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Original article

Fetuin A as a Non-Invasive Serum Biomarker for Diagnosis and Assessment of Severity of Non-Alcoholic Fatty Liver Disease

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is rapidly becoming one of the most common causes of chronic liver disease worldwide. The biopsy is the gold standard tool for disease diagnosis, but it is usually not accepted by the patients due to its invasive nature. The use of non-invasive biomarkers is now attaining a great interest in diagnosis and detection of the disease severity.

Aim of the work: Investigate the role of fetuin A in diagnosis and assessment of severity of non-alcoholic fatty liver disease (NAFLD). **Subjects and methods:** This cross sectional study included 50 patients with NAFLD and 50 healthy control subjects. All cases were subjected to history taking, clinical examination and laboratory investigation (including assessment of fetuin A), abdominal ultrasonography and fibroscan with controlled attenuated parameter (CAP scan). **Results:** The level of fetuin A was statistically significantly higher in the cases group as compared with the control group (1154.85±629.89 and 505.29±150.4 respectively) ($p < 0.001$). Fetuin-A had significant validity in prediction of NAFLD at cut off >702.5 with sensitivity 82%, specificity 90% and accuracy 86%. Also, the marker was related to disease severity as it revealed a significant correlation with ultrasound grading ($p<0.001$) and fibroscan with controlled attenuated parameter ($p= 0.002$). **Conclusion:** Fetuin A could be a potential marker in the diagnosis of NAFLD.

Key words: NAFLD; fetuin A; CAP score; hyperlipidemia.

Introduction:

Nonalcoholic fatty liver disease (NAFLD) has become one of the most common chronic liver diseases in recent years, and the overall prevalence of NAFLD is approximately 25% in the world [(1, 2)].

Whereas simple liver steatosis is regarded as a benign condition, NAFLD can be a progressive liver disease leading to fibrosis and ultimately to cirrhosis. A liver biopsy has long been the only method for NAFLD

diagnosis and for the staging of liver fibrosis. However, there are several drawbacks associated with this procedure. A liver biopsy is an invasive diagnostic method which is distressing to patients, and it has risk of complications [(3)].

Several non-invasive tools and methods have been developed to predict NASH, or to quantify liver fibrosis without having to resort to a liver biopsy. In clinical practice, imaging methods such as ultrasonography, computed tomography, controlled attenuation parameter and magnetic resonance have been used widely for diagnosing NAFLD [(4)].

In addition, many studies have been conducted to explore the valuable serum biomarkers for early diagnosis and progression of NAFLD. Several serum biomarkers, such as alanine aminotransferase, aspartate aminotransferase, gamma-glut amyl trans peptidase, cytokeratin-18 and fibroblast growth factor 21, have been researched in some studies and their potential to serve as the biomarkers in clinical diagnosing of NAFLD have been mentioned [(5)].

Fetuin-A, also known as the 2-Heremans-Schmid glycoprotein, is a phosphorylated glycoprotein and a member of the fetuin group of serum binding proteins that are synthesized primarily by hepatocytes [(6)]. As an endogenous inhibitor of tyrosine kinase, fetuin-A can trigger insulin resistance in the target tissues, such as liver and skeletal muscle [(7)].

Accumulated lines of evidence have reported the significant association between circulating fetuin-A level and the development and progression of NAFLD, but the results have been inconsistent [(8)].

The aim of the current study was to investigate the role of fetuin a in diagnosis and assessment of severity of non-alcoholic fatty liver disease (NAFLD) and disclose the correlation of fetuin A with NAFLD fibrosis score (NFS).

Subjects and methods

This is a cross sectional study conducted at Hepatology and Gastroenterology and Infectious Diseases Department, inpatient and outpatient clinic of Benha University Hospital and El-Mahalla Liver Teaching Hospital, This study was conducted within the period from December 2021 to December 2022. The study was conducted after obtaining approval from the local ethics committee, Faculty of Medicine, Benha University {M.S.1.8.2021}. A written/oral informed consents were obtained from the included cases and accordance with Helsinki Standards as revised in 2013 [(9)].

This study included a total of 100 subjects who were divided into two groups; group I (including 50 cases with NAFLD, diagnosis was based on abdominal U/S and Fibroscan with CAP with or without elevated liver enzymes [(10)]) and group II (50 healthy subjects as a control group with normal liver in transabdominal ultrasonography and normal liver enzymes).

