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Original Article

Effect of diet-induced weight loss on cytokeratin-18 levels in overweight and obese patients with liver fibrosis

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ABSTRACT

Aims: Liver biopsy is currently the gold standard test for NAFLD diagnosis and staging but has many drawbacks. In addition, other tools such as transient elastography are limited to specialized research centers. To assess the usefulness of CK-18 as a non-invasive biomarker for detecting therapeutic responses in patients with liver fibrosis.

Materials and methods: Sixty overweight and obese patients with liver fibrosis were evaluated by a dietitian and given a weight-reducing diet with a calorie deficit of 500–1000 kcal/day over a 6-month period. Controlled attenuation parameter (CAP) and liver stiffness measurement (LSM) both were performed at the beginning and at the end of the trial to determine liver steatosis and liver fibrosis, respectively. Serum CK-18 levels were measured by enzyme linked immune sorbent assay (ELISA) at baseline and at 3 and 6 months after intervention.

Results: Patients experienced a rapid weight loss of −7.6 kg (8.5%) during the trial. Among all participants, liver steatosis decreased from 76.5 ± 12.2% to 51.8 ± 24.4% (baseline to end-point) ($p < 0.001$) and fibrosis score decreased from 9.9 ± 3.7 to 7.2 ± 2.4 ($p < 0.001$) (a 27.2% decrease). Serum CK-18 levels decreased from 290.2 ± 98.1 U/L to 217.6 ± 64.8 U/L ($p < 0.001$) (a 25.0% decrease). ΔCK-18 was found to be significantly associated with delta fibrosis score ($r = 0.25$, $p = 0.05$)

Conclusions: This trial showed a significant positive association between changes in CK-18 levels and changes in liver fibrosis over a 6-month dietary intervention

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a condition defined by excessive fat accumulation (>5%) in the liver in the absence of significant alcohol intake, which affects 20–40% of the world's population [1]. Nonalcoholic steatohepatitis (NASH) is the aggressive form of the disease characterized by liver injury and inflammation which often progresses to liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) [2]. Approximately 3% of individuals in general population may have NASH; although it is more than 25% in patients with obesity [3]. Moreover, liver fibrosis is a multifaceted process characterized by hepatocyte damage, inflammation, matrix deposition and remodeling, and epithelial cell regeneration, with an estimated global prevalence of 2% in the general population [4]. It has been estimated that about 10% of patients with NASH may further progress to cirrhosis, which is the strongest predisposing factor for HCC [5,6].

Liver biopsy is currently the gold standard test for NAFLD diagnosis and staging; however, it has many drawbacks, such as sampling error, cost, and risk of serious complications [7]. Moreover, the grade of steatosis or fibrosis can be estimated using transient elastography (FibroScan), ultrasonography with a specific apparatus, or a magnetic resonance elastography (MRE). However, use of these non-invasive tools is limited to specialized research centers because they are operator-dependent and have high operating costs [8,9].

A growing body of evidence suggests that increased hepatocyte apoptosis has an important role in progression from simple fatty liver to NASH, and correlates with disease severity and hepatic fibrosis [10,11]. Cytokeratin-18 (CK-18) is a type I intermediate

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filament protein of hepatocytes, which is cleaved into proteolytic fragments by caspases and released into the circulation during hepatocyte apoptosis [12]. Thus, the serum level of CK-18 fragment is representative of the degree of hepatocyte apoptosis. Several studies have introduced CK-18 fragment as a useful noninvasive tool for assessing NASH in patients with NAFLD [13–16]. For instance, Feldstein et al. suggested non-invasive monitoring of hepatocyte apoptosis by CK-18 in blood of patients with NAFLD as a reliable tool to diagnose NASH [12]. Using a cutoff value of 150 U/L, Rahman et al. reported a sensitivity and specificity of 85.7% and 80.8% respectively for detection of NASH by CK-18 fragment [14]. However, no previous studies have assessed the usefulness of CK-18 for detecting therapeutic responses in patients with liver fibrosis.

Therefore, the main objective of the present study was to determine the effect of diet-induced weight loss on serum CK-18 levels in overweight and obese patients with liver fibrosis. In addition, we assessed the usefulness of CK-18 as a non-invasive biomarker for detecting therapeutic responses in patients with liver fibrosis.

