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Cathepsin-L as New Diagnostic Biomarker for Detection and Pathogenesis of Fatty Liver Disease

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Abstract

Background: Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease in Western countries, most likely related to the increased incidence of obesity, metabolic illnesses, and exposure to certain environmental chemicals. **Objective:** The aim of this study was to forecast the severity and course of non-alcoholic fatty liver disease (NAFLD) in patients who were in the early stages of the disease., Cathepsins are the most prevalent lysosomal proteases, mostly found in acidic endo/lysosomal compartments, where they play a key role in intracellular protein degradation, energy metabolism, and immunological responses, among a variety of other function., Evaluated demographic factors (Age, BMI,) for the two study groups and insulin ,blood glucose and HOMA IR and lipid profile and liver function test and albumin Also assessing the levels of Cathespin L level, The current study was conducted on 60 Iraqi patients in Najaf Governorate, Iraq, diagnosed with non-alcoholic fatty liver disease (NAFLD), in addition to 30 healthy individuals. The age of study groups ranged from 20 to 60 years. **Methods**: The Cobas C111 device and the sandwich enzyme-linked immunosorbent assay (ELISA) were used to determine the levels of study criteria. **Results** indicated that the body mass index (BMI) of the fatty liver disease that of the healthy group, while HDL and albumin levels decreased. The study also showed group was higher than an increase in LDL and vLDL cholesterol, triglyceride, AST and ALT. The study showed that Cathespin L levels in the serum of patients were significantly higher than those in the healthy group.

Key words: NAFLD, Cathepsins, CTSL, Protease, lysosomal

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, defined by the presence of steatosis in more than 5% of the hepatocytes.[1]. Approximately 25% of people globally suffer from NAFLD at the moment, with estimates ranging from 13% to 32% [2]. The prevalence of non-alcoholic fatty liver disease (NAFLD) has increased significantly due to the global rise in metabolic syndrome, obesity, and diabetes, impacting around 25% of the global population [2]. Nonalcoholic fatty liver disease (NAFLD) it includes a range of histological characteristics, from basic steatosis, which is defined by the accumulation of fat in the liver, to nonalcoholic steatohepatitis (NASH), which is linked to inflammation, fibrosis, and/or hepatocyte ballooning, ultimately leading to liver cirrhosis and hepatocellular carcinoma (HCC) [3]. The initial stage of NAFLD/NASH is characterized by hepatic fat accumulation, a disease that is frequently linked to metabolic syndrome (MetS) characteristics like obesity, type 2 diabetes, dyslipidemia, and hypertension. 2. From a pathological perspective, at least three mechanisms—increasing visceral adipose tissue (AT) lipolysis, hepatic de novo lipogenesis (DNL) activation, and a high calorie and/or fat diet—have been found to be the cause of excessive lipid buildup in the liver [4]. The "multiple hit model" provided a clearer explanation of the complexity of NAFLD: a number of concurrent injuries, such as hepatic steatosis, hepatocyte lipotoxicity, immune-mediated inflammation, mitochondrial dysfunction, and related oxidative stress, work together to cause NAFLD[5]. The diagnosis is based on clinical and histological findings. Imaging modalities used in complementary diagnosis include ultrasound, computed tomography (CT) scans, and magnetic resonance imaging (MRI), which is the most sensitive. A liver biopsy is considered the gold standard for detecting NASH or NAFLD[6].

Cathepsins are a family of lysosomal proteases that have an incredibly wide range of roles. Based on the kind of amino acid at the active site, mammalian proteases are divided into five families: metallo, serine, threonine, aspartic, and cysteine proteases.

All cathepsins belong to three distinct families of proteases.is; serine proteases (cathepsins A and G), aspartic proteases (cathepsin D and E) and eleven cysteine cathepsins (cathepsins B, C, F, H, K, L, O, S, V, X, and W), .CTSL, a lysosomal cysteine protease, is involved in hormone maturation, antigen presentation, and the breakdown of protein substrates inside lysosomes [7]. It is well known that lysosomal cysteine cathepsins play a role in vital signaling pathways like apoptosis and the activation of liver homeostasis maintenance, steatohepatitis non-alcoholic steatohepatitis (NASH)[8]. Because of this and their varied cellular locations, they are able to take part in a variety of cellular functions that are critical for maintaining cellular/tissue homeostasis, including apoptosis, autophagy, matrix remodeling, chemotaxis, and antigen presentation. Thus, it is not unexpected that cathepsins play a role in the emergence of a number of human diseases, such as various liver conditions[9].

