Ref: Ro J Med Pract. 2025;20(3)
DOI: 10.37897/RJMP.2025.3.2

The relationship between cytokeratin 18 and liver steatosis on ultrasound in nonalcoholic fatty liver disease

Douaa S. SALIM, Nazar S. HADDAD

College of Medicine, University of Basrah, Basrah, Iraq

ABSTRACT

Introduction. *Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in Western countries, characterized by increased fat infiltration in the liver without secondary causes.*

Aim. *To correlate cytokeratin 18 (CK-18) levels and NAFLD fibrosis scores with liver steatosis assessed by ultrasound.*

Patients and methods. *A cross-sectional study enrolled 42 individuals with liver steatosis and 18 individuals without liver steatosis by ultrasound, who presented to the Al-Faiha GIT center from March 2020 to May 2021. Ten milliliters of venous blood were drawn from each individual to measure serum cytokeratin 18 levels using immunoassay.*

Results. *In our study, we found that the presence and severity of liver steatosis, as assessed by ultrasound, increased with higher body mass index and advanced age, regardless of gender. A significant association was also found between impaired glucose tolerance and liver steatosis (p = 0.0001). No significant differences were found in lipid profile or liver enzymes between individuals with and without liver steatosis. The study demonstrated a good correlation between cytokeratin 18 levels and liver steatosis grades assessed by ultrasound (P = 0.0001), with a cutoff value of 102.03 pg/ml. However, a poor correlation was observed between the NAFLD fibrosis score and liver steatosis (P = 0.088).*

Conclusion. *Serum cytokeratin 18 levels demonstrated a strong correlation with ultrasound-assessed steatosis grades, in contrast to the NAFLD fibrosis score, which exhibited a weak association. However, the findings should be interpreted with caution due to the relatively small sample size and the absence of histological confirmation.*

Keywords: Cytokeratin 18, liver steatosis, nonalcoholic fatty liver disease, ultrasound

INTRODUCTION

Developed the term nonalcoholic steatohepatitis (NASH) in 1980 to describe the progressive form of fatty liver disease histologically resembling alcoholic steatohepatitis though observed in individuals who denied alcohol abuse [1]. NAFLD is the most common chronic liver disease in the western countries [2,3]. Worldwide, NAFLD instances have risen from 391.2 million in 1990 to 882.1 million in 2017, with the prevalence rate rising from 8.2% to 10.9% over the same time period. The highest prevalence of NAFLD was detected in North Africa and the Middle East, while the greatest increase was seen in Western Europe, followed by Tropical Latin America, then high-income North America [4].

Most patients with NAFLD have fatty infiltration of the liver without any significant inflammation, which is called simple steatosis, whereas about 20% of individuals with NAFLD have non-alcoholic steatohepatitis (NASH), that characterized by lobular inflammation, ballooning of hepatocytes and fibrosis formation [5]. Individuals with NASH can present with progressive fibrosis, which

Corresponding author:
Douaa S. Salim
E-mail: alihuseeinibsh@gmail.com

Article History:
Received: 25 April 2025
Accepted: 22 July 2025

can lead to end stage liver disease manifesting as cirrhosis, liver cancer, and increased risk for liver- related mortality [6,7]. In addition to liver problems NAFLD also increase the risk for cardiovascular disease, malignancy and diabetes [8]. Patients with NAFLD are usually obese and may have hypertension. Most patients presented with fatigue and right upper quadrant pain or dullness, although many patients are usually asymptomatic. Mild to moderate hepatomegaly is one of the most common physical findings [9]. One of many hypotheses for the pathogenesis of NAFLD is the "two-hit" hypothesis proposed by Day and James in 1998 [10]. The presence of excess fat is essential for the subsequent events of NASH. The main feature of NAFLD is the triglyceride (TG) accumulation as fat droplets within the cytoplasm of liver cells. It is defined as more than 10% of liver cells possess fat droplets evident on liver biopsy [9].

