Assessment of the Diagnostic Potential of Lecithin-Cholesterol Acyltransferase in Non-Alcoholic Fatty Liver Disease

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Abstract:

Non-alcoholic fatty liver disease (NAFLD) is a prevalent metabolic liver disorder characterized by fat accumulation in hepatocytes. Lecithin-cholesterol acyltransferase (LCAT) is a liver-derived enzyme involved in lipid metabolism and may serve as a biomarker of hepatic metabolic function. The current study aimed to assess serum LCAT level in NAFLD patients in comparison with matched controls and to estimate its diagnostic potentials for NAFLD and its predictive potentials in relation to steatosis and fibrosis severity.

A case-control study was conducted on 90 individuals, 45 NAFLD patients and 45 age-, sex-, and BMI-matched controls. LCAT level were measured using ELISA. Clinical data, including lipid profile, liver enzymes were recorded. Fibrosis risk was assessed using FIB-4 index, and transabdominal ultrasound was used for steatosis grading. NAFLD patients had significantly lower level of LCAT than control group participants ($p < 0.001$). However, serum LCAT level was not significantly associated with the severity of steatosis or fibrosis risk in NAFLD patients. Receiver operating characteristic curve analysis showed that LCAT has an excellent diagnostic potential, with an AUC of 0.951, sensitivity of 0.978 and specificity of 0.911 ($p < 0.001$). In conclusion, LCAT may serve as promising non-invasive diagnostic biomarkers for NAFLD.

Keywords: Non-alcoholic fatty liver disease (NAFLD), Hepatic fibrosis, Hepatic steatosis, LCAT, Lipid metabolism

تقييم الإمكانات التشخيصية لإنزيم ليسيثين-كوليسترول أسيل ترانسفيراز في مرض الكبد الدهني غير الكحولي

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الخلاصة:
يُعتبر مرض الكبد الدهني غير الكحولي (NAFLD) اضطرابًا شائعًا في الكبد يتميز بتراكم الدهون في خلايا الكبد. إنزيم ليسيثين-كوليسترول أسيل ترانسفيراز (LCAT) هو إنزيم مشتق من الكبد يشارك في عملية الأيض الدهني، وقد يكون بمثابة علامة حيوية لوظيفة الأيض الكبدي. تهدف هذه الدراسة الحالية إلى تقييم مستوى LCAT في مصل الدم لدى مرضى NAFLD مقارنةً بمجموعة الشهود الأصحاء، وتقدير إمكاناته التشخيصية لمرض NAFLD وإمكاناته التنبؤية فيما يتعلق بشدة الدهنيات والتليف. تم إجراء دراسة حالة وشاهد على 90 فردًا، 45 مريضًا بمرض NAFLD و45 شاهدًا متطابقين من حيث العمر والجنس ومؤشر كتلة الجسم. تم قياس مستوى LCAT باستخدام تقنية ELISA. تم تسجيل البيانات السريرية، بما في ذلك ملف الدهون وإنزيمات الكبد. تم تقييم خطر التليف باستخدام مؤشر FIB-4 ، واستخدمت الأشعة فوق الصوتية عبر البطن لتصنيف الدهنيات. كان لدى مرضى NAFLD مستوى LCAT أقل بشكل ملحوظ من المشاركين في مجموعة الشهود ($p < 0.001$). ومع ذلك، لم يكن مستوى LCAT في المصل مرتبطًا بشكل كبير بشدة الدهنيات أو خطر التليف لدى مرضى NAFLD. أظهرت تحليل منحنى التشغيل الاستقبالي أن LCAT يمتلك إمكانات تشخيصية ممتازة، مع AUC قدره 0.951، وحساسية قدرها 0.978 ونوعية قدرها 0.911 ($p < 0.001$). في الختام، قد يكون LCAT علامة حيوية غير جراحية واعدة للتشخيص لمرض NAFLD.

