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ORIGINAL ARTICLE

The predictive value of the hepatorenal index for detection of impaired glucose metabolism in patients with non-alcoholic fatty liver disease

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Abstract

Background/Purpose Non-alcoholic fatty liver disease (NAFLD) patients are at increased risk of liver-related as well as cardiovascular mortality, including diabetes, coronary heart disease, and stroke, independently of traditional cardiovascular risk factors and metabolic syndrome. The aim of this study was to find out the predictive impact of hepatorenal index (HRI) in the detection of impaired glucose metabolism in asymptomatic NAFLD patients.

Methods B-mode ultrasound examinations were performed and ultrasound images from all 89 NAFLD patients aged 50.8 ± 10.1 years were analyzed by *echogenicity* analyzing software and HRI was acquired, and appropriate laboratory tests for liver, glucose, and lipid metabolism were undertaken.

Results The mean HRI was 1.345 ± 0.189. 23.59% of patients had mild NAFLD (HRI = 1.167 ± 0.041), 64.04% moderate (HRI = 1.401 ± 0.102), and 12.36% patients severe NAFLD (HRI = 1.802 ± 0.098). Impaired glucose metabolism was present in 48.31% of patients. A positive correlation was present between HRI and impaired glucose metabolism ($r = 0.335$, $p = 0.001$). The coefficients of determinations R^2 for linear regression for HRI and glycated hemoglobin (HbA1c) and oral glucose tolerance test (GTT) were 0.05841 and 0.07498, respectively. The cutoff values for HRI in the detection of diabetes and prediabetes, and prediabetes only, were 1.4 and 1.38, respectively. In logistic regression, the β coefficients for oral GTT, HbA1c, or HRI were 0.62042 ($p = 0.0002$), 2.18036 ($p = 0.0033$), and 2.36986 ($p = 0.012$). The hazard ratio (HR) coefficients (exp [b]) for HRI, HbA1c, and oral GTT sorted according to their HR strength were 10.6958, 8.8494, and 1.8597, respectively.

Conclusion Ultrasonographically acquired HRI has a significant predictive impact on the detection of prediabetes and diabetes in patients with NAFLD.

Keywords Diabetes · Hepatorenal index · Non-alcoholic fatty liver disease · Ultrasonography

Introduction

Non-alcoholic fatty liver disease (NAFLD) is emerging as a significant health burden raising serious clinical and public health concerns [1], from simple steatosis to more progressive disease with associated hepatitis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma (HCC). NAFLD is characterized by steatosis of the liver, involving greater than 5% of parenchyma, with no evidence of hepatocyte injury [2]. NAFLD patients are at increased risk of liver-related as well as cardiovascular mortality [3], including coronary heart disease and stroke, independently of traditional cardiovascular risk factors and metabolic syndrome [4, 5].

The diagnosis of NAFLD requires the existence of steatosis in the absence of significant alcohol consumption. Because of its high prevalence in the general population, using the routine liver biopsy to diagnose NAFLD is unreasonable [6]. NAFLD is now recognized as one of the most frequent reasons for liver

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Bullet points of the study highlights

What is already known?

- Nonalcoholic fatty liver disease (NAFLD) is a risk factor for the development of type 2 diabetes mellitus.The patients with NAFLD are more likely to have diabetes and metabolic syndrome compared to control healthy subjects.

What are new in this study?

- Detection of prediabetes and diabetes by comparison with liver and right kidney echogenicity called hepatorenal index (HRI) in asymptomatic patients previously diagnosed as NAFLD.

What are the future clinical and research implication of the study findings?

- HRI may be a simple, reliable, and cost-effective screening tool for prediction and detection of impaired glucose metabolism (prediabetes and diabetes) in patients with NAFLD.
- Further studies are needed evaluating the HRI as diagnostic marker for detection of diabetes and its risk when its value is greater than cut-off threshold of 1.4.

enzyme elevation without clinical symptoms. Insulin resistance is considered as having a central role in NAFLD pathogenesis [7]. Therefore, noninvasive methods including imaging techniques as B-mode ultrasound and blood-test-based formulae have been developed to qualify and quantify liver steatosis. Elevated gamma-glutamyl transferase (GGT) has also been reported as a marker of NAFLD [7, 8].

Bedside B-mode ultrasound has been evaluated as a noninvasive method of diagnosing NAFLD; there is a presence of characteristic sonographic findings: bright hepatic echoes, increased hepatorenal echogenicity, diffuse liver echogenicity, subcutaneous tissue thickness [8], and an enlarged liver with the vascular blurring of the portal and hepatic vein [9]. In the prospective study by Dasarathy et al. [10], real-time ultrasound was performed and followed by a liver biopsy to evaluate the accuracy of ultrasound in the detection of steatosis. The B-mode ultrasound technique was able to predict the presence of NAFLD with greater than 90% sensitivity when steatosis was greater than 20% fat on biopsy. Lower levels of fat content resulted in a reduction of sensitivity [10]. An advanced ultrasound imaging method for the detection of NAFLD is based not only on liver echogenicity. Ultrasonographicaly aquired the average liver brighthness was divided by the average kidney brightness to produce hepatorenal index (HRI). The HRI is a simple, reliable, and cost-effective screening tool for identifying patients who should not undergo liver biopsy for evaluation of steatosis [11].

