Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification

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Abstract

AIM: To compare noninvasive methods presently used for steatosis detection and quantification in nonalcoholic fatty liver disease (NAFLD).

METHODS: Cross-sectional study of subjects from the general population, a subgroup from the First Israeli National Health Survey, without excessive alcohol consumption or viral hepatitis. All subjects underwent anthropometric measurements and fasting blood tests. Evaluation of liver fat was performed using four noninvasive methods: the SteatoTest; the fatty liver index (FLI); regular abdominal ultrasound (AUS); and the hepatorenal ultrasound index (HRI). Two of the noninvasive methods have been validated vs liver biopsy and were considered as the reference methods: the HRI, the ratio between the median brightness level of the liver and right kidney cortex; and the SteatoTest, a biochemical surrogate marker of liver steatosis. The FLI is calculated by an algorithm based on triglycerides, body mass index, γ-glutamyl-transpeptidase and waist circumference, that has been validated only vs AUS. FLI < 30 rules out and FLI ≥ 60 rules in fatty liver.

RESULTS: Three hundred and thirty-eight volunteers met the inclusion and exclusion criteria and had valid tests. The prevalence rate of NAFLD was 31.1% according to AUS. The FLI was very strongly correlated with SteatoTest (r = 0.91, P < 0.001) and to a lesser but significant degree with HRI (r = 0.55, P < 0.001). HRI and SteatoTest were significantly correlated (r = 0.52, P < 0.001). The κ between diagnosis of fatty liver by SteatoTest (≥ S2) and by FLI (≥ 60) was 0.74, which represented good agreement. The sensitivity of FLI vs SteatoTest was 85.5%, specificity 92.6%, positive predictive value (PPV) 74.7%, and negative predictive value (NPV) 96.1%. Most subjects (84.2%) with FLI < 60 had S0 and none had S3-S4. The κ between diagnosis of fatty liver by HRI (≥ 1.5) and by FLI (≥ 60) was 0.43, which represented only moderate agreement. The sensitivity of FLI vs HRI was 86.3%, specificity 57.0%, PPV 57.0%, and NPV 86.1%. The diagnostic accuracy of FLI for steatosis > 5%, as predicted by SteatoTest, yielded an area under the receiver operating characteristic curve (AUROC) of 0.97 (95% CI: 0.95-0.98). The diagnostic accuracy of FLI for steatosis
> 5%, as predicted by HRI, yielded an AUROC of 0.82 (95% CI: 0.77-0.87). The $\kappa$ between diagnosis of fatty liver by AUS and by FLI ($\geq$ 60) was 0.48 for the entire sample. However, after exclusion of all subjects with an intermediate FLI score of 30-60, the $\kappa$ between diagnosis of fatty liver by AUS and by FLI either $\geq$ 60 or < 30 was 0.65, representing good agreement. Excluding all the subjects with an intermediate FLI score, the sensitivity of FLI was 80.3% and the specificity 87.3%. Only 8.5% of those with FLI < 30 had fatty liver on AUS, but 27.8% of those with FLI $\geq$ 60 had normal liver on AUS.

CONCLUSION: FLI has striking agreement with SteatoTest and moderate agreements with AUS or HRI. However, if intermediate values are excluded FLI has high diagnostic value vs AUS.

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Key words: Steatosis; Hepatorenal ultrasound index; SteatoTest; Fatty liver index; Screening; Agreement; Sensitivity; Specificity

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is emerging as a significant health burden raising serious clinical and public health concerns. Liver steatosis may predispose the liver to inflammation, fibrosis and eventually cirrhosis and hepatocellular carcinoma[1,2]. Furthermore, it is regarded as the most prevalent chronic liver disease affecting as much as 30% of the adult western population[3-5].

Besides the hepatic damage, in recent years NAFLD has emerged as an independent risk factor for type 2 diabetes and cardiovascular disease[6,7]. Therefore, efforts to prevent NAFLD progression and extrahepatic manifestations must include screening and surveillance strategies. The occult nature of the disease has led to increased efforts in achieving simple and cost-effective diagnostic methods, preferably quantitative, that would be useful for screening, follow-up and evaluation of response to treatment in both clinical practice and research.