2. Materials and methods

2.1. Study population

A total of 60 overweight and obese patients with liver fibrosis [34 (56.7%) males and 26 (43.3%) females] were recruited from a nutrition clinic into a before–after trial. Subjects with autoimmune and infectious diseases, those with viral hepatitis B or C, those with cholestatic liver diseases, those with history of alcohol intake, pregnant women, those taking hepatotoxic medications and those with BMI less than 25 as well as those without liver fibrosis were not included in our study. The mean age of men and women were 43.2 ± 9.1 y and 49.2 ± 11.5 y, respectively. All participants gave informed, written consent to enter the trial, which was reviewed and approved by the ethics committee of Mashhad University of Medical Sciences (MUMS) (Ethics code: IR.MUMS.fm.REC.1395.498).

2.2. Anthropometric measurements

All anthropometric measurements were based on standardized procedures and protocols [17]. Height was measured using a standard stadiometer and the observations were noted to the nearest 0.1 cm. Waist circumference was measured with a flexible steel tape measure at the high point of the iliac crest at minimal respiration while hip circumference was measured at the widest point over the buttocks. Chest circumference was obtained at the level of the nipples and below the inferior angle of the scapula, at the end of a normal expiration. Mid-arm circumference was measured at half the distance between the shoulder (acromion process) and the elbow (olecranon). Wrist circumference was measured at the widest part of the wrist (to the nearest 0.1 cm). The body composition was assessed by means of the bioimpedance analysis (BIA) (Tanita BC-418; Tanita Corp., Tokyo, Japan) in all studied subjects.

2.3. Clinical and laboratory assessments

The systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by sphygmomanometer twice in exactly the same manner. Both SBP and DBP were measured on the left arm when participants remained seated at rest for at least 15 min. We took the third measurement and averaged the two closest readings, if the first two readings differ by more than 15 mmHg in DBP or more than 25 mmHg in SBP. Blood samples were drawn from all participants on the morning after a 12-h fast and fasting blood glucose (FBG) and a complete lipid profile including low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triglyceride (TG) were obtained by standard techniques. Additionally, circulating liver enzymes including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) were measured using standard automated kinetic enzymatic assays.

2.4. Transient elastography

Transient elastography (FibroScan®) was proposed as a tool for determining liver steatosis via controlled attenuation parameter (CAP) and liver fibrosis via liver stiffness measurement (LSM) [18]. CAP is a measure of the liver ultrasound attenuation developed to assess steatosis severity [19]. Several studies have shown a good correlation between CAP values and the amount of hepatic steatosis, even with a small amount [20,21]. CAP results are expressed in decibels per meter (dB/m) and range from 100 to 400 dB/m. In this regards, there are four grades of steatosis, including S0 <237 dB/m, S1: 237–259 dB/m, S2: 259–291 dB/m, and S3: 291–400 dB/m [8]. LSM is an established modality for assessing the extent of liver fibrosis. The LSM results are expressed in kilopascals (kPa). The fibrosis staging was as follows: Up to 7.1 kPa = F0 (absence of fibrosis), 7.1–9.6 kPa = F1 (perisinusoidal or portal fibrosis), 9.6–11.6 kPa = F2 (perisinusoidal and portal fibrosis), 11.6–16.9 kPa = F3 (septal or bridging fibrosis), 16.9–75.0 kPa = F4 (hepatic cirrhosis) [22,23]. FibroScan® was performed by a single physician familiar to the instructions provided by the manufacturer and blinded to patients' data.

2.5. Measurement of CK-18 levels

Serum levels of CK-18 were determined using a commercially available immunoassay [M30-Apoptosense enzyme-linked immunosorbant assay (ELISA) kit, Peviva AB, Bromma, Sweden] according to the manufacturer's instructions. Briefly, in this ELISA, samples were placed into wells coated with a monoclonal antibody as a catcher and a horseradish peroxidase-conjugated antibody (M30) was used for detection. The amount of protein in each sample was determined by a standard curve of optical density values constructed for known concentrations of CK-18 neoepitope M30. Serum levels of CK-18 were expressed in units per liter (U/l).