The prevalence of NAFLD is associated with several risk factors such as obesity, metabolic syndrome, insulin resistance, and diabetes type 2 [12, 13]. An study conducted in Italy with an aim to show the prevalence of NAFLD in type 2 diabetes mellitus gave results which indicated 70% prevalence of NAFLD in type 2 diabetes mellitus patients [14–16]. Beyond that, it is highlighted that the prevalence differed depending on the presence of risk factors such as increased body mass index (BMI), lipid profile, glycated hemoglobin (HbA1c), aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

The aim of this study was to find out the predictive impact of HRI in the detection of impaired glucose metabolism (prediabetes and diabetes) in asymptomatic patients previously diagnosed with NAFLD by ultrasonography.

Methods

Patients

From April 2018 to July 2018, eighty-nine patients (55 male) with the mean age of 50.8 ± 10.1 years and mean BMI of 26.44 ± 4.04 m/kg^2 were ultrasonographically examined and appropriate laboratory tests were performed. More than three hundred consecutive patients with an ultrasound diagnosis of steatosis were selected. Of them, only 89 patients were eligible for entry because they never consumed alcohol and all of them had thicker subcutaneous tissue, with a mean measurement greater than 25 mm ($D > 25$ mm) [8]. Distance D (thickness of subcutaneous tissue) was ultrasonographically measured between the skin and the liver surface and was labeled as the subcutaneous tissue thickness. The patients who were diagnosed earlier as having or medically treated for diabetes mellitus, severe heart disease, kidney failure, or other chronic liver disease, were excluded.

Assessment

Ultrasonography examination

B-mode sonographic examinations were performed with a high-resolution ultrasound machine General Electric Logiq pro 5 (GE Medical Systems, Milwaukee, WI 53215, USA) with convex array 2–5 MHz multi-frequency ultrasound probe GE 3.5C.

Image analysis

All images that have been acquired during the ultrasound examination were imported in JPG (Joint Photographic Experts Group) format. Hepatic (region of interest [ROI] marked with 1) and renal (ROI marked with 2) parenchymal echogenic density on a grayscale (values 0–255) was recorded (Fig. 1). Typically, zero value was taken to be black, and 255 value was taken to be white. The images were analyzed by freeware software from the National Institutes of Health (Bethesda, Maryland, USA, https://imagej.nih.gov/ij) called ImageJ. We used ROI within the liver and kidney on the same image (in the same focus, same gain, same depth of field, and same TGC [time gain compensation]) to provide an average brightness value. Also, Marshall et al. (2012) used the same technique which relied on the same software [11].

The liver ROI can only include homogeneous echotexture (Fig. 1) and could not include large duct or vessels and masses or cysts. Figure 1 present B-mode sonogram of the liver and the right kidney in a 66-year-old man with moderate fatty liver disease which gave graphic presentations of two ROI and calculation of HRI by the ImageJ software. The area of the ROI in the liver and in the kidney was 552 Voxels (unit of graphic information that defines a point in 3D). This area is large enough to calculate an average brightness value and small enough to avoid inclusion of vessels, bile ducts, or outside the structure in thin kidney parenchyma [11, 14].

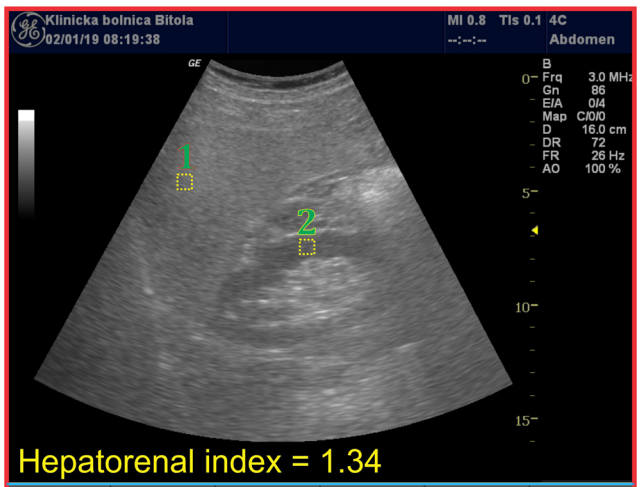
The average liver brightness (115.63 ± 7.54) was divided by the average kidney brightness (86.22 ± 7.95) to calculate the HRI equal to 1.34. Acquiring of ROI and calculations were repeated at 4 different levels in the liver and kidney tissue. The final measured HRI was calculated by the average of these 4 HRI values. Three independent experienced ultrasound examiners performed ultrasound examination and image analysis yielded a Cohen's kappa coefficient (κ) of 0.878, which represents an excellent degree of agreement. HRI is a tool of quantifying the steatosis that is more reliable than subjective assessment alone. In a normal liver, HRI is in the range from 1.00 to 1.04. Liver steatosis was classified according to HRI values as mild (HRI = 1.05–1.24), moderate (HRI = 1.25–1.64), or severe (HRI ≥ 1.65) [17, 18].