Exclusion criteria: Patients below the age of 18 years, patients with history of significant alcohol intake exceeding 40 g/d in males and 20 g/d in females over the past 5 years, presence of concomitant hepatitis B and hepatitis C virus infection, patients with symptoms and signs suggesting (haemochromatosis, Wilson's disease, alpha-one anti-trypsin deficiency and autoimmune hepatitis), patients with

hepatobiliary malignancy and pregnant females (unfit for Fibroscan & CAP). Patients who use these medications; steatogenic drugs (amiodarone, valproic acid, antiretroviral drugs, methotrexate, and tetracyclines), or medicines that are used for management of NAFLD (Vitamin E, metformin, and thiazolidinediones) were excluded from the study.

- The clinical/pathological data of patients were recorded, including age, sex, complete medical history, thorough clinical examination. The cases were subjected to the following; history taking (including the demographic data and history of present illness) and clinical examination with stress on: chronic fatigue, hypertension, manifestations suggesting chronic liver diseases due to viral or other non-viral causes (ascites, LL oedema, jaundice, pruritus, Kyser Fleisher ring by slit lamp examination, clubbing of fingers, signs of chest disease, signs of heart and renal failure) and hepato-biliary malignancy. NAFLD Fibrosis score was calculated according to this formula: -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) [(11)].

Anthropometric evaluation: Weight, height and body mass index (BMI) were measured for each patient. BMI formula uses weight (in kg) and height (in meters) and expressed in kg/m^2 using this formula weight (kg)/[height (m)]2 . BMI results are interpreted as follows: • BMI below 18.5 = Underweight; • BMI 18.5 – 24.9 = Normal weight; • BMI 25.0 – 29.9 = Overweight; • BMI 30.0 and above = Obese [(12)].

Laboratory investigations were done including measurement of serum fetuin-A by using a human fetuin-A sandwich enzyme-linked immunosorbent assay (ELISA) kit. The inter assay coefficient of variation is 5.2%, and the intra assay coefficient of variant is 7.8%.

Imaging:

- **Abdominal ultrasonography:**

Ultrasonography was performed using a 2-5 MHz convex transducer. Various (0-3) grades of steatosis have been proposed based on visual analysis of the intensity of the echogenicity, provided that the gain setting is optimum. When the echogenicity is just increased, it is grade I; when the echogenic liver obscures the echogenic walls of portal vein branches, it is grade II, and, when the echogenic liver obscures the diaphragmatic outline, it is grade III fatty infiltration [(13)].

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

During the hepatology clinic visit, liver stiffness measurement (LSM) and CAP were obtained using FibroScan502 (Echosens, Paris, France). All subjects will be advised to fast for at least 8 h before the procedure.

The LSM score was represented by the median of 10 measurements and was considered reliable only if at least 10 successful acquisitions are obtained and the IQR-to-median ratio of the 10 acquisitions are ≤30%. A CAP measurement was considered reliable and included in the final analysis if 10 successful acquisitions are obtained.

Hepatic steatosis was graded by CAP using the M probe according to published cut-offs

(S1=222–232; S2= 233–289 and S3 ≥290 dB/m) [13].

Probe-specific LSM cut-offs was used to define advanced fibrosis and cirrhosis (M probe F3=9.6–11.4, F4 ≥11.5; XL probe F3=9.3–10.9, F4 ≥11.0 kPa) are derived from previous studies [2].

Statistical analysis

The data collected were coded, processed and analyzed with **SPSS** version 26 for Windows® (Statistical Package for Social Sciences) (IBM, SPSS Inc, Chicago, IL, USA). Qualitative data as number (frequency) and percent was presented. The Chi-Square test (or Fisher's exact test) made the comparison between groups. The Kolmogorov-Smirnov test tested quantitative data for normality. Data was shown as median ± SD.

To compare three or more groups with normally distributed quantitative variables, one-way analysis of the variance (one-way ANOVA) test was used and Kruskal Wallis test was used if the data were abnormally distributed.

Pearson's or Spearman correlation was used to correlate parametric and non-parametric numeric data respectively. The receiver operator characteristic (ROC) curve was used to determine the optimal cutoff value of fetuin-A to distinguish between groups in terms of sensitivity and specificity followed by determining accuracy, NPV and PPV by categorizing the data into two groups according to the cutoff point. For all tests, P values <0.05 are considered significant.