2.6. Experimental design

On enrollment, all patients were evaluated by a dietitian and given a weight-reducing diet with a calorie deficit of 500–1000 kcal/day over a 6-month period. The patients were contacted by phone every week to solve possible problems and to ensure their compliance with the diet by means of a 24-h dietary recall. All anthropometric measurements as well as all the clinical and laboratory data were obtained from the patients at baseline and at 3 and 6 month after dietary intervention. Serum CK-18 was measured at baseline and at 3 and 6 month after dietary intervention, as well. FibroScan® was performed twice at the beginning and the end of the intervention.

2.7. Estimation of dietary intake

Dietary intakes were estimated at baseline, and at 3 and 6 months after enrollment using a 3-day food record which is a standard technique used widely in research as well as in empirically based clinical practice to evaluate recent dietary intake [24,25]. All participants were informed on how to complete the

food record by a dietitian. Each record was analyzed for micro and macronutrient content using the Nutritionist IV software (version 7.0; N-Squared Computing, Salem, OR, USA) which was modified for Iranian food items [26].

2.8. Statistical analysis

Data analysis was carried out using SPSS-18 software (SPSS Inc., IL, USA). The normality of data was evaluated using Kolmogorov–Smirnov test. Descriptive statistics including mean, frequency, and standard deviation (SD) were determined for all variables and expressed as mean ± SD for normally distributed variables. The repeated measure ANOVA was used to assess for significant differences between the various time points (baseline, 3-month and 6-month). Pearson correlation test was used to search for correlations between delta CK-18 (baseline minus end of study) and delta values of anthropometric and biochemical parameters. All the analyses were two-sided and p-value <0.05 was considered as significant.

3. Results

Basal and demographic characteristics of the study population are reported in Table 1. The mean age of the patients was 45.8 ± 10.6 year. The patients' mean weight and height at baseline were 91.2 ± 25.4 kg and 164.0 ± 25.4 cm respectively, yielding a mean BMI of 33.9 kg/m^2. The number of individuals with overweight, obesity class I and obesity class II were 25 (41.7%), 20 (33.3%) and 15 (25.0%), respectively. Approximately 57% (n = 34) of the participants were male and 43% (n = 26) were female. About 23% of subjects had primary education, 35% of subjects were secondary and near 42% were having university education. Among 60 individuals, 5 (8.3%) had grade 2 steatosis and 55 (91.7%) had grade 3 steatosis. Additionally, the number of patients with fibrosis stages 2, 3 and 4 was 38 (63.3%), 20 (33.3%) and 2 (0.4%), respectively (Table 1). Of 60 registered subjects, five did not continue the trial due to personal reasons and were not included in the final analysis.

The anthropometric and biochemical data of study population at baseline and at 3 and 6 months after intervention are presented in Table 2. Patients experienced a rapid weight loss of −6.0 kg (6.6%) in the first 3 months followed by a further decrease of −1.6 kg (1.9%) in the second 3 months of dietary intervention. As expected, BMI (−3.2 kg/m2, p < 0.001), FM (−5.8 kg, p < 0.001), PBF (−4.8%, p < 0.001), TFM (−4.0 kg, p = 0.01), and FFM (−0.7 kg, p = 0.006) showed a significant stepwise decrease during the period of dietary intervention. However, changes in TBW (+0.4 L) were insignificant (p = 0.02). Anthropometric measurements including WC (−8.7 cm), HC (−5.3 cm), CC (−4.7 cm), AC (−1.3 cm) and WrC (−0.6 cm) also showed a significant decrease during the period of trial (p < 0.001). SBP and DBP decreased from 123.5 ± 11.9 mmHg and 86.0 ± 8.6 mmHg at baseline to 119.3 ± 9.1 mmHg and 81.3 ± 7.4 mmHg at the end of the trial. FBG also showed a significant stepwise decrease during dietary intervention (−9.3 mg/dl, p = 0.03). However, changes in lipid profile were insignificant except for HDL-C (p < 0.001), which increased from 41.7 ± 8.3 mg/dl to 45.0 ± 6.5 mg/dl (Table 2).