Clinical and biochemical parameters

Clinical and biochemical parameters (HbA1c, oral glucose tolerance test [GTT]), fasting glucose, insulin, ALT, AST, GGT, total cholesterol, and triglycerides were determined in all participants using standard laboratory procedures performed on a Cobas Mira S Analyzer (Roche Diagnostics, Holliston, MA, USA) in a fasting state.

Statistical analysis

The data were analyzed using MedCalc for Windows, 18.11.6 (MedCalc Software, Ostend, Belgium). The results were presented as mean ± standard deviation (SD), range, or percentage (%). Student's *t* test for unpaired data was used to compare the patients among each of NAFLD subgroups, according to HRI. Bivariate two-tailed Pearson's correlations were calculated to explore the relationships between HRI and other variables, as appropriate. Linear regression analysis was performed and scatter plots were created to show associations between dependent and independent variables. Diagnostic test to make distinctions between NAFLD patients with or without impaired glucose metabolism was assessed by receiver operating characteristic (ROC) curve analysis and appropriate cut-off values. We used binomial logistic regression to identify the independent determinants of impaired glucose metabolism.



	Voxels	Mean	± SD	Min	Max	Mode
1	552	115.63	± 7.54	95	138	120
2	552	86.22	± 7.95	69	110	88

Fig. 1 Analysis of a sonogram by ImageJ software and calculation of the hepatorenal index

Results

Demographic characteristics

During the 4-month period, B-mode ultrasonography and appropriate laboratory test were successfully conducted on 89 patients from general population aged 50.8 ± 10.1 years (range 28–72) with BMI of 26.44 ± 4.04 m/kg^2 (range 18.55–36.06). The demographic and clinical characteristics of the patients are presented in Table 1.

We detected in 21 patients (23.59%) mild NAFLD with a mean HRI = 1.167 ± 0.041 (one patient was prediabetic [4.76%] and 2 patients [9.52%] were diabetic); 57 patients (64.04%) had moderate NAFLD with mean HRI = 1.401 ± 0.102 (23 patients were prediabetic [40.35%] 6 of whom were diabetic [10.53%]; 11 patients [12.36%] had severe NAFLD with mean HRI = 1.802 ± 0.098; (2 patients [18.11%] were prediabetic and 9 [81.81%] patients were diabetic). Therefore, 43/89 patients with NAFLD showed impaired metabolism of diabetes (48.31%). There was a significant difference between the patients with impaired glucose metabolism in mild and severe NAFLD ($p < 0.0001$) and between the patients with moderate and severe NAFLD ($p = 0.004$) according HbA1c and oral GTT, but not between mild and moderate NAFLD ($p = 0.126$). In 17 NAFLD patients with normal value of average BMI = 22.42 kg/m^2, we found mild steatosis (HRI = 1.235). Eight of them had impaired glucose metabolism (prediabetes) with average oral GTT = 7.38 ± 2.01 mmol/L and HbA1c = 5.57 ± 0.47%. We used three colors (pink for mild, blue for moderate, and yellow for severe) to present box plot of the mean for the three different groups of NAFLD according to HRI (Fig. 2).

Pearson's correlation

We found (by bivariate Pearson correlation) a significant positive correlation between the following: age and BMI ($p = 0.232$, $p = 0.029$), HRI and impaired glucose metabolism ($r = 0.335$, $p = 0.001$), HRI and oral GTT ($r = 0.274$, $p = 0.009$), HRI and BMI ($r = 0.302$, $p = 0.004$), and HRI and HbA1c ($r = 0.242$, $p = 0.022$); but not significant correlation between HRI and insulinemia ($r = 0.188$, $p = 0.078$) and HRI and age (0.125, $p = 0.243$) and between other variables. There was no significant correlation between HRI and other variables.

Linear regression and scatter plots

The data from each of the 89 NAFLD patients was displayed as a collection of blue circles determining HRI and HbA1c or oral GTT. Each point (blue circle) had the value of one variable (HbA1c or oral GTT determining the position of the x [horizontal axis] and the value of the other variables [HRI] determining the position on the y [vertical axis]). Figure 3 shows scatter plots of linear regression analysis between HRI and HbA1c and linear regression analysis between HRI and oral GTT.