The accumulation of TG within the cytoplasm of hepatocytes resulted from increased delivery of both free fatty acids (FFA) and TG to the liver, diminished hepatic utilization of FFA, diminished export of TG from the liver, and impaired beta-oxidation of FFA within hepatocytes [11]. In addition, Excess carbohydrates, either from dietary sources or de novo gluconeogenesis within the liver, is also a major stimulus for de novo fatty acid synthesis in the liver. Paradoxically, direct uptake of dietary fat as chylomicron remnants or FFA constitutes a relatively minor contribution to liver fat accumulation [12]. Insulin resistance is a common cause of fat accumulation within the liver. However, a tiny percentage of NAFLD patients do not show any signs of insulin sensitivity impairment [9]. Liver with excess fat may be more vulnerable to the stressors such as reactive oxygen species (ROS), adipokines, and cytokines than a normal liver. The regenerative capacity of a fatty liver is also impaired [13]. Oxidative stress may be other possible second hits, like increased Reactive oxygen Species and antioxidants decline, peroxidation of lipid and reactive metabolites like malondialdehyde and 4-hydroxynonenal, transforming growth factor-β, adipose tissue products, Fas ligand, mitochondrial dysfunction and respiratory chain deficiency, and bacterial overgrowth of small intestine (endotoxins and TNF-α) [9,14].

NAFLD diagnosis require the presence of liver steatosis on imaging or histological examination, and other causes of liver disease or steatosis have been excluded [15]. Individuals with NAFLD are usually asymptomatic, so diagnosis usually follows the incidental finding of abnormal liver enzymes or steatosis on imaging [15]. If abnormal LFTs are detected, there is usually mildly raised transaminases (ALT> aspartate transaminase (AST)) and/or gamma-glutamyl transferase. However, ~80% of patients have normal-range ALT levels (men <40 IU/L and women <31 IU/L), and even if elevated, the ALT typically falls (AST may rise) as fibrosis progresses to cirrhosis. ALT values do not correlate with histological findings and are unhelpful in both the diagnosis of NAFLD and determining disease severity [16,17]. Sensitivity and specificity of ALT for NASH are relatively low (sensitivity 64% and specificity 75%) [18]. Once suspected clinically, hepatic steatosis can be confirmed with imaging. Ultrasonography is usually used as a first-line investigation for hepatic steatosis that provides a qualitative assessment of fatty infiltration of the liver. Ultrasonography is very effective in identifying steatosis where >33% of hepatocytes are steatotic can be unreliable with lesser degrees of steatosis. Therefore, normal finding on ultrasound does not rule out mild fatty infiltration of the liver [19].

Cytokeratin 18 (CK-18) is the major intermediate filament protein in the liver and one of the most prominent substrates of caspases that released during hepatocyte apoptosis. Apoptotic death of hepatocytes is associated with release of caspase-cleaved CK-18 fragments into the bloodstream [20], and several studies have demonstrated elevation of these molecules in the presence of NAFLD [20]. The increased apoptotic rate as a result of the hepatic inflammatory response is reflected by elevation of serum Cytokeratin-18 fragments that may therefore distinguish NASH from simple steatosis [21]. These results have been further confirmed even in NAFLD individuals with normal aminotransferase levels [22]. Aim of the study to correlate the level of cytokeratin 18 with grades of liver steatosis on ultrasound, and correlate NAFLD fibrosis score with grades of liver steatosis on ultrasound.

MATERIALS AND METHODS

Study design and population

It is a cross-sectional study that had been conducted at Al-Faiha GIT center through the period from March 2020 to May 2021, the study was approved by the research ethical committee at Basrah Medical College. 42 individuals with liver steatosis graded on ultrasonography and 18 individuals without liver steatosis on ultrasonography were included. All patients underwent abdominal ultrasound which reveal liver steatosis, then categorized into mild (increased liver echogenicity), moderate (blurring of portal vein branches), and sever (blurring of the diaphragmatic outline) steatosis.

Ultrasonography was done by a single consultant radiologist to omit interobserver bias.

Inclusion criteria

The candidates whom involved in the study should have liver steatosis on ultrasonography.

Exclusion criteria

No history of alcohol intake, no suspicion of Wilson disease, no history of chronic drug intake (ex: steroid, oral contraceptive pills), and Neither history of viral hepatitis nor autoimmune hepatitis.

Laboratory examination

Whole blood (10 mL) was obtained from a peripheral vein into a gel tube labeled with the patient's name and number. The tube was kept on a rack for two hours to clot, then centrifuged at 3000 × g for ten minutes. The serum was transferred into a plain tube labeled with the patient's name, age, and date, and stored at −20°C until the time of cytokeratin-18 measurement.

