

## Study of Serum Osteocalcin Level as a Biomarker of Severity in Patients of NAFLD

Roquiya Begum<sup>1</sup>, Suhail Ahmad<sup>2\*</sup>, Farhan Usmani<sup>3</sup>, Ram Binay Sinha<sup>4</sup>

<sup>1</sup>Tutor, <sup>3</sup>Professor & Head, <sup>4</sup>Professor,

Department of Biochemistry, Patna Medical College, Patna, Bihar, India.

<sup>2\*</sup>Associate Professor,

Department of Pharmacology, Lord Buddha Koshi Medical College, Saharsa, Bihar, India.

### ABSTRACT

**Introduction:** Osteocalcin has been considered an established marker of bone formation; but the newer evidences suggested its role in the obesity, metabolic syndrome, and type 2 diabetes mellitus. Thus, it plays an important part both in bone and energy metabolism.

**Methodology:** This study was conducted in the Department of Biochemistry, Department of Pharmacology & Department of Medicine, PMCH, Patna. Two groups were included in this study. One is Non-NAFLD group and another group is NAFLD. Non-NAFLD group having 130 cases while NAFLD group having 44 cases. The duration of study was over a period of one year.

**Results:** This study revealed that serum osteocalcin levels are inversely associated with NAFLD.

**Conclusion:** This study conclude that, osteocalcin is considered as a biomarker of severity in patients of NAFLD.

**Keywords:** Osteocalcin, Nonalcoholic Fatty Liver Disease, Parameters.

### \*Correspondence to:

**Dr. Suhail Ahmad,**  
Associate Professor,  
Department of Pharmacology,  
Lord Buddha Koshi Medical College,  
Saharsa, Bihar, India.

### Article History:

Received: 27-11-2019, Revised: 24-12-2019, Accepted: 29-01-2020

### Access this article online

Website: <a href="http://www.ijmrp.com">www.ijmrp.com</a>	Quick Response code 
DOI: 10.21276/ijmrp.2020.6.1.032	

### INTRODUCTION

Bone is also an active endocrine organ regulating several metabolic processes via the release of bone-derived hormones, including osteocalcin. Hormone osteocalcin is a small protein which is synthesized by osteoblasts. Though it is principally deposited in the bone mineral matrix, but it is also present in the blood, in both uncarboxylated and fully and partially carboxylated forms. It is involved in bone extracellular matrix mineralization. So far, osteocalcin has been considered an established marker of bone formation; but the newer evidences suggested its role in the obesity, metabolic syndrome, and type 2 diabetes mellitus. Thus, it plays an important part both in bone and energy metabolism.<sup>1-4</sup> Nonalcoholic Fatty Liver Disease (NAFLD) is a common liver disease in adults but not so common in younger age group. NAFLD is a disease, in the absence of alcoholic abuse, with accumulation of fat in the liver (Hepatosteatorosis) with or without associated inflammation (Steatohepatitis) to necrosis, fibrosis and even cirrhosis like alcoholic hepatitis. NAFLD has been reported to be associated with obesity, diabetes mellitus and hypertension the main features of the metabolic syndrome. With the increasing

prevalence of obesity in the younger age group, NAFLD is becoming one of the most common causes of liver diseases in obese children and young adults affecting up to one third of obese children. For the pathogenesis of NAFLD, a "multi-hit" theory has been proposed. As per this theory, the first "hit" was insulin resistance which by lipolysis and hyperinsulinemia, led to the collection of fat within hepatocytes. The second "hit" was the lipid peroxidation, cytokine induction and mitochondrial release of reactive oxygen species (ROS). Patients with chronic NAFLD have been reported to develop metabolic bone disease that may be ascribed to diminished osteocalcin levels. It has been reported that osteocalcin-gene deficiency plays an important role in the pathogenesis of metabolic syndrome in form of insulin resistance (IR), increased fat mass and glucose intolerance.<sup>5-7</sup> In addition, serum osteocalcin levels were significantly associated with plasma adiponectin levels and inversely related to leptin levels, presence of metabolic syndrome.<sup>8</sup> This close relation may lead us to understand the control of metabolism by bone and control of bone by fat.

**MATERIALS & METHODS**

**Study Area:** This study was conducted in the Department of Biochemistry, Department of Pharmacology & Department of Medicine, PMCH, Patna.

