Fetuin-A: a relevant novel serum biomarker for non-invasive diagnosis of metabolic dysfunction-associated steatotic liver disease (MASLD): a retrospective case-control study

Mohamed M. Elhoseeny[1], Badawy A. Abdulaziz[2], Mohamed A. Mohamed[2], Radwa M. Elsharaby[3], Ghadeer M. Rashad[2] and Amira A. A. Othman[1*]

Abstract

Objectives To determine how fetuin-A contributes to diagnosing and assessing MASLD severity.

Methods Fifty MASLD patients and fifty healthy control participants were involved in this retrospective case-control research. Abdominal ultrasonography, fibroscan with controlled attenuated parameter scan (CAP scan), laboratory investigation (including fetuin-A assessment), clinical examination, and history-taking were performed on every case.

Results Fetuin-A level was considerably higher in the Cases group (1154.85 ± 629.89) than in the Control group (505.29 ± 150.4) ($p < 0.001$). Fetuin-A had significant validity in the prediction of MASLD at a cut-off > 702.5 with 82% sensitivity, 90% specificity, and 86% overall accuracy.

Conclusion One possible marker for MASLD diagnosis could be fetuin-A. Furthermore, a substantial association between such marker and the severity of the disease as it revealed a significant correlation with ultrasound grading and fibroscan with controlled attenuated parameters.

Trial registration

1- Pan African Clinical Trial Registry.
Unique Identifying number/registration ID: PACTR202309644280965.
URL: https://pactr.samrc.ac.za/TrialDisplay.aspx?TrialID=26860.
Registration Approval date: 21/09/2023.
2- ClinicalTrials.gov.
Unique Identifying number /registration ID: NCT06097039.
URL: https://clinicaltrials.gov/study/NCT06097039?cond=NCT06097039&rank=1.

*Correspondence:
Amira A. A. Othman
Amira.Othman@med.suezuni.edu.eg
Full list of author information is available at the end of the article

Background

"Non-alcoholic fatty liver disease (NAFLD)" is a broad expression that encompasses a variety of conditions, including "non-alcoholic fatty liver (NAFLD, steatosis)", intrahepatic lipid accumulation without any secondary cause of liver fat accumulation such as heavy alcohol consumption, of at least 5% of the liver weight that causes liver dysfunction and inflammation, and advance to "non-alcoholic steatohepatitis (NASH)", which is distinguished by liver fibrosis, which advances to cirrhosis, and hepatocellular carcinoma in about 30–40% of cases [1]. The principal limitations of the terms NAFLD and NASH are the reliance on exclusionary confounder terms and the use of potentially stigmatizing language. The name chosen to replace NAFLD was metabolic dysfunction-associated steatotic liver disease (MASLD) [2]. MASLD is a serious challenge because of its prevalence, difficult diagnosis, complex etiology, and lack of recognized treatments [3]. It can be a consequence in non-drinker obese or diabetic patients (primary), affecting about 30–40% of the adult population and up to 95% of obese people. It may be a consequence of a toxin or drug (secondary) [4].

Imaging, including "ultrasonography (US)", "computed tomography (CT)", and "magnetic resonance imaging (MRI)", are often used to diagnose MASLD incidentally when routine liver function lab shows abnormal biochemical test results. The controlled attenuation parameter (CAP) represents a US-based quantitative, and non-invasive diagnostic tool for MASLD [5]. A liver biopsy, required to determine the fibrosis advancement, is intrusive and expensive [4], that is why extensive researches have been undertaken to identify relevant blood biomarkers for the early prediction of NAFLD.

Fetuin-A is a plasma carrier glycoprotein that is primarily produced by hepatocytes and is released into the bloodstream to aid in the transportation and availability of a wide range of drugs. It was originally known as phosphoprotein 63 KDa (pp63) or countertrypin. Its potential application as a biomarker for the diagnosis of MASLD has been studied [6]. It is expressed most frequently in adult hepatocytes and embryonic cells, and less frequently in monocytes and adipocytes. Fetuin-A has a wide range of physiological and pathological roles and binds to a variety of receptors. It plays a role in controlling the insulin signaling system, osteogenesis, and calcium metabolism. In addition to these multiple roles, it also plays the roles of an ectopic calcification inhibitor, protease inhibitor, inflammatory mediator, anti-inflammatory partner, atherogenic factor, and adipogenic factor [7]. Additionally, it has been shown that Fetuin-A plays a critical role in the pathophysiology of a number of diseases, including metabolic disorders, nonalcoholic fatty liver disease (NAFLD), insulin resistance (IR), type 2 diabetic mellitus (T2DM), cancers, and brain problems [8].

