Institute, Menoufia University Inpatient and Outpatient Clinic and divided into: (1) Bilharziasis group: 9 patients (males whose age ranged from 6 to 15 years). (2) HBV group: 12 patients (10 males and 2 females whose age ranged from 3 to 15 years). (3) HCV group: 29 patients (22 males and 7 females whose age ranged from 2 to 16 years). (4) Control group: 30 apparently healthy children (22 males and 8 females whose age ranged from 3 to 15 years) with no history or clinical evidence of liver disease or any other diseases. (5) History was obtained from all individuals and clinical examination was carried out.

### Specimen collection

Under complete aseptic condition, 5 mL of blood was taken from all the patients and controls. Each blood sample was divided into two portions: a small portion (1.8 mL) was taken on 3.8% Na-citrate as anticoagulant (0.02 mL) to obtain plasma and a larger portion of blood sample was allowed to clot. Sera were separated and stored at -80°C till being tested, while the plasma sample was used immediately to measure prothrombin time and concentration.

### Serological test

Hepatitis surface antigen (HBsAg), hepatitis B core antibody IgM (HBcAb IgM) and hepatitis B core antibody IgG (HBcAb IgG) were measured by ELISA technique using kits from Dia-Sorin Biomedica Co., according to the methods of Boniolo *et al*<sup>[10]</sup>, Tedder and Wilson<sup>[11]</sup> and Hoofangle *et al*<sup>[12]</sup> respectively.

Anti-HCV was detected by third generation of ELISA using kit from the Biochem ImmunoSystem Inc<sup>[13]</sup>, HCV-RNA extraction was carried out by reverse transcription polymerase chain reaction (RT-PCR) according to the

**Table 1** Serum IGF-1, osteocalcin and PTH levels in patient groups and control group (mean  $\pm$  SE)

	Control (n = 30)	Bilharziasis (n = 9)	HBV (n = 12)	HCV (n = 29)
IGF-1 (ng/mL)	277.1 $\pm$ 12.2	94.0 $\pm$ 4.30 <sup>b</sup>	105.1 $\pm$ 10.3 <sup>b</sup>	230.0 $\pm$ 10.5 <sup>b</sup>
Osteocalcin (ng/mL)	5.7 $\pm$ 0.3	6.8 $\pm$ 0.66	16.5 $\pm$ 0.76 <sup>b</sup>	14.7 $\pm$ 0.54 <sup>b</sup>
PTH (ng/mL)	61.6 $\pm$ 2.7	63.3 $\pm$ 3.03	83.4 $\pm$ 4.30 <sup>b</sup>	70.1 $\pm$ 2.17 <sup>a</sup>

<sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01 vs control group.

method described by Ravaggi *et al*<sup>[14]</sup>. Anti-bilharzial antibodies were detected by indirect haem-agglutination test (IHA) according to the method of Hoshino *et al*<sup>[15]</sup>, using kits of Fumozze-France.

### Liver function tests

Serum AST, ALT, alkaline phosphatase (ALP), GGT, total and direct bilirubin, total protein, and albumin levels were measured using EKTOCHEM 750XRC analyzer. Prothrombin time and concentration were also estimated in plasma according to the method of Poller<sup>[16]</sup>.

### Biochemical markers of bone turnover

Serum insulin like growth factor-1 (IGF-1), osteocalcin and PTH levels were measured by ELISA technique using kits of BioSource-Belgium<sup>[17-19]</sup>.

### Abdominal ultrasonography

To assess the size and echopattern of the liver, the size of the spleen and the presence of ascitis or any other abnormalities in the abdomen were detected using RT-X200 Prob 3.5 MHZ convex of General Electric Company -USA.

### Statistical analysis

The obtained data are presented as mean  $\pm$  SE. The difference between two groups was calculated using unpaired *t*-test, while the difference between more than two groups was calculated using one way analysis of variance ANOVA according to Snedecor and Cochran<sup>[20]</sup>. Least significant difference (LSD) test was used to compare the means of Child classification according to Walter and Duncan<sup>[21]</sup> at probability 0.05.

