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SERUM CYTOKERATIN-18 AS A NON-INVASIVE BIOMARKER IN NON-ALCOHOLIC FATTY LIVER DISEASE DIAGNOSIS

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ABSTRACT

Background: Non-alcoholic fatty liver disease (NAFLD) is a growing global health concern, encompassing a spectrum from simple steatosis to non-alcoholic steatohepatitis (NASH), which may progress to fibrosis, cirrhosis, and hepatocellular carcinoma. Accurate, non-invasive tools are essential for distinguishing NASH from simple steatosis, as early identification significantly impacts clinical outcomes.

Methods: The study included 60 diagnosed cases of NAFLD, including 31 patients with Fatty liver and 29 patients with NASH. Blood samples were collected at admission, and Serum liver function tests were measured. Serum cytokeratin-18 levels were measured using a sandwich enzyme-linked immunosorbent assay (ELISA).

Result: Serum CK-18 levels were significantly higher in the NASH group (mean = 204.85 ± 32.98 U/L) compared to the fatty liver group (mean = 173.97 ± 23.75 U/L), with a statistically significant difference ($P < 0.001$). Receiver operating characteristic (ROC) curve analysis demonstrated that serum CK-18 is a fair discriminatory marker for distinguishing NASH from simple fatty liver, with an area under the curve (AUC) of 0.775 ($P < 0.0001$). The optimal cutoff value identified was 198 U/L, yielding a sensitivity of 65.52% and a specificity of 83.87%.

Conclusion: Serum CK-18 levels can discriminate NASH from fatty liver in NAFLD, its role as a supporting biomarker for disease severity. These findings support the diagnostic utility of CK-18 in clinical assessment of NAFLD, particularly in distinguishing NASH from simple steatosis.

Keywords: Non-alcoholic fatty liver disease, Non-alcoholic steatohepatitis, Cytokeratin-18, receiver operating curve, and area under the curve.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has emerged as the most common chronic liver disorder globally, affecting approximately 25–30% of the population, with increasing prevalence due to the global rise in obesity, type 2 diabetes, and metabolic syndrome.[1] NAFLD encompasses a spectrum of hepatic conditions ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), the

latter being a more aggressive phenotype associated with hepatocellular injury, inflammation, and a higher risk of progression to fibrosis, cirrhosis, and hepatocellular carcinoma.[2]
The differentiation between simple steatosis and NASH is clinically important, as NASH patients have significantly higher risks of liver-related morbidity and mortality.[3] Currently, liver biopsy remains the gold standard for diagnosing and staging NASH. However, its invasive nature, cost, procedural risks, and potential sampling variability make it impractical for widespread clinical use and longitudinal monitoring.[4] Consequently, there is a growing need for accurate, non-invasive biomarkers that can reliably identify NASH and assess disease activity.
Cytokeratin-18 (CK-18), a major intermediate filament protein in hepatocytes, has emerged as a promising non-invasive biomarker. During apoptosis, a key feature in NASH pathogenesis, CK-18 is cleaved by caspases and released into the bloodstream as measurable fragments.[5] Several studies have shown that elevated serum CK-18 levels correlate with histological features of NASH and may serve as a useful marker to distinguish NASH from simple fatty liver disease.[7,8]
This paper aims to evaluate the diagnostic significance of serum CK-18 in NAFLD, focusing on its potential role in identifying NASH and providing a non-invasive alternative to liver biopsy in clinical practice.

MATERIALS AND METHODS: This was a Descriptive, cross-sectional observational study conducted on a total of 60 patients. Patients aged 18 to 60 years patients included according to inclusion and exclusion criteria met in the Gastroenterology department, SMS super specialty hospital, Jaipur, of confirmed NAFLD were included. The diagnosis was made by gastroenterologists using liver USG, ALT, AST, fibro scan, and liver biopsy.
Exclusion criteria included patients with age below 18 and above 60 years, History of alcohol intake >30 gm/day in males and >20gm/day in females, Person refuses to give an informed consent, person has experienced dieting or bariatric surgery and massive rapid weight loss, diabetic and any malignancy and patients with Chronic liver disease due to other causes like viral, autoimmune, metabolic, biliary or drug induced.

Blood Samples
Venous Blood samples(5ml) were collected aseptically into metal-free, anticoagulant-free tubes. After allowing the blood to clot for 20 minutes at room temperature, the samples were centrifuged for 15 minutes. The resulting serum was used for routine biochemical parameters were analyzed using the Beckman Coulter AU 5100 system. Cytokeratin-18 was analyzed in a designated lab using the Elabscience Cytokeratin-18 kit.

STATISTICS AND DATA ENTRY
Quantitative data were summarized as mean and SD, whereas qualitative data were presented as proportion (%). An independent sample t-test was used for comparison of quantitative variables, and a chi-square test and Fisher's exact test were used for analysis of qualitative variables. ROC analysis was done to find out the optimum cut-off value of Cytokeratin-18. P value <0.05 was taken as significant. SPSS 26.0 version software was used for all statistical calculations.

