Pediatr Pol 2020; 95 (3): 141–148
DOI: https://doi.org/10.5114/polp.2020.100990
Submitted: 30.04.2020; Accepted: 12.07.2020; Published: 30.10.2020

PEDIATRIA Polska
PISMO POLSKIEGO TOWARZYSTWA PEDIATRYCZNEGO
Polish Journal of PAEDIATRICS
OFFICIAL JOURNAL OF POLISH SOCIETY OF PAEDIATRICS

ORIGINAL PAPER

The role of serum cytokeratin 18 and platelet count as non-invasive markers in the diagnosis of nonalcoholic fatty liver disease in children with type 1 diabetes mellitus

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ABSTRACT

Introduction: Nonalcoholic fatty liver disease (NAFLD) is the commonest chronic hepatopathy in children and adults.

Aim of the study: To evaluate the role of cytokeratin 18 (CK-18) and platelet count as non-invasive markers in the diagnosis of nonalcoholic fatty liver disease in children with type 1 diabetes mellitus (T1DM).

Material and methods: The study included 71 T1DM patients with age range 6–18 years old and 48 healthy age- and sex-matched volunteers. The T1DM patients were divided into NAFLD(+) (n = 12) and NAFLD(–) (n = 40) groups. Blood samples were collected for assessment of complete blood count, complete lipid profile, glycosylated haemoglobin, liver enzymes, and serum CK-18. Ultrasonography distinctively quantifies visceral fat and subcutaneous fat, ultrasound evaluation of hepatic steatosis, and liver size. Acoustic radiation force impulse elastography was used to evaluate liver fibrosis.

Results: The mean serum cholesterol, triglyceride, and LDL-cholesterol were statistically significantly higher in cases with NAFLD compared to cases without NAFLD and controls (p = 0.033, p = 0.001, p = 0.023, respectively). Comparing with the controls, cases exhibited significantly higher values for platelets count ($p < 0.005$). Regarding the mean level of serum CK-18, it was 168.88 ±96.462 mIU/ml in patients without NAFLD vs. 173.29 ±101.95 mIU/ml in patients with NAFLD and 140.75 ±79.97 mIU/ml in controls. Interestingly, the observed positive correlation between serum CK-18 and platelets counts in diabetic patients was statistically significant (r = 0.230, p = 0.046). Platelets count and visceral fat thickness were statistically significantly predictors of NAFLD. Significantly higher serum CK-18 in cases with NAFLD and liver fibrosis compared with those without liver fibrosis evaluated by acoustic radiation force impulse elastography.

Conclusions: CK-18 and platelet count may be useful markers for predicting liver fibrosis and help in the follow-up regimen of NAFLD in cases with T1DM.

KEY WORDS:

cytokeratin 18, platelets count, nonalcoholic fatty liver disease, liver fibrosis, type1 diabetes mellitus.

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INTRODUCTION

The World Health Organization report in 2017 revealed the fact that about 425 million people are suffering from diabetes. It is believed that this number will increase to 642 million by 2040 [1]. The excess fat in liver cells leads to fatty liver. When fat makes up about 5% of the liver, a benign condition of simple fatty liver usually does not lead to liver damage, while larger amounts of simple fat may lead to scarring, inflammation, and fibrosis of the liver [2]. Hepatic steatosis in type 1 diabetes mellitus (T1DM) in humans could be due to insufficient triglyceride secretion from the liver by very-low-density lipoproteins or hyperglycaemia promotes hepatic lipogenesis. Insulin therapy might cause increased weight gain and obesity-associated metabolic syndrome (MS) [2–4].

Nonalcoholic fatty liver disease (NAFLD) is the commonest chronic hepatopathy in children and adults. Hepatopathies include a wide range of diseases from simple steatosis or NAFLD, through nonalcoholic steatohepatitis (NASH), to cirrhosis [5].

Therefore, there is an urgent need to develop noninvasive biomarkers that can accurately distinguish between NAFLD and NASH. When fat aggregates in hepatocytes and is chronically exposed to oxidative stress, it becomes ballooned, shows disruption in the keratin intermediate filament network, and forms Mallory bodies [6]. A Mallory body contains cytokeratin (CK) 8 and 18 and stress-induced proteins [7], thus becoming susceptible to apoptosis. During apoptosis, CK-18 is cleaved by caspases and released into serum. Such levels of CK-18 released from hepatocytes into peripheral blood may be increased in NASH patients and increase in accordance with disease activity [8, 9].