Results

There were no statistical significance differences between the studied groups as regard age, sex, smoking and BMI. 58% of the studied cases had diabetes mellitus, 42% had hypertension, and 98% had hyperlipidemia. The mean LSM score was 6.58 with range 3.1 to 24.1 and most frequent class in LSM fibro scan was F0 & F1 (38% & 36% respectively). Regarding the CAP score among the studied cases ranged from 222 to 372 with mean 287.5. The most frequent CAP class was S3 (52%). The most frequent US grade among the studied cases was 3 (52%) (Table 1).

(Table 2). There was a statistical significance increase in platelets count, FBS and 2h post prandial blood sugar among cases compared to control group. There was a statistical significance increase in cholesterol, TG, LDL and a statistical significance decrease in HDL among cases compared to control group. Also there was a statistical significance increase in total bilirubin among cases compared to control group. The Fetuin-A was a statistically significantly increase in cases compared to control group.

There was a statistical significance increase in Fetuin-A among S2 & S3 cases compared to S1 and among US grade 3 cases compared to grade 1 & 2 (table 3).

There was a statistical significance positive correlation between Fetuin-A and both CAP score and NAFLD score among the studied cases group. In the control group, there was no statistical significance relation between Fetuin-A and any of the studied parameters among control group (table 4)

Table 1 : Sociodemographic and imaging findings among studied groups.

Variable		**Cases (n=50)**		**Control (n=50)**		**P**
Age: (years)	Mean ± Sd	42.46±10.41		40.62±11.63		0.41
	Range	19-65		22-62		
BMI: (kg/m^2)	Mean ± Sd	35.49±7.02		32.22±10.16		0.06
	Range	26.4-50		18.9-47.8		
Variable		**No**	**%**	**No**	**%**	**P**
Sex:	Male	25	50	24	48	0.84
	Female	25	50	26	52	
Smoking:	N=	9	18	11	22	0.62
Diabetes mellitus	N=	29	58			
Hypertension	N=	21	42			
Hyperlipidemia	N=	49	98			
LSM fibro scan:	F0	19	38			
	F1	18	36			
	F2	4	8			
	F2-3	3	6			
	F3	4	8			
	F4	2	4			
	Mean ± Sd	6.58±3.35				
	Median (Range)	5.7 (3.1-24.1)				
CAP score:	S1	9	18			
	S2	15	30			
	S3	26	52			
	Mean ± Sd	287.5±44.62				
	Range	222-372				
US grade	1	9	18			
	2	15	30			
	3	26	52			

LSM: Liver stiffness measurements **CAP: controlled attenuation parameter** **US: Ultrasound**

Table 2 : Laboratory findings among the studied groups.

Variable		**Cases (n=50)**	**Control (n=50)**	**P**
Hb (g/dl)	Mean ± Sd	13.54±0.98	13.43±1.01	0.60
	Range	11.5-15.2	11.5-15	
WBC (x10^3/mm$^{3)}$	Mean ± Sd	4.72±1.17	4.98±1.63	0.36
	Range	3-8	2.5-8.5	
Platelets (x10^3/mm$^{3)}$	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	Mean ± Sd	176.86±15.63	135.9±17.51	**<0.001**
(mg/dl)	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	
Total Bilirubin (mg/dl)	Mean ± Sd	0.73±0.18	0.66±0.18	**0.04**
	Range	0.4-1	0.45-1	
Direct 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 (g/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	
	Median	879	496	**<0.001**
	Range	592-2400	150-788	
NAFLDF score	Mean ± Sd	-2.10 ±1.66		
	Median	-20.8	-------	------
	Range	-4.5/2.88		

HB: hemoglobin WBCs: White blood cells FBS: Fasting Blood cells
LDL: Low density lipoprotein HDL: High density lipoprotein ALT: Alanine transaminase
AST: aspartate aminotransferase NAFLD: Non-alcoholic fatty liver disease

Table 3: Relation between Fetuin-A and LSM, CAP score and US grade among the studied cases group.

Variable		N	Fetuin-A		P
			Mean±SD	Median	
LSM:	F0	19	1210.13±662.62	893	
	F1	18	1045.73±554.3	829.8	
	F2	4	1357.38±794.39	1206.25	0.26
	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**	
	S2	15	**1232.89±669.74**	**896**	**0.03**
	S3	26	**1271.13±635.3**	**903**	
US grade:	1	9	**1041.36±522.21**	**806**	
	2	15	**1085.22±630.29**	**858**	**0.04**
	3	26	**1343.63±684.08**	**917**	

LSM: Liver stiffness measurements CAP: controlled attenuation parameter US: Ultrasound

Table 4 : Correlation between Fetuin-A and different parameters among the studied cases and control groups.

