

Bone Metabolism Biomarkers as Potential Diagnostic Tools for Non-Alcoholic Fatty Liver Disease: Findings from a Case–Control Study

Sara M. Muhammed Ali^{*,1}, Ali A. Kasim²

and Mohammed H. Al-Rikabi³

¹Department of Need Estimation, Technical Affairs Directorate, Ministry of Health, Baghdad, Iraq.

²Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

³Gastroenterology and Hepatology Teaching Hospital in Medical City, Ministry of Health, Baghdad, Iraq.

*Corresponding author

Received 19/11/2025, Accepted 27/1/2026, Published 28/9/2026



This work is licensed under a Creative Commons Attribution 4.0 International License.

Abstract

Non-alcoholic fatty liver disease (NAFLD) is increasingly recognized as a systemic condition affecting extrahepatic organs, including bone. Alterations in bone metabolism may occur early in the disease course. This study aimed to evaluate the diagnostic and prognostic relevance of selected bone metabolism markers osteocalcin, C-terminal telopeptide of type I collagen (CTX), and fibroblast growth factor 23 (FGF23) in NAFLD patients. A case-control study was conducted involving 45 NAFLD patients and 45 age-, sex-, and body mass index-matched healthy controls. Serum levels of osteocalcin, CTX, and FGF23 were measured using ELISA. The degree of hepatic steatosis was assessed by ultrasonography, while fibrosis risk was evaluated using the FIB-4 index. Statistical analyses included ROC curve analysis, correlation, and group comparisons. NAFLD patients showed significantly lower osteocalcin and higher CTX and FGF23 compared with controls ($p < 0.001$). ROC analysis revealed strong diagnostic accuracy for osteocalcin (AUC = 0.976, sensitivity 97.8%, specificity 86.7%), CTX (AUC = 0.894, sensitivity 91.1%, specificity 93.3%), and FGF23 (AUC = 0.922, sensitivity 97.8%, specificity 84.4%). While these markers correlated with liver enzymes and fibrosis indices, they were not significantly associated with steatosis or fibrosis severity. In conclusion, alterations in osteocalcin, CTX, and FGF23 suggest a metabolic link between liver and bone. These markers may serve as promising non-invasive diagnostic biomarkers for NAFLD, but larger studies with histological validation are needed.

Keywords: Non-alcoholic fatty liver disease (NAFLD), Hepatic fibrosis, CTX, FGF23, Osteocalcin

Introduction

Non-alcoholic fatty liver disease (NAFLD), also referred to as metabolic associated fatty liver disease (MAFLD), is a condition marked by the buildup of excess fat in 5% or more of hepatocytes excluding other identifiable causes, including excessive alcohol intake, viral hepatitis, certain medications (such as methotrexate, steroids, amiodarone, and tamoxifen), and inherited or acquired disorders like Wilson's disease and lipodystrophy⁽¹⁾. It includes a range of liver disorders, from simple steatosis to non-alcoholic steatohepatitis (NASH). NAFLD is frequently linked to metabolic syndrome and conditions like obesity, type 2 diabetes mellitus (T2DM), and dyslipidemia, making it increasingly prevalent due to the rising global rates of these disorders. The global prevalence of NAFLD has increased more than 50% in the last thirty years, rising from 25.3% during the period 1990–2006 reaching up to 38.0% during 2016–2019⁽²⁾. Several risk factors have been

identified to be associated with NAFLD; including genetic, demographic, environmental, clinical, and dietary factors^(3–7). The exact mechanism behind NAFLD is not fully described; yet it involves factors such as fructose and caloric intake, alterations in gut microbiota, lipotoxicity, insulin resistance (IR), oxidative stress, and inflammatory processes⁽⁸⁾. Chronic steatohepatitis transforms hepatic stellate cells into myofibroblasts, which results in hepatic fibrosis. The prognosis of liver fibrosis significantly increases the risk of severe complications, including cirrhosis, hepatic failure, hepatocellular carcinoma, and death^(9, 10).

Liver biopsy as the gold standard for accurate assessment of fibrosis stage in NAFLD patients; however, number of non-invasive techniques have been developed for assessment of hepatic fibrosis, including transient elastography, the fibrosis index based on 4 factors (FIB-4) index, the aspartate aminotransferase/alanine

aminotransferase (AST/ALT) ratio, the AST to platelet ratio index (APRI), and the NAFLD Fibrosis Score (NFS) are widely validated and recommended tests due to their low cost and ease of access in routine clinical practice⁽¹¹⁻¹³⁾.

The liver serves as the central hub of the body's metabolic processes interacting with other tissues, including the bones, through the secretion of hormones and metabolic signaling molecules⁽¹⁴⁻¹⁶⁾. Osteoporosis or diminished bone mineral density (BMD) is a common complication of many types of chronic liver diseases, such as cholestatic liver disease, viral hepatitis and NAFLD⁽¹⁷⁾. There is a significant body of evidence suggesting a detrimental effect of NAFLD on BMD⁽¹⁸⁻²⁰⁾. NAFLD often accompanies several metabolic abnormalities like obesity, IR, and dyslipidemia⁽²¹⁾. These metabolic disorders can interfere with bone metabolism and subsequently leading to reduced BMD. Obesity can increase bone resorption and decreasing bone formation due to the mechanical stress directed on bones⁽²²⁾. IR and dyslipidemia can lead to disturbance of the balance between bone resorption and formation, and hence leading to negative impact on bone health^(23, 24). Additionally, chronic inflammation and oxidative stress that are associated with NAFLD can deteriorate bone health. The inflammatory cytokines such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α) can mediate bone resorption and in the same time inhibiting bone formation⁽²⁵⁾. While, oxidative stress that is characterized by imbalance in reactive oxygen species (ROS) and the antioxidant defenses of NAFLD patients; thus, impairing bone remodeling and reducing BMD^(26, 27). Alterations in adipokine serum levels in NAFLD, particularly adiponectin and leptin that are known to affect bone remodeling may also negatively impacting bone metabolism⁽²⁸⁾. Furthermore, liver dysfunction in NAFLD patients can interfere with vitamin D metabolism and resulting in lower calcium absorption and utilization that further compromising bone health⁽²⁹⁾.