Our objective is to ascertain the extent to which relevant fetuin-A aids in the MASLD diagnosis and severity evaluation.

Methods

Study design and setting

Retrospective case-control research conducted at Benha University's hepatology, gastrointestinal, and infectious diseases departments, Egypt. This investigation was planned to be conducted between December 2021 and December 2022. **The study was conducted after being approved by the Benha University local ethics commission. Approval #: 1.8.19201. All contributors gave their voluntary, written, informed consent for the release of any connected photographs to meet the STROCSS standards** [9].

Subjects

This study included 100 individuals who were divided into two groups: **Group I** "Cases group" consisted of 50 MASLD patients diagnosed using liver biochemistry, abdominal ultrasound, and Fibroscan with CAP, and **Group II** "Control group" consisted of 50 healthy subjects who had normal liver function as determined by transabdominal ultrasonography and normal liver enzyme levels.

Patients with liver cirrhosis were included, they represented ascites, jaundice, enlarged spleen, exhaustion, red palms, and other complications.

Pregnant women, patients under 18 years of age, chronic heavy alcohol drinkers "more than 40 grams a

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day for men and 20 grams a day for women", patients with hepatobiliary cancers, patients with viral hepatitis like HBV or HCV, patients with chronic autoimmune hepatitis, Wilson disease, or hemochromatosis were rolled out from the study. The study also excluded participants who used steatogenic drugs like Amiodarone, Valproic Acid, and Tetracycline, antiretroviral medications, disease-modifying anti-rheumatic medication like methotrexate, or medications used to treat MASLD like vitamin E, and thiazolidinediones. Patients receiving metformin, glucagon-like peptide-1 (GLP-1) agonists, or sodium-glucose cotransporter-2 (SGLT2) Inhibitors were also excluded.

Clinical and biochemical characterization
Clinical examination
Each patient's clinical and pathological data were recorded including a full medical history and a detailed clinical examination, as well as symptoms of chronic fatigue, hypertension, and chronic liver disease.

Anthropometric evaluation
Body mass index "BMI" was calculated from measurements of height and weight. BMI has been graded accordingly into under-weight, optimum-weight, over-weight, and obese "< 18.5, < 24.9, < 25.0–29.9, and < 30.0 respectively" [10].

Lab biochemical assay

- Quantitative measurement of human fetuin-A, in serum, using sandwich enzyme-linked immunosorbent assay "Sandwich-ELISA kit: Epitope Diagnostics Inc.™, CatLog no. KT 800" following the manufacturer operating guidelines.
- MASLD Fibrosis score calculation formula [11]:

  "-1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio − 0.013 × platelet (×109/l) − 0.66 × albumin (g/dl)".

Imaging tests

- *Abdominal ultrasonography*:

A "real-time gray-scale device" with 3.5 MHZ frequency was utilized in ultrasonography patients' examination using. Patients were examined while fasting for 6 h and took anti-flatulent to get rid of gases. Scanning was done while patients were in a supine position.

Steatosis is often classified employing a particular scoring system; "Absent (S0): normal liver echogenicity. Mild (S1): a slight and diffuse increase in liver echogenicity with normal periportal and diaphragmatic

representation; moderate (S2): a moderate, diffuse increase in liver echogenicity with impaired portal vein wall and the diaphragm representation; Severe (S3): a marked diffuse increase in liver echogenicity with obscuring portal vein wall and the diaphragm representation" [12].

- *Fibroscan with Controlled Attenuated Parameter (CAP measurement)*:

The CAP is a novel technique added to the FibroScan® device for the non-invasive steatosis scoring assessment.

