Liver fat content might be an appropriate measure for estimation of cardiovascular disease risk in non-alcoholic steatohepatitis patients

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Abstract
Background: Non-alcoholic steatohepatitis (NASH) is increasing worldwide due to the metabolic syndrome epidemy. According to the current evidence, a higher cardiovascular disease risk (CVDR) is observed in NASH individuals than the general population.
Objective: The relationship between liver fat content (LFC) and CVDR in a cohort of NASH patients was evaluated in this research.
Methods: Consecutively selected patients with increased aminotransferase levels and fatty change in liver ultrasonography were enrolled in the study. Those with known causes of viral hepatitis, any hepatotoxic medications or alcohol consumption, autoimmune hepatitis, cigarette smoking, and ischemic heart disease were excluded from the project. The remaining was presumed to have NASH. The Framingham risk score (FRS) and LFC were calculated by means of an online calculator and a valid formula, respectively. The correlation between LFC and independent variables was measured using the Pearson correlation test. The P-value of less than 0.05 was considered significant. The statistical analysis was performed using SPSS program version 18.
Results: Finally, two hundred NASH patients were included in the study. Considering diabetes mellitus as a confounder, there was a fair relationship between LFC and FRS (R=0.26 and 0.23, respectively, p<0.05) in the second and third visits. Even after adjustment for known cardiovascular risk factors, LFC was associated with increased CVDR (OR=9.181; 95% CI: 2.00-42.14, p=0.01). The cut-off value of 9.1% for LFC had a sensitivity of 92% and a specificity of 87% for discrimination of the FRS >20% and <20%.
Conclusion: LFC might independently be correlated with CVDR in NASH patients. If further research confirmed this relationship, the inclusion of LFC into the FRS formula would provide an appropriate CVDR estimation tool in NASH.

Keywords: Non-alcoholic fatty liver disease, Cardiovascular disease, Fatty liver, Coronary artery disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) includes a range of liver cell damage. It consist of simple liver steatosis, nonalcoholic steatohepatitis (NASH*)*, andcirrhosis (1). The disease burden is growing rapidly due to the epidemic of obesity worldwide (2). Previous leading causes of end-stage liver diseases including viral hepatitis have been well controlled during the recent decades; meanwhile, NASH is the most common cause of cirrhosis and liver transplantation (3-6). NASH is already considered as the hepatic manifestation of insulin resistance syndrome

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↑What is "already known" in this topic:
A higher cardiovascular disease risk (CVDR) is observed in Non-alcoholic steatohepatitis (NASH) individuals than the general population. There is still a paucity of the literature regarding which metabolic index would appropriately estimate the CVDR in NASH patients.

→What this article adds:
This experiment evaluated the liver fat content (LFC) as the possible metabolic index to predict the CVDR accurately. During a short-term follow-up time, the observations showed that LFC might independently be correlated with CVDR in NASH patients.

(7). There is a growing body of evidence suggesting that NASH patients have a higher risk of cardiovascular events in comparison with the normal population. It is worth noting that cardiovascular events are the most causes of mortality and morbidity among NASH patients (8, 9).

There is already no clear guideline regarding cardiovascular disease risk (CVDR) assessment in NASH patients (10-12). It seems to be a reasonable approach to modify risk factors and control cardiovascular morbidity and mortality in such patients. Some studies have shown that NASH subjects have higher 10-year CVDR than individuals without NASH (13, 14). However, there is still a paucity of literature about the association of liver fat content (LFC) and CVDR in NASH. In line with a previous research, we used "NAFLD liver fat score" in this study to estimate the LFC (15). The aim of the current investigation is to assess the correlation between LFC and CVDR in a cohort of NASH subjects during a six-month follow up period.