Numerous metabolic processes, such as insulin signaling, inflammation, and the metabolism of fats and carbohydrates, have been linked to CTSL. Additionally, CTSL is a highly intriguing therapeutic target because it contributes to a number of metabolic-related disorders, including obesity, atherosclerosis, NAFLD, and diabetic kidney disease[10]. Cathespin L, a lysosomal protease, contributes to hepatocyte apoptosis, fibrosis and metabolic dysregulation .Assessing its circulating levels may provide a non-invasive biomarker for disease severity and a potential therapeutic target ,addressing a critical knowledge gap in NAFLD research

Materials and Methods

90 persons were participated in the current study, in this case-control study, covering the period from December 2024 to April 2025, they were classified into two group, patients and healthy individuals. The patient group included 60 patients with non-alcoholic fatty liver disease While the second group included the control group, which included 30 individuals. blood samples were collected from the Digestive System and Liver Hospital and Al-Hakim General Hospital in Najaf Governorate, after obtaining official approvals. All participants fasted for 8–12 hours before blood

collection. Participants' ages ranged from 20 to 60 years. exclusion criteria were detected as having: History of smoking, alcohol drinking, History of heart, kidney, and liver diseases, History of hypothyroidism diseases, Consumption of antioxidant drugs, Women who are pregnant, breastfeeding, or taking contraceptive pills, history of diabetes. lipid profile and liver enzymes were measured in the serum of participants using a Cobas C 111 (Roche, Germany) device. Diagnostic variables, Insulin and cathepsin L levels were estimated using the sandwich ELISA test. SPSS version 27 was used to process and analyze the data of the current study, in addition to the Excel statistical analysis system. "Therefore, parametric tests (independent t-test, Pearson correlation) were applied for normally distributed variables, The mean, standard deviation, and correlation coefficient of the variables were calculated, and the probability of deviation from the controls was considered statistically significant if the p-value <0.05

Results and Discussion

The current results showed no statistically significant differences in age between the NAFLD group of patients and the healthy group (p=0.052). However, the results showed statistically significant differences in BMI, with patients showing a higher BMI than healthy individuals (p=0.01). Patients also showed higher levels of glucose (p=0.001), insulin (p=0.01), and HOMA IR (p=0.001) compared to healthy controls as shown in Table 1, the serum levels of LDL-C, VLDL, cholesterol, and triglycerides in patients with NAFLD were significantly higher than those in the healthy group, with the exception of HDL, whose levels were lower in patients compared to healthy controls, according to the current study. Table 2 presents the statistically significant differences found in the study when compared the levels of AST and ALT enzymes in patients with non-alcoholic fatty liver disease (NAFLD) with healthy control group, while albumin levels were lower in patients compared to healthy individuals, as shown in Table 3. The results also showed higher levels of cathepsin L in a group of patients with NAFLD compared to healthy individuals (P=0.00), and that men and women with NAFLD had higher levels of cathepsin L compared to healthy men and women (P=0.001) for each, as shown in Table 4. Many studies have shown a clear correlation between the development of NAFLD and the disruption of lysosomal cathepsin levels, with the first report on the role of cathepsins in metabolic disorders dating back to 1958.[11]. The cathepsin L (CTSL), it is known that CTSL is linked to a number of metabolic functions, such as, insulin signaling, metabolism of fats and carbohydrates, it is also a very intriguing therapeutic target because it contributes to many metabolic-related disorders, including obesity, atherosclerosis, NAFLD, and diabetic kidney disease[12] Under typical circumstances, macrophages internalize cholesterol produced from lipoproteins and send it to the lysosome for additional processing. Free cholesterol is moved from the lysosome to the cytoplasm following hydrolysis where The free cholesterol is either released through cholesterol efflux or broken down into bile acid precursors. However, it has been demonstrated that lysosomes loaded with cholesterol cause disruptions in the lysosomal enzyme trafficking route, which may result in elevated plasma levels of lysosomal enzymes. These findings imply that cholesterol-rich lipoproteins, such as LDL, enhance the interaction of lysosomes with the plasma, which in turn results in elevated levels of Cathespin plasma, since the plasma membrane is the main location where secretion into plasma occurs. [13]. Lysosomes are best known for its primary role in protein degradation. However, protein degradation during NASH, that cholesterol, seems to be disturbed, with clear association between hepatic inflammation and increased cholesterol accumulation in lysosomes of KCs, this is underlined by the fact that KCs compromise 15–20% of the total number of liver cells and it is responsible for modified lipoproteins rich in cholesterol being mostly absorbed by the liver from the bloodstream.[13,14]. Previous research demonstrated higher cholesterol levels in KCs, as seen by the abundance of cholesterol-filled droplets in the KCs of NASH adults. Growing data today suggests that the lysosomal compartment can be known as vesicles that can secrete their contents, such as lysosomal enzymes, indicating that lysosomal function is not just restricted to the breakdown of proteins but also cholesterol A particular class of lysosomal enzymes known as cathepsins has been demonstrated to have a key role in modulating both cholesterol trafficking and the inflammatory response[15]. Reactive oxygen species (ROS) and cathepsins interact, as seen in regulated cell death (apoptosis, necroptosis, ferroptosis, pyroptosis, and NETosis) and autophagy, in which ROS act as inducers and cathepsins operate as effectors. The interplay of lysosomal cathepsins, oxidative stress, cell death, and autophagy highlights the complexity of cellular responses to environmental cues and stressors. [16]. Oxidative stress can compromise the integrity of lysosomes and rupture them, releasing cathepsins into the cytoplasm, this can contribute to apoptosis, to recap, cathepsins have been shown to regulate oxidative stress via their effect on antioxidant enzymes. In several systems, blocking particular cathepsins has shown promising outcomes in reducing oxidative damage and boosting antioxidant defense mechanisms. Reactive oxygen species has a bidirectional connection with cathepsins, promoting their release from lysosomes and influencing the activity of inhibitors. These interactions have a profound impact on the apoptotic pathway, which ultimately determines cell survival and death[16].Cathepsins contribute to the pathophysiology of lipid-related illnesses as metabolic syndrome and NAFLD/NASH, lipids from non-adipose tissues accumulate in cells and trigger the release of lysosomal enzymes, including cathepsins. This mechanism causes cell death, also known as lipotoxicity[17]. damaged hepatocytes release cathepsins into the cytoplasm or extracellular space which triggers inflammatory signaling (NF-kB,inflammasones), this promotes Kupffer cell(liver macrophage)[18].

