Original Article

Value of Cytokeratin-18 as a non-invasive diagnostic biomarker of non-alcoholic steatohepatitis (NASH)

Alaa Habib, Mahmoud Awad, Dalia Shaheen, Somaia Shehab- Eldeen, Sheren Younes, Esmat Mohammed

Summary

***Background and study aim**: Liver biopsy couldn't be utilized for screening of nonalcoholic steatohepatitis (NASH), as it is considered invasive, and has many complications. The study aims to investigate the value of serum levels of CK -18 fragments as a diagnostic biomarker for NASH and NAFLD and its correlation with severity of NASH as measured by NAFLD activity score (NAS) of liver biopsies. **Patients and methods**: A total number of 46 subjects with biopsy-proven non-alcoholic steatohepatitis (NASH group) and 54 subjects with borderline NASH, simple steatosis and normal liver tissue (non-NASH group) as well as 30 age-matched healthy volunteers were included in the study. Scoring of liver biopsies using the NAFLD activity score (NAS) and measurement of CK-18 in sera was done. **Results**: The serum level of cytokeratin-18 was significantly higher in the NASH group when compared to non-NASH group (P=0.0123) or controls (P=0.00001). Using the ROC curve, the optimal value of cytokeratin-18 was 487U/L, with sensitivity 69 % and specificity 84.5 % in detecting NASH. Serum CK-18 levels were significantly correlated to the disease severity as measured by liver biopsy (degree of steatosis, fibrosis, lobular inflammation, and ballooning) in NASH patients. **Conclusions**: Serum CK-18 could be used as a non-invasive diagnostic serum marker for patients of NAFLD and NASH.*

Keywords: *Cytokeratin-18, NASH, NAFLD*

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is a common and prevalent chronic liver disease worldwide with prevalence ranges from 15% to 30%[1,2]. This prevalence appears to be increasing daily[3]. There is a solid relationship between NAFLD and metabolic syndrome (obesity, insulin resistance, Type 2 diabetes mellitus, hyperlipidemia, and hypertension)[4,5]. Two continuous yet distinct categories of NAFLD exist, which are nonalcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), but in contrast, NASH typically has a progressive nature. Almost 25% of affected patients develop liver cirrhosis which is further complicated with portal hypertension and even hepatocellular carcinoma[6]. The diagnosis of NAFLD depends on the elevation of liver enzymes, the diagnostic ultrasound and pathologic findings of steatosis[1]. Liver biopsy is considered an effective definitive diagnostic tool for NASH, that assists making the diagnosis and provides predictable factors for disease progression, yet it is still of limited use as a screening tool being invasive, and carries the risk of hemorrhage[7]. Non-invasive biomarker tests for fibrosis or steatosis have been developed. It is calculated using the results of other laboratory tests and it is correlated to the degree of fibrosis like Fibro Tests (5), or steatosis like Steato Test (ST)[8]. The balance between cell proliferation and apoptosis on the other side is believed to keep and maintain tissue homeostasis. In liver injury conditions as in NAFLD, the progression of the disease is thought to arise from hepatocyte apoptosis[9]. It is known that the deregulation of tissue homeostasis is likely a cause of several liver diseases. Liver diseases like viral hepatitis, alcoholic hepatitis, NAFLD, liver cirrhosis, cholestatic liver disease, and hepatocellular carcinoma are believed to be related to apoptosis in their development and progression[10]. Both intrinsic and extrinsic pathways of apoptosis are believed to be involved in NASH pathogenesis[11]. Cytokeratin-18 (CK-18) is an intracellular intermediate filament protein. It is abundantly expressed in the liver. It is cleaved in apoptosis and has been implicated in various liver diseases[12]. Many clinical cohorts documented that, in obese patients or patients with insulin resistance, high serum levels of CK-18 fragments were associated with hepatocyte inflammation, necrosis, and fibrosis. Taking the above facts in consideration, CK- 18 could be considered as a potential non-invasive indicator to predict the hepatic histological manifestation of NASH[1,13-16]. The current study aims to address the diagnostic role of serum levels of CK-18 fragments in NASH and address its potential value for differentiating NAFL from NASH and the relation between its serum levels and pathologic criteria of NASH.