Liver biopsy is the gold standard for quantification of liver steatosis in NAFLD[8]. However, it is not routinely performed because it is an invasive procedure with a significant degree of sampling error[9]. In addition, due to the high prevalence of NAFLD in the general population using routine liver biopsy to diagnose NAFLD is unreasonable. Therefore, noninvasive methods including imaging techniques and blood-test-based formulas have been developed to qualify and quantify liver steatosis[10-12]. However, a widely accepted examination that is easy to perform, accurate and inexpensive has yet to be found.

The aim of this study was to compare the fatty liver index (FLI)[13], which has been validated only vs abdominal ultrasound (AUS) with two different reference noninvasive methods for steatosis quantification that were validated vs liver biopsy: the hepatorenal ultrasound index (HRI)[14], and the SteatoTest, a biochemical surrogate marker of liver steatosis[15], providing together a more complete picture of construct validity. We also aimed to compare FLI with regular AUS in a qualitative manner.

MATERIALS AND METHODS

Study population and measurements

A cross-sectional study was performed during 2003-2004, consisting of 375 participants, a subgroup from the First Israeli National Health Survey[16] as described in detail elsewhere[17]. Exclusion criteria were: alcohol consumption $\geq$ 30 g/d in men or 20 g/d in women, presence of hepatitis B surface antigen or anti-hepatitis C virus antibodies, fatty liver suspected to be secondary to hepatotoxic drugs, inflammatory bowel disease, prior surgery that could cause fatty liver, or celiac disease. All patients underwent measurements of weight, height, and waist circumference according to uniform protocols. Blood samples were drawn following a 12-h fast, and tested for liver enzymes, serum lipid profile, and fasting serum glucose and serum insulin levels. Frozen serum samples from all participants were stored at -80 ℃ until the analysis of SteatoTest (BioPredictive, Paris, France). Biomarkers components were analyzed according to published recommendations[18]. A face-to-face interview was carried out with a questionnaire that was assembled by the Israeli Ministry of Health[19] and included demographic data, health status and a detailed questionnaire on alcohol intake.

The study protocol was approved by the institution's human research committee and all participants gave signed informed consent.

AUS for detection of fatty liver and liver fat quantification

Fatty liver was diagnosed qualitatively by AUS using standardized criteria[20]. Ultrasound was performed in all subjects with the same equipment (EUB-8500 scanner; Hitachi Medical Corporation, Tokyo, Japan) and by the same operator (Webb M) as described previously[20]. The radiologist was blinded to the results of the blood tests and the clinical background of the participants, and the calculation of steatosis biomarkers was performed only after the radiological examination.

Furthermore, during AUS, the same single radiologist performed steatosis quantification using the HRI. The HRI has been validated vs liver biopsy and is an objective operator-independent examination[14] (available in 331 subjects). As previously described in detail[14], during ultrasonography, a graphic representation of echo
Statistical analysis

Statistical analyses were performed using SPSS Version 17 (Chicago, IL, United States). Continuous variables are presented as mean ± SD. The Pearson correlation coefficient was used for continuous variables. To test differences in continuous variables between two groups the independent samples t test was performed. For nominal variables, the Pearson χ² test was performed. To test the predictive value of the methods, receiver operating characteristic (ROC) curves were performed with SteatoTest (≥ S2) or HRI (≥ 1.5) as the reference methods, and the area under the ROC (AUROC) curve was recorded. κ was calculated for evaluation of agreement between diagnosis of fatty liver by SteatoTest (≥ S2) or HRI (≥ 1.5) compared to FLI (≥ 60). κ values were interpreted by the following grades: very poor (0.00-0.20), poor (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80), and excellent (0.81-1.00) agreement. P < 0.05 was considered statistically significant.