The results of linear regression analysis represent the relationship between a scalar dependent variable Y (HRI) and an explanatory variable denoted X (HbA1c [%] or oral GTT [mmol/L]). The regression parameter ($b_0 = 0.8018$ for HbA1c and $b_0 = 1.1256$ for oral GTT) showed the expected theoretical value of HRI in case that HbA1c or oral GTT would have value equal to zero. The regression parameter b_1 (0.09506 for HbA1c and 0.0267 for oral GTT) signified that with each increase of unit (% or mmol/L) in HbA1c and oral GTT (respectively), the HRI score increased by 0.09506 or 0.0267, respectively, for HbA1c or oral GTT.

There was a positive correlations between HRI and HbA1c (showed by thick red line, Fig. 3) and HRI and oral GTT (showed by thick red line, Fig. 3). The statistical significance and the ascendency of the linear regression line are more pronounced for oral GTT than HbA1c variable ($p = 0.0094$ vs. $p = 0.0225$). The 95% confidence intervals (CI) of intercept (0.3294 to 1.2743 for HbA1c and 0.9519 to 1.2994 for oral GTT) are shown by the green dashed lines, and the prediction interval is shown by the orange dashed lines. There was a positive correlation between HRI and HbA1c ($r = 0.242$, $R^2 = 0.05841$, $p = 0.0225$) and between HRI and oral GTT ($r = 0.274$, $R^2 = 0.07498$, $p = 0.0094$).

Table 1 Demographic and clinical characteristics of the patients

Characteristics	Values
Age (years)	50.8 ± 10.1
Height (cm)	174.8 ± 10.3
Weight (kg)	80.9 ± 14.6
Body mass index (kg/m^2)	26.44 ± 4.03
Hypertension (n [%])	21 (23.59)
Smokers (n [%])	28 (31.46)
Hepatorenal index	1.345 ± 0.189
Glycated hemoglobin (%)	5.8 ± 0.5
Glucose tolerance test (mmol/L)	8.4 ± 1.9
Glucose fasting (mmol/L)	5.69 ± 0.56
Insulinemia (pmol/L)	111.5 ± 36.5
ALT (U/L)	59.7 ± 33.7
AST (U/L)	48.5 ± 26.6
GGT (U/L)	32.4 ± 16.2
Cholesterol (mmol/L)	5.7 ± 1.1
Triglycerides (mmol/L)	2.3 ± 1.3

Values are presented as mean ± SD or (n [%])

ALT alanine aminotransferase *AST* aspartate aminotransferase *GGT* gamma-glutamyltransferase

The coefficient of determination R^2 (0.05841) showed that 5.841% of the total variability was explained with the linear regression between HRI and HbA1c (%) and that 5.841% from HRI was dependent of HbA1c. Only 5.841% of the changes in HRI were the results of HbA1c value changes, and the remainder of the total variability between these was not explained (94.159% of HRI was dependent on other factors, which were not covered in the regression model).

The coefficient of determination R^2 (0.07498) showed that 7.498% of the total variability was explained by the linear regression between HRI and oral GTT (mmol/L) or that 7.498% from HRI was dependent on an oral GTT. Only 7.498% of the changes in HRI were the results of oral GTT value changes, and the remainder of the total variability between these was not explained (92.502% of HRI was dependent on other factors, which were not covered in the regression model).

Estimation of the cutoff point

We used discriminative ability of the model (estimation of cutoff point) to distinguish between NAFLD patients with or without impaired glucose metabolism (prediabetes and diabetes). We assessed these by ROC curve analysis, a statistical tool for diagnostic test evaluation. The comparative images of two ROC curves for classification variables (diabetes and prediabetes [blue curve] and prediabetes [red curve]) and HRI as a marker for detection of impaired glucose metabolism is shown in Fig. 4.

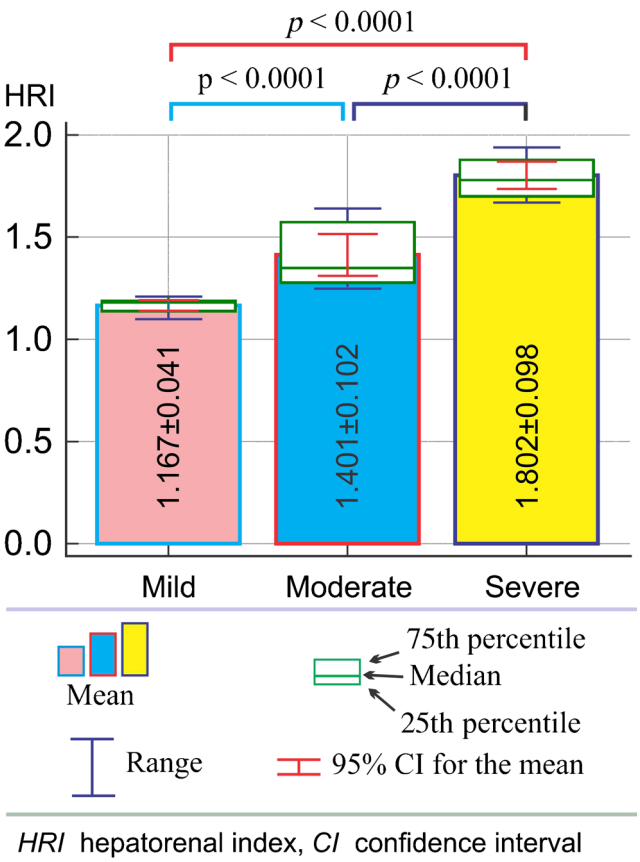


Fig. 2 Box and whisker plot of the mean, range, median, and 25th and 75th percentiles for mild, moderate, and severe non-alcoholic fatty liver disease according to hepatorenal index