## RESULTS

Patients with chronic liver diseases were divided into three groups according to the etiology: group 1 (9 patients with bilharziasis whose mean age was 10.8  $\pm$  1 years), group 2 (12 patients with HBV whose mean age was 11.5  $\pm$  0.6 years, and group 3 (29 patients with HCV infection whose mean age was 11.8  $\pm$  1.5 years). Thirty children whose mean age was 10.5  $\pm$  0.5 years and were sex matched with normal liver served as control group.

Table 1 depicts the results of serum IGF-1, osteocalcin and PTH levels in patients with either bilharziasis or HBV and HCV infections as well as in controls. The results revealed that patients with bilharziasis and those with either HBV or HCV infections showed a significant

**Table 2** Liver function tests in different patient groups and control group (mean  $\pm$  SE)

	Control (n = 30)	Bilharziasis (n = 9)	HBV (n = 12)	HCV (n = 29)
ALT (U/L)	20.5 $\pm$ 0.9	38.0 $\pm$ 6.8 <sup>a</sup>	72.6 $\pm$ 12.4 <sup>a</sup>	107.0 $\pm$ 28.1 <sup>a</sup>
AST (U/L)	18.4 $\pm$ 0.8	46.0 $\pm$ 7.5 <sup>a</sup>	71.2 $\pm$ 10.8 <sup>a</sup>	120.0 $\pm$ 21.8 <sup>a</sup>
ALP (U/L)	74.0 $\pm$ 9.3	82.0 $\pm$ 11.8	214.0 $\pm$ 41.2 <sup>a</sup>	165.0 $\pm$ 28.9 <sup>a</sup>
GGT (U/L)	26.0 $\pm$ 2.1	37.6 $\pm$ 9.9	54.0 $\pm$ 1.5 <sup>a</sup>	59.9 $\pm$ 11.1 <sup>a</sup>
T. bilirubin (mg/dL)	0.84 $\pm$ 0.03	0.9 $\pm$ 0.16	1.8 $\pm$ 0.20 <sup>a</sup>	1.3 $\pm$ 0.13 <sup>a</sup>
T. protein (mg/dL)	6.9 $\pm$ 0.10	7.1 $\pm$ 0.30	6.8 $\pm$ 0.20	6.7 $\pm$ 0.10 <sup>a</sup>
Albumin (mg/dL)	4.0 $\pm$ 0.06	3.9 $\pm$ 0.10	3.5 $\pm$ 0.19 <sup>a</sup>	3.6 $\pm$ 0.10 <sup>a</sup>
Prothrombin (%)	93.0 $\pm$ 1.3	77.0 $\pm$ 4.0 <sup>a</sup>	69.0 $\pm$ 6.0 <sup>a</sup>	65.6 $\pm$ 2.50 <sup>a</sup>

<sup>a</sup>P < 0.05 vs control group.

decrease ( $P < 0.01$ ) in serum IGF-1 level compared to the controls. The most significant decrease in serum IGF-1 was recorded in patients with bilharziasis followed by HBV-infected and HCV-infected patients. Serum osteocalcin level showed a significant increase ( $P < 0.01$ ) in patients with HBV and HCV infection compared to the controls. The most significant increase in serum osteocalcin level was observed in HBV-infected patients. Patients suffering from bilharziasis had no significant ( $P > 0.05$ ) change in serum osteocalcin level compared to the controls. Serum PTH level was significantly increased in HBV-infected patients ( $P < 0.01$ ) and in those with HCV infection ( $P < 0.05$ ) compared to the controls. No significant change in serum PTH level was detected in patients with bilharziasis compared to the controls (Table 1).

Liver function tests in patients with bilharziasis or HBV and HCV infection as well as in the control are presented in Table 2. Serum ALT and AST activities showed a significant ( $P < 0.05$ ) increase in all patient groups compared to the control group. The most significant increase in serum ALT and AST activities was recorded in the patients suffering from HCV infection. The activities of ALP, GGT and total bilirubin in serum revealed a significant increase ( $P < 0.05$ ) in patients with HBV and HCV infection compared to the control, while no significant change ( $P > 0.05$ ) was recorded in patients with bilharziasis as compared to the control group. Serum total protein level showed significant decrease ( $P < 0.05$ ) in patients with HCV infection, whereas patients with either bilharziasis or HBV had no significant ( $P > 0.05$ ) change as compared to the control (Table 2). Serum albumin level revealed a significant ( $P < 0.05$ ) decrease in patients suffering from either HBV or HCV infection compared to the control. No significant change in serum albumin level was observed in patients with bilharziasis compared to the control. Patients suffering from either bilharziasis or HBV and HCV infection had a significant ( $P < 0.05$ ) decrease in

