Original Article

VOLUME 8 | ISSUE 2 | APRIL - JUNE 2017

Evaluation of Different Scores to Predict Non-alcoholic Fatty Liver Disease in patients with type 2 diabetes

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Abstract

Introduction-Aim: Non-alcoholic fatty liver disease (NAFLD) is the commonest cause of abnormal liver function tests and liver disease in the western countries. NAFLD is usually asymptomatic. As a result some non-invasive diagnostic models have been proposed for the diagnosis and staging of NAFLD. The objective of this study was to evaluate whether some of the most common and easily assessed models can also be used to screen for the presence of NAFLD in patients with type 2 diabetes (T2D) in clinical practice.

Patients and Methods: The study population included 110 patients with T2D (28 men) [mean age (±SD) 60.1 ± 9.5 years, HbA1c 6.4 ± 1.0%, body-mass index 28.6 ± 4.8 Kg/m2, duration of diabetes 8.5 ± 4.0 years] attending the outpatient diabetic clinic of our hospital. Anthropometric, clinical, and laboratory data were analyzed during regular health checkups. NAFLD was diagnosed using ultrasound. NAFLD liver fat score, HAIR (Hypertension, ALT, Insulin Resistance), BARD, APRI (AST to Platelet Ratio Index), FIB-4 and LAP (Lipid Accumulation Product) scores were estimated. Discrimination capability was assessed based on the area under the receiver operating characteristic curve (AUC), sensitivity and specificity, positive (PPV) and negative (NPV) predictive values were calculated.

Results: NAFLD, using ultrasound, was diagnosed in 77 patients (70%). Receiver operating characteristic analysis showed that for the NAFLD liver fat score a cut off of ≥-1.44 had a sensitivity of 93%, a specificity of 72%, with an AUC of 0.95 and a PPV of 89% and a NPV of 82%. For the HAIR score a cut off of ≥ 0.50 had a sensitivity of 62%, a specificity of 44%, with an AUC of 0.58 and a PPV of 72% and a

SUBMISSION: 05/07/2017 | ACCEPTANCE: 05/08/2017

Citation

Angelidi A M, Papazafiropoulou A K, Tzouganatou E, et al. Evaluation of Different Scores to Predict Non-alcoholic Fatty Liver Disease in patients with type 2 diabetes. *Hell J Atheroscler* 2017, 8: 103-112

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NPV of 33%. For the BARD score a cut off of ≥ 2.50 had a sensitivity of 51%, a specificity of 64%, with an AUC of 0.59 and a PPV of 77% and a NPV of 36%. For the APRI a cut off of ≥ 0.23 had a sensitivity of 49%, a specificity of 64%, with an AUC of 0.55 and a PPV of 76% and a NPV of 35%. For the FIB-4 score a cut off of ≥ 1.00 had a sensitivity of 53%, a specificity of 54%, with an AUC of 0.52 and a PPV of 73% and a NPV of 33%. For the LAP score a cut off of ≥ 30.93 had a sensitivity of 94%, a specificity of 82%, with an AUC of 0.89 and a PPV of 92% and a NPV of 85%.

Conclusions: The results of the present study showed that NAFLD liver fat and LAP scores showed good sensitivity and specificity for the presence of NAFLD in patients with T2D. Both scores are simple, accurate and non-invasive tools to predict NAFLD. In contrast, HAIR, BARD, APRI and FIB-4 scores showed poor sensitivity and specificity.

Key words: type 2 diabetes mellitus; non-alcoholic fatty liver disease; predicting scores

1. Introduction

Non-Alcoholic Fatty Liver Disease (NAFLD) first appeared in the literature during the 60s, when the paper of Thaler et al. was published.[1,2] NAFLD is defined by the accumulation of fat, in the form of large vacuoles, inside the hepatocytes' cytoplasm, at a percentage higher than the 5% of the liver's weight, as long as other secondary causes of fat accumulation in the liver have been excluded, such as increased alcohol consumption [men: ≤2 drinks/day (140 gr ethanol/week), women: ≤1 drink/day (70gr ethanol/week)] use of medication that cause steatosis such as aspirin, valproate, tetracycline, amiodarone, 5-fluorouracil, methotrexate and tamixofen, to name but a few[3], or finally some hereditary metabolic disorders.[4,5] NAFLD is a slowly progressive disease, that includes a wide range of histopathological findings, starting with non-alcoholic steatosis and steatohepatitis (NASH) and progressing to cirrhosis and hepatocellular carcinoma in a small number of patients.[6,7]