Each point on the ROC curve (green circles on a blue curve) represents a sensitivity/specificity pair corresponding to a particular decision threshold (HRI in detection of diabetes and prediabetes). Each point on the ROC curve (yellow squares on a red curve) represents a sensitivity/specificity pair corresponding to a particular decision threshold (HRI in the detection of diabetes and prediabetes).

The cutoff values, which correspond to the respective values for maximal sensitivity/specificity pair, were HRI = 1.4 (Youden index $J = 0.3872$) for classification variables "diabetes and prediabetes" and HRI = 1.38 (Youden index $J = 0.3668$) for classification variable "prediabetes" which are presented by yellow circle and red square, respectively, in each ROC curves. The J index is the maximum vertical distance between the ROC curve and the diagonal line.

The "prediabetes and diabetes" J index is bigger than the J index of another classification variable "diabetes" (0.3872 vs. 0.3668) which means a more predictable value of classification variable "prediabetes and prediabetes" ($p = 0.0055$) than "prediabetes" ($p = 0.0010$) as classification variable, alone.

The summary image of the three ROC curves for HbA1c, oral GTT, and HRI as a marker for the detection of impaired glucose metabolism (prediabetes) is shown in Fig. 5.

The most powerful predictive value for the detection of prediabetes ($n = 26$) was seen on HRI (AUC = 0.699, $p = 0.001$). The statistical data for markers for detection of diabetes were HbA1c (Youden index 0.195, associate criterion > 5.4, sensitivity = 88.89%, specificity 30.65%, $p = 0.340$); oral GTT (Youden index 0.5615, associate criterion > 7.7, sensitivity = 85.19%, specificity 70.97%, $p = 0.004$); and HRI (Youden index 0.3668, associate criterion > 1.38, sensitivity = 59.26%, specificity 77.42%, $p = 0.001$). We did not find statistically significant predictable value for detection of prediabetes in HbA1c as potentially predictable markers ($p = 0.340$). Pairwise comparison of ROC curves showed high statistical significance between ROC curves for HbA1c and oral GTT ($p = 0.008$) and HbA1c and HRI (0.047). There was no statistically significant difference between ROC curves for oral GTT and HRI (0.744).

The most powerful predictive value for the detection of diabetes ($n = 17$) was seen on oral GTT (AUC = 0.987, $p < 0.0001$) and HbA1c (AUC = 0.954, $p < 0.0001$), but not HRI (AUC = 0.536, $p = 0.728$).

Logistic regression

We used *binomial logistic regression model* because the dependent variable "impaired glucose metabolism" (diabetes and prediabetes) is categorical variable. This model was used

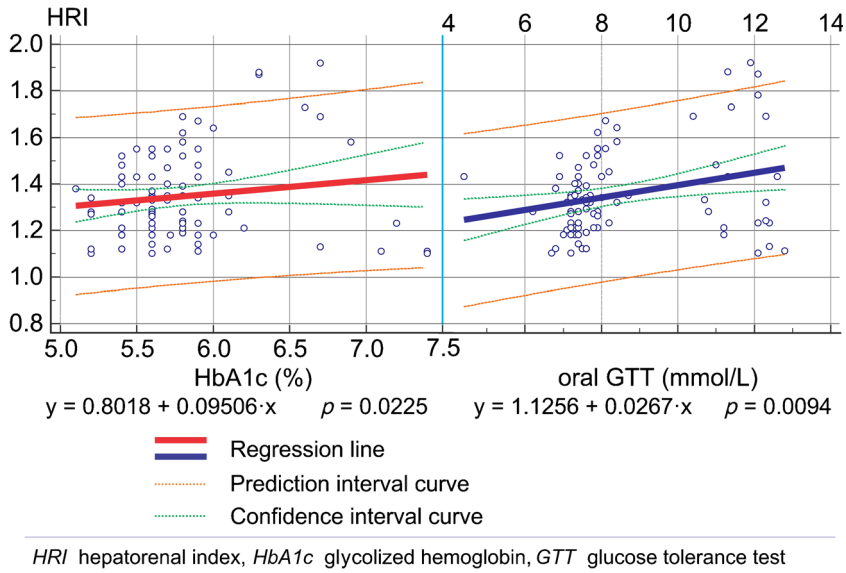


Fig. 3 Comparative linear regression analysis and scatter plots for hepatorenal index and markers of glucose metabolism: glycated hemoglobin, oral glucose tolerance test

to identify the independent determinants of impaired glucose metabolism. The coefficients and standard error parameters (β coefficient, standard error, and Wald and *p*-value) are presented in Table 2. The Wald test (Wald Chi-squared test) is a way to find out if explanatory variables (HRI, oral GTT, and HbA1c) in a model are significant. The Wald values and *p*-values reject the null hypothesis that says: "There is no statistical significance depending on the prevalence of diabetes and prediabetes (as a categorically dependent variable) of HRI and oral GTT (as the continuous independent variable)."