**Table 3** Alterations in serum IGF-1, osteocalcin and PTH levels according to the severity of the disease from class A to C (mean  $\pm$  SE)

Severity	A (n = 24)	B (n = 22)	C (n = 4)	LSD at 0.05
IGF-1 (ng/mL)	152.6 <sup>b</sup> $\pm$ 7	186.8 <sup>a</sup> $\pm$ 20.3	196.0 <sup>a</sup> $\pm$ 36.4	32.67
Osteocalcin (ng/mL)	12.6 <sup>b</sup> $\pm$ 0.9	14.6 <sup>a</sup> $\pm$ 0.9	15.8 <sup>a</sup> $\pm$ 1.2	1.73
PTH (ng/mL)	71.3 <sup>b</sup> $\pm$ 3.0	70.3 <sup>b</sup> $\pm$ 2.7	86.5 <sup>a</sup> $\pm$ 4.3	5.36

Within each row the means followed by different letters are significantly different. LSD: Least significance difference. <sup>a</sup>P < 0.05, <sup>b</sup>P < 0.01 vs control group.

**Table 4** Effect of age and sex on serum IGF-1, osteocalcin and PTH (mean  $\pm$  SE)

	Age (yr)			Sex		
	3-9	10-16	P	Male	Female	P
IGF-1 (ng/mL)	153.2 $\pm$ 59.3	182.6 $\pm$ 84.5	0.268	175.2 $\pm$ 84.2	177.1 $\pm$ 58.4	0.948
Osteocalcin (ng/mL)	13.8 $\pm$ 4.7	13.7 $\pm$ 4.2	0.941	13.1 $\pm$ 4.1	16.4 <sup>a</sup> $\pm$ 4.1	0.037
PTH (ng/mL)	71.1 $\pm$ 14.0	72.3 $\pm$ 13.8	0.786	73.1 $\pm$ 13.8	67.2 $\pm$ 13.1	0.249

<sup>a</sup>P < 0.05 vs males.

prothrombin concentration compared to the control. The most significant decrease in prothrombin concentration was detected in HCV-infected patients (Table 2).

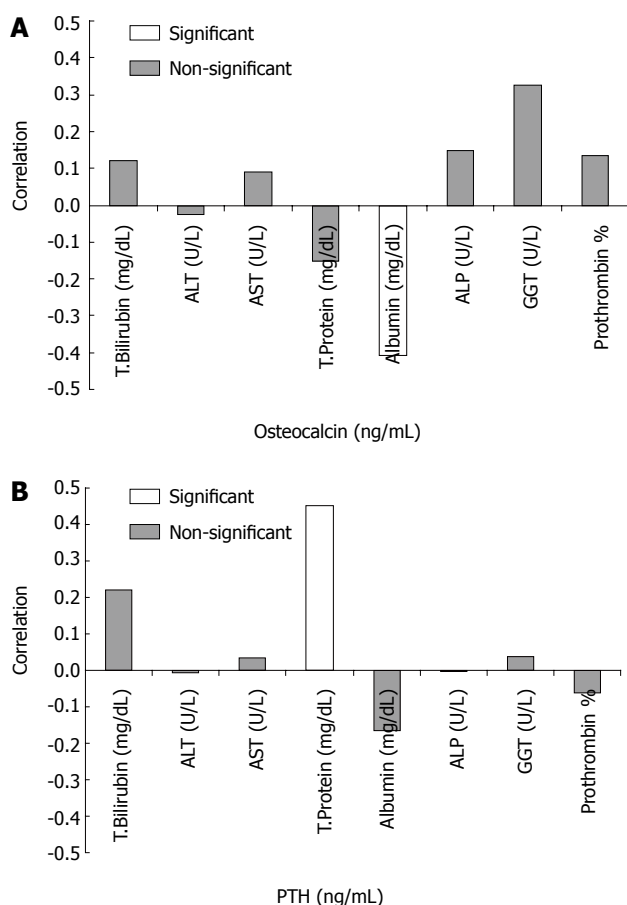
Alterations in serum IGF-1, osteocalcin and PTH level according to the severity of the disease from Child class A to C are depicted in Table 3. Significant ( $P < 0.05$ ) change was detected in the three serum markers (IGF-1, osteocalcin and PTH) in each grade of the disease. The mean values of the three serum markers were markedly increased with the severity of the disease and the highest value was recorded in Child class C of the disease using LSD at probability 0.05.