NAFLD is one of the most common causes of chronic hepatopathy and the incidence of NAFLD is continuously rising worldwide, especially in the countries with high obesity prevalence. The exact prevalence of the disease has not been defined yet and can be estimated around 10-24%, whereas in the diabetic and obese population it approaches 70-90%.[8,9] Most NAFLD patients remain completely asymptomatic, without any signs or symptoms of hepatic disease and without any abnormalities in the routine blood panel, and the disease is often discovered by chance. As a result, NAFLD is often under-diagnosed and the detection of the disease depends on the sensitivity and specificity of the diagnostic criteria that the medical professionals choose to use, as well as on the experience and the sensitization of the medical personnel.

The 25% of NAFLD patients have also type 2 diabetes mellitus (T2D), whereas about 75% of the diabetic population have also NAFLD.[10] The presence of NAFLD in patients with T2D is related with an increase in the mortality from any cause, whereas the presence of T2D triples the risk of fibrosis, doubles the risk of hepatocellular cancer and correlates independently with the overall mortality in patients with NAFLD.[11] Because of the proven and strong bidirectional correlation between T2D and NAFLD, it is now recommended that the patients diagnosed with NAFLD should be also tested for the presence of T2D, whereas, respectively, diabetic patients should be tested for NAFLD regardless of the levels of the liver enzymes, given the very high incidence of NAFLD in the diabetic population.[12]

The diagnosis[13] of NAFLD is based on clinical findings, blood tests (classic and newer biomarkers), imaging studies and liver biopsy. Regarding blood tests, the results could be within normal range and there is not a specific diagnostic marker. An initial

Table. The main baseline characteristics of the study population

Characteristics of the subjects (*n*=110)	Values N (%) or mean ± SD
Male (%)	28 (25.5)
Age (years)	60.1 ± 9.5
Smoking habit (%)	39 (35.5)
Exercise (%)	21 (19.1)
HbA1c (%)	6.4 ± 1.0
Diabetes duration (years)	8.5 ± 4.0
Weight (kg)	89.2 ± 20.4
BMI (kg/m2)	28.6 ± 4.8
Waist circumference (cm)	109.3 ± 17.2
Waist-to-Hip Ratio	0.94 ± 0.11
Systolic Blood Pressure (mmHg)	138.6 ± 15.3
Diastolic Blood Pressure (mmHg)	86.7 ± 9.6
Total cholesterol (mmol/l)	199 ± 44
LDL-C (mmol/l)	127 ± 35
HDL-C (mmol/l)	42 ± 7
Triglycerides (mmol/l)	142 ± 68
hs-CRP (mg/L)	5.62 ± 4.49
HOMA-IR	6.07 ± 3.80

HbA1c, glycosylated hemoglobin; BMI, body mass index; LDL-C, low-density lipoproteincholesterol; HDL-C, high-density lipoprotein-cholesterol; hs-CRP, high sensitivity C-reactive protein; HOMA-IR, Homeostatic model assessment – Insulin Resistance index

mild increase of the liver enzymes can be found, with aspartate transaminase (AST) being predominant over alanine transaminase (ALT). Among the newer biomarkers that are being studied as potentially diagnostic markers, there are Tumor Necrosis Factor alpha (TNF–a), adiponectin, collagen IV 7S, hyaluronic acid, C-reactive protein and CK-18 (caspase-generating cytokeratin-18).[14-17] Imaging studies[18] include liver ultrasound[19], computed tomography, magnetic resonance spectroscopy and elastography. Liver ultrasound is usually the first method used to diagnose NAFLD. However, its sensitivity is low (30%) when only mild steatosis is present. The other imaging studies have considerable limitations because of either their cost or radiation. The gold standard technique not only to diagnose, but also to determine the severity of NAFLD, is liver biopsy and histopathological examination of the specimen.[20,21] Nonetheless, as it is an invasive technique, it has been a debate topic and the most recent guidelines recommend the use of liver biopsy in the diagnosis and staging of NASH.[12] The ideal target would be the discovery of non-invasive scores[22] to better determine the grade of steatosis[23] and fibrosis[24,25] in NAFLD, which would be not only inexpensive, but also easy to perform. For that purpose, many non-invasive scores have been proposed for NAFLD detection and staging.[26, 27]

The objective of this study was to evaluate the sensitivity and specificity of some of the most common non-invasive models for the screening of NAFLD in patients with T2D.