Variable	Fetuin-A [Cases group] (n=50)		Variable	Fetuin-A [Control group] (n=50)	
	r	P		r	P
Age (yrs)	-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
NAFLD score	**0.49**	**<0.001**	**Platelets**	0.08	0.59
Hb (g/dl)	0.03	0.81	**FBS**	0.06	0.71
WBC (x10^3/mm$^{3)}$	0.14	0.35	**Post prandial**	0.13	0.38
Platelets (x10^3/mm$^{3)}$	0.11	0.44	**Cholesterol**	0.13	0.35
FBS (mmol/L)	0.09	0.55	**Triglyceride**	0.09	0.52
Post prandial glucose level (mmol/L)	0.03	0.81	**LDL**	0.22	0.12
Cholesterol (mg/ml)	0.27	0.06	**HDL**	-0.02	0.88
Triglyceride (mg/ml)	0.11	0.44	**ALT**	0.06	0.66
LDL (mg/ml)	0.08	0.60	**AST**	0.04	0.76
HDL (mg/ml)	-0.21	0.14	**T. Bilirubin**	0.09	0.53
ALT (U/L)	0.10	0.48	**D. Bilirubin**	0.03	0.86
AST (U/L)	0.08	0.59	**Albumin**	-0.06	0.70
Total Bilirubin (mg/dl)	0.07	0.65			
Direct Bilirubin (mg/dl)	0.11	0.45			
Albumin (g/dl)	0.05	0.71			

BMI: Body mass index HB: hemoglobin WBCs: White blood cells FBS: Fasting Blood cells LDL: Low density lipoprotein HDL: High density lipoprotein ALT: Alanine transaminase AST: aspartate aminotransferase NAFLD: Non-alcoholic fatty liver disease

Table 5 : Diagnostic performance of Fetuin-A in prediction of non-alcoholic fatty liver among the studied groups.

Cut off	AUC (95%CI)	Sensitivity	Specificity	PPV	NPV	Accuracy	P
>702.89	**0.95 (0.91-0.99)**	**82%**	**90%**	**89.1%**	**83.3%**	**95%**	**<0.001**

AUC: Area under curve CI: Confidence interval PPV: Positive predicted value NPV: Negative predicted value

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

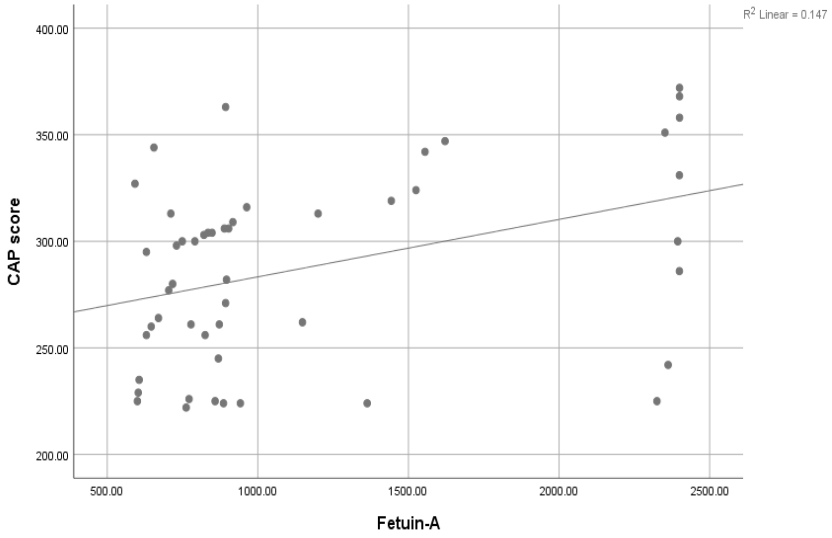

Fig. 1. Correlation between Fetuin-A and CAP score among the studied cases group.