Platelets count has gained attention in NAFLD as a laboratory marker [10–14]. The utility of platelet count was figured out following the observed link in liver cirrhosis patients. Moreover, platelet count has been included in some scoring noninvasive biomarkers of NAFLD [15–17].

The present study aimed to evaluate the role of CK-18 as a non-invasive marker in the diagnosis of NAFLD and its relation to platelet count.

MATERIAL AND METHODS

We conducted a cross-sectional study involving 71 T1DM children with age range 6–18 years (26 male, 45 female), recruited from the Paediatric Diabetic Clinic in Centre of Excellence in National Research Centre during the period 2016 to 2019. The cases were further subdivided into two groups: patients with NAFLD and patients without NAFLD. A control group included 48 participants (9 male, 39 female), who were age-matched healthy subjects. The study protocol was approved by the Human Ethics Committee of the National Research Centre, and written informed consent was obtained from all parent/legal guardians of children, after full acknowledgement about the aims of the study.

Exclusion criteria: Mauriac syndrome, history suggestive of viral hepatitis A, B, or C infection, autoimmune hepatitis, drug-induced hepatitis, genetic disorders, or any other causes of chronic liver diseases.

All children were subjected to history taking and clinical examination to fulfil the required data: insulin therapy, regarding dose in units/kg and type. History suggestive of acute metabolic complications, or chronic complications was included. Blood pressure was measured according to American Heart Association guidelines; three times for patients and controls after a five-min rest in a sitting position with the use of mercury sphygmomanometer. The mean value of second and third measurement was calculated. Systolic blood pressure (SBP) was defined as the onset of the Korotkoff sound (K1), and diastolic blood pressure (DBP) was defined as the fifth Korotkoff sound (K5). Anthropometric indices included the following: body weight measured to the nearest 0.1 kg with a balance scale and height measured to the nearest 0.1 cm. Body mass index was calculated as weight divided by height squared (kg/m^2). Waist circumference (WC) was measured at the level midway between the lowest rib margin and the iliac crest. Hip circumference (HIP C) was measured at the widest level over the greater trochanters in a standing position by the same examiner, then waist to hip ratio (WHR) and waist to height ratio (WHtR) were calculated [18]. The landmarks, instruments used, and techniques followed were those recommended by the international biological program [19].

Using International Diabetes Federation (IDF)-based criteria, MS was defined in patients with diabetes as having two or more of the following criteria: 1) abdominal obesity (waist circumference ≥ the age- and gender specific 90th percentile for this population); 2) high blood pressure (SBP and/or DBP ≥ age and gender and height specific 90th percentile); 3) decreased HDL level (≤ 40 mg/dl); and 4) elevated serum TG (≥ 150 mg/dl).

ABDOMINAL ULTRASONOGRAPHY

In addition to the routine abdominal ultrasound examination based on the clinical indication, ultrasonography (US) distinctively quantifies visceral fat and subcutaneous fat. We measured the maximum visceral fat thickness (VFT) and the minimum subcutaneous fat thickness (SFT) by US. The VFT was measured by 3.5–5 MHz convex-array probe. VFT is the distance between the internal surface of the abdominal surface of the abdominal muscle and the anterior wall of the aorta 1 cm above the umbilicus. The thickness of subcutaneous fat was measured by placement of a 3.75 MHz probe perpendicular to the skin on the epigastrium. Longitudinal scans were obtained along the middle line (linea alba).

The thickness of the subcutaneous fat is defined as the distance between the anterior surface of linea alba and the fat-skin barrier. US evaluation of hepatic steatosis typically consists of a qualitative visual assessment of hepatic echogenicity, measurements of the difference between the liver and kidneys in echo amplitude, evaluation of echo penetration into the deep portion of the liver, and determination of the clarity of blood vessel structures in the liver. The ultrasound apparatus was an SA–R3 (No. S06YM3 HDC00012F) from the Samsung Medison Company – South Korea [20].