الكلمات المفتاحية: مرض الكبد الدهني غير الكحولي (NAFLD)، تليف الكبد، دهون الكبد، LCAT، الأيض الدهن

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a broad clinical condition marked by hepatic fat accumulation in individuals who consume minimal or no alcohol. This condition spans a continuum from benign hepatic steatosis to more progressive forms, including non-alcoholic steatohepatitis (NASH), which involves hepatic inflammation, and may ultimately advance to fibrosis, cirrhosis, or hepatocellular carcinoma (HCC) [1,2]. NAFLD commonly coexists with metabolic comorbidities such as obesity, type 2 diabetes mellitus, and dyslipidemia, which contributes to its rising global prevalence amid the ongoing obesity epidemic [3,4]. The pathogenesis of NAFLD is multifaceted, involving a combination of insulin resistance [5,6], oxidative stress [7], excessive caloric and fructose intake, dysregulation of gut microbiota [8], and chronic low-grade inflammation. These mechanisms are further influenced by a variety of genetic, lifestyle, environmental, and dietary factors [8-12].

The global prevalence of NAFLD has surged by over 50% in the last thirty years, rising from 25.3% during 1990-2006 to 38.0% between 2016-2019 [13]. The diagnosis of NAFLD encompasses a combination of clinical and biochemical assessment, and imaging studies [14], and sometimes liver biopsy [15]. An important diagnostic criterion is to exclude other causes of steatosis (e.g. viral hepatitis, autoimmune liver disease, hemochromatosis and significant alcohol intake) that can cause similar symptoms and liver damage.

Ultrasonography is the most common noninvasive, imaging test used to assess fat in the liver. It can show elevated echogenicity of the liver relatively to the kidney. Computed tomography (CT) and magnetic resonance imaging (MRI) can also be used to assess liver fat content and provide more detailed information about liver structure [16]. While liver biopsy remains the gold standard for assessing fibrosis severity, a range of validated non-invasive methods, including transient elastography, the fibrosis-4 (FIB-4) index, Aspartate aminotransferase (AST) to Alanine aminotransferase ratio, AST-to-platelet ratio index (APRI), and the NAFLD Fibrosis Score (NFS), are widely validated and recommended tests because of their low cost and non-invasiveness [17,18].

NAFLD has been associated with various metabolic disturbances, including alterations in lipid metabolism. One of the key enzymes involved in lipid metabolism is lecithin-cholesterol acyltransferase (LCAT), which

plays a crucial role in the esterification of free cholesterol to cholesteryl esters, facilitating the transport of cholesterol in the bloodstream [19,20].

NAFLD is also associated with chronic inflammation and oxidative stress, which can negatively impact LCAT activity [21]. Inflammatory cytokines may interfere with the normal lipid metabolism by modulating the function of enzymes involved in lipid metabolism. Moreover, NAFLD is closely linked to insulin resistance, which can impair LCAT activity. Insulin resistance usually led to increased free fatty acid level and altered hepatic lipid metabolism that affect LCAT function [21]. Thus, managing NAFLD is not only important for liver health but also for the overall body metabolism.

Taking this into consideration, this study aims to measure the serum levels of LCAT in patients with NAFLD in comparison to its levels in matched controls so as to assess its association with NAFLD. As well as this study aims to assess the correlation of serum LCAT with the steatosis severity and with the fibrosis risk.

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. Ninety participants were recruited in the study; 45 NAFLD patients diagnosed by ultrasonography by a gastroenterologist [22] and 45 subjects who were age-, sex-, and BMI-matched to the NAFLD patient. The ultrasound diagnosis depends on the echogenicity of the liver parenchyma in comparison to the cortex of the right kidney and the sonographer was blinded to the laboratory results. Controls were selected using frequency matching to ensure that the distribution of age, sex, and BMI of the control group is comparable to that of the case group. This involved matching groups based on the overall characteristics rather than individual pairs. The recruited subjects were adults, (18-64) years old, and of both sexes. Verbal consent was obtained from each participant after being informed about the purpose of the study and the expected benefits. Some conditions that might affect LCAT level were excluded from the study including: diabetes mellitus, dyslipidemia that is already existed before NAFLD diagnosis, fish eye disease, use of medications known to affect LCAT level (e.g., Statins) within the last 3 months, alcohol consumption, presence of chronic inflammatory diseases (e.g., rheumatoid arthritis, systemic lupus erythematosus), pregnant or lactating women, and any other significant comorbidity that could interfere with the study outcomes (e.g., severe cardiovascular disease). Furthermore, other conditions that may interfere with NAFLD diagnosis were excluded such as viral hepatitis, certain medications (such as methotrexate, steroids, amiodarone, and tamoxifen), and inherited or acquired disorders like Wilson's disease. In order to control potential confounding factors, information regarding participants' medical history, including the presence of diabetes or the previously mentioned diseases, as well as any medication that could potentially affect our result, was collected through structured interviews and medical records.