2. Patients and Methods

2.1 Subjects

The study population included 110 patients with T2D (28 men) [median age (± standard deviation) 60.1 ± 9.5 y.o], HbA1c 6.4 ± 1.0%, Body Mass Index (BMI) 28.6 ± 4.8 kg/m^2, diabetes duration 8.5 ± 4.0 years] attending

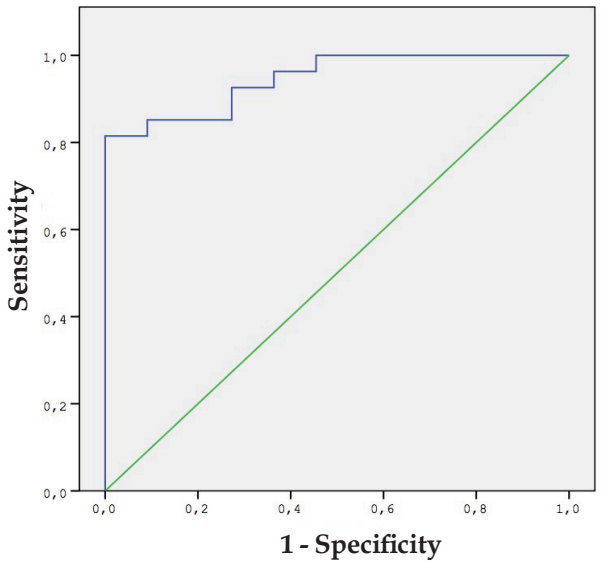

Figure 1. ROC curve for NAFLD liver fat score

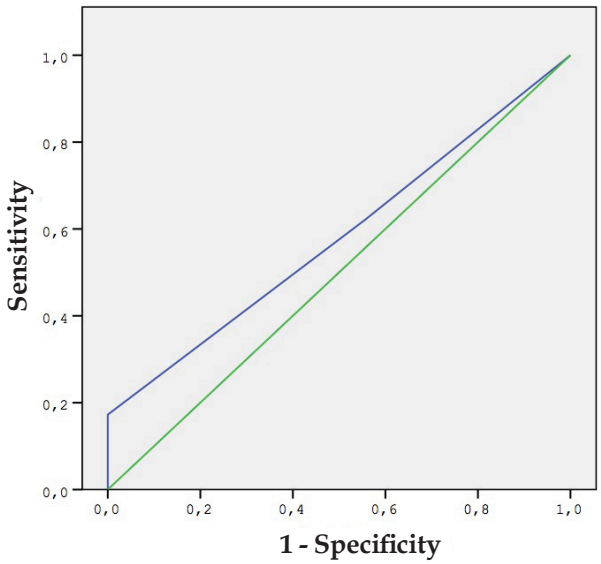

Figure 2. ROC curve for HAIR score

the outpatient diabetic clinic of Tzaneio Hospital.

All patients' anthropometric parameters were registered and a full blood panel was analysed. The **table** summarizes the main characteristics of the study population. In order to confirm or exclude the disease, liver ultrasound was used. The ultrasound is a reliable, easy to use, inexpensive and safe method that has been used in many clinical trials for the screening of NAFLD.[28-30] Moreover, according to the latest recommendations of the EASL (European Association for the Study of Liver), the EASD (European Association for the Study of Diabetes) and the EASO (European Association for the study of Obesity), ultrasound is proposed as a first line diagnostic imaging study.[12]

2.2 Scores

In addition, we calculated the following models: NAFLD liver fat score, HAIR (Hypertension, ALT, Insulin Resistance), BARD, APRI (AST to Platelet Ratio Index), FIB-4 and LAP (Lipid Accumulation Product).