The β coefficient implies that a one unit change in the variables (oral GTT, HbA1c, or HRI) results in a changed (0.62042, 2.18036, and 2.36986, respectively) in the log of the odds. The hazard ratio (HR) coefficients (exp [*b*]) for HRI, HbA1c, and oral GTT, sorted according to their HR strength, were 10.6958, 8.8494, and 1.8597, respectively.

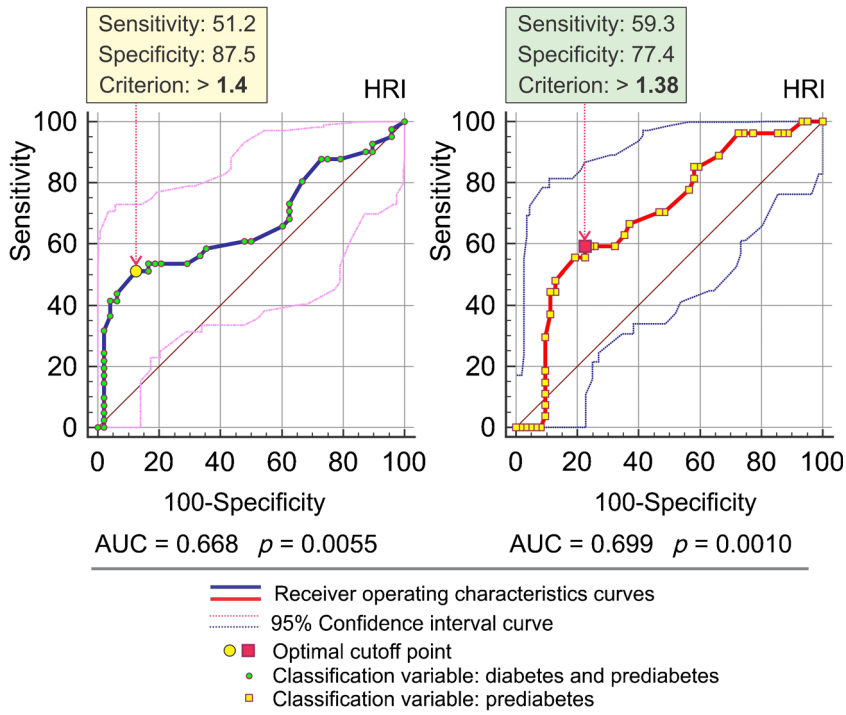


Fig. 4 Receiver operating characteristics curves for the hepatorenal index as a marker for detection of impaired glucose metabolism (prediabetes and diabetes). *ROC* receiver operating characteristics, *HRI* hepatorenal index, *AUC* area under the curve

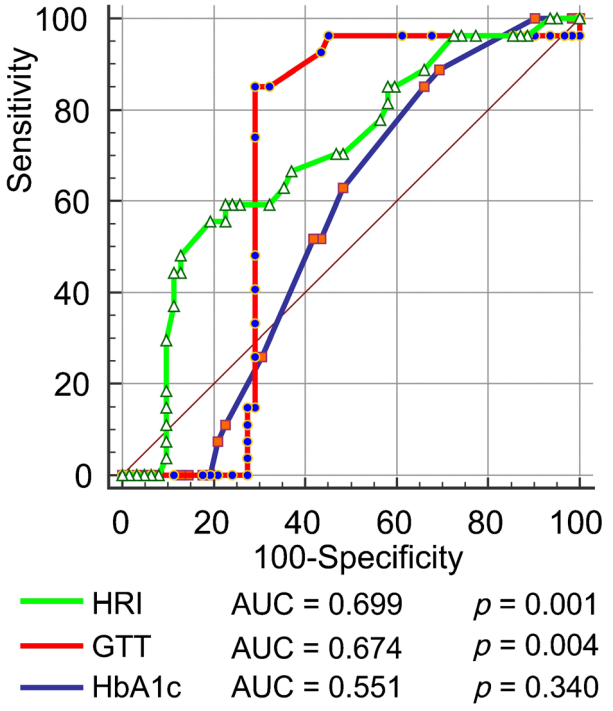


Fig. 5 Receiver operating characteristics curves comparison for glycated hemoglobin, oral glucose tolerance test and hepatorenal index as markers for detection of prediabetes

Discussion

In this prospective longitudinal study, we ultrasonographically examined and laboratory-tested eighty-nine patients with a diagnosis of NAFLD according to appropriate criteria for inclusion. All ultrasound images were analyzed by *echogenicity* analyzing software and HRI was acquired. The purpose of this study was to find the predictive impact of HRI in the detection of impaired glucose metabolism in asymptomatic patients with NAFLD.