Several bone metabolism markers have been studied to assess bone density changes in the course of NAFLD⁽³⁰⁾. Osteocalcin that reflects bone formation has been shown to be decreased in chronic liver dysfunction⁽³¹⁾. Furthermore, C-terminal telopeptide of type I collagen (CTX), a sensitive marker of bone resorption, has potentially linked to systemic inflammation in NAFLD⁽³²⁾. Additionally, fibroblast growth factor 23 (FGF23), which regulates phosphate and vitamin D metabolism has been proposed to represent a metabolic bridge between hepatic and skeletal dysfunction⁽³³⁾.

While several studies have assessed bone density changes in NAFLD, few have simultaneously evaluated the diagnostic potential of osteocalcin, CTX, and FGF23 as non-invasive biomarkers of early NAFLD in an Iraqi population, with careful exclusion of confounding comorbidities. This study aimed to assess the diagnostic relevance of osteocalcin, CTX, and FGF23 in NAFLD patients and to explore their potential as indicators of early metabolic crosstalk between bone and liver.

Materials and Methods

This case-control study was conducted from November 2024 to February 2025 at the Gastroenterology and Hepatology Teaching Hospital in Medical City, Baghdad, Iraq. This hospital is a large tertiary referral hepatology center, where NAFLD is a common diagnosis; participants were drawn from ongoing outpatient visits. Ninety participants were recruited in the study; 45 NAFLD patients diagnosed based on ultrasonography by an expert gastroenterologist according to the European Association for the Study of the Liver (EASL) criteria⁽³⁴⁾, and 45 subjects who were age-, sex-, and BMI-matched to the NAFLD patients, and were apparently healthy to serve as the control group. EASL criteria for the diagnosis of NAFLD include evidence of hepatic steatosis demonstrated by imaging techniques (including abdominal ultrasonography), serum biomarkers/scores or histology; with the exclusion of secondary causes of hepatic fat accumulation, excessive alcohol intake, and other competing liver diseases⁽³⁴⁾. With 45 patients per group, the study had >80% power to detect medium effect sizes (Cohen's $d \geq 0.6$) at $\alpha = 0.05$. The recruited subjects were adult and of both sexes; verbal consent was obtained from each participant after being informed about the purpose of the study and the expected benefits. Subjects with the following conditions were excluded from the study: a history of secondary osteoporosis (e.g., hyperthyroidism, chronic kidney disease, and malignancies), the use of medications known to affect bone metabolism (e.g., corticosteroids, bisphosphonates, hormone replacement therapy) within the last 3 months, alcohol consumption, presence of chronic inflammatory diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus) that may affect bone metabolism, pregnant or lactating women. Severe cardiovascular disease, dyslipidemia, and diabetes patients were also excluded to explore early, non-complicated NAFLD rather than advanced metabolic syndrome-related disease. Participants were informed about the purpose of the study; verbal consent was obtained from each participant before enrollment in the study.

A total of six milliliters of blood was collected from each participant. One milliliter was placed in an ethylenediaminetetraacetic acid (EDTA) tube for platelet counting using a hematology autoanalyzer (Sysmex XP-300; Japan). The remaining blood was placed in a gel tube and allowed to coagulate for 15 minutes before being centrifuged at 4000 rpm for 15 minutes to obtain serum. Approximately 1 milliliter of the serum was used to measure AST and ALT levels directly with COBAS 311 automated analyzer (Roche Diagnostics, Switzerland). The leftover serum sample was stored at -20°C until it was needed for measuring osteocalcin, CTX, and FGF23 levels using enzyme-linked immunosorbent assay (ELISA) (Huma Reader HS, Human; Germany) with the corresponding human ELISA kits obtained from (ELK Biotechnology; USA).

The severity of steatosis in NAFLD patients, assessed through B-Mode transabdominal ultrasound, is categorized as follows: **Mild** when there is a diffuse increase in fine echoes in the liver parenchyma compared to the kidney cortex, with normal visualization of the diaphragm and intrahepatic vessel borders; **Moderate** when there is a diffuse increase in fine echoes with slight impairment of the visualization of intrahepatic vessels and diaphragm; and **Marked** when there is a significant increase in fine echoes with poor or no visualization of the intrahepatic vessel borders, diaphragm, and the posterior right lobe of the liver⁽³⁵⁾.

To assess the risk of hepatic fibrosis in NAFLD patients, FIB-4 index was calculated based on the following equation⁽³⁶⁾:

$$FIB - 4 = \frac{\text{Age (years)} * \text{AST (U/L)}}{\text{Platelet count } (10^9 / \text{L}) * \sqrt{\text{ALT (U/L)}}$$

A FIB-4 score below 1.45 signifies low risk, scores ranging from 1.45 to 3.26 indicate intermediate risk, and scores exceeding 3.26 represent high risk⁽³⁷⁾.