NAFLD liver fat score, that is used to detect steatosis, includes the following parameters: metabolic syndrome, T2D, fasting insulin, AST and ALT.[26] LAP score is a based on a simple equation that includes the waist circumference (WC) and the levels of triglycerides (TG). More specifically for women LAP=(WC[cm]-58)×(Tg [mmol/L]) and for men LAP=(WC[cm]-65)×(Tg [mmol/L]). HAIR score, it is used to detect NAFLD at the stage of steatohepatitis and the result can be between 0 and 3 (a point corresponds to each of the following parameters: hypertension, ALT > 40 U/L and insulin resistant index ≥ 5.0). The BARD score[31] includes: BMI, the ratio AST/ALT and the presence of T2D and it can take values between 0 and 4. For the calculation of APRI score, only the AST and PLTs levels are required. Values < 0.3 indicate strong possibility that hepatic fibrosis is absent, whereas values ≥ 1.5 indicate strong possibility that hepatic fibrosis is present. Finally, for the estimation of FIB-4 score the following parameters are required: Age, PLT number, AST and ALT.[32,33]

2.3 Approval and Consent

The study was approved by the Hospital's Ethics Committee and was performed in accordance with the principles of the Declaration of Helsinki (1975). Written informed consent was obtained from all subjects.

2.4 Statistical Analysis

In order to evaluate the validity of the tests, sensitivity and specificity were calculated, using the receiver-operating characteristics curve (ROC). In addition,