Methods *Study design*

In view of NAFLD mean prevalence in earlier researches in Iran and using this formula: $N=(t/d)2*(1-p)/p$ (t=1.96, p=0.33, and d=0.2), a sample size of 194 subjects was considered in this project (16). In the first phase, consecutively selected patients with increased aminotransferase levels and fatty change in liver ultrasound were enrolled. They were referred to a general hospital gastroenterology clinic from September 2017 to September 2018. At the second phase, individuals with consumption of hepatotoxic medications during the last 6 months, alcohol use or any other known liver diseases, significant systemic comorbidities, cancer, cigarette smoking, and ischemic heart disease were excluded from the study. In the third phase, those with viral hepatitis, autoimmune hepatitis, Wilson disease and hemochromatosis were excluded (17). The remaining participants were presumed as NASH patients. The study measurements, including clinical and laboratory investigations, were performed at baseline, 3 months, and 6 months during the study period.

Evaluation of comorbidities

The ischemic heart disease risk factors and demographic information were evaluated by a questionnaire. Anthropometric indices and body mass index (BMI) were determined. Diabetes mellitus was defined based on the patient declaration, or if fasting blood glucose ≥126 mg/dl (checked twice), or HbA1c ≥ 6.5. Hypertension was defined if the patient notified himself, or the measured blood pressure was higher than 140/90 mmHg (17). Hyperlipidemia was diagnosed if serum fasting triglyceride levels were more than 150 mg/dl, or serum total cholesterol levels were more than 200 mg/dl, or if the patients already used lipid-lowering agents (18). Adult Treatment Panel III (ATPIII) criteria was used for defining metabolic syndrome (19).

Laboratory investigations

Blood samples were collected following a 12-hour overnight fasting. The laboratory measurements were done by ELISA method using Hitachi autoanalyzer 704 (Roche, Switzerland). The Pars Azmoon reagent Kits (Tehran, Iran) were applied for conducting the assessments. These observations were conducted according to the manufacturers' manual based on previous projects (20, 21). ALT levels of more than 40 U/L were considered abnormal according to Merck manual (22).

Framingham risk score

The Framingham risk score (FRS) is a tool for the estimation of 10-year CVDR (23, 24). This score is validated in different worldwide populations, including the Iranian population (25). We used the following web-based calculator to compute FRS in this study: (https://www.qxmd.com/calculate/calculator_252/framing ham-risk-score-2008). Patients with FRS >20% were considered as high-risk groups according to the American Heart Association (AHA) cardiovascular risk-assessment guidelines (23).

Liver fat content (LFC)

Based on large cohort studies with more than 10 years of follow-up, "liver fat score" is one of the best prediction scores for estimating cardiovascular and liver-related mortality (13). This score applies the metabolic syndrome and type 2 diabetes mellitus existence, fasting serum insulin and amino transaminases levels. The mentioned LFC formula seems to be an accurate instrument to detect mild to moderate steatosis (26).

Statistical analysis

The continuous variables were expressed as mean and standard deviation (SD), while the qualitative values are provided as frequencies. The correlation between LFC and independent variables was measured using the Pearson correlation test. Moreover, the relationship between LFC and the 10-year Framingham risk score was calculated by partial correlation considering diabetes mellitus status as a confounder. The LFC changes in different visits were analyzed using ANOVA. Multivariate logistic regression analysis was applied to discriminate which variables were independently related to CVD. Age, gender, waist circumference, HTN, DM and smoking were included in a multivariate linear regression analysis as potential confounders. The Odds ratios (ORs) and their 95% confidence intervals (CI) were defined for each independent variable. Finally, the LFC cut-off values were calculated for discriminating the "low risk" and "high risk" FRS groups, using receiver operating characteristic (ROC) analysis. SPSS program (version 18) was used for statistical analysis.

Ethical considerations

Ethics committee of Tehran University of Medical Sciences approved the current research protocol (No.1396.3404). All participants filled an informed written informed consent before enrollment in the project.