Noteworthy, our results revealed that serum IGF-1, osteocalcin and PTH levels were not age or sex dependent except for osteocalcin level which was higher in females (Table 4).

On correlating the three bone markers with each other and liver function indices using Pearson correlation, there was only significant negative correlation between serum osteocalcin and albumin ( $r = -0.409$ ,  $P = 0.027$ ) and significant positive correlation between PTH and total protein ( $r = 0.451$ ,  $P = 0.014$ ) in patients with HCV infection (Figure 1A and B), while no significant correlation was recorded between IGF-1, PTH or osteocalcin in our patients.

## DISCUSSION

Patients with chronic liver disease are prone to develop hepatic osteodystrophy. In the majority of cases it is



**Figure 1** Correlation coefficient between serum osteocalcin and albumin levels (A) & between serum PTH and total protein levels (B) in patients with HCV infection.

characterized by development of osteoporosis and/or osteomalacia with possible persistence of minor or major disability<sup>[3]</sup>. Osteoporosis reflects loss of bone (both matrix and its mineral) and osteomalacia is due to defective mineralization of osteoid<sup>[22]</sup>. Osteoporosis is a frequent complication of end-stage liver disease irrespective of its etiology. The prevalence varies between 9% and 60%, the highest being observed in cholestatic disorders and alcoholic liver disease<sup>[23]</sup>.

The present study aimed at elucidating the influence of bilharziasis, hepatitis B or C infections on the development of osteoporosis in children. Serum levels of IGF-1, osteocalcin and PTH were measured and correlated with the routine liver functions as well as the child-pough score. The results of the present work showed that serum IGF-1 level was significantly decreased in all children infected with bilharziasis, HBV and HCV, while unexpectedly it showed significant increase with the severity of liver diseases from A to B or C classification but its level was still lower than that in the control.

These results are greatly supported by the finding of Hassan *et al*<sup>[24]</sup> who showed a significant reduction in the level of IGF-1 in children with schistosomiasis with or without hepatic fibrosis. Also, our results are in agreement with the findings of Orsini *et al*<sup>[25]</sup> who found similar results in hepatosplenic schistosomiasis patients and the results of Mazziotti *et al*<sup>[26]</sup> in patients with hepatocellular carcinoma (HCC) and hepatitis C virus-related cirrhosis. The

reduction in IGF-1 level also can precede the diagnosis of HCC<sup>[26]</sup>. Similarly, decreased serum IGF-1 level has been recorded both in patients with viral cirrhosis<sup>[9,27]</sup> and in patients with hepatitis B infection without cirrhosis<sup>[28]</sup>.

In schistosomiasis patients with hepatic fibrosis, the depressed circulation of IGF-1, free T4 and growth hormone may be responsible for stunted stature<sup>[24]</sup>. Moreover, low serum IGF-1 level in hepatosplenic schistosomiasis patients has been found to be related to the clinical form of hepatosplenic schistosomiasis<sup>[25]</sup>. The level of IGF-1 is elevated in patients with schistosomiasis with or without liver fibrosis and relates to its clinical form<sup>[25]</sup>, suggesting that the severity of liver dysfunction can affect the level of IGF-1. As liver function of patients with bilharziasis is usually better than those with cirrhosis due to HBV and HCV infection and usually scored as child A, this may partly explain the relatively unexpected low level of IGF-1 in our patients with less severe liver affection (child A group).

The present study recorded a significant increase in serum osteocalcin level in patients with viral hepatitis infection only. Also a negative correlation was obtained with the level of serum albumin in patients with HCV infection. Moreover, serum osteocalcin level was significantly increased in child B and C patients than in child A patients.