Statistical analysis

The statistical analysis was performed using the Statistical Package for Social Science (SPSS),

version 27. Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess whether variables were normally distributed. Categorical variables were presented as frequency and percentages, and differences between the groups were analyzed using the Chi-square test. Numerical variables were presented as medians and interquartile ranges (IQR), and the differences between the two study groups as well as the association between the bone metabolism markers and fibrosis severity in NAFLD patients were assessed by Mann-Whitney U test. On the other hand, the association between bone metabolism markers and steatosis severity in NAFLD patients was assessed by Kruskal-Wallis test. While, Spearman's correlation was used to assess the correlation among the studied variables. Receiver operating characteristic (ROC) curve analysis was conducted to evaluate the diagnostic performance of the bone metabolism markers in distinguishing NAFLD patients from controls. This included estimating the area under the curve (AUC), sensitivity, specificity, and optimal cut-off values. The AUC ranges from 0 to 1, with values closer to 1 indicating stronger discriminatory ability between NAFLD patients and healthy individuals. A p-value less than 0.05 was considered statistically significant.

Results

The age of NAFLD patients was 37.28(11.47) years, with age range of (18 – 60) year. On the other hand the age of the control subjects was 33(10) year, with age range (20-64) year. About 48.8% of NAFLD patients were male and 51.2% were female; while 51.2% of the control group participants were male and 48.8% were female. Furthermore, the BMI of NAFLD patients and the control group participants were 33.5 (9.75), and 32.3 (4.51) kg/m^2 ; respectively). The lack of statistical difference between the two study groups concerning age, sex, and BMI of participants eliminates the potential effect of those variables on the serum levels of the studied biochemical variables.

Table 1. Demographic characteristics of participants

Variable	NAFLD (n-45)	Control (n-45)	p-value
Age (year)	37.28(11.47)	33(10)	0.133
Sex			
male	22(48.8%)	23(51.2%)	.833
female	23(51.2%)	22(48.8%)	
BMI (kg/m^2)	33.5(9.75)	32.3(4.51)	0.990

NAFLD: Non-alcoholic fatty liver disease, n: Number of participants, BMI: Body mass index.

Sex is presented as frequencies and percentages, and group comparison was performed using the Chi-square test. Age and BMI are presented as median (IQR), and the Mann-Whitney U test was used to evaluate difference between groups.

The serum levels of AST and ALT in NAFLD patients were 26 (17.35) U/L and 34 (31.85) U/L, respectively. These levels were significantly higher compared to those in the control subjects ($p < 0.001$). Likewise, the FIB-4 index was significantly higher in NAFLD patients at 0.88 (0.59) compared to 0.38 (0.2) in the control subjects ($p < 0.001$). Conversely, the platelet count in NAFLD patients was significantly lower than in the control subjects, measuring $200 (105) \times 10^9/L$ compared to $300 (106) \times 10^9/L$, respectively ($p < 0.001$); as shown in Table (2). The highly

significant difference of these variables between the study groups underscores their roles in the pathogenesis of NAFLD.

Approximately 22.22% of NAFLD patients exhibited mild steatosis, 31.12% exhibited moderate steatosis, and 46.66% exhibited marked steatosis; moreover, 91.11% of NAFLD patients were classified as being at low risk for fibrosis, while only 8.89% were categorized as having an intermediate risk and none of the patients were at high risk; as shown in Table (2).

Table 2. Biochemical and clinical characteristics of participants

Variable	NAFLD (n-45)	Control (n-45)	p-value
AST(U/L)	26(17.35)	16.41(4.66)	<0.001
ALT(U/L)	34(31.85)	21.78(8.32)	<0.001
Platelets count ($\times 10^9/L$)	200(105)	300(106)	<0.001
FIB-4 Index	0.88(0.59)	0.38(0.2)	<0.001
Severity of steatosis			
Mild	10(22.22%)	-----	-----
Moderate	14(31.12%)	-----	-----
Marked	21(46.66%)	-----	-----
Risk of Fibrosis			
Low	41(91.11%)	-----	-----
Intermediate	4 (8.89%)	-----	-----
High	0	-----	-----

NAFLD: Non-alcoholic fatty liver disease, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, FIB-4, Fibrosis index based on 4 factors, n: number of participants.

Severity of steatosis and risk of fibrosis are presented as frequencies and percentages. Numerical variables are presented as median (IQR), and the Mann-Whitney U test was used to evaluate difference between groups.

The serum levels of osteocalcin in NAFLD patients were 1.87 (1.56) ng/ml, which were significantly lower compared to the control subjects, who had levels of 4.45 (0.56) ng/ml ($p < 0.001$). On the contrary, the CTX levels in NAFLD patients were significantly higher at 5.26 (0.35) ng/ml compared to 3.07 (1.38) ng/ml in the control

group ($p < 0.001$). Similarly, FGF23 levels were markedly elevated in NAFLD patients, measured at 394.19 (137.08) pg/ml compared to 203.44 (41.71) pg/ml in the control subjects ($p < 0.001$); as shown in Table (3). The findings suggest a complex interplay between liver disease and bone metabolism.

Table 3. Serum levels of bone metabolism markers of participants

Variable	NAFLD (n-45)	Control (n-45)	p-value
Osteocalcin (ng/ml)	1.87(1.56)	4.45 (0.56)	<0.001
CTX (ng/ml)	5.26 (0.35)	3.07(1.38)	<0.001
FGF23(pg/ml)	394.19(137.08)	203.44 (41.71)	<0.001

NAFLD: Non-alcoholic fatty liver disease, CTX: C-terminal telopeptide of type I collagen, FGF23: Fibroblast growth factor 23, n: number of participants

Data are presented as median (IQR) and the Mann-Whitney U test was used to evaluate difference between groups.