Subjects & Methods

This study is a cross-sectional uni-central observational study that was done on a total number of 100 patients, spanning the period between March to December 2017. Cases with a suspected diagnosis of NASH were selected from outpatient clinics of internal medicine department of Riyadh Care Hospital, KSA. Thirty age and sex-matched unaffected individuals were used as a control group.

Inclusion criteria

They included Patients above 18 years, diagnosed with NASH and NAFLD for whom the diagnosis was confirmed by laboratory tests, ultrasonography, and liver biopsy. Patients from both genders were eligible for the study, and written informed consent was signed by each case. The protocol of the study was reviewed and accepted by the local ethics committee of the hospital. It follows the provisions of the World Medical Association (Declaration of Helsinki) for experiments in humans.

Exclusion criteria

They included patients with any liver disease due to any other cause like autoimmune hepatitis, Wilson's disease, chronic viral hepatitis, hemochromatosis, α1-antitrypsin deficiency, primary sclerosing cholangitis and primary biliary cirrhosis, toxins, and medication-induced liver steatosis or hepatotoxicity, and hepatocellular carcinoma. Also, patients with alcoholic fatty liver disease as defined by consumption of alcohol ≥40 g/day for males or ≥20 g/day for females in the past five years or excess alcohol consumption of ≥140 g/week for males or ≥70 g/week for females were excluded as well. None of the patients was pregnant or having evidence of thyroid dysfunctions, type I diabetes or biliary obstruction. All enrolled cases were subjected to detailed history taking, physical examination and investigations including abdominal ultra-sonography, laboratory investigations, and ultr-asonography-guided liver biopsy (except for the control group). Abdominal ultrasonography was done to all individuals by the same operator and steatosis was being graded into 0: absent, 1: mild, 2: Moderate and 3: severe. Liver biopsies were performed by an expert interventional radiologist that sent the samples to an expert pathologist for assessment of the histopathological changes of NASH and excluding other chronic liver diseases in all individuals except the control group. According to NIDDK (National Institutes of Diabetes, Digestive, and Kidney Disease) NASH Clinical Research Network Scoring System[17], our patients with suspected NAFLD can be classified according to their liver histology into four groups: definitive NASH, borderline NASH, simple fatty liver and normal liver tissue. In a secondary analysis, all individuals were subdivided into 3 groups: **Group A:** includes patients with definitive NASH or NASH group. **Group B:** includes patients with borderline NASH, simple fatty liver or normal liver tissue. Collectively named non-NASH group. **Group C:** This included individuals of matched age and sex, of no history of abnormal liver biochemical tests or ultrasonography and currently with normal liver biochemical tests and ultrasonography as a control group.

Clinical data

Collected clinical data included age, gender, height, weight, body mass index (BMI), smoking (as never smoked = 0, ex- smoker = 1, recent smoker = 2).