It has been reported that serum osteocalcin level is higher in cirrhotic patients than in control, suggesting that cirrhotic patients have high bone turnover. In addition, osteodystrophy associated with hepatic cirrhosis is due to a defect in alpha-hydroxylation (1, 25(OH)<sub>2</sub>D) by the kidney due to the decrease in its primary substrate, the liver 25(OH)<sub>2</sub>D. This could be attributed to the reduced availability of vitamin D, inadequate conversion of vitamin D to 25(OH)<sub>2</sub>D, accelerated metabolism of 25(OH)<sub>2</sub>D, and urinary loss of 25(OH)<sub>2</sub>D with its transport protein<sup>[29]</sup>. However, some studies reported that serum osteocalcin level is low in cirrhotic patients and osteopenia in these patients is not due to a decrease in bone formation<sup>[30,31]</sup>, but may be a consequence of hepatic osteodystrophy due to low plasma vitamin D and calcium levels<sup>[31]</sup>.

Concerning the changes in serum PTH level, the present study recorded significant increase in patients with Hepatitis B and C infections. As regard a positive correlation was obtained with serum total protein level among the group of HCV infection. Moreover, serum PTH was significantly increased in class B and C patients than patients of class A.

In hepatocellular dysfunction some previous studies reported that serum PTH level is high in patients with primary biliary cirrhosis<sup>[32]</sup>, liver cirrhosis<sup>[33]</sup>, HBV infection<sup>[34]</sup>, as well as in dogs with schistosomiasis<sup>[35]</sup> and in children with cholestatic and non-cholestatic liver disease<sup>[36]</sup>. On the other hand, other studies reported that PTH is unchanged in patients with chronic viral liver disease<sup>[37]</sup> and post hepatic cirrhosis<sup>[38]</sup>. Kirch and co-workers<sup>[33]</sup> have found a significant correlation between PTH and the parameters of liver functions such as prothrombin, albumin and bilirubin, suggesting that the increasing PTH level is related to liver dysfunction. This may explain why the elevation of PTH level is due



to the impaired liver function rather than secondary hyperparathyroidism<sup>[33]</sup>. Moreover, elevated PTH-related protein was observed with hyperglobulinemia and hypoalbuminemia in dogs with schistosomiasis<sup>[35]</sup>, and this may explain the obtained positive correlation between PTH and serum total protein levels in patients with HCV infection due to hyperglobulinemia observed in our study.

Hepatic osteodystrophy (HOD) begins at the stage of chronic non-cirrhotic liver injury and bone loss is connected with liver damage, suggesting that the principal pathogenesis of HOD is attributed to intestinal Ca-malabsorption as a result of low serum albumin and villous atrophy<sup>[39]</sup>. Lower ionized calcium resulting from deficient intestinal absorption due to low 25(OH)<sub>2</sub>D, leads to increase in PTH level<sup>[36]</sup>.

The significant rise in serum osteocalcin level in our female patients indicated that their bone turnover was accelerated. This result is in agreement with Steinberg *et al*<sup>[40]</sup> who found that female sex is a risk factor for bone turnover and low bone density.

In conclusion, serum IGF-1, osteocalcin and PTH appear to be markers of bone metabolism in children with hepatocellular damage. Low level of IGF-1 seems to play a role in the bone mass loss in patients with chronic liver disease. Elevated biochemical markers of bone remodeling suggest high bone turnover in children with viral infections. Fortification of diet by ergocalciferol is essential in patients with chronic liver disease and regular bone density measurements are necessary in these patients.