The serum levels of osteocalcin in NAFLD patients with mild steatosis were 2.41 (2.18) ng/ml, which were higher compared to those with moderate steatosis at 1.88 (1.51) ng/ml and marked steatosis at 1.71 (0.64) ng/ml, although the differences were not statistically significant ($p = 0.156$). In contrast, the CTX levels among NAFLD

patients showed minimal variation, with mild steatosis at 5.26 (1.66) ng/ml, moderate at 5.29 (0.37) ng/ml, and marked at 5.31 (0.27) ng/ml, indicating no significant differences across groups ($p = 0.852$). Similarly, FGF23 levels were recorded at 403.7 (200.65) pg/ml in mild steatosis, 397.56 (94.47) pg/ml in moderate, and 339.94 (206.23)

pg/ml in marked steatosis, with no statistically significant differences observed ($p = 0.632$); as shown in Table (4). This could imply that the

degree of steatosis does not have a direct or strong impact on bone metabolism markers in NAFLD patients.

Table 4. Serum levels of bone metabolism markers according to severity of steatosis in NAFLD patients

Variable	Severity of steatosis in NAFLD patients			p-value
	Mild (n=10)	Moderate (n=14)	Marked (n=21)	
Osteocalcin (ng/ml)	2.41(2.18)	1.88 (1.51)	1.71(0.64)	0.156
CTX (ng/ml)	5.26 (1.66)	5.29 (0.37)	5.31(0.27)	0.852
FGF23(pg/ml)	403.7 (200.65)	397.56 (94.47)	339.94 (206.23)	0.632

NAFLD: Non-alcoholic fatty liver disease, CTX: C-terminal telopeptide of type I collagen, FGF23: Fibroblast growth factor 23, n: number of patients

Data are presented as median (IQR) and the Kruskal–Wallis test was used to evaluate difference among groups.

The serum levels of osteocalcin in NAFLD patients at low risk of fibrosis were 1.84 (0.69) ng/ml, which were lower compared to those at intermediate risk, who had levels of 3.38 (1.13) ng/ml. Although there was a notable difference, it did not reach statistical significance ($p = 0.056$). In contrast, the CTX levels among NAFLD patients showed minimal variation, with low risk patients at 5.31 (0.33) ng/ml and intermediate risk patients at 5.08 (0.21) ng/ml, indicating no significant differences across groups ($p = 0.091$). Similarly, FGF23 levels were 492 (159.45) pg/ml in low risk

patients, compared to 329.98 (86.65) pg/ml in those at intermediate risk, with no statistically significant differences observed ($p = 0.428$); as shown in Table (5). Serum levels of osteocalcin, CTX, and FGF23 do not appear to be clearly associated with fibrosis risk in this NAFLD sample. However, the limited number of patients in the intermediate-risk group ($n = 4$), along with the absence of patients at high risk of fibrosis, reduces the statistical power of the analysis. Therefore, these associations should be investigated further in larger, more balanced cohorts.

Table 5. Serum levels of bone metabolism markers according to the risk of fibrosis in NAFLD patients

Variable	Risk of fibrosis in NAFLD patients		p-value
	Low (n=41)	Intermediate (n=4)	
Osteocalcin (ng/ml)	1.84 (0.69)	3.38 (1.13)	0.056
CTX (ng/ml)	5.31 (0.33)	5.08 (0.21)	0.091
FGF23 (pg/ml)	492 (159.45)	329.98 (86.65)	0.428

NAFLD: Non-alcoholic fatty liver disease, CTX: C-terminal telopeptide of type I collagen, FGF23: Fibroblast growth factor 23, n: number of patients.

Data are presented as median (IQR) and the Mann-Whitney U test was used to evaluate difference between groups.

The correlation of osteocalcin, CTX, and FGF23 with various studied variables was analyzed. The results showed that there is a significant negative correlation between FGF23 and osteocalcin levels ($\rho = -0.628$, $p < 0.001$). On the contrary, CTX levels showed a significant positive correlation with FGF23 ($\rho = 0.604$, $p < 0.001$). Regarding osteocalcin, the results showed that there is a significant negative correlation with CTX ($\rho = -0.872$, $p < 0.001$). The demographic variables Age, sex, and BMI did not show significant correlation with the studied bone metabolism markers ($p > 0.05$). Furthermore, AST levels exhibited a significant negative correlation with osteocalcin ($\rho = -0.669$, $p < 0.001$) and a significant positive correlation with CTX ($\rho = 0.569$, $p < 0.001$) and FGF23 ($\rho = 0.593$, $p < 0.001$).

Similarly, ALT levels showed a significant negative correlation with osteocalcin ($\rho = -0.513$, $p < 0.001$) and a significant positive correlation with CTX ($\rho = 0.414$, $p < 0.001$) and FGF23 ($\rho = 0.293$, $p < 0.001$). Platelet counts demonstrated a significant positive correlation with osteocalcin ($\rho = 0.450$, $p < 0.001$) and a negative correlation with CTX ($\rho = -0.404$, $p < 0.001$) and FGF23 ($\rho = -0.368$, $p < 0.001$). Lastly, the FIB-4 index showed a significant negative correlation with osteocalcin ($\rho = -0.602$, $p < 0.001$), while correlating positively with both CTX ($\rho = 0.515$, $p < 0.001$) and FGF23 ($\rho = 0.525$, $p < 0.001$); as shown in Table 6. These results show that bone metabolism markers are related to liver function indicators and fibrosis risk in patients with NAFLD.

Table 6. Correlation of bone metabolism markers with the studied variables of participants

Variable	Osteocalcin		CTX		FGF23	
	ρ_s	p-value	ρ_s	p-value	ρ_s	p-value
FGF23	-0.628	<0.001	0.604	<0.001	----	----
Osteocalcin	----	----	-0.872	<0.001	-0.628	<0.001
CTX	-0.872	<0.001	----	----	0.604	<0.001
Age	-0.163	0.124	0.139	0.193	0.082	0.443
Sex	0.167	0.254	0.091	0.245	0.021	0.911
BMI	0.032	0.762	-0.064	0.551	0.101	0.345
AST	-0.669	<0.001	0.569	<0.001	0.593	<0.001
ALT	-0.513	<0.001	0.414	<0.001	0.293	0.005
FIB-4 Index	-0.602	<0.001	0.515	<0.001	0.525	<0.001

FGF23: Fibroblast growth factor 23, CTX: C-terminal telopeptide of type 1 collagen, BMI: Body mass index, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, FIB-4: Fibrosis index based on 4 factors, ρ_s : Spearman correlation coefficient.