Diagnostic kits

Cytokeratin 18 was measured by RayBio® Human cytokeratin 18 ELISA Kit (ab227896) (RAYBIOTECH, USA) which is based on the principle of enzyme-linked immunosorbent assay. It employs antibody specific for human cytokeratin 18 coated on a 96-well plate. Standards and samples are pipetted into the wells and cytokeratin 18 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-human cytokeratin 18 antibody is added. HRP-conjugated streptavidin is pipetted to the wells after washing away unbound biotinylated antibody. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of cytokeratin 18 bound. The stop solution alters the blue color to yellow one, then color's intensity measured at wavelength 450 nm.

Calculation of results

The mean absorbance was calculated for each set of duplicate standards, controls, and samples, and the average zero-standard optical density was subtracted. A standard curve was then plotted with standard concentrations on the x-axis and absorbance values on the y-axis, using log-log graph paper or SigmaPlot software. A best-fit straight line was drawn through the standard points.

Other laboratory test

Aminotransferases, alkaline phosphatase, albumin, bilirubin (total, direct, and indirect), total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, very-low-density lipoprotein (VLDL) cholesterol, triglycerides, viral markers, and random blood sugar were measured using the INTEGRA 400 PLUS analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Platelet count was analyzed using the Sysmex XT-2000i hematology analyzer.

NAFLD fibrosis score

The score was calculated by using the formula of -1.675 + 0.037 age (years) + 0.094 × BMI (kg/m^2) + 1.13 × diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio – 0.013 × platelet (× 10^9/L) – 0.66 × albumin (g/dL). Scores less than -1.455 indicated no advanced fibrosis, while scores greater than 0.676 indicated the existence of advanced fibrosis, and scores in the middle were categorized as indeterminate.

Statistical analysis

The statistical analyses were performed using SPSS software version 22.0. Continuous data presented in mean ± SD while categorical data presented in frequency and percentage. Fisher exact test used for categorical data, while T-student test used for comparison two continuous variables and One-Way ANOVA test used for more than two continuous variables. Pearson's correlation used for two continuous variables. ROC curve used to determine more specific and sensitive cutoff point. p-value less than or equal to 0.05 is considered statistically significant.

RESULTS

This study is a cross-sectional study, which include 60 individuals, 42 (70%) of them diagnosed with liver steatosis on ultrasound and 18 (30%) individuals without liver steatosis on ultrasound. The mean age of individuals whom involved in the study was 43.5 ± 12.2 years old, 32 (53.33%) of them was male, 8 (13.33%) was diabetic. According to body mass index 31 (51.67%) of included individuals were obese, 20 (33.33%) were overweight and 9 (15.00%) individuals had normal BMI.

In regard to laboratory finding, the mean Total serum bilirubin was 0.51± 0.31 mg/dl, mean direct bilirubin 0.14 ± 0.067 mg/dl, mean indirect bilirubin 0.38 ± 0.3 mg/dl, mean AST 32.4 ± 26.7 U/L, mean ALT 36.1± 25.2 U/L, mean AST/ALT ratio 0.91 ± 0.3, mean ALK 75.7 ± 26 IU/L, mean cholesterol 190.8 ± 47.4 mg/dl, mean triglyceride 120.5 ± 98.5 mg/dl, mean HDL 36.9 ± 5.9 mg/dl, mean VLDL 24.1 ± 19.7 mg/dl, mean LDL 129.75 ± 40.3 mg/dl, mean RBS 121.5 ± 52.4 mg/dl, mean albumin 4.22 ± 0.28 g/l, mean platelet count 0.291 ± 0.052 l and mean cytokeratin level 280.55 ± 290.4 pg/ml. There is no significant difference between mean of fatty liver grades and mean of (TSB, DB, IDB, AST, ALT, AST/ALT ratio, ALK, cholesterol, triglyceride, HDL, LDL, VLDL, albumin and platelets) as shown in Table 1.

There is significant difference between mean of age, RBS and fatty liver grades as shown in Table 2.

Table 3 shows the significant difference between mean of Ck-18 and fatty liver grades, but there is no significant difference between mean of NAFLD fibrosis score and fatty liver grades.