## REFERENCES

- Seibel MJ. Bone metabolism, mineral homeostasis and its pharmacological modulation. *Clin Lab* 2004; **50**: 255-264
- Iber F. Bone disease in chronic liver disease. *Am J Gastroenterol* 1989; **84**: 1229-1230
- Rabinovitz M, Shapiro J, Lian J, Block GD, Merkel IS, Van Thiel DH. Vitamin D and osteocalcin levels in liver transplant recipients. Is osteocalcin a reliable marker of bone turnover in such cases? *J Hepatol* 1992; **16**: 50-55
- Ravn P, Fledelius C, Rosenquist C, Overgaard K, Christiansen C. High bone turnover is associated with low bone mass in both pre- and postmenopausal women. *Bone* 1996; **19**: 291-298
- Guañabens N, Parés A, Mariñoso L, Brancós MA, Piera C, Serrano S, Rivera F, Rodés J. Factors influencing the development of metabolic bone disease in primary biliary cirrhosis. *Am J Gastroenterol* 1990; **85**: 1356-1362
- Bonkovsky HL, Hawkins M, Steinberg K, Hersch T, Galambos JT, Henderson JM, Millikan WJ, Galloway JR. Prevalence and prediction of osteopenia in chronic liver disease. *Hepatology* 1990; **12**: 273-280
- Compston JE. Hepatic osteodystrophy: vitamin D metabolism in patients with liver disease. *Gut* 1986; **27**: 1073-1090
- Wu YL, Ye J, Zhang S, Zhong J, Xi RP. Clinical significance of serum IGF-I, IGF-II and IGFBP-3 in liver cirrhosis. *World J Gastroenterol* 2004; **10**: 2740-2743
- Delmas PD, Wahner HW, Mann KG, Riggs BL. Assessment of bone turnover in postmenopausal osteoporosis by measurement of serum bone Gla-protein. *J Lab Clin Med* 1983; **102**: 470-476
- Boniolo A, Dovis M, Matteja R. Use of an enzyme-linked immunosorbent assay for screening hybridoma antibodies against hepatitis B surface antigen. *J Immunol Methods* 1982; **49**: 1-15
- Tedder RS, Wilson-Croome R. IgM-antibody response to the hepatitis B core antigen in acute and chronic hepatitis B. *J Hyg (Lond)* 1981; **86**: 163-172
- Hoofnagle JH, Gerety RJ, Barker LF. Antibody to hepatitis-B-virus core in man. *Lancet* 1973; **2**: 869-873
- Alter HJ, Prince AM. Transfusion-associated non-A, non-B hepatitis: an assessment of the causative agent and its clinical impact. *Transfus Med Rev* 1988; **2**: 288-293
- Ravaggi A, Primi D, Cariani E. Direct PCR amplification of HCV RNA from human serum. *PCR Methods Appl* 1992; **1**: 291-292
- Hoshino S, Camargo ME, da Silva LC. Standardization of a hemagglutination test for schistosomiasis with formalin-treated human erythrocytes. *Am J Trop Med Hyg* 1970; **19**: 463-470
- Poller L. Laboratory control of anticoagulant therapy. *Semin Thromb Hemost* 1986; **12**: 13-19
- Blum WF, Albertsson-Wikland K, Rosberg S, Ranke MB. Serum levels of insulin-like growth factor I (IGF-I) and IGF binding protein 3 reflect spontaneous growth hormone secretion. *J Clin Endocrinol Metab* 1993; **76**: 1610-1616
- Coleman RE, Mashiter G, Fogelman I, Whitaker KD, Caleffi M, Moss DW, Rubens RD. Osteocalcin: a potential marker of metastatic bone disease and response to treatment. *Eur J Cancer Clin Oncol* 1988; **24**: 1211-1217
- Goltzman D, Henderson B, Loveridge N. Cytochemical bioassay of parathyroid hormone: characteristics of the assay and analysis of circulating hormonal forms. *J Clin Invest* 1980; **65**: 1309-1317
- Snedecor GW, Cochran WG. Statistical Methods, 7th ed. Ames, Iowa: The Iowa State University Press, 1980
- Walter A, Duncan DB. Multiple range and multiple test. *Biometrics* 1969; **11**: 1-24
- Sherlock S, Dooley J. Cholestasis. In: Diseases of the liver and biliary system. 10th ed., Sherlock S, Dooley J (editors), Oxford: Blackwell Scientific Publications, 1997: 217-273
- Schiefke I, Fach A, Wiedmann M, Aretin AV, Schenker E, Borte G, Wiese M, Moessner J. 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**: 1843-1847
- Hassan AH, Abd el Moneim MA, Abd el Aal AA, Abou Aly SA, Ahmed SH, Soliman AT, el Kersh MM. Circulating growth hormone, insulin-like growth factor I, cortisol and free thyroxine in children with schistosomiasis with and without hepatic fibrosis. *J Trop Pediatr* 1991; **37**: 25-30
- Orsini M, Rocha RS, Disch J, Katz N, Rabello A. The role of nutritional status and insulin-like growth factor in reduced physical growth in hepatosplenic Schistosoma mansoni infection. *Trans R Soc Trop Med Hyg* 2001; **95**: 453-456
- Mazziotti G, Sorvillo F, Morisco F, Carbone A, Rotondi M, Stornaiuolo G, Precone DF, Cioffi M, Gaeta GB, Caporaso N, Carella C. Serum insulin-like growth factor I evaluation as a useful tool for predicting the risk of developing hepatocellular carcinoma in patients with hepatitis C virus-related cirrhosis: a prospective study. *Cancer* 2002; **95**: 2539-2545
- Gallego-Rojo FJ, Gonzalez-Calvin JL, Muñoz-Torres M, Mundi JL, Fernandez-Perez R, Rodrigo-Moreno D. Bone mineral density, serum insulin-like growth factor I, and bone turnover markers in viral cirrhosis. *Hepatology* 1998; **28**: 695-699
- Chui SH, Chan K, Chui AK, Shek LS, Wong RN. The effects of a Chinese medicinal suppository (Vitaliver) on insulin-like growth factor 1 and homocysteine in patients with hepatitis B infection. *Phytother Res* 2005; **19**: 674-678
- Suzuki K, Arakawa Y, Chino S, Yagi K. Hepatic osteodystrophy. *Nihon Rinsho* 1998; **56**: 1604-1608
- Resch H, Pietschmann P, Krexner E, Woloszczuk W, Willvonseder R. Peripheral bone mineral content in patients with fatty liver and hepatic cirrhosis. *Scand J Gastroenterol* 1990; **25**: 412-416
- Crosbie OM, Freaney R, McKenna MJ, Hegarty JE. Bone density, vitamin D status, and disordered bone remodeling in end-stage chronic liver disease. *Calcif Tissue Int* 1999; **64**: 295-300