Table (5) represents the relationship between age, BMI, glucose, insulin, and triglyceride with Cathespin which had a strong positive association, according to Pearson's correlation coefficient.

Present study has good area under the curve for Cathespin as illustrated in Figure:1 which was 0.963 in NAFLD disease, that is mean a strong performance but many have some errors, also have sensitivity (80%) and the specificity was very high which was (100%), as show in Table (7) for this may be consider Cathespin as candidate diagnosis marker for NAFLD disease, also use as predictor of disease progression and surveillance marker of disease severity.

Table -1 Demographic characteristic, Blood glucose, Insulin AND HOMA IR of patients and control subjects

Variable	Subject	Control Mean ± SD	MAFLD Patients Mean ± SD	P -value
Sex No .	Total	30	60	-
	Male	17	32	-
	Female	13	28	-
Age (year)	Total	46.67±6.030	48.75±10.507	0.052
	Male	45.69±8.528	46.03±10.917	0.086
	Female	43.41 ±9.586	45.19±10.052	0.062
BMI (Kg\m)	Total	25.93 ± 2.990	28.97 ±3.076	0.01
	Male	25.670 ±2.900	27.558±3.1532	0.01
	Female	24.5972 ±3.070	28.670 ±2.476	0.001
Blood Glucose (mg/dL)	Total	79.17±11.24	103.80±10.538	0.001
	Male	78.92±14.009	101.38±10.153	0.001
	Female	79.35±9.117	106.77±10.61	0.001
Insulin (pmol/L)	Total	12.9905±2.272	20.3164±2.2968	0.001
	Male	13.0304±2.642	20.776±2.526	0.001
	Female	12.960±2.030	19.7205±1.8347	0.001
HOMA IR	Total	2.4893±0.2591	5.1817±0.58613	0.01
	Male	2.4663±0.2660	5.1741±0.5959	0.001
	Female	2.5054±0.2630	5.1833±0.5893	0.001