RESULTS

Characteristics of the study population and comparison between subjects with FLI ≥ 60 and < 60

Three hundred and forty-nine volunteers met the inclusion criteria. Three hundred and forty serum samples were available for the SteatoTest (9 were either missing or hemolysed). Two subjects had a high risk of a false-positive FibroTest and thus were omitted from analysis, leaving a sample size of 338 subjects. Detailed information on the study population has been described elsewhere. The main relevant characteristics of the study sample and comparison between the subjects with FLI ≥ 60 and and < 60 are depicted in Table 1. Subjects with FLI ≥ 60 were older, had a higher percentage of men, higher BMI, and higher serum fasting levels of liver enzymes, glucose, triglycerides, insulin and ferritin.

Distribution of steatosis as predicted by different methods in the entire sample and by FLI

Applying AUS as the reference method, most subjects with FLI ≥ 60 had fatty liver on AUS, yielding a positive predictive value (PPV) of 72.2%. Most subjects with FLI < 60 had normal liver on AUS, yielding a negative predictive value (NPV) of 81.5%. Applying HRI as the reference method, only 57.0% (PPV) of subjects with FLI ≥ 60 had HRI ≥ 1.5, but 86.1% (NPV) of the subjects with FLI < 60 also had HRI < 1.5. Applying the SteatoTest as the reference method, most subjects with FLI ≥ 60 had a SteatoTest of ≥ S2, yielding a PPV of 74.7%, and the majority of those with FLI < 60 had a SteatoTest < S2, yielding an NPV of 96.1%. Most subjects (84.2%) with FLI < 60 had S0 and none had S3-S4 (Table 1).

Correlation between FLI and SteatoTest or HRI

FLI was very strongly correlated with SteatoTest (r = 0.91, P < 0.001) and to a lesser but significant degree with HRI (r = 0.55, P < 0.001) (Figure 2). HRI and SteatoTest were also significantly correlated (r = 0.52, P < 0.001).

Furthermore, when testing the distribution of SteatoTest by FLI categories, FLI value above or below 60 discriminated the SteatoTest values with no overlap between the box plots (interquartile range). Similarly, the HRI values were discriminated by FLI categories, but to
a lesser extent (Figure 3). The mean levels of SteatoTest and HRI were significantly different between FLI categories of above or below 60 (Table 1).

**Concordance between FLI and SteatoTest or HRI in diagnosis of steatosis**

The $\kappa$ between diagnosis of fatty liver by SteatoTest ($\geq S2$) and by FLI ($\geq 60$) was 0.74, which represented good agreement (Table 2). The sensitivity of FLI vs SteatoTest was 85.5% (59/69) and the specificity 92.6% (249/269). The $\kappa$ between diagnosis of fatty liver by HRI ($\geq 1.5$) and by FLI ($\geq 60$) was 0.43, which represented only moderate agreement (Table 2). The sensitivity of FLI vs HRI was 56.3% (45/80) and the specificity 86.5% (217/251).

![Figure 2](https://example.com/figure2.png)  
**Figure 2** Correlation of fatty liver index with the reference methods. A: SteatoTest; B: Hepatorenal ultrasound index (HRI). FLI: Fatty liver index.

### Table 1  Characteristics of the study sample, distribution of steatosis as predicted by different methods, and comparison between subjects with fatty liver index $\geq 60$ and $< 60$