The "FibroScan® 502 device, Echosens, Paris, France" equipped with either the 3.5 MHz M-probe or 2.5 MHz XL-probe developed recently for obese subjects, is used to assess CAP and liver stiffness measurement (LSM). The value is expressed in decibels per meter (dB/m), ranging from 100 to 400 dB/m, and kilopascal (kPa) ranging from 2.5 to 75 kPa. A median CAP of at least 236 dB/m indicates liver steatosis. A reliable LSM measurement is obtained when having at least 10 valid shots and a success rate of ≥60% and an interquartile range (IQR)<30% of the median LSM value [13]. In liver biopsies, three grades of steatosis can be determined according to the level of CAP: "absent (S0<236 dB), mild (S1≥236 dB), moderate (S2≥270), and severe (S3≥302 dB)" [13]. The optimal cut-off LSM values to define advanced fibrosis (≥F3) and cirrhosis (F4), varied considerably according to the probe used: "F3=9.6–11.4 kPa, F4≥11.5 kPa" using M-probe, and "F3=9.3–10.9 kPa, F4≥11.0 kPa" using XL-probe [14].

Statistical analysis
"SPSS®" program (IBM, SPSS Inc, USA) was used to code, process, and analyze the data. "Median±SD" was used to display the data. "Numbers (frequency)" and "percentages" representing qualitative data were displayed. "The chi-square test" was used to compare the groups. "The Kolmogorov-Smirnov test" examined the normality of quantitative data. If the data were abnormally distributed, then "the Kruskal Wallis test" was applied; otherwise, "one-way ANOVA test" was used to compare normally distributed quantitative variables of the groups. "The receiver operator characteristic curve (ROC)" is used to define "the true-positive rate" (Sensitivity) as a function of "the false-positive rate" (Specificity). $P<0.05$ is considered significant for all tests.

Results
There were no statistically significant variations in age, or sex, among the studied groups, indicating that they were homogeneous. In terms of BMI, the studied groups differed statistically significantly. In the Cases group, 58% of

Table 1 Demographic data of the studied groups

Parameter		Cases group $N=50$	Control group $N=50$	P
Sex	Female	25(50%)	26(52%)	0.08
	Male	25(50%)	24(48%)	
Age (years)		42.46±10.41	40.62±11.63	0.06
BMI (kg/m2)		46.49±7.02	32.22±10.16	0.61
Diabetes mellitus		29(58%)	0	
Hypertension		21(42%)	0	
Hyperlipidemia		49(98%)	0	

Table 2 Imaging findings among studied groups

Parameter		Cases group $N=50$	Control group $N=50$
LSM fibroscan	F 0	19 (38%)	
	F 1	18 (36%)	
	F 2	4 (8%)	
	F 2–3	3 (6%)	
	F 3	4 (8%)	
	F 4	2 (4%)	
	Mean±Sd	6.58±3.35	
	Median (Range)	5.7 (3.1–24.1)	
CAP score	S 1	9 (18%)	
	S 2	15 (30%)	
	S 3	26 (52%)	
	Mean±Sd	287.5±44.62	
	Range	222–372	
US grade	1	9 (18%)	
	2	15 (30%)	
	3	26 (52%)	

Table 3 Laboratory findings among the studied groups

Parameter		Cases group $N=50$	Control group $N=50$	P
Hb (gm/dl)	Mean±Sd	13.54±0.98	13.43±1.01	0.60
	Range	11.5–15.2	11.5–15	
WBC (x10³/mm3)	Mean±Sd	4.72±1.17	4.98±1.63	0.36
	Range	3–8	2.5–8.5	
Platelets (x10³/mm3)	Mean±Sd	269.86±68.76	240.08±50.85	0.02
	Range	160–400	150–350	
FBS (mmol/l)	Mean±Sd	117.4±27	82.86±7.77	<0001
	Range	75–170	75–120	
2 h Post prandial (mmol/l)	Mean±Sd	193.42±67.81	110.24±14.03	<0.001
	Range	90–320	90–160	
Cholesterol (mg/dl)	Mean±Sd	193.8±26.53	164.2±17.25	<0.001
	Range	150–250	124–195	
Triglyceride (mg/dl)	Mean±Sd	176.86±15.63	135.9±17.51	<0.001
	Range	140–220	100–180	
LDL (mg/dl)	Mean±Sd	100.57±14.28	81.04±14.61	<0.001
	Range	75–140	40–100	
HDL (mg/dl)	Mean±Sd	44.82±12.84	65.36±7.92	<0.001
	Range	24–85	45–80	
ALT (U/L)	Mean±Sd	19.06±8.6	18.28±6.74	
	Median	16	18	0.92
	Range	8–42	10–34	
AST (U/L)	Mean±Sd	19.4±7.84	18.5±5.79	
	Median	12	18	0.48
	Range	7–38	10–35	
T. Bilirubin (mg/dl)	Mean±Sd	0.73±0.18	0.66±0.18	0.04
	Range	0.4-1	0.45-1	
D. Bilirubin (mg/dl)	Mean±Sd	0.36±0.12	0.34±0.11	0.29
	Range	0.1–0.6	0.2–0.6	
Albumin (gm/dl)	Mean±Sd	4.09±0.47	4.22±0.56	0.20
	Range	3.2-5	3-5.1	
Fetuin-A (ng/ml)	Mean±Sd	1154.85±629.89	505.29±150.4	<0.001
	Median	879	496	
	Range	592–2400	150–788	
MASLD F score	Mean±Sd	-2.10±1.66	-------	------
	Median	-20.8		
	Range	-4.5/ 2.88		