The amount of steatosis and fibrosis as well as liver enzymes and CK-18 levels at baseline and at 3 and 6 months after intervention are reported in Table 3. Among all 55 patients, liver steatosis decreased from 76.5 ± 12.2% to 51.8 ± 24.4% (baseline to end-point) (p < 0.001). Mean values for the total fibrosis score also decreased from 9.9 ± 3.7 to 7.2 ± 2.4 (a 27.2% decrease) (p < 0.001). Circulating liver enzymes including ALT (−42.0 U/L, p < 0.001), AST (−24.5 U/L, p < 0.001) and GGT (−5.3 U/L, p = 0.008) showed a significant decrease during the trial. However, final ALP (167.5 ± 44.5 U/L) did not differ significantly from baseline values (170.9 ± 44.3 U/L, p = 0.3). As reported in Table 3, serum CK-18 levels decreased from 290.2 ± 98.1 U/L to 254.9 ± 62.0 U/L in the first 3 months followed by a further decrease in the second 3 months reaching 217.6 ± 64.8 U/L at the end of the dietary intervention (a 25.0% decrease).

Energy and dietary intake of study participants at baseline and at 3 and 6 months after intervention are presented in Table 4. The amount of energy intake decreased from 2211 ± 503 kcal at baseline to 1322 ± 256 kcal at the end of the dietary intervention. There was no significant difference in carbohydrate percentage of energy intake at baseline and 3 and 6 month after intervention (p = 0.6). Protein percentage of energy intake significantly increased from 13.8 ± 3.3 to 17.5 ± 4.0 (p < 0.001), while fat percentage of energy intake significantly decreased from 31.7 ± 10.1 to 29.3 ± 9.1 (p = 0.02). As expected, the dietary intake of carbohydrate, protein and fat significantly decreased during dietary intervention (p < 0.001). However, changes in cholesterol (p = 0.9), lactose (p = 0.1) and dietary fiber (p = 0.6) intake were insignificant during the trial (Table 4).

Correlations between delta CK-18 (baseline minus end of study) and delta values of anthropometric and biochemical parameters are reported in Table 5. Delta CK-18 levels was positively correlated with delta fibrosis score (r = 0.25, p = 0.05), delta WrC (r = 0.38, p = 0.004) and delta DBP (r = 0.31, p = 0.02), and negatively correlated with delta FM (r = −0.30, p = 0.02).

Table 1
Basal and demographic characteristics of the study population.

	Total subjects (n = 60)
Age (year)	45.8 ± 10.6
Height (cm)	164.0 ± 25.4
Weight (kg)	91.2 ± 14.9
Weight status n(%)	
Overweight	25 (41.7)
Obesity class 1	20 (33.3)
Obesity class 2	15 (25.0)
Sex n(%)	
Male	34 (56.7)
Female	26 (43.3)
Education n(%)	
Primary	14 (23.3)
Secondary	21 (35.0)
Tertiary (higher)	25 (41.7)
Smoking n(%)	
Yes	3 (5.0)
No	57 (95.0)
Steatosis	
Grade 2	5 (8.3)
Grade 3	55 (91.7)
Fibrosis	
Stage 2	38 (63.3)
Stage 3	20 (33.3)
Stage 4	2 (0.4)

Values are expressed as mean±SD for continuous variables and number of subjects (%) for categorical variables.

4. Discussion

Our results suggest that diet-induced weight loss results in a significant reduction in liver steatosis (as assessed by CAP) and fibrosis (as assessed by LSM) as well as serum CK-18 levels. Moreover, changes in CK-18 levels over a 6-month period was significantly associated with changes in liver fibrosis. Furthermore, ΔCK-18 was positively correlated with ΔWrC and ΔDBP, and negatively correlated with ΔFM.

Currently, no previous studies have examined the effect of diet-induced weight loss on CK-18 levels. However, in agreement with our findings, several studies have shown a positive relationship

Table 2
Anthropometric and biochemical parameters at baseline and at 3 and 6 months after intervention.