| Characteristics/method | Range | Total ($n = 338$) | FLI $< 60$ ($n = 259$) | FLI $\geq 60$ ($n = 79$) | $P$ value |
|------------------------|-------|------------------|------------------------|------------------------|----------|
| Age (yr)               | 50.8 ± 10.4 | 50.0 ± 10.4 | 53.3 ± 10.0 | 0.01 |
| Male %                 | 53.0 | 48.3 | 68.4 | 0.002 |
| BMI (kg/m$^2$)         | 27.2 ± 4.4 | 25.6 ± 3.2 | 32.4 ± 3.9 | $< 0.001$ |
| ALT (U/L)              | 22.1 ± 9.6 | 20.6 ± 7.4 | 26.9 ± 13.5 | $< 0.001$ |
| AST (U/L)              | 23.0 ± 5.5 | 22.4 ± 4.8 | 24.7 ± 7.0 | 0.008 |
| GGT (U/L)              | 16.0 ± 12.1 | 13.9 ± 11.1 | 22.7 ± 12.7 | $< 0.001$ |
| Glucose (mg/dL)        | 1.3 ± 0.3 | 1.2 ± 0.3 | 1.6 ± 0.4 | $< 0.001$ |
| Insulin (μU/mL)        | 24.2 | 13.9 | 57.0 | $< 0.001$ |
| Triglycerides (mg/dL)  | 195 ± 10.0 | 195 ± 10.0 | 31.5 ± 12.3 | $< 0.001$ |
| Ferritin (ng/mL)       | 71.5 ± 5.3 | 64.6 ± 5.3 | 100.1 ± 6.7 | $< 0.001$ |
| FL on AUS % ($n = 338$) | 31.1 | 18.5 | 72.2 | $< 0.001$ |
| HRI ($n = 331$)        | 1.3 ± 0.4 | 1.2 ± 0.3 | 1.6 ± 0.4 | $< 0.001$ |
| SteatoTest ($n = 338$) | 0.3 ± 0.2 | 0.2 ± 0.1 | 0.6 ± 0.1 | $< 0.001$ |
| S $\geq 2$ (FL) %      | 20.4 | 3.9 | 74.7 | $< 0.001$ |
| SI %                   | 66.9 | 84.2 | 10.1 | $< 0.001$ |
| S1 %                   | 1-5 | 12.7 | 12.0 | 15.2 | 0.45 |
| S2 %                   | 6-33 | 13.0 | 3.9 | 43.0 | $< 0.001$ |
| S3-S4 %                | 34-100 | 7.4 | 0 | 31.6 | $< 0.001$ |
| FLI ($n = 338$)        | 36.7 ± 27.7 | 24.2 ± 17.1 | 77.8 ± 11.1 | $< 0.001$ |
| FLI $> 60$ (FL) %      | 23.4 | NA | NA | NA |
| FLI $< 30$ %           | 48.8 | NA | NA | NA |

Data are mean ± SD or proportion. BMI: Body mass index; ALT: Alanine amino transferase; AST: Abstract syntax tree; GGT: Gamma-glutamyl transpeptidase; FL: Fatty liver; FLI: Fatty liver index; AUS: Abdominal ultrasound; HRI: Hepatorenal ultrasound index; NA: Not available.

Concordance between FLI and regular AUS in diagnosis of steatosis

The $\kappa$ between diagnosis of fatty liver by AUS and by FLI ($\geq 60$) was 0.48 (data not shown). A validation study of FLI has suggested that FLI $< 30$ rules out and FLI $\geq 60$ rules in fatty liver$^{[13]}$, therefore, further analysis was performed after exclusion of all subjects with an intermediate FLI score of 30-60 (27.8%). The $\kappa$ between diagnosis of fatty liver by AUS and by FLI either $\geq 60$ or $< 30$ was 0.65, which represented good agreement (Table 2). The sensitivity of FLI was 80.3% (57/71) and the specificity 87.3% (151/173).

Only 8.5% of those with FLI $< 30$ had fatty liver on AUS, but 27.8% of those with FLI $\geq 60$ had normal liver on AUS.
Diagnostic accuracy of FLI for detection of steatosis > 5% in comparison to SteatoTest or HRI

The diagnostic accuracy of FLI for steatosis > 5%, as predicted by SteatoTest, yielded an AUROC of 0.97 (95% CI: 0.95-0.98). The diagnostic accuracy of FLI for steatosis > 5%, as predicted by HRI, yielded an AUROC of 0.82 (95% CI: 0.77-0.87) (Figure 4).