RESULTS:
The present study was conducted on 60 patients with NAFLD and classified according to their clinical diagnosis. Out of these 31 (51.67%) were diagnosed with fatty liver, and 29 (48.33%) with NASH. Demographic, clinical, and Laboratory Features of the patients are presented in the Table 1.

Table 1. Baseline Clinical, Biochemical characteristics of the study population across NAFLD

	Fatty liver (n=31)	NASH (n=29)		P
Clinical data				

Age, years (Mean)	42.74 ± 11.62	49.14 ± 9.67		0.025
Body mass index, kg/m^2 (Mean)	26.38 ± 2.05	27.10 ± 2.19		0.192
Hypertension n (%)	7 (46.67%)	8 (53.33%)		0.769
Smoking n (%)	5 (33.33%)	10 (66.67%)		0.139
Biochemical data				
Liver function test				
SGOT (U/L)	35.44 ± 9.32	63.93 ± 36.20		<0.001
SGPT (U/L)	49.56 ± 20.77	103.90 ± 54.57		<0.001
T. Bil (mg/dl)	0.91 ± 1.04	1.39 ± 0.70		0.042
ALP (IU/L)	83.00 ± 43.59	124.03 ± 91.36		0.029
TP (gm/dl	6.97+-0.96	6.73+-0.76		0.306
Alb (gm/dl)	4.12 ± 0.53	3.78 ± 0.65		0.034
Glob (gm/dl)	2.85+-0.65	2.95+-0.60		0.509
CK-18(U/L)	173.97 ± 23.75	204.85 ± 32.98		<0.001

Results

The mean age of patients with Fatty Liver was (42.74 ± 11.62 years) and NASH (49.14 ± 9.67 years), which was significantly higher than fatty liver (p= 0.025).

The mean BMI was higher in the NASH group (27.10 ± 2.19 kg/m²) compared to the Fatty Liver group (26.38 ± 2.05 kg/m²), but the difference was not statistically significant. (P= 0.192).

Among hypertensives 46.67% were diagnosed with Fatty Liver and 53.33% with NASH; the difference was not statistically significant (p = 0.769).

Among smokers, 33.33% with Fatty Liver and 66.67% with NASH. The association between smoking and disease type did not reach statistical significance (p = 0.139).

SGOT and SGPT levels were significantly elevated in NASH patients (SGOT: 63.93 ± 36.20 U/L; SGPT: 103.90 ± 54.57 U/L) compared to those with Fatty Liver (SGOT: 35.44 ± 9.32 U/L; SGPT: 49.56 ± 20.77 U/L), with both significant differences (p < 0.001).

T.BIL was also significantly higher in the NASH group (1.39 ± 0.70 mg/dL) compared to the Fatty Liver group (0.91 ± 1.04 mg/dL. (p = 0.042).

Alkaline Phosphatase levels were elevated in NASH patients (124.03 ± 91.36 U/L) relative to Fatty Liver patients (83.00 ± 43.59 U/L), showing statistical significance (p = 0.029).

Albumin levels were significantly lower in NASH patients (3.78 ± 0.65 g/dL) compared to the Fatty Liver group (4.12 ± 0.53 g/dL. (p = 0.034).

Total Protein and Globulin showed no statistically significant differences between the groups (p = 0.306, 0.509).

Cytokeratin-18 levels were elevated in the NASH group (mean = 204.85 ± 32.98 U/L) compared to the fatty liver group (mean = 173.97 ± 23.75 U/L), also showing a statistically significant difference (P < 0.001).

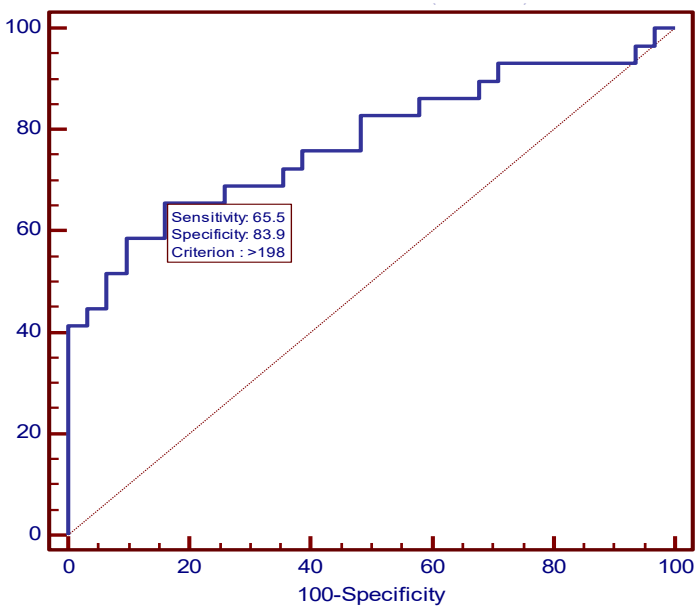

Figure 2 : ROC curve analysis of serum Cytokeratin-18 level to differentiate NASH and fatty liver