The study was conducted according to the principles of the Helsinki Declaration and was approved by the Research Ethics Committee of the College of Pharmacy, University of Baghdad. Verbal consent was obtained from all participants after explaining the objectives of the study and its expected benefits.

Six milliliters of blood was collected from each fasting 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. Part of the serum was used to measure AST, ALT level and lipid profile directly with COBAS 311 automated analyzer (Roche Diagnostics, Switzerland). The remaining serum was aliquoted to avoid repeated freeze-thaw cycles; samples were kept frozen (–20 °C) until time of analysis. Serum LCAT level was measured using enzyme-linked immunosorbent assay (ELISA) (Huma Reader HS, Human; Germany) with the corresponding human ELISA kits (ELK Biotechnology; USA); according to the manufacturers' instructions.

In the current study, we used a commercially available ELISA kit (Catalog Number: ELK3186) specialized for the quantitative measurement of human LCAT. The detection range was [0.47 to 30 ng/mL], with a sensitivity limit of detection (LOD) of [0.19 ng/mL]. Each sample was analyzed in duplicate to confirm accuracy. Intra-assay and inter-assay coefficients of variation (CVs) were determined to be <8% and <10%, respectively.

The procedure included pre-coating each well of a microtiter plate with a monoclonal antibody specific for human LCAT. Following the addition of either standards or diluted samples to the wells, a biotin-labeled secondary antibody specific for LCAT was added to bind to the captured enzyme. Subsequently, avidin conjugated to horseradish peroxidase (HRP) was added, which strictly binds to the biotin-labeled antibody. After an incubation period of 50 minutes, a tetramethylbenzidine (TMB) substrate solution was introduced to initiate a colorimetric reaction catalyzed by HRP, resulting in the appearance of a blue color in wells containing the full LCAT-antibody-avidin-HRP complex. This enzymatic reaction was terminated by the addition of a sulfuric acid stop solution, which changed the color from blue to yellow.

The absorbance was measured at 450 nm using a microplate reader, and the optical density (OD) for each sample was compared with a standard curve which is generated from known concentrations of LCAT to assess the enzyme concentration in the test samples. The results were expressed in ng/mL, and all the values were within the assay's validation range.

Body mass index was calculated for each participant using the following formula [23]:

$BMI = Weight(kg.)/Hight^2(in\ meteres)$

The severity of steatosis in NAFLD patients that was assessed using B-Mode transabdominal ultrasound, is classified 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 [24].

Fibrosis-4 index was calculated to evaluate the risk of liver fibrosis in NAFLD patients, by the following formula [25]:

$FIB\text{-}4 = [Age\ (years) \times AST\ (U/L)] / [Platelet\ count\ (10^9/L) \times \sqrt{ALT\ (U/L)}]$. [25]

According to the European Association for the Study of the Liver (EASL) criteria: scores < 1.30 indicate low risk of fibrosis, scores 1.30–2.67 indicate intermediate risk, and scores > 2.67 represent high risk (with higher rule-out thresholds such as <2.0 in patients >65 years) [26].

Statistical analysis

The statistical analysis was performed using SPSS, version 27. The normality of data distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests.

Categorical variables were presented as frequencies and percentages, with their differences analyzed using the Chi-square test. Numerical variables were expressed as medians with interquartile range(IQR). Mann-Whitney U test was applied to compare differences between the two study groups. Meanwhile, the Kruskal–Wallis test was utilized to evaluate the difference of LCAT level among NAFLD patients with mild, moderate and severe steatosis, also it is used to compare serum LCAT levels among NAFLD patients with mild, intermediate, and high hepatic fibrosis risk. Spearman's correlation was employed to evaluate the correlation among the studied variables. Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic potential of LCAT for distinguishing NAFLD patients from controls. ROC analysis included estimating the area under the curve (AUC), sensitivity, specificity, and optimal cut-off values.