Table 2 Levels (Mean ± SD) of Lipid Profile in Sera of Study Groups

Variable	Subject	Control Mean ± SD	MAFLD Patients Mean ± SD	P -value
Triglyceride mg/dL	Total	93.67 ± 28.787	162.75±30.895	0.001
	Male	90.158±28.823	165.823±34.571	0.001
	Female	97.882±28.037	158.2308±25.157	0.001
HDL-C mg/dL	Total	45.29±9.809	32.77 ±6.282	0.001
	Male	44.307±9.4097	33.0559±5.887	0.001
	Female	46.0471±10.323	32.7423 ±6.5809	0.001
LDL-C mg/dL	Total	110.33 ±26.321	167.3917±29.536	0.001
	Male	18.038±5.764	33.167±6.914	0.001
	Female	19.5765±5.607	31.646±5.0312	0.001
VLDL-C mg/dL	Total	18.91 ±5.631	32.55±6.179	0.001
	Male	118.892±28.89	162.367±28.898	0.001
	Female	103.788±23.213	173.471±29.471	0.001
Cholesterol mg/dL	Total	159.17±28.415	225.01±27.670	0.001
	Male	181.230±27.059	228.588±26.088	0.001
	Female	169.411±26.002	237.80±25.491	0.001

The difference is considered significant at p<0.05

Table 3Levels (Mean ± SD) of Liver Function Test Concentration (mg/dL) and Albumin Sera of Study Groups.

Variable	Subject	Control Mean ± SD	MAFLD Patients Mean ± SD	P -value
ALT (mmol/L)	Total	26.366±7.248	50.533±14.092	0.001
	Male	19.615±6.0231	44.250±6.094	0.001
	Female	18.4118±7.1592	42.8846±9.754	0.001
AST (mmol/L)	Total	18.933±6.660	43.474±7.584	0.001
	Male	25.46±7.178	52.264±13.07	0.001
	Female	27.058±7.152	49.6907±15.679	0.001
Albumin (mmol/L)	Total	4.04±0.5648	2.878±0.4851	0.001
	Male	4.135±0.692	2.850±0.477	0.001
	Female	3.964±0.4526	2.8923±0.516	0.001

The difference is considered significant at $p<0.05$

Table 4Levels (Mean ± SD) of Cathespin L Concentration (mg/dL) Sera of Study Groups.

Variable	Subject	Control Mean ± SD	MAFLD Patients Mean ± SD	P -value
Cathespin (pg\mm)	Total	15.544±2.154	40.013±11.725	0.001
	Male	14.407±2.293	34.532±9.384	0.001
	Female	13.612±1.5438	32.187±7.427	0.001