Results

Two hundred patients (50% male and 50% female) participated in the study with an age range of 40 to 65 years

Table 1. Patient charachteristics during the study period

Variable	First Visit		Second Visit		Third Visit		
	Mean±SD	Min-Max	Mean±SD	Min-Max	Mean±SD	Min-Max	p
Systolic Blood Pressure (mmHg)	140.2±5	130-160	121±7	110-140	121±7	110-150	< 0.001
Body Mass Index(Kg/m²)	32±4.9	18.6-48.9	31.7±4.8	18.3-48	27.8±5.9	6.4-449	< 0.001
Waist Circumference (cm)	118.2±13	88-149	114.8±11.7	87-146	113.0±11.9	87-144	< 0.001
Fasting Blood Sugar (mg/dL)	106.2±14.9	75-144	99.5±10.8	70-126	97.1±10.9	68-121	< 0.001
Fasting Serum Insulin (Miu/L)	20.6±5.7	10-32	19.4±5.1	10-29	19.0±5.2	9-30	< 0.001
Triglyceride (mg/dL)	164.3±93	43-531	130.5±66.1	40-410	126.8±60.9	43-400	< 0.001
Cholesterol (mg/dL)	182.3±36	107-387	172.6±29.1	114-326	170.0±28.5	112-324	< 0.001
Low Density Lipoprotein (mg/dL)	105.7±31.8	40-308	99.9±26.5	44-250	96.4±26.4	40-246	< 0.001
High Density Lipoprotein (mg/dL)	34.6±3.6	27-55	33.5±3.0	27-49	33.2±2.9	28-49	< 0.001
Aspartate Aminotransferase(U/L)	26.7±13.4	12-99	27.3±13.2	9-134	29.3±13.2	9-103	0.001
Alanine Aminotransferase (U/L)	52.7±51.7	10-345	44.8±36.9	9-325	43.8±32.8	12-248	< 0.001
Alkaline Phosphatase (U/L)	271.7±170.6	72-1184	239.4±126.9	34-796	221.5±127.0	60-956	< 0.001
Liver Fat Content (%)	12.9±6.5	2.1-43.8	11.9±5.4	2.5-28.0	11.9±5.6	2.5-40	0.001
Framingham Risk Score	33.2±16	3.8-78.1	24.4±13.0	3.0-69.6	24.4±13.1	2.9-69.8	< 0.001

Table 2. Partial correlation analysis between liver fat content and 10-Year Framingham risk score

Visit	Gender	R	p
First	Male	0.381	< 0.001
	Female	0.072	0.472
Second	Male	0.381	< 0.001
	Female	0.442	< 0.001
Third	Male	0.383	< 0.001
	Female	0.402	< 0.001

Table 3. Comparison of liver fat content in patients with Framingham risk score of > 20% and < 20% during the study period

	Visit	FRS ≥ 20% (Mean±SD)	FRS < 20% (Mean±SD)	p
Liver Fat Content	First	14.825±5.977	6.334±3.792	< 0.001
	Second	14.345±4.478	8.457±4.891	< 0.001
	Third	14.532±4.854	8.421±4.518	< 0.001

FRS: Framingham risk score

(mean±SD:51.6±2.7). The summary of clinical features, laboratory data, FRS, and LFC in the study population is shown in Table 1. The metabolic syndrome was found in 80% of participants. In particular, DM and/or hypertension were present in 75% of the population. The calculated 10-year CHD risk ≥ 20% in patients with NAFLD was 77.5%.

Correlation between LFC and FRS

Considering diabetes mellitus as a possible confounder and using a partial correlation test, no significant relationship was found between LFC and FRS in the first visit. Meanwhile, there was a fair relationship between the mentioned variables in the subsequent second and third visits (Table 2). The mean LFC was higher in high-risk group patients (FRS > 20%) than those with the low-risk group (FRS < 20%) in all visits (Table 3). In multivariate regression analysis, after adjustment for possible cardiovascular risk factors (DM, HTN, age, gender, waist circumference and smoking), LFC was found to be associated independently with an increased risk of cardiovascular diseases (OR=9.183; 95% CI: 2.001-42.143, p=0.011).