Results

The age of NAFLD patients was 36.22 (11.32) year (median, IQR), and their age range was (18 – 60) year; whereas the age of the control group subjects was 33(10)-year (median, IQR), and the age range (20-64) year. Sex distribution was nearly identical between groups, with males comprising 48.8% of the NAFLD group and 51.2% of the control group, and females comprising 51.2% and 48.8%, respectively ($p = 0.833$). BMI of NAFLD patients was 33.02 (9.75) (median, IQR) and that of the control group participants was 32.04 (4.76) kg/m^2 (median, IQR), this difference was not statistically significant ($p = 0.990$).

Overall, there were no significant differences in age, sex, or BMI between the two study groups, indicating successful matching and minimizing potential confounding effects from these variables.

Significant differences were observed between the NAFLD and control groups across multiple biochemical and hematological parameters. Serum level of total cholesterol (TC) in NAFLD patients was 193(44) mg/dl, which is significantly higher than that of the control group subjects which was 166(25) mg/dl (p is 0.004). Similarly, the triglyceride (TG) level was markedly elevated in the NAFLD group at 187 (136) mg/dl, compared to 129 (27) mg/dl in the control group ($p < 0.001$). Low-density lipoprotein-cholesterol (LDL-c) was also significantly higher in NAFLD patients, measuring 111.8 (39) mg/dl versus 78.5 (27.47) mg/dl in the control group ($p < 0.001$). Conversely HDL-cholesterol (HDL-c) level was significantly lower in NAFLD patients than in the controls, measuring 41(15.21) and 52.09(15.7); respectively ($p<0.001$).

The serum level 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) × 109/L compared to 300 (106) × 109/L, respectively ($p < 0.001$); as presented in Table (1). The highly significant difference of these variables between the study groups underscores their association with NAFLD.

Approximately 22.22% of NAFLD patients exhibited mild steatosis, 31.12% exhibited moderate steatosis, and 46.66% exhibited marked steatosis; moreover, 82.22% of NAFLD patients were classified as being at low risk for fibrosis, while 15.55% were categorized as having an intermediate risk and only 2.22% of the patients were at high risk; as presented in Table (1).

Table 1. Biochemical and hematological characteristics of participants

Variable	NAFLD (n=45)	Control (n=45)	p-value
TC (mg/dl)	193(44)	166(25)	0.004
TG (mg/dl)	187(136)	129(27)	<0.001
LDL-c (mg/dl)	111.8(39)	78.5(27.47)	<0.001
HDL-c (mg/dl)	41(15.21)	52.09(15.7)	<0.001
AST (U/L)	26(17.35)	16.8(6.15)	<0.001
ALT (U/L)	34(31.85)	23.4(10.10)	<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	37(82.22%)	-----	-----
Intermediate	7 (15.55%)	-----	-----
High	1(2.22%)	-----	-----

NAFLD: Non-alcoholic fatty liver disease, TC: Total cholesterol, TG: Triglycerides, LDL-c: Low-density lipoprotein-cholesterol, HDL-c: High-density lipoprotein-cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, FIB-4: Fibrosis 4 index, n: Number of participants.

Severity of steatosis and risk of fibrosis are expressed as frequencies and percentages. TC, TG, LDL-c, HDL-c, ALT, AST, platelete count and FIB-4 Index are expressed as median (IQR), and the Mann-Whitney U test was used to evaluate difference between groups.

The serum level of LCAT in patients with NAFLD was significantly lower, averaging 1.7 (0.27) ng/ml, compared to 3.02 (0.8) ng/ml in the control subjects ($p < 0.001$); as shown in Table (2). The finding suggests an association of LCAT with the disrupted lipid metabolism that is associated with the disease.