	Baseline (n = 55)	3-month (n = 55)	6-month (n = 55)	P-value
Weight (kg)	90.9 ± 14.5	84.9 ± 14.3	83.3 ± 13.7	<0.001
BMI (kg/m^2)	31.9 ± 3.9	29.8 ± 3.9	28.7 ± 4.6	<0.001
FM (kg)	31.3 ± 10.0	26.7 ± 9.9	25.5 ± 12.0	<0.001
PBF (%)	34.3 ± 9.0	31.0 ± 9.3	29.5 ± 9.4	<0.001
TFM (kg)	17.4 ± 5.0	15.8 ± 10.8	13.4 ± 4.9	0.01
FFM (kg)	59.1 ± 12.0	58.3 ± 11.3	58.4 ± 11.4	0.006
TBW (L)	43.3 ± 9.3	42.7 ± 8.2	43.7 ± 10.2	0.2
WC (cm)	108.8 ± 9.7	102.5 ± 10.3	100.1 ± 10.1	<0.001
HC (cm)	111.9 ± 8.7	107.8 ± 9.4	106.6 ± 9.1	<0.001
CC (cm)	110.3 ± 7.1	107.3 ± 7.0	105.6 ± 7.2	<0.001
AC (cm)	34.5 ± 2.8	33.5 ± 2.6	33.2 ± 2.7	<0.001
WrC (cm)	17.1 ± 1.2	16.6 ± 1.1	16.5 ± 1.1	<0.001
SBP (mmHg)	123.5 ± 11.9	119.6 ± 11.4	119.3 ± 9.1	<0.001
DBP (mmHg)	86.0 ± 8.6	81.8 ± 9.4	81.3 ± 7.4	<0.001
FBG (mg/dl)	106.7 ± 32.5	99.7 ± 19.3	97.4 ± 22.7	0.03
LDL (mg/dl)	93.0 ± 35.6	98.5 ± 29.6	100.1 ± 26.5	0.4
HDL (mg/dl)	41.7 ± 8.3	46.8 ± 8.4	45.0 ± 6.5	<0.001
TC (mg/dl)	167.3 ± 38.6	171.5 ± 38.9	171.9 ± 35.1	0.5
TG (mg/dl)	128.3 ± 49.4	131.5 ± 50.6	133.1 ± 49.5	0.7

Values are expressed as mean±SD. BMI: body mass index; FM: fat mass; PBF: percent body fat; TFM: trunk fat mass; FFM: fat free mass; TBW: total body water; WC: waist circumference; HC: hip circumference; CC: chest circumference; AC: arm circumference; WrC: wrist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood pressure; LDL: low-density lipoprotein: HDL: high-density lipoprotein; TC: total cholesterol; TG: triglyceride.

Table 3
Comparison of disease severity, liver enzymes and cytokeratin-18 levels at baseline and at 3 and 6 months after intervention.

	Baseline (n = 55)	3-month (n = 55)	6-month (n = 55)	P-value
Steatosis (%)	76.5 ± 12.2	–	51.8 ± 24.4	<0.001
Fibrosis score	9.9 ± 3.7	–	7.2 ± 2.4	<0.001
Steatosis n(%)				
Grade 0	0	–	2 (3.6)	<0.001
Grade 1	0	–	11 (20.0)	
Grade 2	5 (9.0)	–	23 (41.8)	
Grade 3	50 (91.0)	–	19 (34.5)	
Fibrosis n(%)				
Stage 0	0	–	10 (18.2)	<0.001
Stage 1	0	–	25 (45.5)	
Stage 2	34 (61.9)	–	14 (25.5)	
Stage 3	19 (34.5)	–	5 (9.0)	
Stage 4	2 (3.6)	–	1 (1.8)	
ALT (U/L)	68.0 ± 23.5	25.7 ± 11.9	26.0 ± 13.8	<0.001
AST (U/L)	48.1 ± 26.5	23.7 ± 11.2	23.6 ± 8.0	<0.001
ALP (U/L)	170.9 ± 44.3	163.1 ± 45.7	167.5 ± 44.5	0.3
GGT (U/L)	36.0 ± 17.6	30.4 ± 14.1	30.7 ± 14.3	0.008
CK-18 (U/L)	290.2 ± 98.1	254.9 ± 62.0	217.6 ± 64.8	<0.001

Values are expressed as mean±SD for continuous variables and number of subjects (%) for categorical variables. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: γ-glutamyltransferase; CK-18: cytokeratin-18.

Table 4
Energy and dietary intake of study participants at baseline and at 3 and 6 months after intervention.