The difference is considered significant at $p<0.05$

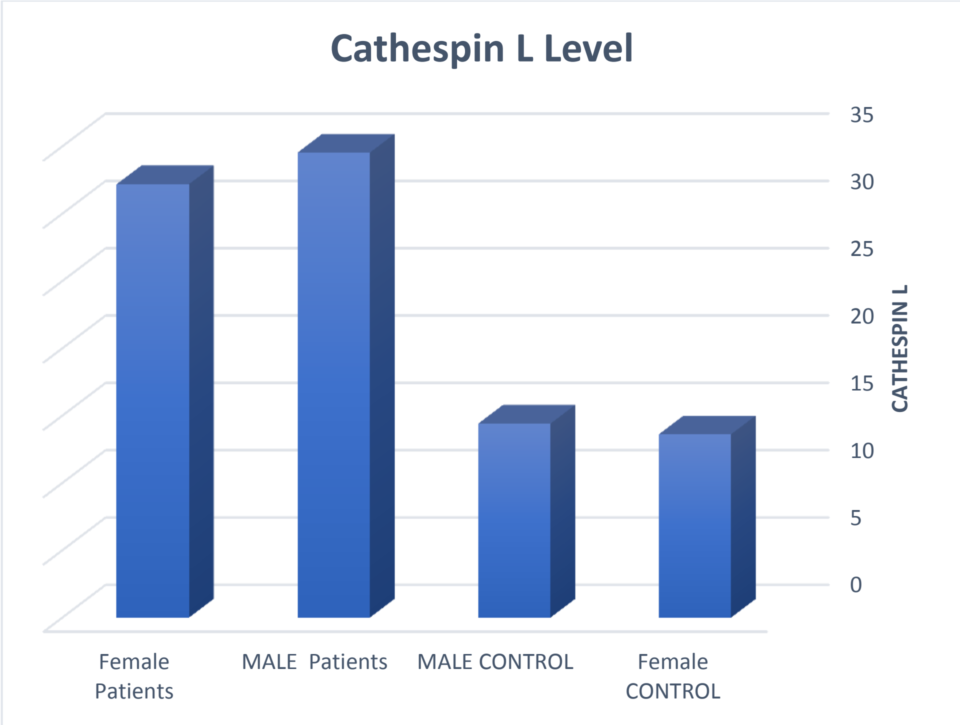

Figure 1: Levels of CATHESPIN L in Sera of the Study Groups

Table 5: Table Correlation Cathespin L with Study variables in NAFLD Diseases

Parameters	Pearson coefficient (r)	p-value
Age	0.641**	0.001
BMI	0.304*	0.023
Blood Glucose	0.278*	0.031
Insulin	0.841*	0.001
HOMA IR	0.174	0.183
Triglyceride	0.335*	0.020

Correlation is significant at the 0.05 level

Table: 6 Analysis of Cathespin L Receiver Operating Characteristic in NAFLD Disease.

Parameter	AUC	S. E	P-value	Cutoff value	Sensitivity %	Specificity %	Cl (95%) of AUC
CATHESPIN L	0.968	0.015	0.000	29.6316	0.800	1.000	0.983-0.999

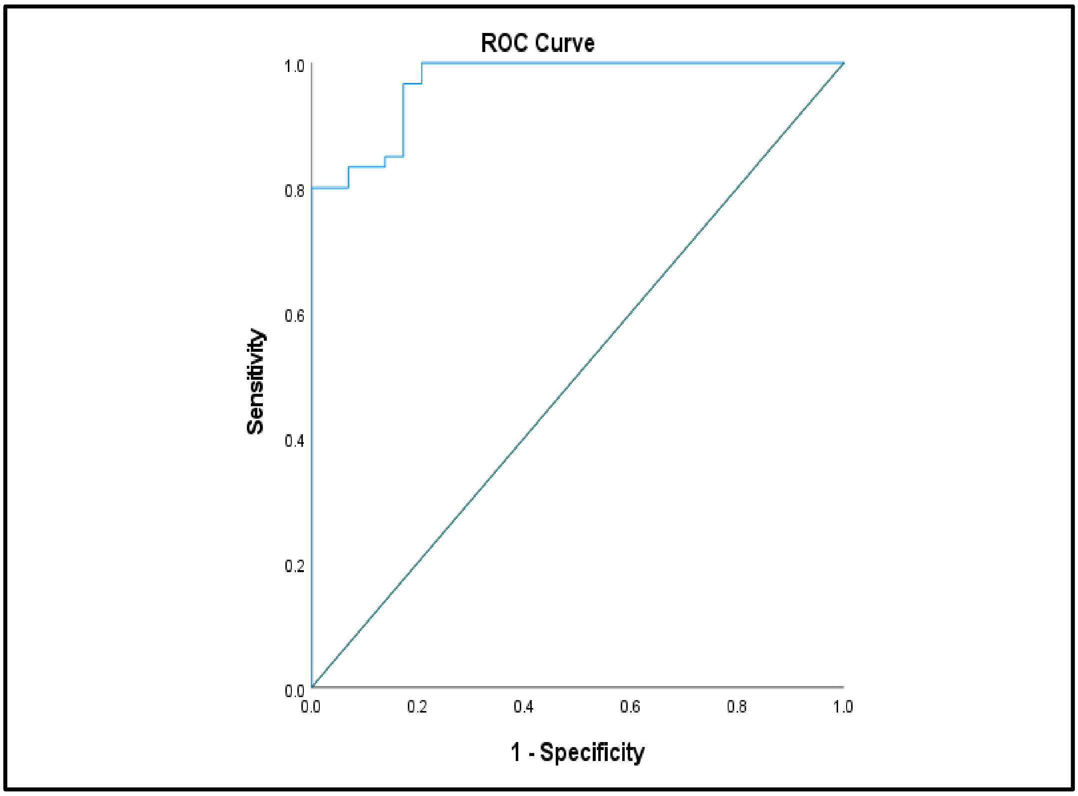

Figure 2: Curve of CATHESPIN L Receiver Operating Characteristic in non-alcoholic fatty liver disease

Conclusions

Present study reached to many important conclusions which that cathepsin –L expression and activity are increased in hepatocyte cells during fatty liver, reflecting lysosomal stress and lipid overload, in addition to elevated cathepsin –L, activates pro-apoptotic pathways and enhances NF- kB- mediated inflammatory signaling, this links steatosis to liver injury and the progression from simple steatosis to NASH.

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