ROC curve analysis of serum cytokeratin-18 level reveals it as a fair discriminatory marker of NASH with fatty liver, with an AUC of 0.775 and a P value <0.0001 optimum cut-off level obtained was <198U/L, with the highest Youden index, with sensitivity of 65.52% and specificity of 83.87%. Cytokeratin-18 (CK-18) is an intermediate filament protein expressed in hepatocytes and plays a significant role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), particularly in the transition to non-alcoholic steatohepatitis (NASH). Therefore, serum CK-18 levels not only reflect hepatocellular injury but also provide insight into disease activity and progression in NAFLD.[9]

In this study, the mean Cytokeratin-18 levels were significantly higher in the NASH group (mean = 204.85 ± 32.98 U/L) compared to the fatty liver group (mean = 173.97 ± 23.75 U/L), showing a statistically significant difference (P < 0.001). However, among patients with Fatty Liver, 77.42% had high CK-18 levels, and among those with NASH, 93.10% had high CK-18 levels, showing a higher proportion of NASH patients exhibited elevated CK-18 levels compared to those with Fatty Liver, but we did not find any significant difference between the both groups.(p>0.05). The ROC curve analysis was done for finding the efficiency of CK-18 in discriminating NASH from Fatty liver, and it was found that CK-18 was found to be a fair and acceptable marker in discriminating NASH patients from Fatty liver patients with an AUC 0.775 and a model having a highly significant P value of <0.0001. The optimum cut-off level at the highest Youden index obtained was 198U/L with a sensitivity of 65.52% and specificity of 83.87%.
Albeltazi et al.[10] performed a retrospective case-control study on NAFLD patients (steatosis and NASH case group) and compared with the control group and found the mean level of serum CK18 in NASH cases (247.7±66.3 U/L) was significantly higher than in steatosis (168.7±51.1 U/L) and control (94.9±43.1 U/LL) groups(P < 0.001). ROC curve of CK-18 for predicting NAFLD showed AUC 0.921 with sensitivity 77.1%, specifically 96.6% and optimal cut-off value for the CK18 test as 161 U/L. So they found CK-18 as an excellent marker of the severity of NAFLD with good AUC, but in our study, we got the AUC of 0.775, which can be taken as a fair or acceptable value for its discriminating ability, and the significance level was <0.0001. Though we found a bit higher cut-off value of 198U/L compared to 161 U/L, showing the test could not diagnose the NASH cases at a level less than 198U/L, which can be explained by its low sensitivity of 65.52%.
Pagano et al.[11] in a similar study on CK18 (including M65 and M30 forms) and found that both CK18 M30 and M65 levels were significantly higher in subjects with FLI ≥ 60 (NAFLD) than in subjects with FLI < 60. Moreover, the FLI ≥ 60 group had significantly higher values of FRS

(Framingham risk score) and SCORE2 compared to the FLI < 60 group. Statistics analyses showed that both M30 and M65 as continuous variables had a significant discriminant accuracy in predicting an FLI ≥ 60, with AUCs of 0.702 ($p < 0.0001$) and 0.657 ($p < 0.0001$), respectively. Adjusted logistic regression analyses indicated that only M30 was an independent predictor of FLI ≥ 60. Values above the predefined cut-off of 200 U/L were associated with an independent 3-fold increased risk of NAFLD (FLI ≥ 60). At this cutoff, the sensitivity (SE), specificity (SP), and positive and negative predictive values (PPV, NPV) were 57.1%, 72.5%, 54.0%, and 75.0%, respectively. So they found that both components of CK-18 were able to predict the severity of NAFLD with fair AUC, and the AUC nearly matches with our study (0.775), showing its utility.

STUDY LIMITATION:

Despite demonstrating the diagnostic significance of serum Cytokeratin-18 (CK-18) levels in distinguishing NASH from simple fatty liver disease, this study has several limitations. The sample size was relatively small, which may limit the generalization of the findings to the broader NAFLD population. CK-18 showed moderate sensitivity and good specificity, it should be interpreted cautiously when used alone, as it may not fully capture the complexity of NAFLD pathogenesis. Lastly, confounding factors such as coexisting metabolic disorders, medication use, and genetic variations were not fully controlled, which may have influenced CK-18 levels.

CONCLUSION: This study demonstrates that serum Cytokeratin-18 levels were significantly elevated in patients with non-alcoholic steatohepatitis (NASH) compared to those with simple fatty liver, indicating its potential as a non-invasive biomarker for diagnosing NASH. CK-18 may serve as a useful adjunct in the non-invasive assessment of NAFLD severity. However, due to moderate sensitivity and limitations such as small sample size, CK-18 should be interpreted alongside other clinical and biochemical parameters. Further large-scale, multi-center studies are warranted to validate its utility in routine clinical practice.

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