Table 2. Serum LCAT level of participants

Variable	NAFLD (n-45)	Control (n-45)	p-value
LCAT (ng/ml)	1.7(0.27)	3.01(0.8)	<0.001

NAFLD: Non-alcoholic fatty liver disease, LCAT: Lecithin-cholesterol acyltransferase, n: number of participants

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

Serum LCAT levels were analyzed according to the severity of hepatic steatosis in NAFLD patients. The serum LCAT level was 1.69 (0.38) ng/ml in patients with mild steatosis, 1.61 (0.31) ng/ml in those with moderate steatosis, and 1.76 (0.21) ng/ml in the marked steatosis group. Despite the observed variations, the differences were not statistically significant ($p = 0.33$), indicating that serum LCAT concentration may not be strongly influenced by the degree of fat accumulation in the liver; as presented in Table (3).

Table 3. Serum LCAT level according the severity of steatosis in NAFLD patients

Variable	Severity of steatosis in NAFLD patients			p-value
	Mild (n=10)	Moderate (n=14)	Marked (n=21)	
LCAT (ng/ml)	1.67(0.44)	1.61(0.31)	1.76(0.21)	0.329

NAFLD: Non-alcoholic fatty liver disease, LCAT: Lecithin-cholesterol acyltransferase, n: number of patients.
Data are presented as median (IQR), and the Kruskal–Walli's test was used to evaluate difference among groups.

Serum LCAT level was evaluated based on the hepatic fibrosis risk classification among NAFLD patients. The levels were 1.68(0.28) ng/ml in the low-risk group, 1.82(0.11) ng/ml in the intermediate-risk group and 1.54 ng/ml in the high-risk group. The difference among the groups did not reach statistical significance ($p = 0.07$); as presented in Table (4). The findings are indicating that serum LCAT level did not show a clear association with fibrosis risk. However, the small number of participants classified within the intermediate and high risk categories (n = 7, 1; respectively) limit the statistical strength of this analysis. Consequently, further research involving larger and more evenly distributed populations is needed to clarify these potential associations.

Table 4. Serum LCAT level according the hepatic fibrosis risk in NAFLD patients

Variable	Risk of hepatic fibrosis in NAFLD patients			p-value
	Low (n=37)	Intermediate (n=7)	High (n=1)	
LCAT (ng/ml)	1.68(0.28)	1.82(0.11)	1.54	0.07

NAFLD: Non-alcoholic fatty liver disease, LCAT: Lecithin-cholesterol acyltransferase, n: number of patients.
Data are presented as median (IQR) and the Kruskal–Wallis test was used to evaluate difference among groups.

Correlation analyses revealed several significant relationships between LCAT with various parameters; as presented in Table (5). Serum LCAT level showed significant negative correlation with AST ($\rho = –0.589$, $p < 0.001$), ALT ($\rho = –0.441$, $p < 0.001$), FIB-4 index ($\rho = –0.508$, $p < 0.001$), and LDL-c ($\rho = –0.342$, $p < 0.001$). Conversely, LCAT showed positive correlation with platelet count ($\rho = 0.355$, $p < 0.001$), and HDL-c ($\rho = 0.418$, $p < 0.001$). On the other hand LCAT showed no significant correlation with each of age, sex, BMI or TG ($p>0.05$ for all).

Table 5. Correlation of LCAT with the studied variables of participants

Variable	Ps	p-value
Age	-0.171	0.106
Sex	0.071	0.304
BMI	0.037	0.73
Total cholesterol	-0.118	0.266
TG	-0.18	0.089
LDL-c	-0.342	<0.001
HDL-c	0.418	<0.001
AST	-0.589	<0.001
ALT	-0.441	<0.001
Platelets count	0.355	<0.001
FIB-4 Index	-0.508	<0.001

LCAT: Lecithin-cholesterol acyltransferase, BMI: Body mass index, TG: Triglycerides, LDL-c: Low-density lipoprotein-cholesterol, HDL-c: High-density lipoprotein-cholesterol, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, FIB-4: Fibrosis 4 index, ρs: Spearman correlation coefficient.