Laboratory assessment

Blood samples were drawn from patients then stored at -80 °C. This was done at the same time with the liver biopsy. The laboratory assessment for all patients included: **1-** Liver function tests including serum aspartate transferase (AST), serum alanine transferase (ALT)[18], alkaline phosphatase (ALP)[19], gamma-glutamyl transferase (GGT)[20], and bilirubin[21], and serum albumin. **2-** Viral B & C hepatitis screen via HBs Ag & Anti- HCV antibody was assayed by ELISA. **3-** Renal function tests including creatinine and uric acid (UA) were assessed using Reflotron plus. **4-** Measurements of total, HDL cholesterol, and triglycerides[22]. **5-** Quantitative measurement of C-reactive protein (CRP)[23]. **6-** International normalized ratio (INR), platelets, white blood cell (WBC) and hemoglobin were assessed. **7-** Anti-nuclear (ANA), anti-smooth muscle (ASMA) and anti-liver/kidney microsomal (LKMA) antibodies were assayed by ELISA. **8-** Quantitative measurement of ferritin was done using an immunoturbidimetric. assay (Kamiya Biomedical Company, Seattle, WA). **9-** Alpha-1 antitrypsin concentrations were determined using immunoturbidimetric assay kits (DiaSorinInc, Stillwater, MN). **10-** Ceruloplasmin was detected colorimetrically. **11-** Diagnosis of diabetes mellitus (DM) was depending on a history of hypoglycemic medications and/or fasting glucose (FG) of 126 mg dL-1 or more and diagnosis of hypertension was based on a history of blood pressure medications or twice/ day BP at ≥140/90 mmHg. Dyslipidemia was assessed by medications history and/or fasting total cholesterol >200 mg dL-1 and/or fasting TG >150 mg dL-1.

Cytokeratin-18 (CK-18) Fragments (M30 ANTIGEN)

Sera for CK18 fragments of the patients and the healthy control group were assessed in duplicate for CK18–Asp196 by means of the M30-Apoptosense ELISA (PEVIVA AB, Bromma, Sweden), (M30 Apoptosense® ELISA (PEVIVA®).

Histopathology

Liver biopsies of the patients were obtained under moderate sedation by a 16- gauge Klatskin needle. A 2.5 cm is the minimal length of the specimen. Fixation of the biopsies was done by 10% neutral buffered formalin solution. Then the sections were concurrently stained with Hematoxylin-Eosin and Masson's trichrome. Histopathological examination was done by an experienced pathologist and scoring of the liver biopsies was done according to the NAFLD activity score (NAS) in which: steatosis score from 0 to 3, lobular inflamemation score from 0 to 3, ballooning score from 0 to 2 and fibrosis was scored from 0 to 4, fig_s (1 & 2)[17]. The histological NASH was defined as the sum of all these scores (steatosis, lobular inflammation, cell ballooning, and fibrosis). Patients with nonalc-oholic fatty liver (NAFLD) have NAS score of <3, patients with borderline nonalcoholic steatohepatitis (NASH) have a score of 3-4, and patients with NASH have a score of ≥5 corresponds [17].

Statistical Analysis

It was done using the SPSS (Statistical Package for the Social Sciences) software version 20.0 for Windows (SPSS, Inc., Chicago, IL). Data were expressed as median (25^{th} & 75^{th} centiles for non-normally distributed data) and as mean ± SD for normally distributed variables. The χ^2 test used to compare proportions and categorical variables. Normality of distribution of continuous variables was tested using a Kolmogorov-Smirnov test. Mann-Whitney test and Kruskal-Wallis tests were used in group comparisons for non-normally distributed variables. Analysis of variance (ANOVA) and unpaired Student's *t*-test was used for normally distributed variables.

Results

The clinical and laboratory parameters of the pati-ents (NASH and non-NASH) and the control group are displayed in tab. (1). Our current study included 54 patients with non-NASH with mean age 46.7±13.6 years, 31 were males and 23 were females, with BMI of 36.23±5.9 kg/m^2. The NASH group included 46 patients, 27 were males and 19 were females, with mean age 57.9±15.3 years with BMI of 39.76±6.9 kg/m^2. The control group included 30 volunteer individuals, 19 males and 11 females, with mean age 55.65±9.52 years and BMI was 55.65±9.52 kg/m^2. Of the 54 non-NASH patients, 14 (25.6%) patients had type 2 diabetes mellitus, 13 (24%) patients had hypertension and 34 (62.9%) patients had dyslipidemia. Of the 46 NASH patients, 13 (28.3%) patients had type 2 diabetes, 12 (26.1%) had hypertension and 30 (65.2%) patients had dyslipidemia. Of the control group, 3 patients (10%) had diabetes, 7 pati-ents (23.3. %) had hypertension and 10 patients (33.3%) had dyslipidemia. There were significant statistical differences in the prevalence of diabetes, dyslipidemia, and obesity among non-NASH and NASH groups compared to the control group. There were significant statistical differ-ences in AST, ALT, GGT, FBG, TC, TG, HDL, BMI and of HOMA-IR of both NASH and non-NASH groups compared to the control group. AST, Gamma-GT, HOMA- IR, ferritin and hc-CRP showed statistically significant differences between NASH and non-NASH groups. Ferritin and HDL showed statistically significant differences between NASH and control groups.