- 32 **Fonseca V**, Epstein O, Gill DS, Menon RK, Thomas M, McIntyre N, Dandona P. Hyperparathyroidism and low serum osteocalcin despite vitamin D replacement in primary biliary cirrhosis. *J Clin Endocrinol Metab* 1987; **64**: 873-877
- 33 **Kirch W**, Höfig M, Ledendecker T, Schmidt-Gayk H. Parathyroid hormone and cirrhosis of the liver. *J Clin Endocrinol Metab* 1990; **71**: 1561-1566
- 34 **Wiczowski A**. The influence of hypervolemia on the secretion of atrial natriuretic peptide, the renin-angiotensin-aldosterone system's activity and concentration of vasopressin, parathormone and calcitonin in hepatitis B virus infected patients with chronic liver diseases. *Przegl Epidemiol* 1994; **48**: 433-440
- 35 **Fradkin JM**, Braniecki AM, Craig TM, Ramiro-Ibanez F, Rogers KS, Zoran DL. Elevated parathyroid hormone-related protein and hypercalcemia in two dogs with schistosomiasis. *J Am Anim Hosp Assoc* 2001; **37**: 349-355
- 36 **Mehany NL**, Mostafa AA, El-karakasy H, El-Asrag HA and El-Bayoumy AS. Vitamin D metabolism and related biochemical parameters in children with chronic liver diseases. *Arab J Lab Med* 2000; **26**: 331-348
- 37 **Duarte MP**, Farias ML, Coelho HS, Mendonça LM, Stabnov LM, do Carmo d Oliveira M, Lamy RA, Oliveira DS. Calcium-parathyroid hormone-vitamin D axis and metabolic bone disease in chronic viral liver disease. *J Gastroenterol Hepatol* 2001; **16**: 1022-1027
- 38 **Karan MA**, Erten N, Tascioglu C, Karan A, Sindel D, Dilsen G. Osteodystrophy in posthepatitic cirrhosis. *Yonsei Med J* 2001; **42**: 547-552
- 39 **Nakano A**, Kanda T, Abe H. Bone changes and mineral metabolism disorders in rats with experimental liver cirrhosis. *J Gastroenterol Hepatol* 1996; **11**: 1143-1154
- 40 **Steinberg KK**, Bonkovsky HL, Caudill SP, Bernhardt RK, Hawkins M. Osteocalcin and bone alkaline phosphatase in the serum of women with liver disease. *Ann Clin Lab Sci* 1991; **21**: 305-314

S- Editor Liu Y L- Editor Wang XL E- Editor Ma WH