the patients had diabetes mellitus, 42% had hypertension, and 98% had hyperlipidemia (Table 1).

The mean LSM score was 6.58 with a range of 3.1 to 24.1 and the most frequent class in LSM fibroscan was F0 & F1 (38% & 36% respectively). The CAP score among the studied cases ranged from 222 to 372 with a mean of 287.5. The most frequent CAP class was S 3 (52%). The most frequent US grade among the studied cases was 3 (52%) (Table 2).

The cases group had a statistically significant higher platelet count, FBS, and 2-hour postprandial blood sugar than the control group. When compared to the control group, cases showed a statistically significant increase in cholesterol, TG, and LDL, as well as a statistically significant drop in HDL. In addition, the cases group had a statistically significant higher total bilirubin level than the control group. There was a statistically significant increase in cases group fetuin-A when compared to the control group (Table 3).

Fetuin-A levels were statistically significantly higher in S2 and S3 cases than in S1, as well as in US grade 3 cases than in grades 1 and 2 (Table 4).

Among the cases group, there was a statistically significant positive connection between Fetuin-A and both the CAP and MASLD scores. There was no statistically significant relationship between Fetuin-A and any of the examined parameters in the control group (Table 5).

Fetuin-A had a significant validity in the prediction of the non-alcoholic fatty liver at cut-off >702.89 with a sensitivity of 82%, specificity of 90%, and accuracy of 86% (Table 6).

The study found a correlation between Fetuin-A and CAP score among the studied cases (Fig. 1, A), a correlation between Fetuin-A and MASLD score among the studied cases (Fig. 1, B), and the Roc curve analysis showed the role of Fetuin-A in the prediction of MASLD among the studied groups (Fig. 1, C).

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Table 4 Relation between Fetuin-A and LSM, CAP score, and US grade among the studied cases group

Parameter		N	Fetuin-A		P
			Mean ± SD	Median	
LSM	F0	19	1210.13 ± 662.62	893	0.26
	F1	18	1045.73 ± 554.3	829.8	
	F2	4	1357.38 ± 794.39	1206.25	
	F2-3	3	1873.4 ± 870.16	2351.7	
	F3	4	884 ± 221.42	891	
	F4	2	670.45 ± 110.95	670.45	
CAP score	S1	9	808.96 ± 623.27	806	0.03
	S2	15	1232.89 ± 669.74	896	
	S3	26	1271.13 ± 635.3	903	
US grade:	1	9	1041.36 ± 522.21	806	0.04
	2	15	1085.22 ± 630.29	858	
	3	26	1343.63 ± 684.08	917	

Table 5 Correlation between Fetuin-A and different parameters among the studied cases and control groups