Cytokeratin-18 (Ck-18) In studied groups

Table (2) shows serum CK-18 levels in the patients (NASH and non-NASH) and the control group. The mean CK-18 of the NASH group was 754±467.4 U/L, the non-NASH group was 443±267.7 U/L, and the control group was 154±67.3 U/L. Serum CK-18 was statistically significant higher in non-NASH and NASH groups in relation to the control group (P values were 0.0001 and 0.00001 respectively). Moreover, it was statistically significant higher in the NASH group in relation to the non-NASH group (P value was 0.0123).

Receiver Operating Characteristic (Roc) Curve

Figure (3) showed ROC curve showing the diagnostic sensitivity and specificity of CK-18 in diagnosing NASH. ROC curve was performed to have a cut-off value of CK-18 and to detect the predictive discriminative value of CK-18 in NASH diagnosis. At the optimal value of 487U/L, the area under the curve (AUC) of CK-18 was 0.81 with sensitivity 69 % and specificity of 84.5 % in detecting NASH. The positive predictive value (PPV) and negative predictive value (NPV) at the cut-off of 487U/L for CK-18 for the diagnosis of NASH were 83% and 71% respectively. The relationships between CK-18 and clinical and laboratory and histopathologic parameters of the NASH patients: Table (3) showed the correlations of CK-18 to the clinical and laboratory parameters of the NASH patients. Multiple regression analyses were done and resulted in significant positive associations between serum CK-18 and BMI (r= 0.71, p <0.001), FBG (r= 0.312, p= 0.017), HOMA-IR (r= 0.179, p= 0.021), hc-CRP (r= 0.64, p <0.001), degree of steatosis (r= 0.421, p <0.001), degree of fibrosis (r= 0.74, p <0.001), lobular inflammation (r= 0.56, p <0.001) and ballooning (r= 0.39, p <0.001) in our NASH patients.

Table (1) Clinical and laboratory parameters of the patients (NASH and non-NASH) and the control group