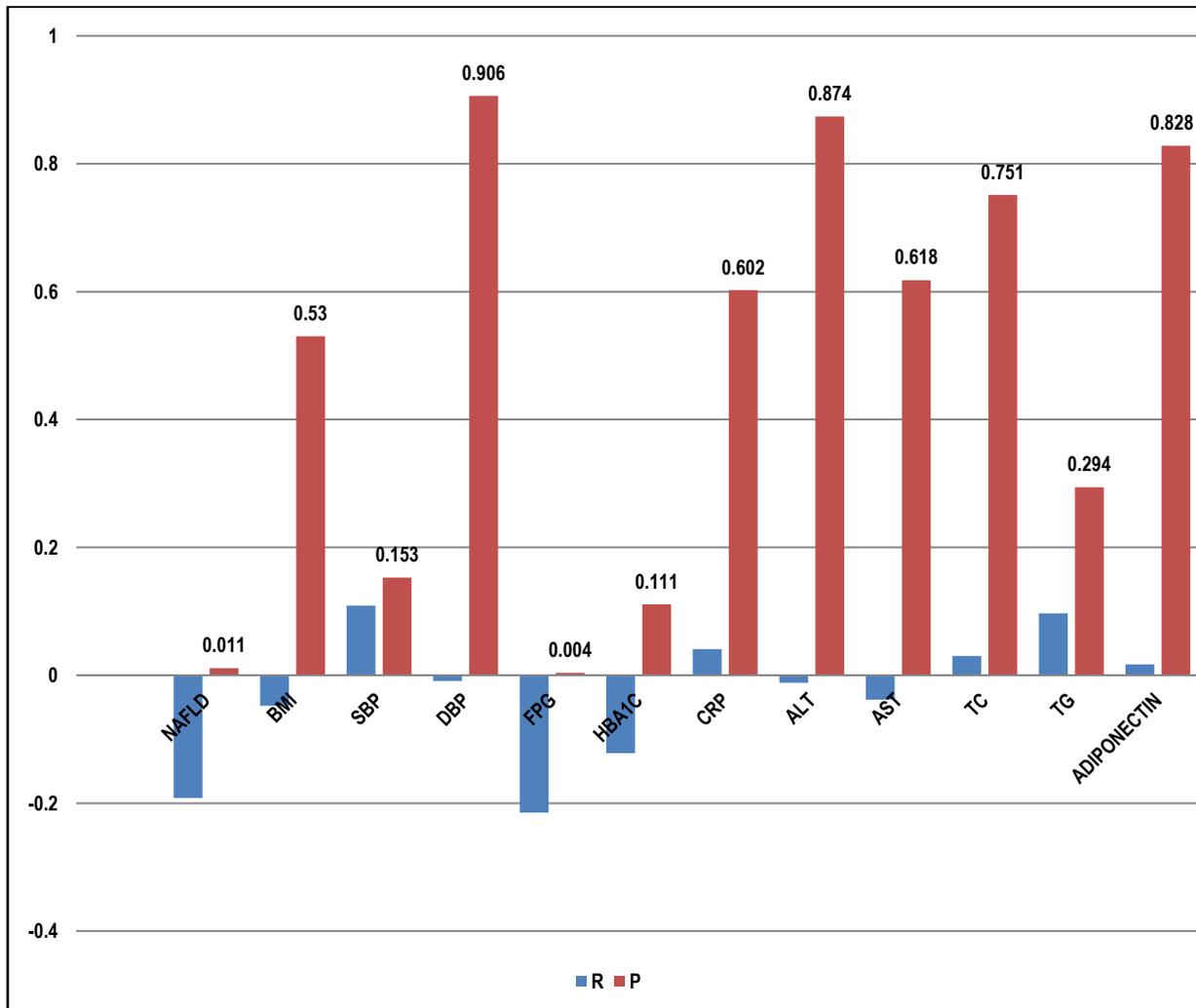
**Study Population:** Two groups were included in this study. One is Non-NAFLD group and another group is NAFLD. Non-NAFLD group having 130 cases while NAFLD group having 44 cases.

**Study Duration:** The duration of study was over a period of one year.

**Data Collection:** Consent were taken from all study groups. Weight (in kg) and height (in m) were recorded and used to calculate the Body Mass Index (BMI; kg/m<sup>2</sup>). Waist circumference (W) was measured at the midpoint between the inferior border of the lowest rib and the upper margin of the iliac crest, in the mid-axillary line. Blood pressure (BP) was measured with a sphygmomanometer manometer. Patients were instructed to fast overnight (10 h), then fasting and 2-h-postprandial venous blood

samples were collected. Fasting plasma glucose (FPG) and 2-h-postprandial glucose (2 h PG) levels were measured using the glucose oxidase method. Fasting serum insulin (FINS) levels were determined by radioimmunoassay and used to calculate  $\beta$  as follows: auto-analyzer was used to measure the levels of the liver function markers aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transpeptidase (GGT) (by enzymatic methods); and lipid levels including those of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) (again by enzymatic methods). C-reactive protein (CRP) levels were determined via particle-enhanced immunonephelometry using the Cardiophase hs-CRP Reagent. Serum adiponectin levels were measured with a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). Total serum osteocalcin levels were quantified via electrochemiluminescent.