Parameter	Fetuin-A [Cases group] (n = 50)		Parameter	Fetuin-A [Control group] (n = 50)	
	r	P		r	P
Age	-0.05	0.74	Age	0.10	0.51
BMI	0.25	0.08	BMI	0.11	0.44
LSM fibroscan	0.07	0.65	Hb	0.03	0.82
CAP score	0.34	0.02	WBC	0.09	0.52
MASLD score	0.49	< 0.001	Platelets	0.08	0.59
Hb	0.03	0.81	FBS	0.06	0.71
WBC	0.14	0.35	Post prandial	0.13	0.38
Platelets	0.11	0.44	Cholesterol	0.13	0.35
FBS	0.09	0.55	Triglyceride	0.09	0.52
Post prandial	0.03	0.81	LDL	0.22	0.12
Cholesterol	0.27	0.06	HDL	-0.02	0.88
Triglyceride	0.11	0.44	ALT	0.06	0.66
LDL	0.08	0.60	AST	0.04	0.76
HDL	-0.21	0.14	T. Bilirubin	0.09	0.53
ALT	0.10	0.48	D. Bilirubin	0.03	0.86
AST	0.08	0.59	Albumin	-0.06	0.70
T. Bilirubin	0.07	0.65			
D. Bilirubin	0.11	0.45			
Albumin	0.05	0.71			

Discussion

MASLD is diagnosed using three criteria: the patient must not be an alcoholic, steatosis must be detected using imaging or histology, and other liver disorders must be ruled out. "Liver biopsy" remains the most reliable way of diagnosing NASH. However, there are several downsides to liver biopsy [15]. Non-invasive,

blood-based biomarkers with prognostic value in studies of MASLD patients: NAFLD fibrosis score (NFS), Fibrosis-4 (FIB-4), aspartate aminotransferase (AST) to platelet ratio index (APRI), enhanced liver fibrosis (ELF™), BARD (BMI, AST/ALT (alanine aminotransferase) ratio, diabetes), Hepamet Fibrosis Score (HFS), liver enzymes (AST + ALT), alpha-fetoprotein, platelet count, neutrophil to lymphocyte ratio (NLR), Lysyl oxidase-like (LOXL) 2, miR-122, liver stiffness, MEFIB (liver stiffness measured with magnetic resonance elastography (MRE) + FIB-4), and PNPLA3 GG genotype [16, 17].

Elevated fetuin-A levels have been associated with the development of a variety of liver-related metabolic issues, including IR, adipocyte inflammation, hepatocyte fibrosis, dyslipidemia, progressive macrophage infiltration, and increased toll-like receptor-4 (TLR4) expression resulting in obesity [18]. Recent clinical studies have found that a confirmed MASLD biopsy is related to elevated fetuin-A serum levels in conjunction with several MetS including poor glucose and lipid metabolism [19, 20]. Such findings unambiguously demonstrate that elevated fetuin-A levels in MASLD patients may be a helpful blood biomarker for early diagnosis [21, 22].

The current investigation found a statistically significant difference in BMI between cases and control groups, with mean values of 46.49 and 32.22 kg/m2, respectively. The rise in obesity, MetS, and T2D prevalence coincides with an increase in the incidence of NAFLD. Substantial prospective epidemiological studies have found a strong link between type 2 diabetes (T2D), obesity, and NAFLD. The three disorders are routinely treated as co-morbidities in various biomedical research settings. Patients with MASLD and NASH exhibited overall obesity rates of 51% and 81%, respectively with 60 to 95% prevalence [23].

Our study found that MASLD cases had a significantly higher platelet count than controls (269.86 vs. 240.08, respectively, but both groups were within normal ranges). Previous research supported our findings, as platelet count increased significantly in conjunction with MASLD compared to controls (251.36 vs. 246.22 respectively) [24, 25].

The elevated platelet count could indicate an inflammatory condition linked with MASLD [18, 19]. Platelets play a function in liver regeneration following liver damage, but they can also contribute to the development of liver fibrosis [24]. MASLD is characterized by "subclinical inflammation, and white blood cells (WBCs) and platelets" are frequent blood components used to detect

Table 6 Diagnostic performance of Fetuin-A in the prediction of non-alcoholic fatty liver among the studied groups

Cut off	AUC (95%CI)	Sensitivity	Specificity	PPV	NPV	Accuracy	P
> 702.891	0.95 (0.91–0.99)	82%	90%	89.1%	83.3%	86%	< 0.001