Receiver operating characteristic (ROC) analysis was used to examine whether osteocalcin, CTX, and FGF23 are capable of discriminating NAFLD patients from controls. Osteocalcin with an AUC = 0.976 and a cut-off of 3.51 ng/ml showed 97.8% sensitivity and 86.7% specificity ($p < 0.001$). Similarly, CTX showed excellent ability in this regard, with an AUC of 0.894. CTX showed strong potential as a diagnostic biomarker for NAFLD with a sensitivity of 91.1% and specificity

of 93.3%, at a cut-off 4.62 ng/ml and AUC=0.894 ($p < 0.001$). FGF23 also showed good potentials, showing an AUC of 0.922 with a cut-off value 240.25 pg/ml, and a sensitivity and specificity of 97.8% and 84.4%; respectively ($p < 0.001$), suggesting highly sensitivity and moderately specificity for identifying NAFLD patients. These findings suggest that osteocalcin, CTX and FGF23 may serve as promising diagnostic markers for NAFLD; as shown in Table (7).

Table 7. The receiver operating characteristic analysis of bone metabolism markers in identifying NAFLD

Variables	AUC	Cut-off	Sensitivity	Specificity	p-value
Osteocalcin (ng/ml)	0.976	3.51	0.978	0.867	$p < 0.001$
CTX (ng/ml)	0.894	4.62	0.911	0.933	$p < 0.001$
FGF23 (pg/ml)	0.922	240.25	0.978	0.844	$p < 0.001$

CTX: C-terminal telopeptide of type I collagen, FGF23: Fibroblast growth factor 23.

Discussion

The present study aimed to investigate the serum levels of the bone metabolism markers osteocalcin, CTX, and FGF23 in newly diagnosed NAFLD patients, and to evaluate potentials of these bone metabolism markers as diagnostic and prognostic biomarkers. The findings revealed significant differences in bone metabolism markers between NAFLD patients and healthy controls, suggesting a complex interaction between liver function and bone metabolism markers. The most important finding was the elevated levels of CTX and FGF23 and the reduced levels of osteocalcin in NAFLD patients. These results support that NAFLD may be associated with increased bone resorption and impaired bone formation and mineralization^(16, 20, 38).

The potential association between NAFLD and decreased BMD is still under debate. A clear relationship between NAFLD and BMD has been described particularly in patients with advanced

fibrosis^(16, 20, 38); however, some studies did not show such relationship^(39, 40). This controversy may be attributed to the differences in study designs, the dependence on cross-sectional findings, the fact that most of the studies did not use liver biopsy for accurate NAFLD diagnosis, and the presence of confounding factors such as comorbidities and medications. Despite these challenges, the large mass of evidence suggest that NAFLD is associated with alterations in bone metabolism, where bone resorption increases over the expense of bone formation, and may thus represent a risk factor for osteoporosis in NAFLD patients.

The diagnostic performance of osteocalcin, CTX, and FGF23 in distinguishing NAFLD patients from healthy controls that has been reported in the present study, go in accordance with the emerging evidence linking altered bone metabolism with metabolic liver disorders; a relationship potentially mediated IR, inflammation, systemic mineral metabolism disturbances and vitamin D deficiency⁽⁴¹⁻⁴⁵⁾. Taking into

consideration the reported high diagnostic potentials of the studied bone markers, then these markers may complement existing non-invasive tools for NAFLD detection, particularly when imaging or liver biopsy is limited. However, despite their promising diagnostic potentials, the results in this regard should be interpreted cautiously as external validation in independent cohorts is required to confirm their strength and generalizability.

Although serum levels of osteocalcin, CTX, and FGF23 in the present study were significantly different between NAFLD patients and controls, it is interesting that they were not significantly associated with the degree of hepatic steatosis or fibrosis risk in NAFLD patients. This may indicate that the disturbance in bone metabolism occurs early in the course of NAFLD and bone markers levels are not directly proportional to NAFLD severity. Alternatively, the limited number of patients with advanced fibrosis in the present cohort may reduce the ability to detect such associations. The observed near-significant association between osteocalcin and fibrosis risk suggests a potentially important association that should not be overlooked and deserves confirmation in larger and well-characterized study populations.

Correlation analyses provided additional insight into the biological relevance of these markers. The inverse relationship between osteocalcin and both CTX and FGF23 reflects a disruption in the balance between bone formation and resorption, while the associations of CTX and FGF23 with liver enzymes and the FIB-4 index suggest that bone metabolism alterations parallel markers of hepatic injury and fibrosis risk. These findings support the concept of a shared pathological axis linking liver dysfunction and skeletal metabolism, likely mediated by systemic inflammation, IR, and metabolic stress rather than fibrosis stage alone. (16, 20, 34)

The observed discrepancy between significant correlations with the continuous FIB-4 index and the lack of significant differences across fibrosis risk categories may be explained by methodological factors. Correlation analyses capture subtle associations across the full spectrum of values, whereas categorical subgroup analyses reduce statistical sensitivity, particularly in imbalanced samples. Moreover, changes in bone metabolism markers may represent early systemic responses to hepatic metabolic dysfunction that precede or occur independently of advanced fibrotic changes, especially when fibrosis is assessed using indirect indices rather than histological confirmation.