Factor		Non-NASH (no=54)	NASH (no=46)	Control (no=30)	P1 Non-NASH Vs Control	P2 NASH Vs Control	P3 NASH Vs Non NASH
Age (years)		46.7±13.6	57.9±15.3	55.65±9.52	0.0312	0.8586	0.0765
Gender (M/F)		31/23	27/19	19/11			
BMI (kg/m^2)		36.23±5.9	39.76±6.9	30.23±6.7	0.045	0.035	0.79
Smoking habits	0	19 (35.2%)	20 (43.5%)	17 (56.6%)			
	1	11 (20.4%)	13 (28.3%)	6 (20%)			
	2	24 (44.4%)	13 (28.3%)	7 (23.4%)			
SBP (mmHg)		133±29.7	128±23.9	135±19.6	0.675	0.876	0.566
DBP (mmHg)		85±18.7	88±19.8	83±20.4	0.886	0.976	0.676
AST (U/L)		39.66±35.34	65.65±49.3	22.23±6.65	˂0.001	<0. 001	0.0456
ALT (U/L)		65.54±56.34	77.56±54.67	19.54±5.74	<0.001	<0.001	0.564
Total bilirubin (mg/dl)		0.79±0.29	0.81±0.18	0.75±0.18	0.876	0.785	0.675
ALK (IU/L)		79.44±12.8	86.32±17.4	69.9±13.9	0.765	0.675	0.735
γ-GT (IU/L)		59.56±43.44	89.45±99.34	23.6±17.5	<0.001	˂0.001	0.0345
ALB (g/dl)		3.8±0.2	4.1±0.5	4.3±0.8	0.553	0.481	0.345
WBC (10^9/L)		5.8±2.3	6.3±3.3	6.±2.4	0.345	0.654	0.436
HGB (gm/dl)		147.7±13.4	149.7±12.4	150.7±13.7	0.356	0.445	0.345
Platelets (10^9/L)		210.7±41.4	226.5±46.7	216.4±53.3	0.546	0.237	0.498
Creatinine(mg/dl)		77.4±16.5	72.5±14.5	71.4±12.9	0.342	0.765	0.654
UA (mg/dl)		5.3±1.7	5.0±1.4	5.5±1.3	0.087	0.098	0.076
INR		1.02±0.2	1.12±0.3	1.0±0.1	0.554	0.543	0.643
hs-CRP		1.9 (1.4 , 2.6)	2.1 (1.7 , 2.7)	2.0 (1.6 , 2.5)	0.156	0.064	0.04
Ferritin (ng/ml)		113.76±44.6	165±44.7	119±33.4	0.876	0.0342	0.0234
Iron (µgm/dl)		109±67	118±75	111±65	0.435	0.543	0.654
FBG (mg/dl)		112.6±18.5	116±23.7	78.4±9.2	0.023	0.0123	0.435

TC (mg/dl)	197±35.9	207±39.9	157±15.9	0.0345	0.0234	0.654
TG (mg/dl)	157±75.9	167±85.6	127±35.9	0.0453	0.0332	0.564
HDL (mg/dl)	47.6±18.5	53.6±23.5	69.6±18.5	0.0132	0.0386	0.931
HOMA-IR	3.1±0.87	4.3±0.37	1.5±0.07	0.003	0.0001	0.0321
DM	14 (25.6%)	13 (28.3%)	3 (10%)	0.026	0.014	0.651
HTN	13 (24%)	12(26.1%)	7(23.3%)	0.764	0.546	0.654
DLP	34 (62.9%)	30 (65.2%)	10 (33.3%)	0.002	0.001	0.231

***BMI**: body mass index, **SBP**: systolic blood pressure, **DBP**: diastolic blood pressure, **AST**: aspartate transferase, **ALT**: alanine transferase, **ALK**: alkaline phosphatase, **Gamma-GT**: gamma glutamyl transpeptidase, **ALB**: albumin, **WBC**: white blood cells, **HGB**: haemoglobin, **UA**: uric acid, **INR**: international normalization ratio, **hs-CRP**: high-sensitivity C-reactive protein, **FBG**: fasting blood glucose, **TC**: total cholesterol, **TG**: triglycerides, **HDL**: high density lipoprotein, **HOMA-IR**: Homeostatic Model Assessment of Insulin Resistance, **DM**: diabetes mellitus, **HTN**: hypertension, **DLP**: dyslipidemia.*

Table (2) Serum CK-18 levels in patients (NASH and non-NASH) and the control group

Factor	Non-NASH (no=54)	NASH (no=46)	CONTROL (no=30)	P1 Non- NASH VS CONTROL	P2 NASH VS CONTROL	P3 Non- NASH VS NASH
CK-18 (U/L)	**443±267.7**	**754±467.4**	**154±67.3**	**0.0001**	**0.00001**	**0.0123**

***CK-18:** cytokeratin 18*

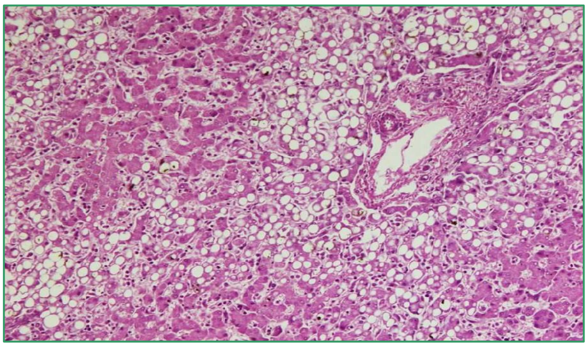

Figure (2) Liver biopsy from NAFLD patient shows extensive periportal steatosis (Original magnification X200)