AUC Area under curve, *CI* Confidence interval, *PPV* Positive predicted value, *NPV* Negative predicted value
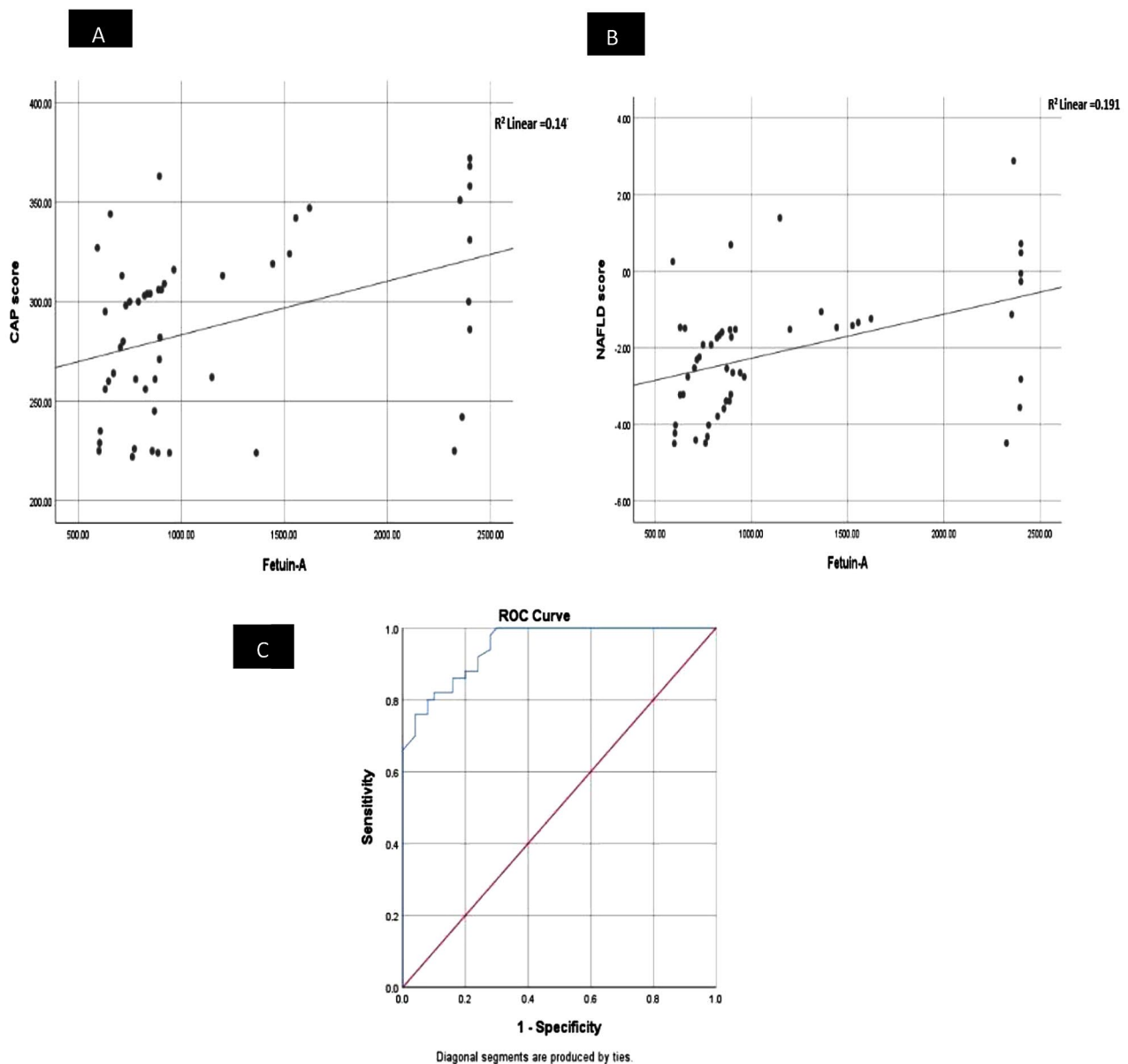
Fig. 1 (**A**): Correlation between Fetuin-A and CAP score among the studied cases group, (**B**): Correlation between Fetuin-A and MASLD score among the studied cases group, (**C**): Roc curve analysis for Fetuin-A in the prediction of non-alcoholic fatty liver among the studied groups

systemic inflammation in such cases. Recently, "mean platelet volume (MPV), lymphocyte-monocyte ratio.

(LMR), platelet-to-lymphocyte count ratio (PLR), and neutrophil-to-lymphocyte ratio (NLR)" have all been postulated as potential novel biomarkers of the inflammatory process. It is still unclear whether inflammatory markers and MASLD are connected although numerous studies have found an association between MASLD and WBC count, MPV, and PLR [26].