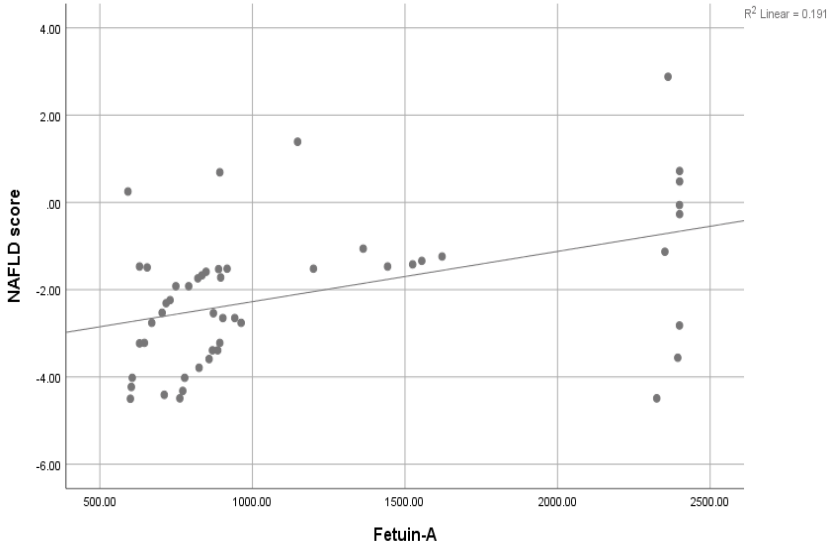

Fig. 2. Correlation between Fetuin-A and NAFLD score among the studied cases group.

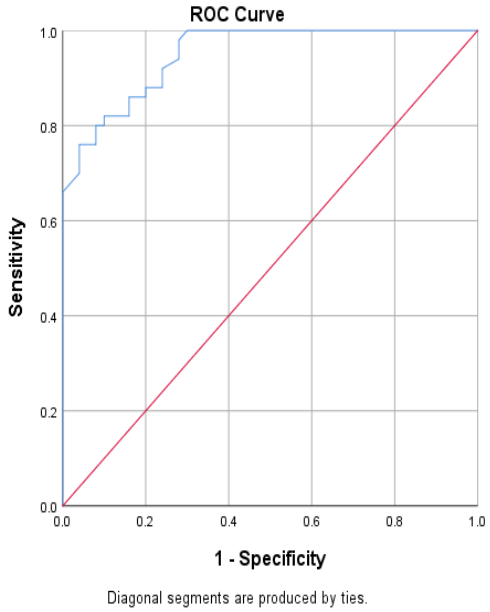

Fig. 3. ROC curve analysis for Fetuin-A in prediction of non-alcoholic fatty liver among the studied groups.

Discussion:

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common chronic liver diseases in recent years, and the overall prevalence of NAFLD is approximately 25% in the world [(4)].

Several non-invasive tools and methods have been developed to predict NASH, or to quantify liver fibrosis without having to resort to a liver biopsy. In clinical practice, imaging methods such as ultrasonography, computed tomography, controlled attenuation parameter and magnetic resonance have been used widely for diagnosing NAFLD [(14)].

Several serum biomarkers, such as alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltranspeptidase, cytokeratin-18 and fibroblast growth factor 21, have been researched in some studies and their potential to serve as the biomarkers in clinical diagnosing of NAFLD have been mentioned [(5)].

Fetuin-A, is a phosphorylated glycoprotein and a member of the fetuin group of serum binding proteins that are synthesized primarily by hepatocytes, As an endogenous inhibitor of tyrosine kinase, fetuin-A can trigger insulin resistance in the target tissues, such as liver and skeletal muscle [(7)].

In previous studies, it was studied that people with hepatic fat steatosis had higher levels of circulating Fetuin A [(15)].

In the present study, there was no statistical significant difference between the cases and control groups regarding BMI, with mean values of 35.49 and 32.22 kg/m^2 respectively. One should notice the increased BMI in association with NAFLD despite the absence of any statistical significance.

The current study revealed that there was a significant increase in platelet number in NAFLD cases compared to controls

(269.86 vs. 240.08 respectively but within normal level in both groups). Despite the statistical difference between the two groups, most of the recorded values were within the normal platelet range. The increased platelet could reflect the inflammatory state associated with NAFLD [15, 16]. Platelets also play a role in liver regeneration after liver damage; however, they can also trigger the pathogenesis of liver fibrosis [17]. By producing transforming growth factor-beta and platelet-derived growth factor, which activate fibro competent cells, notably hepatic stellate cells [1].