ACOUSTIC RADIATION FORCE IMPULSE ELASTOGRAPHY

Acoustic radiation force impulse elastography (ARFI) was performed for all subjects with a Siemens Acuson S3000 Virtual Touch ultrasound system (Siemens AG, Erlangen, Germany) with a 6CI transducer. The principle underlying ARFI elastography is that sharing of the examined tissue induces a strain in the tissues. An acoustic "push" pulse is automatically produced by the ultrasound probe and directed to the side of a region of interest (ROI), which is where the speed of the shear wave is measured. This ROI has a predefined size, provided by the system (10 mm long and 5 mm wide). The acoustic "push" pulse generates shear waves that propagate into the tissue, perpendicular to the "push" axis. Detection waves are also generated by the transducer to measure the propagation speed of these shear waves, which increases with fibrosis severity [21]. The speed of the shear waves, measured in metres per second, as well as measurement depth, is displayed by the system. For each patient, 10 valid ARFI measurements were performed under fasting conditions, with the patient in supine position with the right arm in maximum abduction, by the intercostal approach in the right liver lobe, 1–2 cm under the liver capsule. Minimal scanning pressure was applied by the operator, and the patient was asked to stop normal breathing for a moment to minimise breathing motion. The mean of 8–10 valid measurements was calculated and considered indicative of the severity of fibrosis. The operators were blinded to any clinical or elastographic data.

LABORATORY MEASUREMENTS

Ten millilitres of peripheral venous blood were withdrawn under complete aseptic precautions from fasting subjects (12–14 hours). Three millilitres of blood were anticoagulated with EDTA for CBC and HBA_{1c} assessment, and the rest of the sample was left to clot at room temperature for 15 min then centrifuged. Sera were then separated, labelled uniquely, and aliquoted for evaluation of the following parameters: 1) complete lipid profile (serum total cholesterol, triglycerides, HDL, LDL) and liver enzymes (aspartate aminotransferase [AST] and alanine aminotransferase [ALT]) were carried out using an Olympus AU 400 supplied from Olympus Life and Material Science (Europe GmbH, Wendenstraße, Hamburg, Germany). 2) Viral markers: HBs Ag, HCV Ab, and HIV Ab (to exclude any viral hepatitis in those patients) using PRECHECK Kit (USA). 3) CK-18 levels were estimated by Elabsciensce Eliza kit (USA) Cat: E-EL-H2072 Lot: 3BLG4RW72R, which employs a competitive enzyme-linked immunosorbent technique.

STATISTICAL ANALYSIS

The standard computer program Statistical Package for the Social Sciences (SPSS) for Windows, release 17.0 (SPSS Inc., USA) was used for data entry and analysis. All numeric variables were expressed as mean ±standard deviation (SD). Comparison between groups was made using Student's t-test for continuous variables and χ^2 tests for categorical variables. Pearson's and Spearman's correlation tests (r = correlation coefficient) were used for correlating normal and non-parametric variables, respectively. Assessing normality was done with the concept of the bell curve. Linear multiple regression was run to predict NAFLD. The receiver operating characteristic (ROC) curve was generated to detect the predictive capabilities of CK-18 in detecting liver fibrosis. $p < 0.05$ was considered statistically significant.

RESULTS

A total of 119 participants were enrolled in the study. The age range of T1DM patients was 6–18 years, 36.6% were boys, and 63.4% were girls. The mean ins/kg doses were 1.28 ±0.63, and the mean disease duration was 5.52 ±3.23 years. No cases had treated hypertension or associated autoimmune or celiac diseases.

According to the result of abdominal ultrasound, 12 (23.9%) cases had NAFLD. ALT > 25 IU/l was considered abnormal in boys and > 22 IU/l in girls; only three female cases had mild elevation (4.2%).

Table 1 and 2 show a comparison of the mean of different parameters between the studied groups. Comparing studied groups, patients with NAFLD exhibited significantly higher mean values of body mass index, waist circumference, liver span, and VFT ($p < 0.001$).

Considering HbA_{1c}% mean levels, there was a statistically significant difference between the three groups ($p < 0.001$). As regard the mean levels of serum cholesterol, triglyceride, and LDL-cholesterol, there were statistically significantly higher levels in the NAFLD group compared to the other two groups ($p = 0.033$, $p = 0.001$, $p = 0.023$, respectively). However, there was a statistically significantly higher level of HDL in the controls ($p = 0.014$). ALT was statistically significantly higher in patients with NAFLD ($p = 0.011$). Compared with the controls, the patients exhibited significantly higher values of platelet count ($\times 10^9$/l)