In the present study, comparing cases to the control group, there was a significant rise of serum cholesterol (193.8 vs. 164.2 mg/dl respectively), serum

TG (176.86 vs. 135.9 mg/dl respectively), serum LDL (100.57 vs. 81.04 mg/dl respectively). In addition to a significant decline of serum HDL (44.82 vs. 65.36 mg/dl respectively).

Dyslipidemia is characterized by "abnormally raised cholesterol, TG, and low-density lipoprotein cholesterol (LDL-C), as well as abnormally declined high-density lipoprotein cholesterol (HDL-C)" [27]. About 20–80% of MASLD patients have "atherogenic dyslipidemia, characteristically seen in patients with obesity, MetS, IR, and T2D, which is distinguished by increased blood levels of TG, LDL-C, and decreased blood levels of HDL-C" [28].

Extra fat builds up in hepatocytes as lipid droplets covered with a variety of structural proteins that may have a role in the etiology of liver disorders. Intrahepatic lipid buildup in MASLD is caused by anomalies in lipid metabolism, including reduced triglyceride (TG) export, increased liver uptake of free fatty acids, increased whole-body lipolysis, and increased synthesis of very low-density lipoproteins. These changes in lipid metabolism are associated with aberrant synthesis of adipokines (as adiponectin, resistin, and leptin) that impact signaling pathways, causing inflammatory - oxidative stress [29–31].

In this study, comparing cases vs. control group, there was a substantial rise in serum ALT (19.06 vs. 18.28 u/l) and serum AST (19.4 vs. 18.5 u/l) respectively.

Patients with MASLD may have "mild or moderate elevations in serum transaminases (ALT and AST), although normal aminotransferase levels do not rule out NAFLD" [32]. A high permeability of.

the hepatocyte cell membrane permits transaminases to seep into the bloodstream, as seen by higher levels of these enzymes, which are very vulnerable to liver injury. While AST elevation could be implicated in cardiac, hepatic, and skeletal muscle injuries, ALT is liver connected. Because of this, serum transaminases have been used as substitute markers for NAFLD. Higher hepatic fat fraction and intraabdominal visceral adipose tissue have been associated with higher blood transaminase concentrations [33]. As a result, individuals with "increased serum transaminases and fatty liver" have more severe "steatosis and visceral obesity" because of "insulin resistance-related metabolic abnormalities". When insulin resistance first appears, affected individuals have normal serum transaminases and mild steatosis. Patients with advanced insulin resistance show substantial steatosis, elevated blood transaminases, and visceral adiposity [34].

In the present study, comparing cases to the control group, there was a significant increase of serum fetuin-A (1154.85 vs. 505.29 respectively).

Although a wide range of laboratory testing, imaging studies, and combinations of clinical and blood test data are nowadays available as non-invasive diagnostic markers for NAFLD, they are not sensitive enough or specific enough to differentiate between NAFL and NASH or to identify the progress of fibrosis [35]. Unfortunately, "liver biopsy" is still considered the "golden standard" for MASLD diagnosis and assessment of fibrosis progression [36]. Fetuin-A represents one of the newly adopted serum biomarkers for MASLD diagnosis of severity assessment [37]. Fetuin-A has been incriminated in the causation of several metabolic disorders for instance, dyslipidemia and hepatic steatosis [38–40]. Furthermore, it has been discovered that obesity [41], hypertriglyceridemia [42], T2D [43], and IR [44] are linked to elevated

fetuin-A levels. As a result, the data presented above show that fetuin-A may be employed as a unique biological marker for the diagnosis of several metabolic illnesses including dyslipidemia, poor glycemic control, which all share responsibility for mitochondrial dysfunction, which increases reactive oxygen species (ROS) and eventually leads to MASLD [45].

In our investigation, we employed a cutoff value of 702.5 for serum fetuin-A, which resulted in 82% and 90% sensitivity and specificity, respectively, for identifying MASLD cases, with 86% diagnostic accuracy.

It is expected to find differences among different studies regarding the diagnostic ability of fetuin-A in MASLD cases diagnosis. These differences could be attributed to different sample sizes and methods of fetuin-A assessment in different ethical populations. The best "cutoff value" to distinguish between the MASLD group and control group regarding fetuin-A level was found to be " > 500 with a sensitivity of 96.67%, a specificity of 100.0%, and an area under the curve (AUC) of 97.7%" in a prior similar study [46].