It was confirmed the current findings, as platelet count showed a significant increase in association with NAFLD compared to controls (251.36 vs. 246.22 respectively – $p = 0.002$) [17].

On the other hand, it was reported that there was no significant difference between NAFLD and controls regarding platelet count ($p = 0.058$), that had mean values of 327.37 and 300.9 in the two groups respectively [18].

The present study revealed a significant increase of serum cholesterol in cases compared to controls (193.8 vs. 164.2 mg/dl respectively), this was in agreement with the study done in 2013 by a group of researchers who disclosed in their study that the mean values of cholesterol in NAFLD cases and controls were 198.9 and 190.1 mg/dl in respectively with a significant rise in association with NAFLD ($p = 0.011$) [19].

In the current study, there was a significant rise of serum TGs level in NAFLD cases compared to controls (176.86 vs. 135.9 mg/dl respectively).

In line with the previous findings, serum TGs had mean values 166.36 and 134.95 mg/dl in NAFLD cases compared to controls respectively, with a significant rise in NAFLD ($p = 0.001$) Other authors confirmed the same findings [20].

In the current study, there was a significant increase of serum LDL levels in the cases compared to controls (100.57 vs. 81.04 mg/dl respectively). Also noted a significant increase of serum LDL in association with NAFLD (159.22 vs. 108.6 mg/dl in controls – $p < 0.001$) [21].

The current study revealed a significant decline in serum HDL in NAFLD patients compared to controls (44.82 vs. 65.36 mg/dl respectively), this is in a agreement with who reported a significant drop of serum HDL in association with NAFLD (42.53 vs. 50.27 in controls – $p = 0.013$) [22].

On the other hand, it was reported that HDL had mean values of 41.31 and 41.98 mg/dl in cases and controls respectively, which was comparable on statistical analysis ($p = 0.43$) [23].

In the current study, both hepatic transaminases showed statistically comparable levels between cases and controls. ALT had mean values of 19.06 and 18.28 u/l, whereas AST had mean values of 19.4 and 18.5 u/l in cases and controls respectively.

On the other hand, it was declared that there was a significant elevation of both hepatic transaminases in NAFLD cases. AST had mean values of 26.9 (U/L) and 23.7 (U/L), while ALT had mean values of 31.1 (U/L) and 21 u/l (U/L) in cases and controls respectively [19].

Patients with NAFLD may have mild or moderate elevations in the aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) [24]. Although normal aminotransferase levels do not exclude NAFLD [25]. Changes in the grade of NAFLD among studies could explain the previous heterogenicity.

In the present study, serum fetuin A had mean values of 1154.85 ng/mL and 505.29 ng/mL in cases versus controls respectively, with a significant increase in association with NAFLD ($p < 0.001$).

Because fetuin-A is a pro-inflammatory protein secreted by hepatocytes, the inflammation in NAFLD-affected liver would enhance hepatic fetuin-A production [26].

This is in concordant with the recently published meta-analysis which confirmed that circulating levels of fetuin-A in patients with NAFLD were significantly higher than in healthy controls [7].

Additionally, researchers reported that the same marker had median values of 72.3 and 67.4 mcg/ml in NAFLD and controls respectively, with a significant elevation in NAFLD ($p = 0.012$) [27].

Haukel and and his coworkers reported that circulating fetuin A levels were markedly elevated in NAFLD patients (324 mg/l) compared with controls (225 mg/l), and that difference was statistically significant between the two groups regarding all BMI classes [14].

Furthermore, it was reported that this studied marker had mean values of 816 and 653 mcg/ml in NAFLD patients versus controls respectively, which was highly significant between the two groups [28].

Although previous researchers reported that NAFLD cases had a mean serum fetuin A value of 195.8 mcg/ml versus 182.9 mcg/ml in controls, that difference was insignificant in the statistical analysis ($p < 0.07$) [29]. Another study reported that serum fetuin A levels were comparable between NAFLD cases and controls (853.3 and 807.4 mcg/ml respectively – $p > 0.05$) [30].

Contrarily, reported a significant decline of serum fetuin A in NAFLD cases compared to controls (0.27 vs. 0.32 g/l respectively – $p < 0.05$) [31].