TABLE 1. Comparison of clinical and ultrasonography data of the studied groups

Parameter	Studied groups	Mean ±SD	*p*
BMI (kg/m^2)	NAFLD(–) (*n* = 53)	19.58 ±4.40	< 0.001
	NAFLD(+) (*n* = 17)	25.10 ±6.20	
	Controls (*n* = 48)	18.87 ±4.20	
Waist circumference (cm)	NAFLD(–) (*n* = 54)	68.69 ±11.0	< 0.001
	NAFLD(+) (*n* = 17)	81.59 ±12.3	
	Controls (*n* = 48)	66.81 ±12.48	
Liver span (cm)	NAFLD(–) (*n* = 52)	12.80 ±1.38	< 0.001
	NAFLD(+) (*n* = 17)	15.9235 ±0.68	
	Controls (*n* = 48)	12.2813 ±1.60	
Visceral fat thickness (cm)	NAFLD(–) (*n* = 48)	2.9271 ±0.93	< 0.001
	NAFLD(+) (*n* = 16)	4.0688 ±1.08	
	Controls (*n* = 48)	2.5125 ±0.75	

NAFLD – nonalcoholic fatty liver disease

TABLE 2. Comparison of laboratory data of the studied groups

Parameter	Groups			*p*
	NAFLD(–) (*n* = 54)	NAFLD(+) (*n* = 17)	Controls (*n* = 48)	
HbA_{1c}%	7.98 ±0.70	8.41 ±0.88	5.52 ±0.68	< 0.001
Cholesterol (mg/dl)	154.65 ±17.75	158.76 ±28.99	139.88 ±26.69	0.033
Triglycerides (mg/dl)	59.35 ±21.01	78.89 ±39.22	36.94 ±10.41	0.001
HDL cholesterol (mg/dl)	39.43 ±6.91	37.41 ±5.22	47.00 ±19.15	0.014
LDL cholesterol (mg/dl)	60.93 ±9.38	66.29 ±24.36	53.25 ±8.35	0.023
AST (IU/l)	16.78 ±3.52	15.65 ±2.55	14.81 ±2.46	0.075
ALT (IU/l)	17.68 ±4.44	17.78 ±2.63	14.31 ±3.32	0.011
Platelet count (×10^9/l)	263.17 ±77.24	337.41 ±94.59	259.94 ±82.70	< 0.005
CK-18 (mIU/ml)	168.88 ±96.46	173.29 ±101.95	140.75 ±79.97	0.090

NAFLD – nonalcoholic fatty liver disease, HbA_{1c} – glycosylated haemoglobin, HDL – high-density lipoprotein, LDL – low-density lipoprotein, ALT – alanine aminotransferase, AST – aspartate aminotransferase, CK-18 – cytokeratin 18. Values are given as mean ±SD

($p < 0.005$). The mean levels of serum CK-18 were 168.88 ±96.462 in patients without NAFLD, 173.29 ±101.945 in patients with NAFLD, and 140.75±79.974 in controls, which was statistically insignificant ($p = 0.090$).

Interestingly, the observed positive correlation between serum CK-18 and platelet count in diabetic patients was statistically significant ($r = 0.230$, $p = 0.046$). NAFLD showed statistically significantly positive correlation to duration of T1DM, liver span, platelet count, HbA_{1c}%, and VFT ($r = 0.342$, $r = 0.737$, $r = 0.366$, $r = 0.239$, and $r = 0.460$; $p = 0.004$, $p = 0.000$, $p = 0.002$, $p = 0.044$, and $p = 0.000$, respectively). Details are included in Figure 1 and Table 3.

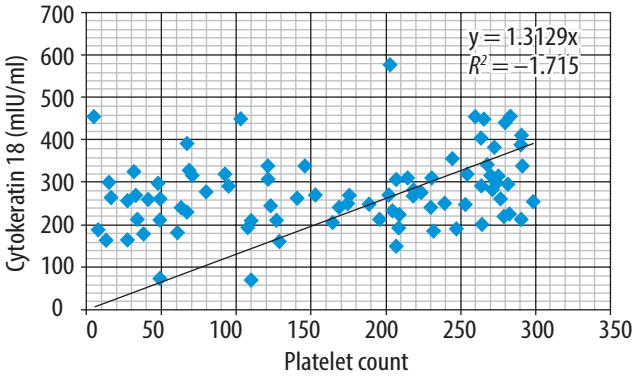

FIGURE 1. Relation between cytokeratin 18 and platelet count

Linear multiple regression was applied to predict NAFLD; platelet count (× 10^9/l) and VFT were the main predictors of NAFLD, as shown in Table 4.