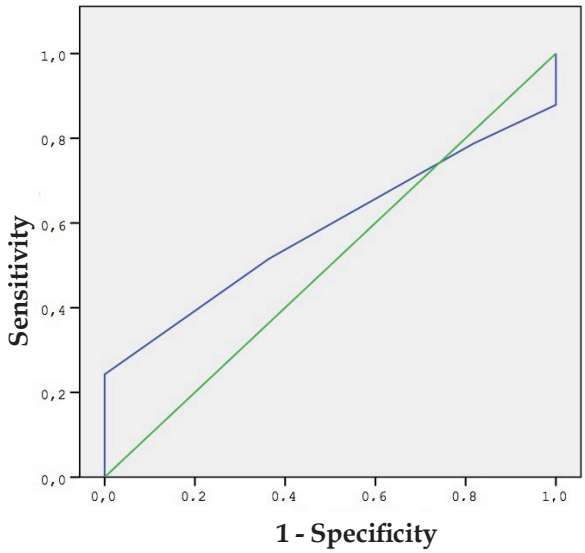

Figure 3. ROC curve for BARD score

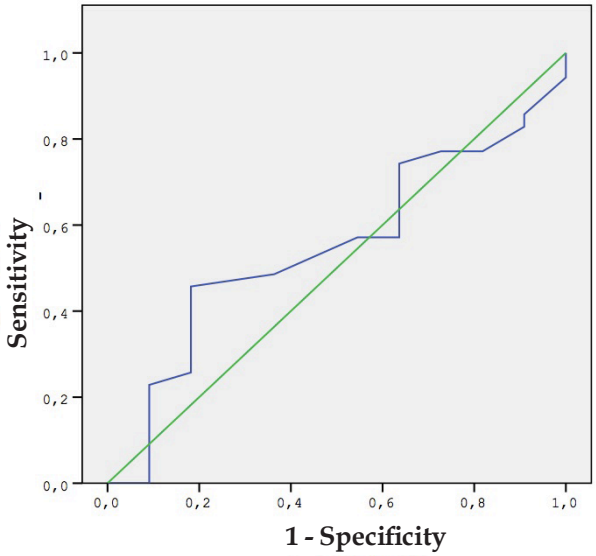

Figure 4. ROC curve for APRI score

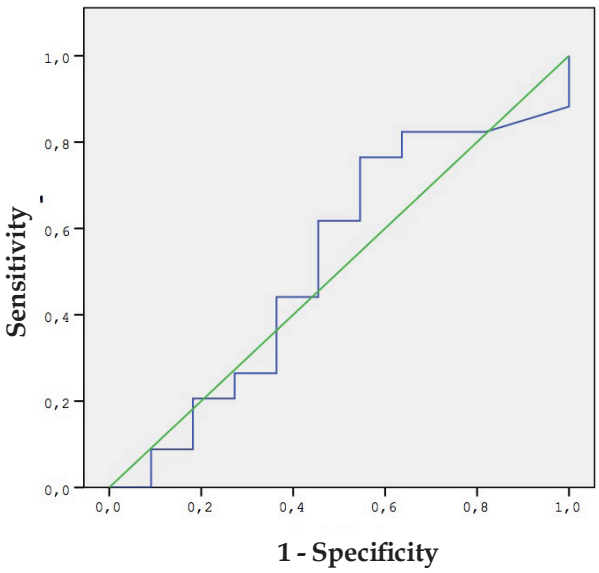

Figure 5. ROC curve for FIB-4 score

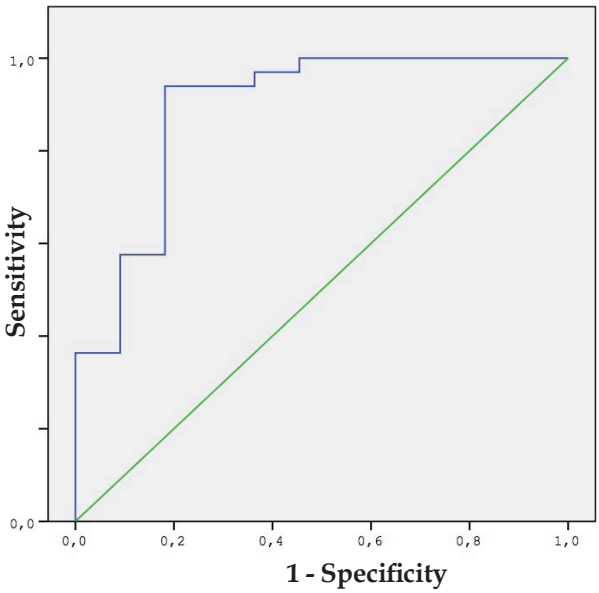

Figure 6. ROC curve for LAP score

the Area Under the Curve (AUC) was estimated, as well as the Positive Prognostic Value (PPV) and the Negative Prognostic Value (NPV). Data were analysed using SPSS (SPSS 20.0, Chicago, IL, USA).

3. Results

NAFLD was detected in 77 patients (70%), using the ultrasound as a screening tool. The analysis with the use of the ROC curve showed that a NAFLD liver fat score of ≤ -1.44 had 93% sensitivity, 72% specificity, 89% PPV and 82% NPV for the diagnosis of NAFLD (**Figure 1**).

As for the HAIR score, it was found that values ≥ 0,50 had 62% sensitivity and 44% specificity (AUC: 0.58), 72% PPV, 33% NPV **(Figure 2)**.

A BARD score ≥2.50 had 51% sensitivity, 64% specificity (AUC: 0.59), 77% PPV and 36% NPV **(Figure 3)**.

An APRI score ≥ 0.23 had 49% sensitivity, 64% specificity (AUC: 0,55), 76% PPV, 35% NPV (**Figure 4).**

Regarding to FIB-4 score, it was estimated that values ≥ 1.00 had 53% sensitivity, 54% specificity (AUC: 0.52), 73% PPV, 33% NPV for the diagnosis of NAFLD (**Figure 5**).

Finally, a LAP score ≥ 30.93 had 94% sensitivity, 82% specificity (AUC: 0.89), 92% PPV, 85% NPV (**Figure 6**).

4. Discussion

According to the results of the present trial, NAFLD liver fat score and LAP have high sensitivity and specificity for the screening of NAFLD in patients with T2D. These scores are quite simple to use, which makes them especially attractive in the clinical practice. Another noteworthy result of the present study is that the incidence of NAFLD in T2D patients is 70%, which is in accordance with the results of other studies. [34, 35]

NAFLD liver fat score has been estimated that for a lower cut-off value of -1.413, the score has 95% accuracy to detect increased fatty infiltration (95% sensitivity, 56% specificity), and for a higher cut-off value of 1.257 it has about 95% accuracy to exclude fatty infiltration (59% sensitivity, 94% specificity).[6] NAFLD liver fat score and LAP score are mainly used to detect the presence of NAFLD at the stage of hepatic steatosis.[26, 36]

According to a recent analysis, with a population of 5000 white, non-Hispanic persons, the AUC for NAFLD liver fat score and LAP was 0.78 and 0.767 respectively.[37] In our study, the values were higher (0.95 and 0.89 for NAFLD liver fat score and LAP respectively). Furthermore, in the previous analysis the sensitivity and specificity of NAFLD liver fat score were 73.95 and 67.94 as compared to 93% and 74% of the present study. These differences could be explained by the different size of these two studies' population. More specifically, the present study includes only patients with T2D, whereas the other includes people of the general population.