The best LFC cut off value for differentiating CVDR groups

TheLFC optimal cut-off value for discrimination of "low-risk" and "high-risk" FRS groups is illustrated in Figure 1. The LFC cut-off value of 9.1% had a sensitivity of 92% and a specificity of 87% for the discrimination

oflow and high CVDR groups (Fig. 2).

Discussion

This study evaluated the correlation between LFC and the Framingham 10-year CVDR score in a group of NASH outpatients visited in a referral hospital. The main
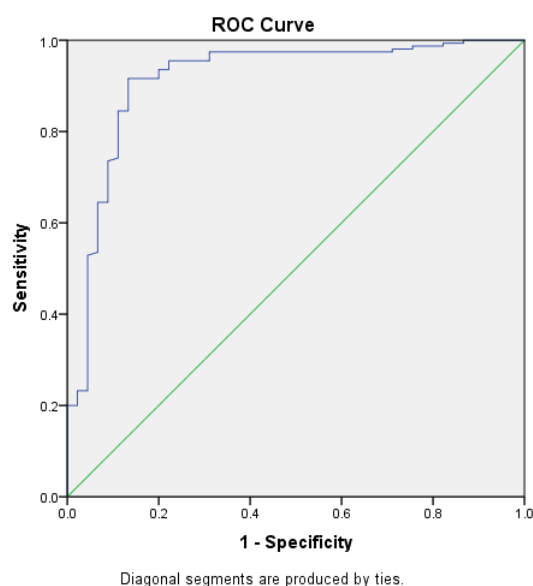

Fig. 1. Receiver operating characteristic (ROC) curve of liver fat content for prediction of high cardiovascular risk group. [Area Under Curve (AUC): 0.91 (0.86-0.97), p< 0.001].
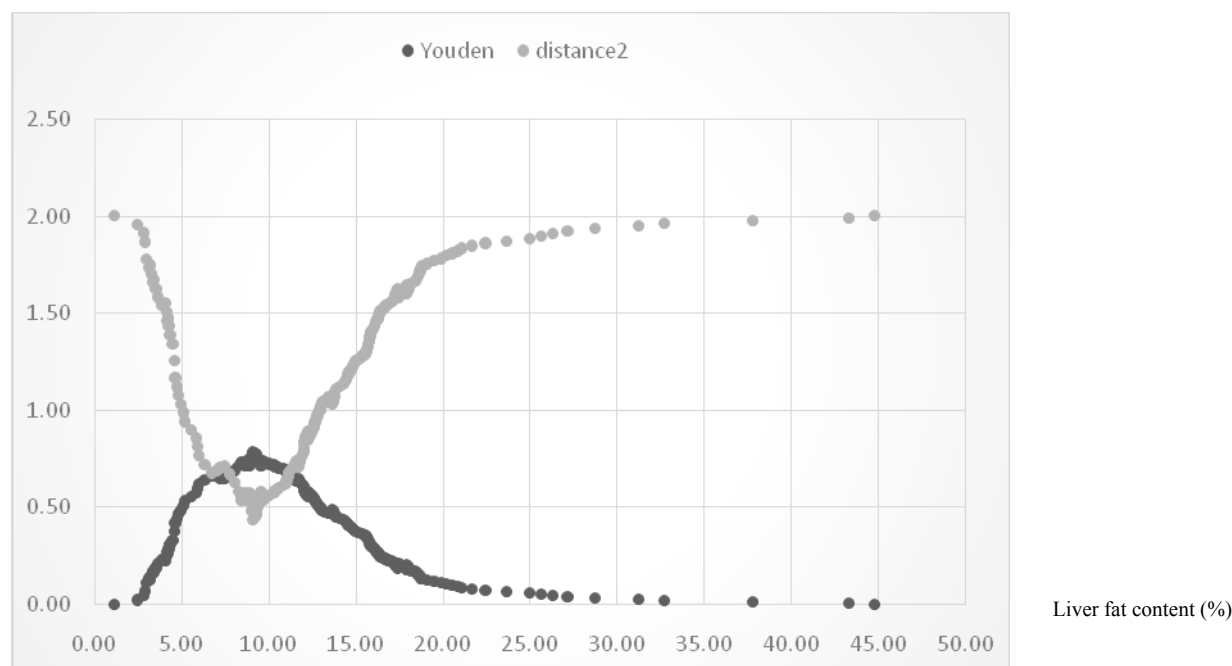
Fig. 2. The optimal cut point of liver fat content for differentiating high-risk cardiovascular disease from low-risk group (cutoff = 9.1%).