Out of the 71 T1DM patients, 52 children only were evaluated by both abdominal ultrasonography and FibroScan because of non-compliance to the appointment. Table 5 shows different stages of fibrosis diagnosed by ARFI FibroScan compared to NAFLD diagnosed by abdominal ultrasonography. Most T1DM children had stage 1 ($n = 16$), a few had stage 2 ($n = 5$) or 3 ($n = 3$), and one had stage 4 fibrosis. There was a significant difference between proportions using Pearson χ^2 test in ARFI Fibro-

TABLE 3. Correlation between cytokeratin 18 (CK-18), nonalcoholic fatty liver disease (NAFLD), and different variables in type 1 diabetes mellitus (T1DM) patients

Parameter		CK-18 (mIU/ml)	Duration of T1DM (years)	TG (mg/dl)	Liver span (cm)	Platelet count ($\times 10^9$/l)	HbA_{1c}%	VFT (cm)
CK-18 (mIU/ml)	*r*	1	0.123	0.314**	0.040	0.230*	0.099	0.086
	p		0.309	0.008	0.742	0.046	0.411	0.500
NAFLD	*r*	0.123	0.342**	0.241*	0.737**	0.366**	0.239*	0.460**
	p	0.309	0.004	0.043	0.000	0.002	0.044	0.000

*TG – triglycerides, HbA_{1c} – glycosylated haemoglobin, VFT – visceral fat thickness, * significant , ** highly significant*

TABLE 4. Linear multiple regression to predict nonalcoholic fatty liver disease (NAFLD)

Parameter	Unstandardised coefficients		Standardised coefficients	*t*	*p*
	B	Std. error	β		
Constant	–0.728	0.188		–3.861	0.000
Platelets count ($\times 10^9$/l)	0.001	0.001	0.301	2.815	0.007
Duration (years)	0.026	0.015	0.200	1.691	0.096
Visceral fat thickness (cm)	0.128	0.047	0.318	2.718	0.009

Dependent variable – cases positive NAFLD

TABLE 5. Comparison between abdominal ultrasonography and acoustic radiation force impulse (ARFI) elastography stages of fibrosis

Liver ultrasonography	ARFI FibroScan stages of fibrosis, *n* (%)					Asymp. sig. (2-sided)	
	Normal	1	2	3	4	Pearson χ^2	LBLA
NAFLD(–) (*n* = 40)	24 (60.0)	12 (30.0)	3 (7.5)	1 (2.5)	0	0.048	0.003
NAFLD(+) (*n* = 12)	3 (25.0)	4 (33.3)	2 (16.7)	2 (16.7)	1 (8.3)		
Total (*n* = 52)	27 (52)	16 (31)	5 (9)	3 (6)	1 (2)		

NAFLD – nonalcoholic fatty liver disease, LBLA – linear-by-linear association test

Scan stages between patients with NAFLD by abdominal ultrasonography and NAFLD-free patients. Linear-by-linear association of the Mantel-Haenszel test of trend among χ^2 test statistics showed high significance ($p = 0.003$).

To evaluate the possible effect of several parameters on serum CK-18 in T1DM patients, they were subdivided as displayed in Table 6. Regarding the effect of other parameters, our study detected insignificantly higher serum CK-18 levels between MS cases, obese patients, cases with NAFLD, uncontrolled patients, and diabetes duration ≥ 5 years. Serum CK-18 levels were significantly higher in patients with hepatic fibrosis ($p = 0.041$).

The ROC curve of platelet count for diagnosing NAFLD was performed. The area under the curve was found to be 0.749 ($p = 0.002$) as shown in Table 7 and Figure 2.

An ROC curve was done to test the sensitivity and specificity of CK-18 for diagnosing liver fibrosis. The area under the curve of CK-18 was found to be 0.752 ($p = 0.033$), indicating that overall diagnosing of CK-18 of liver fibrosis is significantly good. Figure 3 and Table 7 show the area under the curve of CK-18 for diagnosing liver fibrosis.