It appears that the current literature is full of heterogenicity regarding fetuin A levels in NAFLD patients. Hypothesis has been raised referred to decease-increase trend in serum fetuin A level as NAFLD exacerbated. First, Fetuin A was indeed an aggravated element in NAFLD, and down-regulation might be explained by feed-back protection mechanism when steatosis first appeared and cell injury confined to mild range. Second, as disease severity increased, compensatory pathways weakened, and Fetuin A as well as other elevated.

In the current study, using a cut-off value of 702.5ng/mL for serum fetuin A, it had sensitivity and specificity of 82% and 90% for the detection of NAFLD cases, with a diagnostic accuracy of 86%.

In a previous similar study, the receiver operating characteristic curve (ROC) showed that the best cutoff point to differentiate between NAFLD group and control group regarding fetuin A level was found > 500 ng/mL with sensitivity of 96.67%, specificity of 100.0% and area under curve (AUC) of 97.7% [32].

It is expected to find some differences among studies regarding the diagnostic ability of the previous marker in NAFLD cases. These differences could be attributed to different sample size, and

methods of fetuin A assessment in different ethical population

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

Given the well-known association between insulin resistance and fibrosis stage in NAFLD patients [(33)]. **O**ne could expect fetuin A, as a marker of insulin resistance, to increase in parallel with the degree of liver fibrosis.

On the other hand, fetuin A is a known extracellular inhibitor of transforming growth factor [(34)]. the key profibrogenic stimuli in chronic liver disease [(35)]. Accordingly, elevated fetuin A could represent a counteracting and protective mechanism against development of liver fibrosis. At present, no firm conclusions can be drawn about the role of fetuin A in liver fibrogenesis in NAFLD.

In agreement with the current findings, it was reported that bivariate analyses in patients with NAFLD showed a statistically significant association between serum fetuin A levels and the liver fibrosis score index ($r = 0.36$, $P < 0.001$). However, other authors found no association between the fetuin A/α2HS-glycoprotein (AHSG) and NASH scores [(36)].

Furthermore, it was reported that mild NAFLD and moderate NAFLD had significantly lower levels of fetuin A, while serum fetuin A level tended to increase with the severity of NAFLD [(31)].

On the other hand, it was proved that serum fetuin A has a significant negative correlation with NAFLD score ($R = -0.25$, $P < 0.01$) [(29).] The previous authors demonstrated that the serum fetuin-A concentration decreases with the progression of liver and vascular fibrosis in NAFLD patients.

Other authors noted no significant association between serum fetuin A levels and fibrosis stage, or the degree of liver steatosis [(14).] The lack of association between fetuin-A and hepatic inflammation suggests that cytokines are not involved in the modulation of fetuin-A release and that fetuin-A is not involved in the progression of liver damage. However, these data need to be confirmed in view of previous discordant results.

In the current study, serum fetuin A did not have any significant correlation with any of the collected numerical variables in NAFLD cases, apart from the two scores (NAFLD and CAP score).

In the study conducted on serum fetuin-A level, it was found to be negatively and significantly correlated with age and mean carotid intima media thickness (CIMT). In addition, the serum fetuin-A level was positively and significantly correlated with AST, ALT, GGT and platelet count [(29)].

Additionally, reported that fetuin-A levels were found to be positively correlated with TGs, HOMA-IR and CIMT levels in NAFLD group. On the other hand, it was negatively correlated with HDL-C and adiponectin levels [(27)].

Other authors reported that fetuin-A levels correlated significantly ($P < 0.05$) with waist circumference ($r = 0.45$), systolic blood pressure ($r = 0.50$), diastolic blood pressure ($r = 0.41$), insulin resistance index

HOMA (r = 0.28), HDL-cholesterol (r = −0.31), but not to LDL-cholesterol, triglycerides, transaminases, and age [37].

The current findings also showed the significant rise of serum fetuin in smokers versus non-smokers in the cases group. It had mean values of 1205.76 and 922.89 in the previous two groups respectively. This significant association has not been reported in the previous literature, and that needs to be further studies in future studies.

The current study has some limitations. First of all, it included a relatively small sample size. Also, all cases were collected from a single Hepatology center and the impact of serum fetuin A on patient prognosis should have been assessed. The previous drawbacks should be discussed in the upcoming studies

Conclusion

Fetuin A could be a potential marker used in the diagnosis of nonalcoholic fatty liver disease (NAFLD). In addition, the marker was connected to the activity of the disease because it demonstrated a significant correlation with the ultrasound grading and the fibroscan using the controlled attenuated parameter.

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