According to previously established criteria for sonographic quantification of liver steatosis [8], we divided the cohort into three subgroups: mild NAFLD, moderate NAFLD, and severe NAFLD. Among these three subgroups with a different stage of NAFLD and appropriately different HRI, there was a significant difference between the patients with impaired glucose metabolism in mild and severe NAFLD, and between the patients with moderate and severe NAFLD, according to glycated hemoglobin and oral GTT. There was no statistically significant difference in glycated hemoglobin and oral GTT between mild and moderate NAFLD.

Due to the relatively high percentage (48.31%) of NAFLD patients with impaired glucose metabolism, we examined and proved a significant positive correlation between glucose metabolism markers (oral GTT and HbA1c) and ultrasound indicator for NAFLD (HRI). The correlation between diabetes mellitus and HRI in patients with NAFLD, metabolic syndrome, and insulin resistance was previously investigated and has been proven as positive in other studies [19–21], very close to our results. The positive correlation between HRI and BMI in our study is also confirmed by the results of other studies [22–24]. They found that risk for NAFLD is 50% higher in BMI > 30 kg/m^2 and approximately double in those with diabetes. The results in our study showed that NAFLD has occurred even in patients with normal BMI (< 25 kg/m^2). They are correlated with results of a study [23] which conclude that NAFLD may develop even in individuals with normal BMI too. The increased BMI is one of the strong risk factors, which causes fatty liver, that further progress to NAFLD. Patients with NAFLD are commonly insulin-

Table 2 Logistic regression of categorical dependent variable (diabetes and prediabetes) in dependency of hepatorenal index, oral glucose tolerance test, and glycated hemoglobin

Logistic regression					
Dependent	Impaired glucose metabolism (diabetes and prediabetes)				
Method	Backward, positive cases 43 (48.31%), negative cases 46 (51.68%)				
Coefficients and standard errors					
Variable	β coefficient	Std. error	Wald	*p*	Exp (*b*)
Oral GTT	0.62042	0.16911	13.4599	0.0002	1.8597
HbA1c	2.18036	0.74146	8.6474	0.0033	8.8494
HRI	2.36986	0.94338	6.3106	0.012	10.6958
Constant	− 16.041				
Full model − 2 log likelihood	86.949				
Cox and Snell R2	0.3318	Significance level $p < 0.0001$			
Area under the receiver operating characteristics curve			0.857		

HRI hepatorenal index, *GTT* glucose tolerance test, *HbA1c* glycated hemoglobin

resistant. On the other hand, a large number of patients with impaired glucose metabolism and type 2 diabetes mellitus develop NAFLD with its inflammatory complication, non-alcoholic steatohepatitis (NASH) [24]. Early ultrasound detection of liver steatosis and its gradation by HRI can prevent the development of NAFLD and NASH in patients with impaired glucose metabolism to further complications, such as liver cirrhosis and HCC, which are increasingly recognized [25, 26].

There is a clear association between diabetes and NAFLD. Studies over recent years have shown that NAFLD predicts the development of diabetes and vice versa [27] and that each condition serves as a progression factor for the other [27, 28]. NAFLD and type 2 diabetes share multiple cardiometabolic risk factors and proinflammatory pathophysiological pathways which suggest that there is a bidirectional relationship between NAFLD and type 2 diabetes and that NAFLD may precede and/or promote the development of type 2 diabetes [28].

We found by linear regression analysis a positive association between diabetes (variables: HbA1c and oral GTT) and NAFLD (variable: HRI). Based on regression parameter b_1 for HbA1c and oral GTT, we estimated that HRI index increased by 0.09506 with each increase of unit (%) in HbA1c, as well as that HRI index increased by 0.0267 with each increase of unit (mmol/L) in oral GTT (according to linear regression equation). Based on the coefficient of determination (R^2), we measured that 5.841% of the changes in HRI were the results of HbA1c value changes, and 7.498% from HRI changes were dependent of an oral GTT. In approaching the relationship between NAFLD (diagnosed by HRI) and diabetes (diagnosed by HbA1c and oral GTT) from another perspective, multiple studies supported by a meta-analysis have shown that NAFLD is associated with impaired glucose metabolism (prediabetes and diabetes) and that the presence of NAFLD presence predicts the development of diabetes [27, 29].

NAFLD prevalence is increased in patients with diabetes mellitus. On the other hand, patients with NAFLD have an elevated prevalence of prediabetes and type 2 diabetes [27, 28, 30]. There is a high prevalence of diabetes among NAFLD patients in our study, and 48.31% of patients showed impaired metabolism of glucose (prediabetes 29.2% and diabetes 19.1%). The prevalence of impaired glucose metabolism in other studies [31, 32] varied between 45% and 75% according to degree of NAFLD and it is similar to the results of our study. Consistent with this finding, newly diagnosed prediabetes was considerably more common in patients with NAFLD than in those without NAFLD (up to 75% vs. up to 25%, $p < 0.001$) [31].