TABLE 6. Comparison between the mean serum cytokeratin 18 (CK-18) and several parameters in type1 diabetes mellitus (T1DM) cases

T1DM patients	Mean ±SD	*p*
Without MS (*n* = 57)	170.60 ±95.40	0.926
With MS (*n* = 14)	173.29 ±101.95	
Non-obese (*n* = 56)	180.11 ±93.07	0.778
Obese (*n* = 15)	188.27 ±93.07	
Without NAFLD (*n* = 54)	164.57 ±96.40	0.309
With NAFLD (*n* = 17)	191.94 ±115.46	
HbA_{1c}% < 7.50 (*n* = 14)	158.17 ±107.70	0.364
HbA_{1c}% ≥ 7.50 (*n* = 57)	186.19 ±95.32	
T1DM < 5 years (*n* = 40)	153.40 ±92.28	0.079
T1DM > 5 years (*n* = 31)	194.00 ±97.28	
Without fibrosis (*n* = 45)	179.67 ±99.64	0.041
With fibrosis (*n* = 7)	258.00 ±47.88	

MS – metabolic syndrome, NAFLD – nonalcoholic fatty liver disease, HbA_{1c} – glycosylated haemoglobin

TABLE 7. ROC curve of platelet count for diagnosis accuracy of nonalcoholic fatty liver disease and cytokeratin 18 for diagnosis accuracy of liver fibrosis

Variable	Area	SD	Asymptotic sig.	Asymptotic 95% CI		Cut off	Sensitivity	Specificity
				Lower bound	Upper bound			
Platelet count*	0.749	0.064	0.002	0.623	0.875	239,5	94	61
Cytokeratin 18**	0.752	0.092	0.033	0.572	0.933	121	70,5	61

** null hypothesis: true area = 0.5, ** null hypothesis: true area = 0*

DISCUSSION

NAFLD includes a broad spectrum of pathological changes, ranging from steatosis (NAFLD) to NASH and liver fibrosis [22]. The liver biopsy is the gold standard for diagnosis of NAFLD in children, although parents may be unwillingly to accept a biopsy, being an invasive procedure. Imaging techniques, such as hepatic ultrasound or FibroScan can give insight into the extent of liver involvement in NAFLD, and distinguish effectively between NAFLD and NASH. Additional non-invasive measures of liver fibrosis are under investigation, including levels of serum CK-18 fragments [23].

According to the result of abdominal ultrasound, 12 (23.9%) patients had NAFLD. Other causes of chronic liver disease were excluded from the study, such as Mauriac syndrome and viral hepatitis A.

Although estimation of serum ALT level is usually performed as a part of the hepatic panel, the significance of this is controversial [24]. According to new evidence-based standards for normal ALT in children: serum ALT level > 25 IU/l in boy and > 22 IU/l in girls was considered abnormal [24]; only three female patients had mildly elevated ALT level. In general, the mean serum ALT level was at a high normal level. Many studies reported that the full histological spectrum of NAFLD could be reported in individuals with normal ALT values [25–27].

We found also that the NAFLD group had significantly higher fasting lipid profile (cholesterol, triglyceride and LDL), which agreed with another study that emphasised that the patients with T1DM are at particularly high risk of cardiovascular disease [28].

Platelet count was significantly higher in our NAFLD patients. Other ultrasonography-based studies also reported that with the increase in steatosis grade, platelet count increased [29, 30]. Several studies have suggested this as a marker in alcoholic liver disease and a predictor of fibrosis severity (grade) in NAFLD and NASH because the platelet count later decreases in liver cirrhosis patients as well as pathophysiologic changes that occur including splenomegaly and sequestration of platelets that end in thrombocytopaenia [12, 31]. The ROC curve of platelets count for diagnosis accuracy of NAFL was < 0.749 in the present study.

Hepatocyte apoptosis may play an important role in liver injury and disease progression in NAFLD [32]. Increased apoptosis in NAFLD might lead to an increase