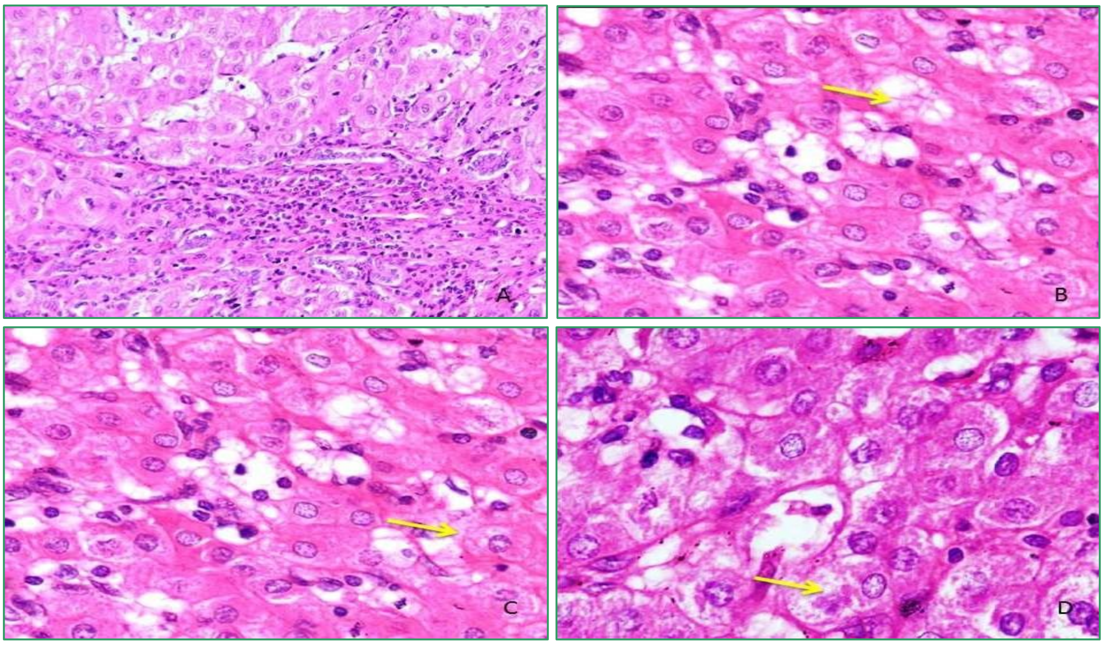

Figure (3) Different pathologic features of NAFLD; (A): portal inflammation, (B): focalmicrovesicular steatosis, (C): Ballooned hepatocytes, (D): Ballooned hepatocytes with intracytoplasmic inclusions "Mallory Denk Bodies"

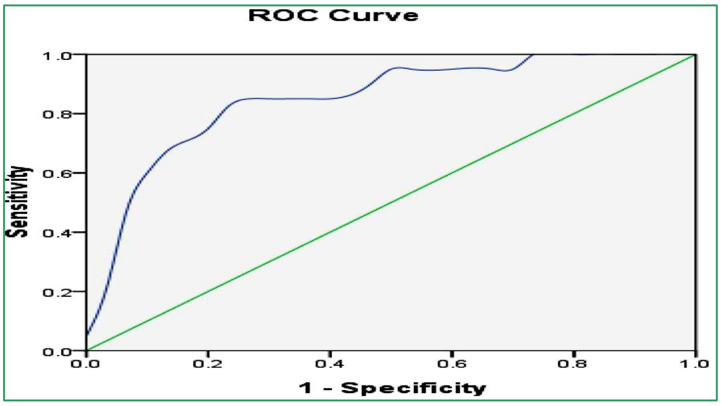

Figure (3) ROC curve showing the diagnostic sensitivity and specificity of CK-18 in diagnosing NASH