To minimize the influence of between-group differences, correlation analyses between LCAT and clinical variables (such as AST, ALT, FIB-4, and lipid profile) were performed separately within the NAFLD cohort (n=45) to identify true prognostic associations.

The receiver operating characteristic (ROC) analysis was used to assess the potential of LCAT to distinguish between NAFLD patients and the control group subjects. LCAT demonstrated an excellent diagnostic performance, with an area under the curve (AUC) of 0.951. At a cut-off value of 1.907ng/ml which is calculated by Youden Index, LCAT achieved a sensitivity of 97.8% and a specificity of 91.1% ($p < 0.001$). In our ROC analysis, we had calculated the area under the curve (AUC) to assess the diagnostic performance of the model. The reported p-value tests the null hypothesis that the AUC is equal to 0.5, which indicates no discriminative capacity. A p-value less than 0.05 suggests that the AUC is significantly different from 0.5, suggesting that the model has a statistical significance to discriminate between the positive and negative classes. These findings suggest that serum LCAT level may serve as promising diagnostic marker for NAFLD; as shown in Table (6) and figure (1).

Table 6. The receiver operating characteristic curve analysis of LCAT in identifying NAFLD

Variables	AUC	Cut-off value	Sensitivity	Specificity	Interpretation	p-value
LCAT	0.951	1.90	0.978	0.911	Excellent	<0.001

LCAT: Lecithin-cholesterol acyltransferase, AUC: Area under the curve.

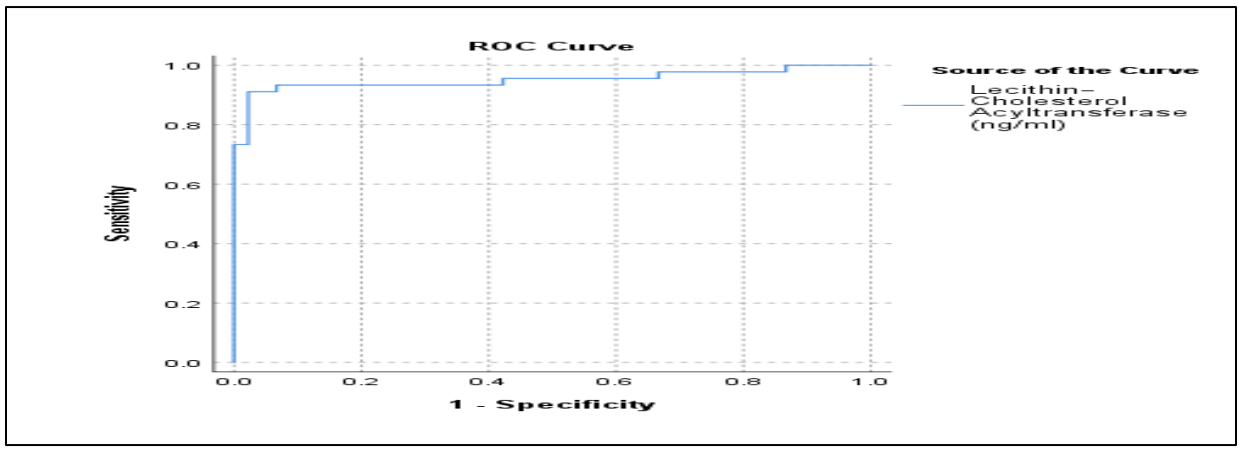

Figure 1: Receiver operating characteristics curve of lecithin cholesterol acyltransferase

Discussion

The comparison between NAFLD patients and matched controls revealed significant differences in several liver function, hematological, and lipid profile parameters, supporting the systemic nature of NAFLD and its metabolic and hepatic implications. The serum levels of both AST and ALT were significantly higher in the NAFLD patients compared to the controls. This elevation is highly expected with the ongoing hepatocellular injury and inflammation accompanying NAFLD [27]. Moreover, the FIB-4 index that is a non-invasive fibrosis score, was also significantly higher in NAFLD patients compared to the controls suggesting a greater risk of liver fibrosis in NAFLD patients [28]. However, a significantly lower platelet count was recorded in the NAFLD patients compared to controls. This may be attributed to platelet sequestration in the spleen and decreased thrombopoietin production in in NAFLD and in hepatic fibrosis [29, 30].