**Data Analysis:** Data was analyzed by using Microsoft excel & statistics.



**Chart 1: Association between serum osteocalcin levels and parameters**

**RESULTS**

In this study we included two groups. One group has 130 cases, which were non-NAFLD group. While, another group has 44 cases, which were NAFLD group. In Non-NAFLD group 89 male & 41 were female out of 130 cases. Whereas, in NAFLD group 34 were male & 10 were female. We found that serum osteocalcin

levels were inversely related with NAFLD, BMI, SBP, FPG & HB1AC. We show that different parameters in both group in table 3. We also showed association between serum osteocalcin levels and parameters in chart 1. We found that serum osteocalcin levels were negatively associated with NAFLD, BMI, DBP, FPG, HBA1C, ALT & AST.

**Table 1: Distribution of cases according to groups**

GROUPS		No. of cases
Group I	Non-NAFLD	130
Group II	NAFLD	44
Total		174

**Table 2: Distribution of cases according to gender**

GENDER	Non-NAFLD	NAFLD
MALE	89	34
FEMALE	41	10
TOTAL	130	44

**Table 3: This table showing different parameters in Non-NAFLD & NAFLD group**

PARAMETERS	Non-NAFLD	NAFLD
BMI	23.4±2.7	26.5±2.9
SBP	130	140
DBP	75	80
FPG	5.3	6.4
HBA1C	6.1	6.9
TC	4.1±1.0	4.7±1.2
TG	1.4	2.3
HDL-c	1.1	0.9
LDL-c	2.8±0.9	3.1±1.0
CRP	0.9	2.1
ALT	16	26
AST	20	22
GGT	21	35
ADIPONECTIN	8.2	5.6

**Table 4: Distribution of cases according to drug in both groups**

	Non-NAFLD	NAFLD
ANTI-DIABETIC DRUG	31	13
ANTI-HYPERTENSIVES	60	16
STATINS THERAPY	39	15
TOTAL	130	44

**Table 5: Serum osteocalcin levels**

Serum osteocalcin	Mean ± SD
Non-NAFLD	48.3 ± 7.8
NAFLD	52.8 ± 7.6

**DISCUSSION**

It has been reported in a case-control study that the serum levels of osteocalcin were significantly lower in NAFLD while some other studies refuted these results. It has been reported previously that the serum levels of osteocalcin were inversely correlated with NAFLD (P<0.01) but without any statistical significance.<sup>9</sup> Same claims have been done by some other authors<sup>10,11</sup> who worked on

severely obese patients and/ or in pre- and post-menopausal females with osteopenia or osteoporosis. In another study with hepatocyte ballooning in NALFD patients, serum osteocalcin levels were inversely associated with both serum ALT and AST levels.<sup>12</sup> Lower serum levels of osteocalcin were found to be associated with NAFLD in a large-scale population-based study.<sup>13</sup> Improvement in hepatic steatosis was reported in some animal studies in which subcutaneous infusion or intermittent intraperitoneal injection of osteocalcin were given to wild-type mice.<sup>14-17</sup> In the same studies, osteocalcin was also reported to improve NAFLD by reducing the endoplasmic reticulum stress via the NF-κB signaling pathway and the levels of pro-inflammatory and pro-fibrotic genes . It has been reported in some recent studies with mice that the osteocalcin was associated with glucose and lipid metabolism<sup>18</sup> showing glucose tolerance, insulin sensitivity and increased insulin secretion. The same findings have been reported in various human epidemiological studies. Similarly, in the present study, serum levels of osteocalcin have been found to be associated with FPG and HbA1c levels. The body mass, TG and HbA1c levels were reported to be independently correlated with NAFLD in CAD patients. Thus, the serum levels of osteocalcin levels were found to be significantly lower in CAD patients with NAFLD than those without.<sup>8,18,19</sup>

**CONCLUSION**

Though, it can be suggested by the present study that the serum levels of osteocalcin were independently and negatively associated with the presence of NAFLD in CAD patients. So, osteocalcin is considered as a biomarker of severity in patients of NAFLD.