**DISCUSSION**

In view of the public health issue of the increasing prevalence of NAFLD and its hepatic and extrahepatic consequences, the development of simple cost-effective screening methods has become extremely important.

In the present study, the agreement between different potential noninvasive screening methods was evaluated. This is believed to be the first study to evaluate the agreement between FLI and SteatoTest and between FLI and quantitative ultrasound methodology (HRI).

We found a striking agreement between SteatoTest and FLI, which were very highly correlated. A less impressive but still high correlation was found between FLI and HRI. The $\kappa$ between diagnosis of fatty liver by SteatoTest and by FLI was 0.73, which represented good agreement. The $\kappa$ between diagnosis of fatty liver by HRI and by FLI was 0.44, which represented only moderate agreement.

Although evaluation and quantification of steatosis does not provide a complete reflection of severity of
NAFLD, such evaluation is important for several reasons. As much as 23% of patients with simple steatosis may still develop nonalcoholic steatohepatitis (NASH) and fibrosis progression, as demonstrated in a recent 3-year follow-up of NAFLD patients\[22]. Furthermore, recent literature indicates that NAFLD predicts the tendency to develop both diabetes mellitus\[23,24] and cardiovascular disease\[25-27]. Therefore, it is not surprising that patients with NAFLD have increased mortality and morbidity compared with the general population\[28,29]. Moreover, NAFLD patients seem to have diminished quality of life\[30], which is manifested by increased fatigue with impairment in physical function\[31] and over-representation of depressive and anxiety disorders\[32]. From the economic point of view, the healthcare costs were demonstrated to be significantly higher for individuals with NAFLD and increased serum ALT levels by 33%, controlling for BMI, lifestyle and comorbid conditions\[33].

The limitation of this study was that it had no liver biopsy as a gold standard because it could not be obtained in a population-based screening study for NAFLD. Therefore, no inference can be made as a criterion validity of the FLI. Two quantitative methods were developed and validated against liver biopsy: the HRI, a radiological method\[34], and the SteatoTest\[35] based on biochemical markers. Therefore, both methods were used as the best available reference for steatosis quantification, in the absence of biopsies, in the present population-based study. For that reason, the correlations presented here can only provide construct validity to the FLI. In fact, for a broader use of both SteatoTest and HRI, more validation studies including liver biopsy are warranted because only one has been performed for the HRI\[36] and two for the SteatoTest in liver disease patients\[37], and recently in patients with morbid obesity treated with bariatric surgery\[38]. The HRI has been used in very few studies so far\[35,39], probably because it requires special ultrasonographic equipment, and a dedicated ultrasonographer. In contrast, there have been more studies using the SteatoTest\[17,37-39] providing it with some construct validity.

The FLI is a continuous measure that has been validated against AUS for the qualitative detection of NAFLD and has never been validated as liver biopsy. However, the presence of quantitative reference methods in the current study has enabled the testing of FLI also in a quantitative manner. The FLI has recently been used as a surrogate for NAFLD in large epidemiological studies. In a large European cross-sectional population-based study, FLI was associated with insulin resistance, higher Framingham risk score, and increased intima-media thickness\[40]. More importantly, the predictive validity of FLI was demonstrated in two large cohorts. In the French general population cohort, FLI was an independent predictor for diabetes in a 9-year follow-up\[41], as would be expected from ultrasound-diagnosed NAFLD\[42]. In an Italian population cohort, after 15 years follow-up, FLI was independently associated with liver-related mortality\[43].

The commonest noninvasive method for the evaluation of fatty liver is AUS\[44,45]. AUS is the modern diagnostic test of choice for NAFLD in epidemiological surveys because it is noninvasive, safe, widely available, and with a reasonable sensitivity and specificity\[42,46,47]. In a recent meta-analysis, the overall sensitivity and specificity of ultrasound for the detection of moderate-severe fatty liver compared to histology were 84.8% and 93.6%, respectively\[48]. We demonstrated only moderate agreement between diagnosis of fatty liver by AUS and by FLI (≥ 60) (κ = 0.48), but after exclusion of all subjects with a intermediate FLI score of 30-60, κ increased to 0.65, representing good agreement, and the sensitivity and specificity of FLI were 80.3% and 87.3%, respectively. This however was at the cost of leaving almost 30% of the study population undiagnosed.