This study has several strengths, including careful matching of participants for age, sex, and

BMI, strict exclusion of confounding conditions affecting bone metabolism, and the simultaneous evaluation of multiple bone turnover markers reflecting distinct aspects of skeletal physiology. Nevertheless, several limitations should be acknowledged. The observational design precludes causal inference, and the relatively small sample size, particularly in higher fibrosis risk categories, limits statistical power. The absence of liver biopsy and unmeasured factors such as vitamin D status, dietary intake, and physical activity may also have influenced the findings. Additionally, the diagnostic performance of the biomarkers was evaluated within a single cohort, underscoring the need for external validation.

Conclusion

In patients with NAFLD, serum osteocalcin levels were significantly reduced, while CTX and FGF23 levels were markedly elevated compared with healthy controls. These alterations in bone metabolism markers suggest their potential utility as non-invasive biomarkers for NAFLD diagnosis and highlight the complex interplay between bone and liver metabolism. However, validation through larger, multi-center longitudinal studies with histological confirmation is warranted to establish their prognostic significance and further elucidate their mechanistic role in NAFLD pathophysiology.

Acknowledgment

The authors would like to thank the staff of the Gastroenterology and Hepatology Teaching Hospital in Baghdad for their support during sample collection

Conflicts of Interest

The authors declare no conflict of interest.

Funding

No funding sources.

Ethics Statements

The study was performed according to the Helsinki Declaration; and was approved by the Research Ethics Committee of the College of Pharmacy, University of Baghdad (Approval Number RE052428C).

Author Contribution

Authors contributed equally to this work.

References

1. Habibullah M, Jemmieh K, Ouda A, Haider MZ, Malki MI, Elzouki AN. Metabolic-associated fatty liver disease: a selective review of pathogenesis, diagnostic approaches, and therapeutic strategies. *Front Med (Lausanne)*. 2024 Jan 23;11:1291501.
2. Younossi ZM, Golabi P, Paik JM, Henry A, Van Dongen C, Henry L. The global epidemiology of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis

- (NASH): a systematic review. *Hepatology*. 2023 Apr 1;77(4):1335-47.
3. Sabah YH, Hassan F. The Link between NAFLD and Colorectal Cancer: Insights from Liver Function Enzymes and Serum Biomarkers in a Case-Control Study. *Journal of Biotechnology Research Center*. 2025 Jun 3;19(2):96-103.
 4. Alrubaie AL, Rezaqallah RE, Ahmed MA, Musa S, Al-Qanbar MH. Correlation between Body Mass Index and Nonalcoholic Fatty Liver Disease. *AL-Kindy College Medical Journal*. 2022 May 5;18(1):56-9.
 5. Abdul Sattar A, T. Al-Sa'adi Wa. Evaluation of Adiponectin, Irisin, Vitamin D Levels and Their Relation in Iraqi Patients with Non alcoholic Fatty Liver Disease. *Ibn AL-Haitham Journal For Pure and Applied Sciences*. 2023 Apr 20;36(2):241-50.
 6. ShojaeiBaghini M, Fattahi M, Mohammadi M, Hamzavi F. Risk Factors for Non-alcoholic Fatty Liver Disease from the Perspective of Medical Professionals: A Systematic Review and Expert Opinion. *Middle East J Dig Dis*. 2025 Jan;17(1):54-9.
 7. Abdulrahman NM, Shatha Hussein A. Polymorphism of Vitamin D Receptor Gene Taq1 (rs731236) and its Effect on Bone Turnover Markers in Iraqi Postmenopausal Osteoporotic Women After Vitamin D Supplementation. *Iraqi Journal of Pharmaceutical Sciences*. 2025 Jun 25;34(2):175-83.
 8. Savari F, Mard SA. Nonalcoholic steatohepatitis: A comprehensive updated review of risk factors, symptoms, and treatment. *Heliyon*. 2024 Mar 25;10(7):e28468.
 9. Zhang CY, Yuan WG, He P, Lei JH, Wang CX. Liver fibrosis and hepatic stellate cells: Etiology, pathological hallmarks and therapeutic targets. *World J Gastroenterol*. 2016 Dec 28;22(48):10512-22.
 10. Somnay K, Wadgaonkar P, Sridhar N, Roshni P, Rao N, Wadgaonkar R. Liver Fibrosis Leading to Cirrhosis: Basic Mechanisms and Clinical Perspectives. *Biomedicine*. 2024 Sep 30; 12(10):2229.
 11. Sanyal AJ, Castera L, Wong VW. Noninvasive Assessment of Liver Fibrosis in NAFLD. *Clin Gastroenterol Hepatol*. 2023 Jul;21(8):2026-39.
 12. Wang J, Qin T, Sun J, Li S, Cao L, Lu X. Non-invasive methods to evaluate liver fibrosis in patients with non-alcoholic fatty liver disease. *Front Physiol*. 2022 Dec 14;13:1046497.
 13. Kazi IN, Kuo L, Tsai E. Noninvasive Methods for Assessing Liver Fibrosis and Steatosis. *Gastroenterol Hepatol (NY)*. 2024 Jan;20(1):21-9.
 14. AlKenany Q, K. Rasheed M, A. J. AlKazraj K. The Predictive Role of Osteopontin Level in Patients with Type 2 Diabetes Mellitus without Fatty Liver Disease. *Journal of the Faculty of Medicine Baghdad*. 2024 Apr 1;66(1):93-8.
 15. Khammas I, Ali Abdullhussain K, Bassam F. Serum Levels of Advanced Oxidation Protein Products and some Liver Function Markers in Chronic Myeloid Leukemia-Chronic Phase Patients Receiving Imatinib or Nilotinib. *Iraqi Journal of Pharmaceutical Sciences*. 2025 Jun 25;34(2):40-6.
 16. Zhao J, Lei H, Wang T, Xiong X. Liver-bone crosstalk in non-alcoholic fatty liver disease: Clinical implications and underlying pathophysiology. *Front Endocrinol (Lausanne)*. 2023 Mar 10;14:1161402.
 17. Danford CJ, Trivedi HD, Bonder A. Bone Health in Patients With Liver Diseases. *J Clin Densitom*. 2020 Apr-Jun;23(2):212-22.
 18. Vachliotis ID, Anastasilakis AD, Goulas A, Goulis DG, Polyzos SA. Nonalcoholic fatty liver disease and osteoporosis: A potential association with therapeutic implications. *Diabetes Obes Metab*. 2022 Sep;24(9):1702-20.
 19. Hassan AM, Haridy MA, Shoaier MZ, Abdel-Aziz TM, Qura MK, Kenawy EM, *et al*. Non-alcoholic fatty liver disease is associated with decreased bone mineral density in upper Egyptian patients. *Sci Rep*. 2023 Mar 16;13(1):4353.
 20. Drapkina OM, Elkina AY, Sheptulina AF, Kiselev AR. Non-Alcoholic Fatty Liver Disease and Bone Tissue Metabolism: Current Findings and Future Perspectives. *Int J Mol Sci*. 2023 May 8;24(9):8445.
 21. Sun Y, Dai W, Liang Y, Yang P, Yang Q, Liang M, Xia N. Relationship between nonalcoholic fatty liver disease and bone mineral density in adolescents with obesity: a meta-analysis. *Diabetes Metab Syndr Obes*. 2019 Jan 30;12:199-207.
 22. Piñar-Gutierrez A, García-Fontana C, García-Fontana B, Muñoz-Torres M. Obesity and Bone Health: A Complex Relationship. *Int J Mol Sci*. 2022 Jul 27;23(15):8303.
 23. Zhou R, Guo Q, Xiao Y, Guo Q, Huang Y, Li C, Luo X. Endocrine role of bone in the regulation of energy metabolism. *Bone Res*. 2021 May 20;9(1):25.
 24. Zamil A, Amin S. Insulin Resistance in Association with Metabolic Syndrome in Wasit University Students in Iraq. *Iraqi Journal of Pharmaceutical Sciences*. 2023 Sep 27;32(2):65-73.
 25. Amarasekara DS, Yu J, Rho J. Bone Loss Triggered by the Cytokine Network in Inflammatory Autoimmune Diseases. *J Immunol Res*. 2015;2015:832127.