There is no significant association between cytokeratin-18, gender, BMI and diabetes, as shown in Table 4.

At specificity (100%) and sensitivity (100%), the cut-off point of cytokeratin 18 level is 102.03 pg/ml as shown in Table 5.

When the calculated NAFLD fibrosis score was correlated with cytokeratin 18 level, the result was significantly positive, in comparison between controls and

TABLE 1. *The difference between mean of fatty liver grades and mean of (TSB, DB, IDB, AST, ALT, AST/ALT ratio, ALK, cholesterol, triglyceride, HDL, LDL, VLDL, albumin and platelets)*

Dependent variable	Grade	N	Mean	Std. deviation	p-value
TSB	normal	18	0.6	0.37	0.466
	grade 1	17	0.4	0.22	
	grade 2	18	0.5	0.34	
	grade 3	7	0.4	0.15	
	Total	60	0.5	0.30	
DB	normal	18	0.1	0.08	0.434
	grade 1	17	0.1	0.06	
	grade 2	18	0.1	0.04	
	grade 3	7	0.1	0.07	
	Total	60	0.1	0.06	
IDB	normal	18	0.4	0.37	0.505
	grade 1	17	0.3	0.22	
	grade 2	18	0.4	0.34	
	grade 3	7	0.2	0.18	
	Total	60	0.4	0.31	
AST	normal	18	27.88	13.47	0.441
	grade 1	17	34.11	31.90	
	grade 2	18	29.88	18.37	
	grade 3	7	46.71	50.09	
	Total	60	32.45	26.72	
ALT	normal	18	29.83	12.35	0.354
	grade 1	17	36.94	32.55	
	grade 2	18	36.33	14.14	
	grade 3	7	50.14	45.50	
	Total	60	36.16	25.20	
AST/ALT ratio	normal	18	0.99	0.41	0.350
	grade 1	17	0.95	0.24	
	grade 2	18	0.82	0.23	
	grade 3	7	0.86	0.20	
	Total	60	0.91	0.30	
ALK	normal	18	65.38	18.43	0.130
	grade 1	17	78.29	16.24	
	grade 2	18	77.44	33.21	
	grade 3	7	91.42	35.29	
	Total	60	75.70	26.05	
Cholesterol	normal	18	173.50	43.27	0.225
	grade 1	17	194.52	58.31	
	grade 2	18	195.55	40.15	
	grade 3	7	214.28	39.72	
	Total	60	190.83	47.48	
Triglyceride	normal	18	94.944	48.4677	0.440
	grade 1	17	145.941	125.7694	
	grade 2	18	129.389	118.7370	
	grade 3	7	101.571	45.9233	
	Total	60	120.500	98.4661	
HDL	normal	18	34.556	6.8275	0.174
	grade 1	17	37.176	4.3191	
	grade 2	18	38.389	6.1657	
	grade 3	7	39.143	5.2418	
	Total	60	36.983	5.9332	
VLDL	normal	18	18.989	9.6935	0.440
	grade 1	17	29.188	25.1539	
	grade 2	18	25.878	23.7474	
	grade 3	7	20.314	9.1847	
	Total	60	24.100	19.6932	
LDL	normal	18	119.9556	37.61286	0.286
	grade 1	17	128.1647	50.20260	
	grade 2	18	131.2889	30.99292	
	grade 3	7	154.8286	39.00178	
	Total	60	129.7500	40.34727	
Albumin	normal	18	4.283	0.2728	0.652
	grade 1	17	4.182	0.2877	
	grade 2	18	4.194	0.2859	
	grade 3	7	4.286	0.3132	
	Total	60	4.228	0.2823	
Platelet	Normal	18	0.3061	0.04906	0.348
	grade 1	17	0.2862	0.06020	
	grade 2	18	0.2920	0.04849	
	grade 3	7	0.2653	0.04548	
	Total	60	0.2915	0.05214	

p-value ≤ 0.05 was considered statistically significant

TABLE 2. *The difference between mean of age, RBS and fatty liver grades*

Dependent variable		N	Mean	Std. deviation	p-value
Age	normal	18	39.3	10.7	0.002
	grade 1	17	41.2	12.1	
	grade 2	18	43.7	10.2	
	grade 3	7	59.1	10.3	
	Total	60	43.5	12.2	
RBS	normal	18	93.3	7.3	0.0001
	grade 1	17	112.7	24.8	
	grade 2	18	131.5	56.3	
	grade 3	7	189.7	88.6	
	Total	60	121.5	52.3	

p-value ≤ 0.05 was considered statistically significant

patients with grade 1 liver steatosis in respect to cytokeratin 18, NAFLD score and AST/ALT ratio, the result is represented in the following Table 6.