We assessed, by ROC curves, a diagnostic test to evaluate the HRI as a marker for detection of impaired glucose metabolism. Any increase of the HRI over 1.38 increases the sensitivity and specificity of the HRI as a diagnostic test for the detection of prediabetes and diabetes, and the cutoff value of 1.40 is a threshold value for detection of diabetes. Isaksen et al. (2016) provide similar threshold HRI cutoff values (HRI = 1.42) for detection of diabetes, with results of sensitivity/specificity very close to ours. They found that HRI cutoff values corresponding to mild steatosis showed high sensitivity but a relatively low specificity. However, in moderate steatosis, HRI threshold values got higher specificity, but lower sensitivity [17, 18]. The high AUC and its statistical significance give us the right to use the HRI cutoff value as a threshold point for detection of impaired glucose metabolism (prediabetes and diabetes). Comparing the three ROC curves for HbA1c, oral GTT, and HRI as markers for the detection of prediabetes, we found powerful predictable value of oral GTT ($p = 0.004$) and HRI ($p = 0.001$), but not in the HbA1c ($p = 0.340$). The ROC curve of oral GTT in detection of prediabetes was more predictable than the ROC curve of HRI; however, there were no statistically significant difference between these, which in some way equates their predictor's impact.

In detection of diabetes, the impact of HRI as potential predictor is on the last stair, after oral GTT and HbA1c. Most studies that investigated the use of HbA1c values against oral GTT as a diagnostic tool for diabetes mellitus have found reduced prevalence by HbA1c criteria compared with the oral GTT criteria [32–35]. The high prevalence of diabetes in general population when using HbA1c values in some studies (but not in our own) may reflect a high chronic glycemic burden in patients with peripheral arterial disease [36]. Bearing in mind the foregoing, we do not intend to diminish the significance of the traditional indicators for the detection of prediabetes and diabetes (HbA1c and oral GTT), but we want to emphasize the role of the HRI, which in no way lags behind them: neither on the AUC surface nor on the statistical significance nor on the Youden index, etc., especially in the detection of the prediabetes. Many studies have proven a strong association between NAFLD and diabetes risk. An individual's risk of developing diabetes is increased approximately fivefold if they have NAFLD, although this is dependent on the population studied, duration of follow up, and methodology used to diagnose NAFLD [37–39].

We rejected the null hypothesis: "There is no statistical significance depending on the prevalence of diabetes and prediabetes of HRI and oral GTT." There is high statistical significance of HRI (HR = 10.69) and oral GTT (HR = 1.86) impact on impaired glucose metabolism (diabetes and prediabetes), and HbA1c (HR = 8.85), too. Musso et al. (2011) reported 3.51 HR for incident diabetes in patients with evidence of ultrasonographic NAFLD [27], and the other studies showed that multivariate-adjusted HR for developing diabetes in subjects who had NAFLD on ultrasound and impaired fasting glucose at baseline was 8.95 (95% CI of 6.49 to 12.35) at 4 years [38, 39].

Study limitations

Several limitations to this study should be considered. NAFLD is closely correlated with abdominal obesity [38, 39]. We did not measure adjust the analyses for waist circumference which reflects abdominal obesity. The relatively small sample size (89 participants) is a weakness of this study, especially in the subgroup of severe NAFLD ($n = 11$). Lastly, a potential weakness of this study is that we had no data on insulin resistance (homeostasis model assessment–estimated insulin resistance [HOMA-IR] index) or fasting insulin which are strongly associated with NAFLD [39]. Our results of fasting insulin do not significantly correlate with NAFLD, how they could provide valid data.

In conclusion, HRI has a significant predictive impact in the detection of prediabetes and diabetes in asymptomatic patients previously diagnosed with NAFLD by ultrasonography, not less important than other markers for detection of diabetes such as HbA1c and oral GTT. This study shows the high potential value of ultrasonographically acquired HRI as a simple, noninvasive, accurate, and cost-effective diagnostic method for screening patients for steatosis and NAFLD, as well as to determine the increased risk for diabetes in patients who exceed the HRI cutoff threshold value of 1.4. By establishing HRI, our goal was not to completely replace conventional markers for diabetes (HbA1c and oral GTT) but to select NAFLD patients with an increased risk of diabetes and to direct them for glucose metabolism tests through appropriate laboratory investigations.

Compliance with ethical standards

Conflict of interest PA, MA, ZN, BI, KS, and ES declare that they have no conflict of interest.

Ethics statement The study was performed conforming to the Helsinki declaration of 1975, as revised in 2000 and 2008 concerning human and animal rights, and the authors followed the policy concerning informed consent as shown on Springer.com.

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