26. Bonaccorsi G, Piva I, Greco P, Cervellati C. Oxidative stress as a possible pathogenic cofactor of post-menopausal osteoporosis: Existing evidence in support of the axis oestrogen deficiency-redox imbalance-bone loss. *Indian J Med Res.* 2018 Apr;147(4):341-51.
27. Fairouz Aj, Jamal My, Alkhalidi Nm. Therapeutic Effects of Vitamin E in Non-alcoholic Fatty Liver Disease: An Open-Labeled Clinical Trial. *Iraqi Journal of Pharmaceutical Sciences.* 2022 Dec 24;31(2):135-43.
28. Tariq S, Baig M, Valjevac A. Editorial: Osteoporosis and adipokines: the potential for future treatment. *Front Endocrinol (Lausanne).* 2024 Apr 10;15:1405412.
29. Filip R, Radzki RP, Bieńko M. Novel insights into the relationship between nonalcoholic fatty liver disease and osteoporosis. *Clin Interv Aging.* 2018 Oct 4;13:1879-91.
30. Li C, Cui Y, Zhou W, Zhang Y, Huang X, Yu F. Bone Turnover Markers in Adults with Nonalcoholic Fatty Liver Disease: A Systematic Review and Meta-Analysis. *Int J Endocrinol.* 2023 Jul 19;2023:9957194.
31. Xia M, Rong S, Zhu X, Yan H, Chang X, Sun X, et al. Osteocalcin and Non-Alcoholic Fatty Liver Disease: Lessons From Two Population-Based Cohorts and Animal Models. *J Bone Miner Res.* 2021 Apr;36(4):712-728.
32. Deng H, Dai Y, Lu H, Li SS, Gao L, Zhu DL. Analysis of the correlation between non-alcoholic fatty liver disease and bone metabolism indicators in healthy middle-aged men. *Eur Rev Med Pharmacol Sci.* 2018 Mar;22(5):1457-1462.
33. Xie Y, Su N, Yang J, Tan Q, Huang S, Jin M, et al. FGF/FGFR signaling in health and disease. *Signal Transduct Target Ther.* 2020 Sep 2;5(1):181.
34. European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016 Jun;64(6):1388-402.
35. Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology.* 2002 Sep;123(3):745-50.
36. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D, Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology.* 2006 Jun;43(6):1317-25.
37. Sumida Y, Yoneda M, Tokushige K, Kawanaka M, Fujii H, Yoneda M, et al. FIB-4 First in the Diagnostic Algorithm of Metabolic-Dysfunction-Associated Fatty Liver Disease in the Era of the Global Metabodemic. *Life (Basel).* 2021 Feb 14;11(2):143.
38. Huang Q, Guo J, Zhao H, Zheng Y, Zhang Y. The associations of alcoholic liver disease and nonalcoholic fatty liver disease with bone mineral density and the mediation of serum 25-Hydroxyvitamin D: A bidirectional and two-step Mendelian randomization. *PLoS One.* 2023 Oct 19;18(10):e0292881.
39. Mantovani A, Dauriz M, Gatti D, Viapiana O, Zoppini G, Lippi G, Byrne CD, Bonnet F, Bonora E, Targher G. Systematic review with meta-analysis: non-alcoholic fatty liver disease is associated with a history of osteoporotic fractures but not with low bone mineral density. *Aliment Pharmacol Ther.* 2019 Feb;49(4):375-88.
40. Hansen SG, Wernberg CW, Grønkjær LL, Jacobsen BG, Caterino TD, Krag A, Juhl CB, Lauridsen MM, Shanbhogue VV. Are Nonalcoholic Fatty Liver Disease and Bone Mineral Density Associated? - A Cross-Sectional Study Using Liver Biopsy and Dual-Energy X-Ray Absorptiometry. *JBMR Plus.* 2023 Jan 11;7(3):e10714.
41. Guedes JAC, Esteves JV, Morais MR, Zorn TM, Furuya DT. Osteocalcin improves insulin resistance and inflammation in obese mice: Participation of white adipose tissue and bone. *Bone.* 2018 Oct;115:68-82.
42. Wu XL, Zou XY, Zhang M, Hu HQ, Wei XL, Jin ML, Cheng HW, Jiang S. Osteocalcin prevents insulin resistance, hepatic inflammation, and activates autophagy associated with high-fat diet-induced fatty liver hemorrhagic syndrome in aged laying hens. *Poult Sci.* 2021 Jan;100(1):73-83.
43. He X, Shen Y, Ma X, Ying L, Peng J, Pan X, Bao Y, Zhou J. The association of serum FGF23 and non-alcoholic fatty liver disease is independent of vitamin D in type 2 diabetes patients. *Clin Exp Pharmacol Physiol.* 2018 Jul;45(7):668-674.
44. Nysather J, Kaya E, Manka P, Gudsoorkar P, Syn WK. Nonalcoholic Fatty Liver Disease and Chronic Kidney Disease Cross Talk. *Adv Kidney Dis Health.* 2023 Jul;30(4):315-335.
45. Hanudel M, Jüppner H, Salusky IB. Fibroblast growth factor 23: fueling the fire. *Kidney Int.* 2016 Nov;90(5):928-930.