NAFLD patients in the present study exhibited significantly higher total cholesterol, triglycerides, and LDL-cholesterol, and significantly lower HDL-cholesterol compared to controls. The altered lipid metabolism is actually a one hallmark of NAFLD [31]. Atherosclerosis is quite common in NAFLD patients mediated by the altered lipid metabolism in those patients; this occurs in accordance with existing evidence that links NAFLD to increased cardiovascular disease risk [32-34].

Overall, these findings highlight that NAFLD effect is not confined to hepatic injury and increasing the risk of hepatic fibrosis but also dysregulation of different metabolic pathways.

The present study demonstrated that serum levels of LCAT were significantly lower in NAFLD patients compared to matched controls. The fact that lipid metabolism dysregulation is a characteristic of NAFLD is consistent with the lower LCAT level, since LCAT has central role in the HDL particles maturation and in the esterification of free cholesterol, which are essential for reverse cholesterol transport and lipid homeostasis [35]. The lower LCAT levels in NAFLD patients may reflect lipids accumulation in hepatocytes and thus hepatic steatosis and progression of liver injury [36]. The lower LCAT levels in NAFLD patients reported in the present study is consistent with the findings of previous studies that linked the reduced LCAT activity with several metabolic disturbances and with liver dysfunction [37].

A previous investigation in cows has highlighted the association between LCAT activity and hepatic disorders. Nakagawa *et*

al. observed a marked reduction in LCAT activity in dairy cows that developed spontaneous fatty liver disease. This was accompanied by lower serum levels of a key physiological activator of LCAT, apolipoprotein A-I. Nakagawa *et al.* suggested that lower LCAT activity may be the result of impaired hepatic synthesis and secretion or from reduced plasma activation of LCAT due to decreased apolipoprotein A-I levels [36].

In a separate study, He *et al*. investigated LCAT expression in HCC tissues and found that its expression was significantly downregulated, and this reduction in LCAT expression was negatively associated with HCC prognosis, suggesting that LCAT may not only serve as a metabolic enzyme but also hold prognostic relevance in liver malignancies [38].

In contrast to our findings, a previous study conducted by Nass *et al.* reported that plasma LCAT activity was elevated in individuals at high risk for NAFLD, as defined by a fatty liver index (FLI) ≥ 60, and remained significantly associated with NAFLD even after adjusting for key metabolic variables including T2DM, obesity, and insulin resistance [39]. This discrepancy between the present study and Nass *et al.* study may be attributed to several methodological and population-based differences. To begin with, the present study directly measured serum LCAT concentrations using a quantitative ELISA method, whereas the referenced study evaluated LCAT activity using an exogenous substrate-based assay, which may not reflect actual circulating levels of LCAT. Moreover, participants in the present study were recruited from a well-characterized cohort, and NAFLD diagnosis in those participants was performed using abdominal ultrasonography by expert gastroenterologists in accordance with EASL criteria; whereas the other study used a surrogate index that is FLI. Furthermore, the present study used a very strict exclusion of individuals with comorbidities that may confound LCAT expression. Additionally, differences in genetic background, environmental exposures, diet, and ethnicity between the Middle Eastern population in the present study and the European cohort studied by Nass *et al.* may influence LCAT regulation. Given these opposing findings, further studies are required to examine the role of LCAT in biopsy-confirmed NAFLD, particularly across populations of different genetic backgrounds and using standardized measurement techniques.

In the present study, serum LCAT levels were analyzed in relation to both the severity of hepatic steatosis and the risk of fibrosis among patients with NAFLD. The results showed that LCAT levels were comparable throughout different steatosis stages, as well as they were comparable throughout the fibrosis risk groups. This indicates that serum LCAT levels were not clearly associated with steatosis severity or fibrosis risk in this cohort.

Nevertheless, the study reported a significant negative correlation between serum LCAT levels and the FIB-4 index, indicating that lower LCAT levels may be associated with more advanced liver fibrosis. This inverse relationship suggests a potential association of LCAT with the progression of liver fibrosis, even if such associations were not evident through categorical group comparisons.