Regarding to HAIR score, for a value ≥ 2, the score has specificity 89% and 80% sensitivity for the screening of NAFLD at the stage of steatohepatitis.[38] When it comes to the BARD, APRI and FIB-4 score, they have been used to screen for NAFLD and more specifically to screen for the presence or absence of hepatic fibrosis. For BARD score, values as high as 2 to 4 the model has remarkable high NPV (about 96%) for the absence of hepatic fibrosis (which means a very low number of false negative results) and a mediocre PPV.[31]

According to a study with a population of 104 patients diagnosed with NAFLD by biopsy, BARD score's sensitivity and specificity were 86.7% and 72.7% respectively, with an AUC value of 0.821.[39] Moreover, the NPV was 97%, whereas the PPV only 35.1%.[39] These values are higher than the values estimated in our study. The higher accuracy of the BARD score in the study mentioned above could be explained by the fact that it was about the detection of advanced stages of liver fibrosis, in contrast with the present study that examined the validity of the score to screen for NAFLD in general, among patients with confirmed diagnosis by means of ultrasound.

It should be mentioned that in one trial that used APRI score the AUC value for the screening of hepatic fibrosis was 0.564 [40], similarly to the results of the present study that was about the detection of NAFLD independently of disease stage. Moreover, according to a trial, in which FIB-4 score was used to detect fibrosis among NAFLD patients, for values < 1.30 the score has high NPV (90%), moderate specificity (71%) and sensitivity (74%), low PPV (43%) and AUC 0.802 for the detection of hepatic fibrosis. Furthermore, for values > 2.67, the score has 98% sensitivity, 33% specificity, 83% NPV and 80% PPV.[41] The higher accuracy of FIB-4 score in the study mentioned above could be justified by the fact that the study was about the detection of fibrosis among NAFLD patients.

4.1 Limitations and strengths

Some of the potential limitations of the study are mainly the small number of included participants and the lack of biopsy specimens in order to determine the stage of the disease.

A significant advantage of the present study is the simultaneous evaluation and comparison of several non-invasive valid scores regarding their prognostic accuracy in order to early identify NAFLD among patients with T2D. Additionally, the included

models are quite simple to calculate and could be included as a useful everyday tool even in primary the clinical practice.

Finally, it should be noted that various studies have demonstrated the association of NAFLD with both cardiovascular morbidity and mortality, while it is considered an independent predictor of arterial stiffness.[42-44] Furthermore, distinct stages of NAFLD seem to be associated, to a different extent, with CVD development and mortality.[44, 45] Additionally, several therapeutic measures and specific pharmacological treatments with a potential benefit have been examined and proposed for patients for NAFLD non-alcoholic steatosis and steatohepatitis.[44, 46]

Taking all the aforementioned into deep consideration it is suggested that the early identification of NAFLD is of paramount importance given the association of the latter with cardiovascular disease and mortality as well as all-cause death.

5. Conclusion

In the present study, the authors concluded that NAFLD liver fat score and LAP have high sensitivity and specificity for NAFLD screening in T2D patients. As they are easy to perform, non-invasive and reliable tools, they could be established for the early diagnosis of NAFLD in this patient population. In contrast, HAIR, BARD, APRI and FIB-4 scores have low specificity and sensitivity for the detection of NAFLD in T2D patients. However, there is a need for more trials with larger populations to draw final conclusions for the use of these scores in clinical practice.

Overall, NAFLD is not only linked to cardiometabolic disorders, but also seems to aggravate insulin resistance and T2D development, while its presence is independently associated with a higher cardiovascular risk. Therefore, the prompt recognition of this disease with the variable clinical presentation is utterly important for the detection of patients that need further testing, alterations in their lifestyle towards a healthier way of life and/or medical intervention.

Conflict of Interest

All authors declare no conflict of interest.

Acknowledgement

Financial disclosure: None

Περίληψη

Συγκριτική μελέτη μη επεμβατικών συνδυαστικών μοντέλων για την ανίχνευση της Μη Αλκοολικής Νόσου του Ήπατος

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Εισαγωγή-Σκοπός: Η μη αλκοολική λιπώδης νόσος του ήπατος (non alcoholic fatty liver disease – NAFLD) αποτελεί την πιο συχνά απαντόμενη νόσο του ήπατος στον δυτικό κόσμο και συνήθως είναι ασυμπτωματική. Καθώς όμως οι περισσότερες μέθοδοι είναι παρεμβατικές, έχουν αναπτυχθεί μαθηματικά μοντέλα με στόχο την έγκαιρη ανίχνευση και σταδιοποίηση της νόσου. Σκοπός της παρούσας μελέτης ήταν η αξιολόγηση της ευαισθησίας και ειδικότητας συγκεκριμένων αποδεκτών και εύχρηστων συνδυαστικών μη παρεμβα-

Περίληψη *(συνέχεια)*

τικών μοντέλων για τη διάγνωση της NAFLD σε άτομα με σακχαρώδη τύπου 2 (ΣΔτ2).