Table (3) The correlations between CK-18 and clinical and laboratory and histopathologic parameters of the NASH patients

The parameter	R	P
Age (years)	0.051	NS
BMI (kg/m2)	0.71	< 0.001
FBG (mg/dl)	0.312	0.017
MOMA-IR	0.179	0.021
AST (U/L)	0.16	NS
ALT (U/L)	0.14	NS
Gamma GT (U/L)	0.18	NS
Total cholesterol (mg/dl)	0.028	NS
Triglycerides (mg/dl)	0.083	NS
HDL (mg/dl)	0.021	NS
hc-CRP	0.64	<0.001
Ferritin (ng/ml)	0.042	NS
Steatosis	0.421	< 0.001
Fibrosis	0.74	< 0.001
Lobular inflammation	0.56	< 0.001
Ballooning	0.39	<0.001

Discussion

Non-alcoholic fatty liver disease (NAFLD) and its variant, non-alcoholic steatohepatitis (NASH) are steatotic liver diseases in the absence of significant alcohol intake. They are considered a hepatic sign of the metabolic syndrome, characterized by a spectrum of morphologic changes starting by macro vesicular steatosis, progressing to portal inflammation, ending up in liver cirrhosis. NAFLD is a common disease in the US and worldwide. Studies in the US found a significant association with obesity and diabetes, and it is even more common than alcoholic liver disease and hepatitis C with estimated 5 to 6 fold higher prevalence than that of chronic hepatitis C[24]. NASH is thought to eventually progress to liver cirrhosis, failure, and even carcinoma, unlike simple steatosis that is morphologically similar but harbors much better prognosis[25]. The diagnosis of NAFLD is based on clinical, radiologic, and morphologic characteristics. It combines the observation of persistently elevated serum aminotransferases with confirmatory imaging studies and supported by histopathological findings. The diagnosis is made in the setting of insignificant alcohol use, and any other chronic liver diseases. Liver biopsy is mandatory not only for confirming the diagnosis of NAFLD but for providing clues on the disease progression and grading of severity. It is also helpful in excluding the possibility of other chronic liver diseases. Despite being a major diagnostic tool for NAFLD, liver biopsy still has some limitations including the invasive nature of the procedure, being costly and requires hospitalization with the possibility of bleeding, the impact of biopsy size on the adequacy of biopsy for making the definitive diagnosis, and the pathologist experience role. The complexity of disease as captured in