In summary, the present study provides construct validity to simple, inexpensive surrogate markers of NAFLD. FLI highly correlates and has good agreement with SteatoTest, perhaps because both are calculated measures based on overlapping parameters. FLI has moderate agreement with ultrasonographic methods; either regular AUS or HRI. These noninvasive diagnostic methods for liver steatosis should be further validated in different populations, preferably by criterion (vs liver histology) and predictive validity.

NAFLD has become one of the most important public health issues today. Although NASH is more relevant for the development of life-threatening liver disease, such as cirrhosis and hepatocellular carcinoma\[49-51], it has now become clear from population studies that steatosis is relevant for the development of extrahepatic life-threatening diseases\[52], such as diabetes\[53] and cardiovascular disease\[26,27]. Therefore, there is an urgent need for well-validated, quantitative, cost-effective, noninvasive methods for evaluation of steatosis in clinical practice, and epidemiological and clinical research when liver biopsy is not feasible.

**COMMENTS**

**Background**
Nonalcoholic fatty liver disease (NAFLD) is regarded as the most prevalent chronic liver disease affecting as much as 30% of the adult western population. Besides hepatic damage, in recent years, NAFLD has emerged as an independent risk factor for type 2 diabetes and cardiovascular disease.

**Research frontiers**
Liver biopsy is the gold standard for detection and quantification of liver steatosis in NAFLD. However, it is not routinely performed because it is an invasive procedure with a significant degree of sampling error. In addition, due to the high prevalence of NAFLD, using routine liver biopsy to diagnose or screen for NAFLD is unreasonable and also unethical in epidemiological population-based studies. Therefore, noninvasive methods including imaging techniques and blood-test-based formulas have been developed to qualify and quantify liver steatosis. However, a widely accepted examination that is easy to perform, accurate and inexpensive has yet to be found.

**Innovations and breakthroughs**
In view of the increasing prevalence of NAFLD and its hepatic and extrahepatic consequences, the development of simple cost-effective screening methods has become extremely important. In the present study, the agreement between different potential noninvasive screening methods was evaluated. This is be-
lied to be the first study to evaluate the agreement between fatty liver index (FLI) and two quantitative methods, the steatoTest and the hepaticorenal index (HRI), which have been validated so far only in one study.

Applications
Efforts to prevent NAFLD progression and extrahepatic manifestations must include screening and surveillance strategies. The present study provides validity to FLI, a simple, inexpensive surrogate marker of NAFLD. Validated, quantitative, cost-effective methods for evaluation of steatosis can help in repeated evaluation of treatment efficacy during follow-up in clinical practice and clinical trials. Furthermore, in large epidemiological population-based studies, when liver biopsy is not feasible, noninvasive methods may serve as an alternative. Knowing the agreement and disagreement between the different noninvasive methods would help in the interpretation of results from studies using different methods. Further validation of FLI in comparison with liver biopsy is still warranted.

Terminology
Steatosis is fatty infiltration of the liver, mainly triglycerides. Fatty liver is defined as steatosis exceeding 5%-10% of its weight. NAFLD may predispose the liver to inflammation, fibrosis and eventually cirrhosis and hepatocellular carcinoma. The HRI is a quantitative ultrasound methodology. The SteatoTest and the FLI are biochemical surrogate markers of liver steatosis based on calculated algorithms. All these are noninvasive methods presently used for steatosis detection and quantification.

Peer review
The subject of the article is of interest and importance. This was a good descriptive study in which the authors compared noninvasive methods presently used for steatosis detection and quantification in NAFLD. The results are interesting and suggest that FLI has striking agreement with SteatoTest and moderate agreements with AUS or HRI.

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