finding in this research was the association between LFC and increased risk of CVD in 6 months' follow up period. Our results are in parallel with the previous studies that suggested NASH as an independent risk factor for coronary artery disease and cardiac-related mortality in Asia and Europe (27-31). Meanwhile, a large prospective study of patients with the acute coronary syndrome concluded that NAFLD independently increased the risk of coronary artery disease (32). Moreover, another investigation demonstrated the association between FRS and NASH fibrosis score (33).

On the other hand, the Diabetes Heart Study suggested that fatty liver is less likely to be a direct mediator of CVD. It proposed that fatty liver is an epiphenomenon in metabolic syndrome (34). At the same time, a population-based study concluded that fatty liver is not a causal factor for the development of IHD (35).

The controversy in the result of the mentioned investigations might be encountered due to several reasons. First, we should pay attention to the study group characteristics' regarding CVD risk factors. A complete panel of IHD risk factors (including age, low physical activity, smoking, and components of metabolic syndrome) must be addressed in the methodology. The difference in checking these confounding variables describes the discrepancies in the results of correlation analysis. In addition, the method of LFC estimation might also be responsible for the diversity of previous research results.

LFC was independently associated with CVDR in our project after adjusting for the effect of diabetes mellitus as a possible confounding factor. Meanwhile, some explorations recommended that NASH could induce endothelial damage unrelated to CVDR factors (36, 37). Additionally, NASH may cause coronary artery calcifications, lead to an increase in carotid intima-media thickness, and generalized arterial stiffness (38). It was anticipated as a subclinical indicator of atherosclerosis, independent of other risk factors for CVD (39).

LFC decreased during the second and third visits in this study. This is probably due to lifestyle change, DM and HTN control. The management of co-morbidities (such as hypertension, dyslipidemia, and lifestyle change) is an effective method for NASH treatment (40).

Finally, the LFC cut-off value for differentiating between "low-risk" and "high-risk" FRS groups was calculated by ROC analysis. The LFC cut-off value of 9.1% had a sensitivity of 92% and a specificity of 87% for discrimination of low and high CVDR groups.

The strength of our study is the utilization of "Liver Fat Score", a highly specific and sensitive tool for LFC calculation. We also considered the FRS for the estimation of 10-year CVDR in our study. This score confirmed to be a strong model (C index of 0.83 in women and 0.79 in men) for predicting CVDR in a cohort of Iranian patients (25). This is in line with the previous exploration that emphasized FRS could accurately predict the 10-year CVDR in NASH subjects (41). We tried to include a complete panel of CVD risk factors to empower the accuracy of our correlation analysis outputs.

The first limitation of the study was the shortage of checking for storage diseases, primary sclerosing cholangitis, and α-1 antitrypsin, since their prevalence is too low to affect our results. The second limitation was the selection of a specific group of NAFLD patients with elevated aminotransferase levels, called as NASH. Therefore, the results could not be generalized to all NAFLD patients including those with normal aminotransferase levels, regarded as simple fatty liver. The third limitation of the

study was the short period of follow up. Finally, we could not evaluate the effect of LFC on CVDR due to the cross-sectional design of the study. Further, clinical trials regarding the response to this issue are recommended.If further research confirmed this relationship, the inclusion of LFC into the FRS formula would provide an appropriate CVDR estimation tool in NASH.

Conclusion

LFC might be correlated to CVDR in NASH patients independent of known CVD risk factors. To lower the CVDR in NASH patients, LFC reduction in accompany with other CVDR modification is recommended.

Acknowledgment

Conflict of Interests

The authors declare that they have no competing interests.

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