morphology and the similarity of alcoholic and NAFLD in terms of morphology are also limiting factors of liver biopsy[13,26]. Taking the above factors in consideration, there is a necessity to find non-invasive serological markers, efficiently considered as screening tests for NAFLD presence and to add to the prognostic criteria of NAFLD to identify patients likely to have NASH is mandatory[27].
A large body of evidence confirms the fundamental role of hepatocytes apoptosis in the pathogenesis of NAFLD and NASH[11,28]. CK-18 is an intermediate filament protein that constitutes almost 5% of total liver proteins. It is considered a biomarker of hepatocellular apoptosis as it is cleaved, during apoptosis, at Asp 396 and recognized by M30 antibody specific for C-terminus[29]. A high prevalence of type 2 DM, dyslipidemia, obesity, and insulin resistance were noticed in our NAFLD and NASH patients compared to the control group. A result that was expected and supports other previous studies[30-32] In NASH cases, significantly higher insulin resistance is found compared to cases with simple steatosis. This observation was similarly noted in different previous studies that declared the significance of insulin resistance in the pathogenesis and progress of NASH[33,34]. Our study showed significantly higher levels of aminotransferases (AST and ALT) in patients of simple steatosis and NASH compared to the healthy individuals. The relationship between elevation of aminotransferases and the progression of NASH to the fibrotic stage is somewhat complicated. Although elevated serum concentrations of ALT is known as a biomarker of liver injury and damage in NAFLD[33,34], it does not necessarily indicate progression to the fibrotic stage. It was found that even NAFLD patients with normal ALT concentrations might still progress to NASH and advanced stage of fibrosis[35]. In the current study, significantly higher levels of AST were recorded in NASH patients in relation to the non-NASH group, a finding that was similarly supported by the study of Feldstein et al[1]. Accordingly, we can tell that patients with NAFLD associated with increased ALT levels are at increased risk to develop NASH along the disease course, but even in those patients with normal aminotransferases levels, NASH cannot be ruled out[36]. Hc-CRP as an inflammatory factor was elevated in our NASH patients compared to the control group, supported by Park et al., who reported hc-CRP as an independent risk factor in non-alcoholic liver disease[37]. A significant elevation of serum level of ferritin in NASH patients was noted in our study, compared to both the control and the simple steatosis groups. A similar study conducted in Japan by Yoneda et al. supports the current result as it has stated that the serum level of ferritin was related to insulin resistance in NASH patients[38]. High levels of serum ferritin are not only simply explained by iron overload and liver accumulation but it could be also caused by hepatic inflammation as an inflammatory marker, oxidative stress and highly expressed cytokines[39]. There is strong evidence that serum CK- 18M65 is released in NAFLD patients as a biomarker of apoptosis in NASH patients[40,41]. The current study showed significantly higher serum levels of CK-18 in our patients with NAFLD and NASH compared to healthy group and in NASH compared to NAFLD group. Similarly, Wiekowska et al. has found that significantly higher levels of serum CK-18 are detected in NASH patients than NAFLD patients and healthy individuals, as an indicator of hepatic inflamemation[7]. Essentially, CK-18 is found to be generated by caspase-3, which in turn is activated in the hepatocytes of NASH patients, and it is also found to be elevated in NASH compared to non-NASH patients[28]. The current results add to the growing body of evidence supporting the role of CK-18 in apoptosis induction and in NASH development. It is now unequivocally confirmed that CK-18 is an indicator of apoptosis and it is activated in NASH liver. Those findings along with our findings support the possibility of using CK-18 as a non-invasive marker for diagnosis and differentiation between NAFL and NASH[42-45]. A significantly positive association was discovered between serum CK-18 levels and all of fasting plasma glucose, body mass index, insulin resistance and C– reactive protein in NASH patients enrolled in the current study. This result was explained by Chitturri et al., who found a significant relationship between CK-18 and insulin resistance and confirmed its role in the pathogenesis and progression of NAFLD[33]. Similarly, Miyasato et al., observed a significant correlation between CK-18 and BMI in type 2 diabetic patients with NASH[46]. However,

we failed to find significant correlations between serum CK-18 levels and aminotransferases, serum ferritin, the age of the patients and lipid profile in our NASH patients, the results suggested by Yilmaz et al., showed a weak correlation between serum CK-18 and the aminotransferases[47]. Serum CK-18 of our NASH patients correlates significantly with NAS scoring, the degree of steatosis, ballooning, lobular inflammation and fibrosis in our study. This is supported by Feldstein et al. and Wieckowska et al[1,13]. The results of Diab et al. and Vuppalanchi et al support our results about the association between serum levels of CK-18 and NAS score in their patients[48]. Our study has limitations, like the small number of patients, uni-central study and absence of liver biopsy in the control group. So, a larger number multi-centric study can be done in the future.

Conclusions

In conclusion, serum CK-18 could be used as a non-invasive biomarker in NAFLD and NASH.

List of Abbreviations

CK -18: Cytokeratin-18, **NAFL**: Nonalcoholic fatty liver, **NASH**: nonalcoholic steatohepatitis, **NAFLD**: Nonalcoholic fatty liver disease, **NAS**: **NAFLD** Activity Score.

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