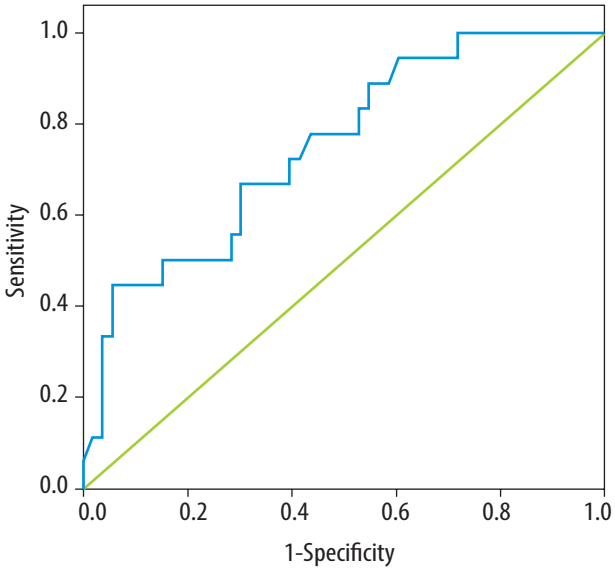

FIGURE 2. The receiver operating characteristic (ROC) curve of platelet count for nonalcoholic fatty liver disease

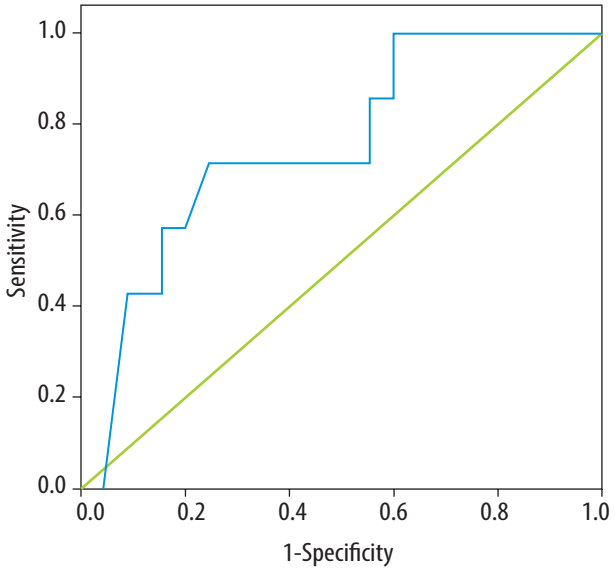

FIGURE 3. The receiver operating characteristic (ROC) curve of cytokeratin 18 for liver fibrosis

in CK-18 in the serum of NAFLD patients. We found an increase in serum CK-18 level, although this trend was not statistically significant; this result was similar to that of a previous study [32]. A prediction model for the presence of NAFLD was generated by using linear multiple regression; platelet count ($\times 10^9$/l) and VFT were statistically significant predictors of NAFL. Our result is in concordance with other studies [30, 33]

ARFI FibroScan staging of fibrosis showed that four patients (8.0 %) had liver fibrosis (stage 3 and stage 4). Ultrasonography could evaluate hepatomegaly and diffuse increase in echogenicity of the liver parenchyma but not fibrosis. AFRI FibroScan has the advantage that it is a non-invasive method to assess liver fibrosis. Data for CK-18 and liver fibrosis in T1DM patients with NAFLD are limited. In our study, significantly higher serum CK-18 was seen in children with NAFLD and liver fibrosis compared with those without liver fibrosis evaluated by AFRI FibroScan. Other studies showed the same result [34–36]. The ROC curve of CK-18 for diagnosis accuracy of liver fibrosis was < 0.752 in the present study; a similar result was reported by Mandelia *et al.* [35].

Obesity has been found to be highly prevalent among children with T1DM [37]. We reported 15/71 obese children with non-significantly higher serum CK-18 in obese children; this was previously reported by Mandelia *et al.* [35].

NAFLD is considered as a hepatic manifestation of MS. Using IDF-based criteria, MB was defined in T1DM patients as having two or more MS criteria; out of our 71 T1DM patients, 14 patients showed features of MS. The non-significantly higher CK-18 levels in children with MS than in those without MS is in agreement with a study offered by Yilmaz *et al.* [38]. As expected, patients experiencing longer duration of T1DM and uncontrolled patients had higher levels of serum CK-18. This indicates that the increased serum CK-18 in diabetics is involved in the process of disease progression and is related to uncontrolled and duration of diabetes. CK-18 may be a useful marker in the follow-up of T1DM patients with NAFLD and in the assessment of disease severity.

There were some limitations to the study. Firstly, the cross-sectional nature of the study did not allow us to monitor CK-18 and the disease progress over time. Secondly, the sample size was small. Lastly, the gender proportions in the study and control group were not similar.

CONCLUSIONS

CK-18 and platelet count may be useful markers for predicting liver fibrosis and may help in the follow-up of NAFLD in children with T1DM.

DISCLOSURE

The authors declare no conflict of interest.

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