In comparison between patients with grade 1 and grade 2 liver steatosis in respect to cytokeratin-18, NAFLD score and AST/ALT ratio, the result is represented in the following Table 7.

In comparison between patients with grade 2 and grade 3 liver steatosis in respect to cytokeratin-18, NAFLD score and AST/ALT ratio, the result is represented in the following Table 8.

The exceptionally high AUC values (1.000) observed for cytokeratin-18 may be influenced by the limited sample sizes within subgroups, particularly grade 3 (n = 7), and potential overfitting. Therefore, these results should be interpreted with caution.

TABLE 3. *The difference between mean of cytokeratin-18, NAFLD fibrosis score and fatty liver grades*

Dependent variable	Fatty liver grade	N	Mean	Std. deviation	p-value
Cytokeratin-18	normal	18	66.7	16.4	0.0001
	grade 1	17	152.95	24.9	
	grade 2	18	349.6	108.7	
	grade 3	7	962.9	229.5	
	Total	60	280.6	290.4	
NAFLD fibrosis score	normal	18	0.7	0.94	0.088
	grade 1	17	1.2	0.99	
	grade 2	18	1.1	1.14	
	grade 3	7	1.9	0.81	
	Total	60	1.1	1.04	

p-value ≤ 0.05 was considered statistically significant

TABLE 4. *Association between cytokeratin-18, gender, BMI and diabetes*

Dependent variable		Cytokeratin 18		p-value
		Negative (<102.03)	Positive (≥102.03)	
Gender	female	6	22	0.26
		33.3%	52.4%	
	male	12	20	
		66.7%	47.6%	
	total	18	42	
		100.0%	100.0%	
BMI	Normal	5	4	0.164
	%	27.8%	9.5%	
	Overweight	6	14	
	%	33.3%	33.3%	
	Obese	7	24	
	%	38.9%	57.1%	
	total	18	42	
	%	100.0%	100.0%	
DM	non diabetic	18	34	0.091
	%	100.0%	81.0%	
	diabetic	0	8	
	%	0.0%	19.0%	
	total	18	42	
	%	100.0%	100.0%	

p-value ≤ 0.05 was considered statistically significant

TABLE 5. *The cutoff point of cytokeratin-18 level among different sensitivity and specificity*

Cutoff point of cytokeratin	Sensitivity	Specificity
102.03	100%	100%
106.09	97%	100%
112.32	95%	100%

TABLE 6. *Results of ROC curve for comparison between controls and grade 1 liver steatosis*

Test result variable (s)	Area	Std. error	Asymptotic sig.[b]	Asymptotic 95% Confidence Interval	
				Lower bound	Upper bound
Cytokeratin-18	1.000	0.0001	0.0001	1.000	1.000
NAFLD score	0.618	0.095	0.235	0.431	0.805
AST/ALT ratio	0.539	0.101	0.692	0.342	0.737

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) is the liver pandemic in this 21st century, affecting 20-45% population around the world [23]. Liver biopsy is the gold standard for liver steatosis and fibrosis assessment; however, because of its several limitation and invasiveness, it is rarely done [24]. Hepatic steatosis and fibrosis are now being assessed by using noninvasive tech-

TABLE 7. *Results of ROC curve of grade 1 and grade 2 liver steatosis patients*

Test result variable	Area	Std. error	Asymptotic sig.	Asymptotic 95% Confidence interval	
				Lower bound	Upper bound
Cytokeratin	1.000	0.0001	0.0001	1.000	1.000
NAFLD score	0.444	0.099	0.575	0.250	0.639
AST/ALT ratio	0.350	0.095	0.129	0.163	0.537