Υλικό - Συμμετέχοντες: Ο πληθυσμός της μελέτης περιελάμβανε 110 ασθενείς με ΣΔτ2 (28 άνδρες) μέσης ηλικίας (± SD): 60,1 ± 9,5 έτη, HbA1c: 6,4 ± 1,0%, δείκτη μάζας σώματος: 28,6 ± 4,8 Kg/m^2 και διάρκεια ΣΔτ2: 8,5 ± 4,0 έτη. Η παρουσία NAFLD ελέγχθηκε με τη χρήση υπερηχοτομογραφήματος. Τα μοντέλα που υπολογίσθηκαν ήταν τα: NAFLD liver fat score, HAIR (Hypertension, ALT, Insulin Resistance), BARD, APRI (AST to Platelet Ratio Index), FIB-4 και LAP (Lipid Accumulation Product). Για την αξιολόγηση της εγκυρότητας των διαφόρων μοντέλων μετρήθηκαν η ευαισθησία και η ειδικότητα με τη χρήση της καμπύλης ROC (receiver-operating characteristics curve). Επίσης, υπολογίσθηκε το εμβαδόν της περιοχής κάτω από την καμπύλη (Area Under the Curve, AUC), η θετική (positive predictive value, PPV) και αρνητική προγνωστική τους αξία (negative predictive value, NPV). Η στατιστική ανάλυση έγινε με τη χρήση του στατιστικού προγράμματος SPSS statistical package (SPSS, Chicago, IL, ΗΠΑ).

Αποτελέσματα: Διάγνωση NAFLD, βασιζόμενη στην υπερηχοτομογραφική μελέτη, τέθηκε σε 77 (70%) συμμετέχοντες. Από την ανάλυση των δεδομένων, με τη χρήση της καμπύλης ROC, φάνηκε πως το NAFLD liver fat score για όριο ≥ -1,44 εμφάνιζε 93% ευαισθησία, 72%, ειδικότητα AUC 0,95, PPV 89% και NPV 82%. Το μοντέλο HAIR για τιμές ≥ 0,50 είχε ευαισθησία 62%, ειδικότητα 44%, AUC 0,58, PPV 72% και NPV 33%. Το μοντέλο BARD για όριο ≥ 2,50 εμφάνισε 51% ευαισθησία, 64% ειδικότητα, AUC 0,59, PPV 77% και NPV 36%. Το μοντέλο APRI για τιμές ≥ 0,23 παρουσιάζει 49% ευαισθησία, 64% ειδικότητα, AUC 0,55, PPV 76% και NPV 35%. Αντίστοιχα για το FIB-4 για όριο ≥ 1,00 είχε 53% ευαισθησία, 54% ειδικότητα, AUC 0,52, PPV 73% και NPV 33%. Τέλος το μοντέλο LAP για τιμές ≥ 30,93 εμφάνιζε 94% ευαισθησία, 82% ειδικότητα, AUC 0,89, PPV 92% και NPV 85%.

Συμπεράσματα: Σύμφωνα με τα αποτελέσματα της παρούσας μελέτης τα μοντέλα NAFLD liver fat score και LAP εμφανίζουν υψηλή ευαισθησία και ειδικότητα για τη διάγνωση της NAFLD σε άτομα με ΣΔτ2. Καθώς αποτελούν εύχρηστα, αξιόπιστα και μη παρεπεμβατικά εργαλεία θα μπορούσαν να καθιερωθούν για την έγκαιρη διάγνωση της NAFLD στα άτομα με ΣΔτ2. Αντίθετα, τα μοντέλα HAIR, BARD, APRI και FIB-4 παρουσιάζουν χαμηλή ευαισθησία και ειδικότητα για τη διάγνωση της NAFLD σε άτομα με ΣΔτ2.

Λέξεις ευρετηρίου: σακχαρώδης διαβήτης τύπου 2, μη αλκοολική λιπώδης νόσος του ήπατος, προγνωστικά μοντέλα

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