The observed discrepancy could be largely attributed to the small number of patients in the intermediate and high-risk fibrosis group (n = 7, 1; respectively), so the patients were not evenly distributed throughout the fibrosis risk categories. This limitation likely reduced the statistical power to identify meaningful differences across fibrosis stages. These findings suggest the value of using both continuous and categorical methods when analyzing how biomarkers relate to disease. They also highlight the need for studies with larger sample size and more evenly

distributed groups across the steatosis severity or fibrosis stages, in order to better understand the association of LCAT with liver damage linked to NAFLD.

In the present study, several significant correlations were observed between serum level of LCAT and the studied variables. LCAT was inversely associated with liver enzymes (AST, ALT), FIB-4 index, and atherogenic lipids such as triglycerides and LDL-cholesterol, while showing a positive correlation with HDL-cholesterol and platelet count. These findings reinforce the potential hepatoprotective and anti-inflammatory role of LCAT in NAFLD, where lower LCAT levels may reflect more advanced hepatic dysfunction or systemic metabolic stress. Notably, human studies have reported reduced LCAT activity or concentration in individuals with NAFLD, particularly in those with higher liver fat or more advanced fibrosis. One cohort observed decreased LCAT activity, measured indirectly via cholesterol ester/free cholesterol ratios, in patients with moderate to severe steatosis, while another found lower HDL-bound LCAT levels in fibrosis-positive NAFLD patients, even after adjustment for HDL particle count [40, 41]. These findings mirror our results and further suggest that diminished LCAT may reflect greater hepatic dysfunction or lipid regulatory impairment in NAFLD.

The ROC curve analysis conducted in this study demonstrated that serum level of LCAT exhibits significant diagnostic value in distinguishing NAFLD patients from matched controls. The diagnostic performance of LCAT possibly reflects the disturbances in lipid processing commonly observed in the disease [42, 43]. Thus, measurement of the serum level of LCAT may offer valuable non-invasive tool for the early detection of NAFLD. Incorporating such biomarker into clinical practice could enhance diagnostic precision, especially in resource-limited settings where imaging or biopsy may not be feasible.

This study has several notable strengths. First, it is one of the few case-control studies conducted to explore the association of LCAT with NAFLD, contributing to an emerging area of interest in metabolic and hepatic research. Second, the study carefully controlled for confounding variables by matching participants for age, sex, and BMI, thereby enhancing the validity and reliability of group comparisons. Lastly, the application of strict exclusion criteria helped minimize the influence of other factors known to affect lipid metabolism.

Despite the promising findings, this study is subject to several limitations. The control group participants were not screened by ultrasound to exclude the subclinical NAFLD; therefore, future studies should perform an imaging-based confirmation of the control group. The observational nature restricts the ability to draw causal inferences between NAFLD and LCAT. In addition, the relatively small sample size, particularly the low number of participants in the higher fibrosis risk category, limits the statistical power and generalizability of the results. Another limitation is the absence of liver biopsy, which remains the gold standard for assessing hepatic steatosis and fibrosis. Instead, the study relied on non-invasive tools that are the FIB-4 index and ultrasound, which may have introduced classification bias.

Conclusion

In summary, this study demonstrates that patients with non-alcoholic fatty liver disease (NAFLD) show significantly lower serum levels of lecithin-cholesterol acyltransferase (LCAT) when compared to matched control group. This finding suggests a potential cross-talk between NAFLD and the disturbances in lipid metabolism. In addition, our receiver operating characteristic (ROC)

analysis suggests that LCAT has a strong diagnostic potential for discriminating NAFLD from healthy individuals. However, it is important to note that LCAT levels did not express a significant correlation with the degree of hepatic steatosis or fibrosis, indicating its limited prognostic potential in assessing the disease progression.

These results suggest that although LCAT may serve as a promising non-invasive diagnostic marker for NAFLD, it should not be served for evaluating the severity of the disease. Future studies are warranted for further investigation of the underlying mechanisms linking LCAT to NAFLD and to explore other biomarkers that may provide better insights into disease progression.

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