In our study, there was a significant positive correlation between serum fetuin-A and both CAP and MASLD scores, indicating the value of serum fetuin-A not only in the diagnosis but also in the assessment of the severity of MASLD cases. The more increased fetuin-A levels, the more hepatic steatosis is expected.

fetuin-A is produced by steatotic hepatocytes at early timepoints in MAFLD and correlates with insulin resistance both in mice and humans. In NASH, fetuin-A also co-localizes with activated liver macrophages and could be interpreted as a signal released by damaged hepatocytes [47]. Both preclinical and clinical research have demonstrated that elevated fetuin-A level is an indicator for several metabolic illnesses, such as obesity, T2D, NAFLD, NASH, IR, etc. [48, 49], which in turn cause several hepatic-related complications to progress to cirrhosis [50]. Fetuin-A modulation is associated with a number of pathophysiological variables that cause liver problems, including insulin receptor signaling deficiencies, adipocyte dysfunction, hepatic inflammation, fibrosis, triacylglycerol production, macrophage invasion, and TLR4 activation [35]. Furthermore, fetuin-A is essential for the pathophysiology of several inflammatory and metabolic diseases. It has recently been demonstrated that elevated fetuin-A levels induce mice's adipocytes and macrophages to produce more inflammatory cytokines, which accelerates the development of many liver-related problems [48]. Hepatic sensitivity to high doses of pro-inflammatory cytokines leads to the histopathological changes seen in NASH [51]. This shows that hepatic cytokines play an important role in the transition from steatosis to NASH. One strategy to halt the progression

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of MASLD is to lower blood fetuin-A levels, which can be accomplished by treating a variety of conditions [52].

In the current study, serum fetuin-A did not have any significant correlation with any of the collected numerical variables in MASLD cases, apart from the two scores (MASLD and CAP score). Fetuin-A could be a promising non-invasive marker for the diagnosis of MASLD with a sensitivity of 82%, specificity of 90%, and accuracy of 86%.

Our study has some limitations. First of all, it included a relatively small sample size. Also, the impact of serum fetuin-A on patient prognosis should have been assessed. Finally, the effect of nutritional status on serum fetuin-A level was not investigated. The previous drawbacks should be discussed in the upcoming studies.

Conclusion

Fetuin-A could be a reliable marker diagnostic biomarker for NAFLD. In addition, the marker was connected to the severity of the disease because it demonstrated a significant correlation with the ultrasound grading and the FibroScan using the controlled attenuated parameter.

Abbreviations

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ANOVA	Analysis of Variance
AUC	Area Under Curve
BMI	Body Mass Index
CAP scan	Controlled Attenuated Parameter scan
CIMT	Carotid Intima-Media Thickness
CK18	Cytokeratin-18
CT	Computed tomography
ELISA	Enzyme-Linked Immunosorbent Assay
F	Fibrosis
FBS	Fasting Blood Sugar
FGF-21	Fibroblast Growth Factor-21
GGT	Gamma-Glutamyl Transpeptidase
HDL	High-Density Lipoprotein
IFG	Impaired fasting glycaemia
IQR	Interquartile Range
LDL	Low-Density Lipoprotein
LSM	Liver Stiffness Measurement
MetS	Metabolic Syndrome
MRI	Magnetic Resonance Imaging
NAFLD	Non-Alcoholic Fatty Liver Disease
NFS	NAFLD fibrosis score
ROC Curve	Receiver Operator Characteristic Curve
S	Steatosis
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
T2D	Type 2 diabetes
TG	Triglycerides

Declarations

Ethics approval and consent to participate
Benha University Scientific Research and Publications Ethics Committee approved the study (Approval Number: 1.8.19201). All subjects were informed and gave their voluntary, written, informed consent. It is declared that: All methods were carried out in accordance with relevant guidelines and regulations. It was performed according to the recommendations of Good Clinical Practice and the Declaration of Helsinki (2013).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Internal Medicine, Faculty of Medicine, Suez University, Suez, Egypt
[2]Department of Hepatology, Gastroenterology, and Infectious Diseases, Faculty of Medicine, Benha University, Benha, Egypt
[3]Department of Clinical Pathology, Faculty of Medicine, Tanta University, Tanta, Egypt

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