TABLE 8. *Results of ROC curve of grade 2 and grade 3 liver steatosis patients*

Test result variable	Area	Std. error	Asymptotic sig.	Asymptotic 95% Confidence interval	
				Lower bound	Upper bound
Cytokeratin	1.000	0.0001	0.0001	1.000	1.000
NAFLD score	0.714	0.109	0.102	0.500	0.928
AST/ALT ratio	0.556	0.120	0.672	0.320	0.792

niques like imaging and biomarkers. Abdominal ultrasonography represents a useful screening tool for NAFLD, mainly due to its short examination time and noninvasiveness. Hepatic steatosis on ultrasound demonstrated as increase echogenicity of the hepatic parenchyma, giving a brighter image when compared to renal cortex on the same side [25]. Cytokeratin 18 is a noninvasive biomarker that is currently under study to asses NASH and liver fibrosis [26]. In our study we found that the prevalence of liver steatosis increases with advanced age (mean 43.5 ± 12.2) years, and this finding is consistent with the study done by P. Golabi et al. [27]. This is may be explained by functional and anatomical changes in the liver (elderly lose nearly one third of their liver volume and perfusion) resulting in decrease regenerative capacity of the liver [28]. In addition to accumulation of the lipid within the liver and increase the oxidative stress which promote NAFLD progression [29]. S. Kosasih et al study demonstrates no association between age and liver steatosis [24].

The result of our study did not show significant differences between male (47.61%) and female (52.38%) patients, while the study done by Lonardo et al. [30] showed that the prevalence of NAFLD is higher in males than females in reproductive age and this is may be due to hormonal factors [30]. S. Kosasih et al study showed no correlation between liver steatosis and gender [24]. The result of this study indicates that the prevalence of liver steatosis increases with increased BMI. This result confirmed the association between obesity and NAFLD as studied by E. Fabbrini [31], this is may be due to insulin resistance and dyslipidemia [31]. The result of this study showed that the prevalence of liver steatosis is higher among patients with impaired glucose tolerance with mean (121.5 ± 52.3) mg/dl, (p-value 0.0001), and this finding matches those observed in the study done by E. Hatziagelaki et al. [32]. Also, there is a study done by H. J. Cho et al. [33] demonstrating that development of new NAFLD could increase the risk of diabetes mellitus development in the future, while resolution of NAFLD could decrease it [33]. There are several possible explanations for this result like metabolic derangement and insulin resistance which produce stress on beta cells of the pancreas that eventually fail to correspond the increase in insulin requirements [34].

The current study found that there is no significance difference between normal individuals and those with liver steatosis in respect to TSB, DB, IDB, and these findings did not match those observed in the prospective cohort study done by J. Tian et al. which demonstrated that levels of direct bilirubin were inversely related to NAFLD risk [35] this may be explained by the protective and antioxidant effect of bilirubin. The study done by L. Luo et al. showed that elevated plasma bilirubin level was not associated with a decreased risk of NAFLD [36]. The current study did not show significant difference in the level of albumin and platelets count among controls and patients with liver steatosis, this is consistent with the finding of S. Kosasih et al. study [24]. Regarding lipid profile, our study did not show significant difference among controls and patients with liver steatosis, this finding is inconsistent with D. U. Mahaling et al study that indicate S.TG, total cholesterol, LDL and VLDL were raised among NAFLD cases [37].

The current study did not show significant difference in AST, ALT and AST/ALT ratio among different grades of liver steatosis and controls, this finding is consistent with the study of S. Gawrieh et al which demonstrated normal serum level of aminotransferases enzyme among NAFLD patients, and it did not correlate with the severity of the disease [38]. Our finding was inconsistent with S. Kosasih et al. study that found significant differences in AST, ALT and AST/ALT ratio among patients and control [24]. Our study found that there is no significant correlation between NAFLD fibrosis score and liver steatosis on ultrasound (p-value 0.08) and this

is consistent with S. Kosasih et al. study finding [24]. One study done by E. Kaya et al. [39] showed that NFS had acceptable diagnostic performance in the exclusion of advance fibrosis in both individuals with normal and high aminotransferase.