**REFERENCES**

- Musso G, Paschetta E, Gambino R, Cassader M, Molinaro F. Interactions among bone, liver, and adipose tissue predisposing to diabetes and fatty liver. *Trends Mol Med* 2013; 19: 522-35.
- Cairns JR, Price PA. Direct demonstration that the vitamin K-dependent bone Gla protein is incompletely gamma-carboxylated in humans. *J Bone Miner Res* 1994; 9: 1989-97.
- Brown JP, Delmas PD, Malaval L, Edouard C, Chapuy MC, Meunier PJ. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. *Lancet* 1984;1:1091-3.
- Ducy P, Desbois C, Boyce B, Pinero G, Story B, Dunstan C, Smith E, Bonadio J, Goldstein S, Gundberg C, Bradley A, Karsenty G. Increased bone formation in osteocalcin-deficient mice. *Nature* 1996; 382: 448-52.
- Pardee PE, Dunn W, Schwimmer JB. Non-alcoholic fatty liver disease in association with low bone mineral density in obese children. *Alimentary Pharmacology & Therapeutics* 2012; 35: 248-54.
- Lee NK, Karsenty G. Reciprocal regulation of bone and energy metabolism. *Trends in Endocrinology & Metabolism* 2008;19:161-6.
- Ducy P. The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. *Diabetologia* 2011; 54: 1291.
- Saleem, U., Mosley, T.H., Jr, and Kullo, I.J. Serum Osteocalcin Is Associated With Measures of Insulin Resistance, Adipokine Levels, and the Presence of Metabolic Syndrome. *Arterioscler. Thromb. Vasc. Biol.* 2010; 30(7): 1474–8. doi:10.1161/ATVBAHA.110.204859. PMID:20395593.

9. Dou J, Ma X, Fang Q, Hao Y, Yang R, Wang F, Zhu J, Bao Y, Jia W. Relationship between serum osteocalcin levels and non-alcoholic fatty liver disease in Chinese men. *Clin Exp Pharmacol Physiol* 2013; 40: 282-8.
10. Sinn DH, Gwak GY, Rhee SY, Cho J, Son HJ, Paik YH, Choi MS, Lee JH, Koh KC, Yoo BC, Paik SW. Association between serum osteocalcin levels and non-alcoholic fatty liver disease in women. *Digestion* 2015; 91: 150-7.
11. Díez Rodríguez R, Ballesteros Pomar MD, Calleja Fernández A, Calleja Antolin S, Cano Rodríguez I, Linares Torres P, Jorquera Plaza F, Olcoz Goñi JL. Vitamin D levels and bone turnover markers are not related to non-alcoholic fatty liver disease in severely obese patients. *Nutr Hosp* 2014; 30: 1256-62.
12. Yilmaz Y, Kurt R, Eren F, Imeryuz N. Serum osteocalcin levels in patients with nonalcoholic fatty liver disease: association with ballooning degeneration. *Scand J Clin Lab Invest* 2011; 71: 631-6.
13. Liu JJ, Chen YY, Mo ZN, Tian GX, Tan AH, Gao Y, Yang XB, Zhang HY, Li ZX. Relationship between serum osteocalcin levels and non-alcoholic fatty liver disease in adult males, South China. *Int J Mol Sci* 2013; 14: 19782-91.
14. Ferron M, McKee MD, Levine RL, Ducy P, Karsenty G. Intermittent injections of osteocalcin improve glucose metabolism and prevent type 2 diabetes in mice. *Bone* 2012; 50: 568-75.
15. Zhou B, Li H, Xu L, Zang W, Wu S, Sun H. Osteocalcin reverses endoplasmic reticulum stress and improves impaired insulin sensitivity secondary to diet-induced obesity through nuclear factor- $\kappa$ B signaling pathway. *Endocrinology* 2013; 154: 1055-68.
16. Gupte AA, Sabek OM, Fraga D, Minze LJ, Nishimoto SK, Liu JZ et al. Osteocalcin protects against nonalcoholic steatohepatitis in a mouse model of metabolic syndrome. *Endocrinology* 2014; 155: 4697-705.
17. Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, Confavreux C, Dacquin R, Mee PJ, McKee MD, Jung DY, Zhang Z, Kim JK, Mauvais-Jarvis F, Ducy P, Karsenty G. Endocrine regulation of energy metabolism by the skeleton. *Cell* 2007; 130: 456-69.
18. Zhou M, Ma X, Li H, Pan X, Tang J, Gao Y, Hou X, Lu H, Bao Y, Jia W. Serum osteocalcin concentrations in relation to glucose and lipid metabolism in Chinese individuals. *Eur J Endocrinol* 2009; 161: 723-9.
19. Yeap BB, Chubb SA, Flicker L, McCaul KA, Ebeling PR, Beilby JP, Norman PE. Reduced serum total osteocalcin is associated with metabolic syndrome in older men via waist circumference, hyperglycemia, and triglyceride levels *Eur J Endocrinol* 2010; 163: 265-72.

**Source of Support:** Nil.

**Conflict of Interest:** None Declared.

**Copyright:** © the author(s) and publisher. IJMRP is an official publication of Ibn Sina Academy of Medieval Medicine & Sciences, registered in 2001 under Indian Trusts Act, 1882. This is an open access article distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

**Cite this article as:** Roquiya Begum, Suhail Ahmad, Farhan Usmani, Ram Binay Sinha. Study of Serum Osteocalcin Level as a Biomarker of Severity in Patients of NAFLD. *Int J Med Res Prof.* 2020 Jan; 6(1):130-33. DOI:10.21276/ijmrp.2020.6.1.032