	Baseline (n = 55)	3-month (n = 55)	6-month (n = 55)	P-value
Energy (kcal)	2211 ± 503	1283 ± 285	1322 ± 256	<0.001
Carbohydrate (% of energy)	54.5 ± 10.4	54.5 ± 9.7	53.2 ± 9.0	0.6
Protein (% of energy)	13.8 ± 3.3	18.0 ± 4.6	17.5 ± 4.0	<0.001
Fat (% of energy)	31.7 ± 10.1	27.5 ± 8.7	29.3 ± 9.1	0.02
Carbohydrate (g)	298.4 ± 81.1	172.8 ± 39.3	174.3 ± 38.9	<0.001
Protein (g)	75.1 ± 20.4	57.4 ± 19.3	57.1 ± 14.4	<0.001
Fat (g)	79.6 ± 32.6	40.2 ± 17.9	44.0 ± 18.4	<0.001
Saturated fat (g)	20.5 ± 9.8	11.6 ± 4.9	12.6 ± 5.0	<0.001
PUFA (g)	27.6 ± 15.9	11.8 ± 7.2	12.6 ± 8.5	<0.001
MUFA (g)	22.7 ± 10.1	12.1 ± 7.1	13.8 ± 7.1	<0.001
Cholesterol (g)	187.9 ± 132.9	181.0 ± 172.9	182.4 ± 163.3	0.9
Total sugars (g)	52.0 ± 32.8	38.6 ± 17.0	41.3 ± 18.3	0.006
Glucose (g)	11.3 ± 10.4	7.9 ± 6.1	8.0 ± 6.3	0.04
Fructose (g)	12.2 ± 11.8	8.1 ± 6.6	8.5 ± 6.9	0.03
Lactose (g)	3.2 ± 5.0	4.1 ± 3.4	4.5 ± 4.0	0.1
Sucrose (g)	14.0 ± 20.7	7.2 ± 6.5	9.0 ± 5.1	0.03
Dietary fiber (g)	13.6 ± 4.3	13.3 ± 5.6	12.7 ± 5.3	0.6

Values are expressed as mean±SD. PUFA: poly-unsaturated fatty acid; MUFA: mono-unsaturated fatty acid.

Table 5
Correlations between the change (Δ) in CK-18 levels (baseline minus end of study) and delta values of anthropometric and biochemical parameters.

	Δ CK-18 (U/L)	P-value
Δ Steatosis (%)	0.20	0.1
Δ Fibrosis score	0.25	0.05
Δ ALT (U/L)	0.09	0.4
Δ AST (U/L)	0.02	0.8
Δ ALP (U/L)	0.09	0.5
Δ GGT (U/L)	0.12	0.3
Δ Weight (kg)	0.15	0.2
Δ BMI (kg/m^2)	0.10	0.4
Δ FM (kg)	−0.30	0.02
Δ PBF (%)	0.02	0.9
Δ FFM (kg)	0.09	0.5
Δ TBW (L)	0.12	0.1
Δ WC (cm)	0.09	0.5
Δ HC (cm)	0.01	0.9
Δ CC (cm)	0.18	0.2
Δ AC (cm)	0.11	0.4
Δ WrC (cm)	0.38	0.004
Δ SBP (mmHg)	0.08	0.5
Δ DBP (mmHg)	0.31	0.02
Δ FBG (mg/dl)	−0.05	0.7
Δ LDL (mg/dl)	0.11	0.4
Δ HDL (mg/dl)	−0.22	0.1
Δ TC (mg/dl)	0.02	0.8
Δ TG (mg/dl)	0.006	0.9

The correlation coefficients obtained from Pearson statistical test. ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; GGT: γ-glutamyltransferase; BMI: body mass index; FM: fat mass; PBF: percent body fat; TFM: trunk fat mass; FFM: fat free mass; TBW: total body water; WC: waist circumference; HC: hip circumference; CC: chest circumference; AC: arm circumference; WrC: wrist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; FBG: fasting blood pressure; LDL: low-density lipoprotein: HDL: high-density lipoprotein; TC: total cholesterol; TG: triglyceride; CK-18: cytokeratin-18.