المؤشرات الحيوية لتمثيل العظام كأدوات تشخيصية محتملة لمرض الكبد الدهني غير الكحولي: نتائج من دراسة حالة- شاهد

سارة مازن محمد علي^{١*}، علي عبدالحسين قاسم^٢ و محمد هادي الركابي^٣

^١ قسم تقدير الحاجة، دائرة الأمور الفنية، وزارة الصحة، بغداد، العراق.

^٢ فرع العلوم المختبرية السريرية، كلية الصيدلة، جامعة بغداد، بغداد، العراق.

^٣ مستشفى أمراض الجهاز الهضمي والكبد التعليمي، مدينة الطب، بغداد، العراق.

الخلاصة

أن مرض الكبد الدهني غير الكحولي (NAFLD) هو حالة تصيب الكبد ولكنها تؤثر على الأعضاء خارج الكبد أيضاً، بما في ذلك العظام. قد تحدث التغيرات في أيض العظام في مراحل مبكرة من تطور المرض. هدفت هذه الدراسة إلى تقييم القيمة التشخيصية والتنبؤية لعدد من مؤشرات أيض العظام المختارة، وهي أوستيوكالسين (Osteocalcin)، والجزء النهائي الكربوكسي من الكولاجين النوع الأول (CTX)، وعامل نمو الأرومة الليفية^{٢٣} (FGF23)، لدى مرضى NAFLD. أجريت دراسة حالة-شاهد رصدية وشملت ٤٥ مريضاً مصاباً بـ NAFLD و ٤٥ شخصاً سليماً مناظرين في العمر والجنس ومؤشر كتلة الجسم. تم قياس مستويات أوستيوكالسين و CTX و FGF23 في مصل الدم باستخدام تقنية الإليزا. تم تقييم درجة التكتس الدهني الكبدي باستخدام التصوير بالموجات فوق الصوتية، بينما تم تقدير خطر التليف الكبدي باستخدام مؤشر التليف (FIB-4). شملت التحليلات الإحصائية تحليل منحنى ROC، وتحليل الارتباط، والمقارنات بين المجموعات.

أظهر مرضى NAFLD انخفاضاً معنوياً في أوستيوكالسين وارتفاعاً ملحوظاً في CTX و FGF23 مقارنةً بالأصحاء ($p < 0.001$). أوضح تحليل ROC دقة تشخيصية عالية لأوستيوكالسين و CTX و FGF23 (مساحة تحت المنحنى = 0.976، الحساسية ٩٧.٨٪، النوعية ٨٦.٧٪) و (مساحة تحت المنحنى = 0.894، الحساسية 91.1٪، النوعية 93.3٪) و (مساحة تحت المنحنى = 0.922، الحساسية ٩٧.٨٪، النوعية 84.4٪) على التوالي. ورغم ارتباط مؤشرات أيض العظام المقاسة بإنزيمات الكبد ومعامل FIB-4، فإنها لم تُظهر علاقة معنوية مع شدة التكتس الدهني أو التليف.

يمكن الاستنتاج أن التغيرات في مستويات أوستيوكالسين و CTX و FGF23 بين مجموعتي الدراسة تشير إلى وجود ترابط أبيض بين الكبد والعظام. وتُعد هذه المؤشرات واسمات حيوية واعدة للتشخيص غير الباضع لـ NAFLD، لكن هناك حاجة إلى دراسات أوسع مع تأكيد نسبي. الكلمات المفتاحية: مرض الكبد الدهني غير الكحولي، التليف الكبدي، الجزء النهائي الكربوكسي من الكولاجين النوع الأول، عامل نمو الأرومة الليفية^{٢٣}، أوستيوكالسين