In the current study, cytokeratin 18 showed a good correlation with the grades of hepatic steatosis as assessed by ultrasound (p-value 0.0001). Cytokeratin 18 level is not influenced by gender, BMI and diabetes. The cutoff point of cytokeratin level at which sensitivity and specificity equal to 100%, is 102.03 pg/ml, while at cytokeratin level equals to 106.09 pg/ml, sensitivity declines to 97%, and cytokeratin level equals to 112.32 pg/ml, sensitivity becomes 95%. These findings warrant validation in larger, independent cohorts to establish their generalizability. Furthermore, interpretation of the proposed cutoff should consider potential influences such as limited sample size, population-specific characteristics, and inter-assay variability. Until externally validated, this threshold should not be regarded as a definitive diagnostic criterion. When we correlated between cytokeratin 18 level and NFS, we found there is significant correlation with p-value 0.031. S. Kosasih et al. study also found good correlation with cutoff value equal to 194 unit/l with sensitivity and specificity (70%, 82.6%) respectively in grade 2 liver steatosis, and cutoff value equal to 345 unit/l with sensitivity and specificity (66.7%, 91.8%) respectively in grade 3 liver steatosis.

In comparison between control and patients with grade 1 liver steatosis regarding CK-18, there is significant difference between the two groups with AUC 1.000 while there is no significant difference in respect to NFS and AST/AST ratio with AUC 0.618, 0.539 respectively. In comparison between patients with grade 1 and grade 2 liver steatosis regarding CK-18 there is significant difference between the two groups with AUC 1.000 while there is no significant difference in respect to NFS, AST/AST ratio with AUC 0.444, 0.350 respectively. In comparison between patients with grade 2 and grade 3 liver steatosis regarding CK-18 there is significant difference between the two groups with AUC 1.000 while AUC for NFS and AST/ALT ratio are 0.714, 0.556 respectively. CK 18 plays an important role in NAFLD as a noninvasive biomarker which can distinguish NASH from simple steatosis (this required CK18 measurement in patients who had histological finding of NAFLD) [40].

However, it is important to interpret the perfect AUC values of 1.000 for cytokeratin 18 with caution, as such perfect discrimination is uncommon in clinical biomarker studies. These results may be influenced by the relatively small sample size, especially in advanced steatosis grades, and potential overfitting. Therefore, further validation in larger, independent cohorts is necessary to confirm the robustness and generalizability of cytokeratin 18 as a diagnostic biomarker for NAFLD severity.

This study provides valuable insight into the potential role of CK18 as a non-invasive biomarker for hepatic steatosis. One of the key strengths of this work is the use of objective imaging modalities and quantitative biochemical measures. In addition, the consistent association between CK18 levels and steatosis grades across subgroups strengthens the internal validity of the findings.

However, several limitations should be acknowledged. First, the relatively small sample size may limit the statistical power and generalizability of the results. Second, the absence of liver biopsy – the gold standard for diagnosing and staging NAFLD – means that histological confirmation of steatosis was not possible. Third, the study population may be subject to selection bias, particularly if participants were recruited from a single center or shared specific clinical characteristics not representative of the broader population. Finally, potential confounding variables such as medication use, dietary intake, or physical activity were not fully accounted for and may influence both CK18 levels and liver fat content.

To strengthen the clinical applicability of these findings, future research should aim to validate the proposed CK18 cutoff in larger, diverse, and independent cohorts. Incorporating liver histology where ethically feasible would further clarify the diagnostic accuracy of CK18. Additionally, longitudinal studies are warranted to assess whether CK18 levels can predict disease progression or response to therapy.

CONCLUSION

The study concluded that cytokeratin 18 was relatively well correlated with grades of hepatic steatosis as assessed by ultrasound. The study concluded that NAFLD fibrosis score was poorly correlated with grades of hepatic steatosis as assessed by ultrasound. The study concluded that AST/ALT ratio did not differ significantly among grades of liver steatosis. The study concluded that cytokeratin 18 cutoff value, at which the sensitivity is 100% and the specificity is 100% was 102.03 pg/ml.

Conflict of interest

Author's declare no conflict of interest.

Author's contributions

Conceptualization, D.S.S. and N.S.H.; methodology, D.S.S.; software, N.S.H.; validation, N.S.H.; formal analysis, N.S.H.; investigation, N.S.H.; resources, D.S.S.; data curation, D.S.S.; writing—original draft preparation, N.S.H.; writing—review and editing, D.S.S.; visualization, D.S.S.; supervision, N.S.H.; project administration, D.S.S.; funding acquisition, N.S.H. All authors have read and agreed to the published version of the manuscript.

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