between CK-18 levels and liver fibrosis [27–30]. In a study conducted on 99 patients, serum CK-18 levels were found to be significantly higher in subjects with moderate to severe fibrosis when compared with those who had no or mild fibrosis. Moreover, bariatric surgery resulted in a significant decrease in BMI and serum CK-18 levels in most patients 6 months after intervention [27]. A study on ninety-five patients with NAFLD (44 NASH and 51 non-NASH) showed that NASH patients have significantly higher serum CK-18 fragments. Additionally, CK-18 levels were positively correlated with liver histologic manifestations such as steatosis, lobular inflammation, and fibrosis [28]. In another study conducted on 83 patients with suspected NAFLD, serum levels of two soluble forms of extracellular CK-18, M30-antigen and M65-antigen showed the ability to distinguish between advanced fibrosis and early-stage fibrosis with a sensitivity of 64.7% and 70.6%, and a specificity of 77.3% and 71.2%, respectively [29]. Furthermore, Kobayashi and colleagues reported that CK-18 levels have good diagnostic ability not only for NASH overall, but also the form of the disease with mild fibrosis [30]. Considering the hepatocyte damage that occur during liver fibrosis, it is likely that the reduction in serum CK-18 levels is largely due to weight loss-induced improvement in liver fibrosis.

Several investigations have suggested that CK-18 is a non-invasive diagnostic and prognostic biomarker for NASH [12–16]. In a study by Arab et al., CK-18 levels were significantly higher in patients with NASH versus those without NASH, suggesting CK-18 as a good non-invasive marker for NASH [13]. Feldstein et al. also suggested that non-invasive monitoring of hepatocyte apoptosis is a reliable tool to diagnose NASH in patients with suspected NAFLD, supporting the potential usefulness of CK-18 in clinical practice as a non-invasive biomarker of NASH [12]. Likewise, Rahman et al. studied 40 patients with NAFLD and found the sensitivity and specificity for detection of NASH by CK-18 to be 85.7% and 80.8%, respectively [14]. In another study conducted on 147 patients diagnosed with NAFLD, Kawanaka et al. calculated a cut-off value of 375 U/L with a specificity and sensitivity of 81.5 and 65%, respectively, for the diagnosis of NASH [15]. Moreover, on a biopsy-proven cohort, Huang et al. reported that CK-18 is a non-invasive biomarker for prediction of disease severity in NASH patients [16]. Therefore, CK-18 can be considered as an ideal biomarker for either diagnosis of NASH or prediction of disease severity.

A positive but insignificant correlation was observed between ΔCK-18 levels and ΔBMI over the period of this trial. There are some conflicting results in the literature concerning the association between CK-18 levels and BMI [31–34]. Miyasato et al. evaluated the three-month change in the CK-18 concentration and found that ΔCK-18 was significantly associated with ΔBMI in type 2 diabetic patients with NAFLD [31]. Del Ben et al. also found a positive correlation between CK-18 and BMI and a negative correlation between CK-18 and HDL-C in 209 patients with NAFLD [32]. However, Zwolak and colleagues observed that serum concentrations of CK-18 fragments correlated with the severity of NAFLD, but not with obesity [33]. Similarly, a study conducted on 424 middle-aged subjects showed that plasma CK-18 is correlated with ALT, adipose tissue insulin resistance, steatosis, lobular inflammation, and fibrosis, but not with ballooning, BMI, metabolic syndrome or type 2 diabetes mellitus [34].

To our knowledge, this is the first trial to determine the effects of diet-induced weight loss on CK-18 levels in a sample of overweight and obese subjects with liver fibrosis. The trial was performed for 6 months in a before–after fashion. All anthropometric and biochemical parameters were measured at three different time points (at baseline, and at 3 month and 6 month after intervention). However, the uncontrolled nature of the study, small number of patients treated, and using transient elastography instead of liver biopsy are among limitations of the trial to be mentioned.

In conclusion, this trial showed a significant positive association between changes in CK-18 levels and changes in liver fibrosis over a 6-month dietary intervention. Every 1% reduction in liver fibrosis was accompanied by about 1% decrease in serum CK-18 levels. These findings suggest that CK-18 is a promising biomarker for detecting therapeutic responses in patients with liver fibrosis.

Declarations

Ethics approval and consent to participate

The study protocol, informed consent form and other study-related documents were reviewed and approved by the Human Research Ethics Committee of Mashhad University of Medical Sciences (MUMS). All patients were able to read and understand and were willing to sign the informed consent form.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Authors' contributions

Mojtaba Shafiee and Sepideh Mohammadpour collected the data and wrote the paper; Mohammad Safarian and Mohsen Nematy designed the study and supervised the data collection; Anvar Soleimani analyzed the data, Azita Ganji and Ali Bahari extensively reviewed and edited the manuscript; and all authors were involved in interpretation of results and revision of the manuscript and approved the final version of the manuscript.

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