Cairo University Fibrosis Index (CUFI): A MicroRNA Based Score for Accurate Prediction of Hepatic Fibrosis: A Biopsy Controlled Study

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Abstract

Background: The exact identification of liver fibrosis is mandatory for customizing therapy and controlling complications. The aim of the current study is to measure the ability to circulate miRNAs; in the diagnosis of hepatic fibrosis.

Methods: Routine laboratory, liver biopsy, and histopathological investigations were done on seventy HCV patients. In addition, estimation of HCV-RNA, serum micro RNA (miRNA)-122, 221, 192, 224, 375, and 885 were done by PCR. To assess the ability of each miRNA, the area under the receiver operating characteristics (AUC) curves were plotted. Significant miRNA were analyzed by linear regression analysis in a stepwise pattern to develop a score for pointing out significant fibrosis. Moreover, the following scores were calculated: aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio (AAR), aspartate to platelet ratio index (APRI), FIB-4 score, Hui index, Fibrosis Index (FI), Fibro-Q, Fibro-Alfa Biotechnology Research Center (BRC) score, and Goteborg University Cirrhosis Index (GUCI).

Results: Patients with significant and advanced fibrosis have significantly lower miR-122. MiR-122, FIB4, total bilirubin, and miR-855 proved to be independent predictors of significant fibrosis in univariate analysis. A novel score; Cairo University Fibrosis Index (CUFI) based on microRNA 122, FIB4, bilirubin, and microRNA 855 were formulated for the identification of significant liver fibrosis. The AUC of the score, for predicting significant and advanced hepatic fibrosis was 0.85 and 0.81. This AUC was higher than those of other common fibrosis scores.

Conclusion: Cairo University Fibrosis Index proved to be better than other existing scores in assessing fibrosis in chronic hepatitis C (CHC) patients.

Introduction

Liver fibrosis induced by Hepatitis C virus (HCV) is a dynamic, wound healing, process that results from ongoing damage to the hepatocytes from direct viral cytopathic effect or due to immunological response with subsequent activation of hepatic stellate cell and changes in extracellular matrix formation and degradation, including deposition of collagen [1]. In the era of treatment of chronic HCV using highly efficient direct-acting antivirals (DAAs), hepatic fibrosis staging as a triage for therapy indication is no longer as crucial as once believed [2-6]. Rather, precise identification of advanced hepatic fibrosis as well as cirrhosis is highly required for adjusting HCV therapy, tracking possible regression of liver fibrosis after achieving sustained virological response, and proper prevention and treatment of HCV-related end-stage liver disease complications [7-10]. Histopathology is the traditional benchmark for the determination of liver fibrosis stage, but needle liver biopsy procedure is invasive and has some limitations including cost, risk of complications, sampling errors, inaccurate representation of unevenly distributed liver disease as the sample of the liver biopsy parallels a fraction of 1/50,000th of the whole liver [5]. Noninvasive methods for hepatic fibrosis staging in chronic HCV patients including physical (imaging techniques) and biological (serum biomarkers) have become increasingly available over the last few decades [7,8,10-14].

Mi-RNA are small non-coding RNAs with an average length of 22 nucleotides that control the translation and transcription of many genes [15]. Mi-RNAs are crucial for the regulation of inflammatory diseases and are considered predictive biomarkers for liver diseases [16, 17]. Scarce studies explored the potential role of circulating miRNAs in the prediction of liver fibrosis stage. The present study was conducted to estimate the accuracy of microRNAs; 122,224, 192, 375, 221, and 855 in predicting the severity of liver fibrosis. Moreover, we tried to formulate a score and validate it against other noninvasive fibrosis biomarkers; AST/ALT ratio (AAR), FIB-4 score, APRI, Fibrosis Index (Fi), Hui index, Fibro-Alfa, Fibro-Q, Biotechnology Research Center (BRC) score, and GUCI using liver biopsy as the gold standard.

Material And Methods

Study population

In this cohort study, 70 consecutive patients suffering chronic hepatitis C joined from Theodor Bilhariz research institute. Adult patients (18 – 70 years) with virological proof of chronic HCV infection by HCV antibodies and real-time polymerase chain reaction (RT-PCR) were included. Exclusion criteria included other causes of liver disease e.g. α 1 anti-Trypsin deficiency, Wilson disease, hemochromatosis, co-infection, with hepatitis B virus (HBV), and non-alcoholic fatty liver disease.

Laboratory assessments

Laboratory investigations included full blood count, liver biochemical profile was done. HCV-RNA using reverse transcriptase-polymerase chain reaction (RT-PCR) (The Abbott RealTime HCV assay) with detection limit < 10 IU/ml was done. All testing was performed at a single reference laboratory.

miRNA extraction

Serum miRNA 122,192, 885, 375, 224, and 221 were evaluated by PCR (Sera had been stored at -80 °C till use and RNA separated using TriHReagentLS (Sigma-Aldrich, St. Louis, MO), chloroform, and the mirVanaTM RNA isolation kit (Ambion-ABI, Austin, TX). Total RNA was eluted in 100 μ l and stored at -20C. Five μ l of RNA reverse transcribed with the TaqMan miRNA reverse transcription kit and the TaqMan miRNA assay-specific RT primers for miRNAs 122,192,885,375,224 and 221 according to the instructions of the manufacturer (ABI). Real-time PCR was done with three μ l of each cDNA on a Step One TM Plus Real-Time PCR System (ABI) in duplicates. Fractional threshold cycles (CT) expressed the initial concentration of the target sequence. Relative mRNA quantification was calculated using the arithmetic formula $2^{-\Delta\Delta C_T}$, where $\Delta\Delta C_T$ is the difference between the CT of a given target cDNA and an endogenous reference cDNA. Thus, this value yielded the amount of the target normalized to an endogenous reference.

Liver biopsy

Liver biopsy was performed under ultrasound guidance using a disposable automatic Guillotine tru-cut needle (16-gauge). The acceptable sample was at least 10 mm in length and contained at least 5 portal

tracts. The fibrosis stage was determined according to the METAVIR group scoring system [18]. The present study was organized in accordance with the Declaration of Helsinki 1973 and Good Clinical Practice guidelines. The Ethics Committee of Endemic Medicine and the Hepato-gastroenterology Department reviewed and approved the protocol. Every patient signed informed consent before taking part in the study.

Statistical analysis

Results were examined utilizing SPSS software package version 25.0 (SPSS Inc., Chicago, IL). Numerical data were demonstrated as mean $\pm$ SD and differences were carried out using analysis of variance (ANOVA) or Student's t-test. Tests were significant when the P-value < 0.05 . Patients were classified into 2 groups; significant liver fibrosis (F2–F4) and advanced liver fibrosis (F3–F4). Noninvasive hepatic fibrosis indexes were calculated; table 1. To assess the ability of each biomarker for differentiating between the two groups, the AUC curves were plotted; significant biomarkers were selected and analyzed by linear regression analysis in a stepwise pattern to develop a score that combined the independent factors for pointing out significant fibrosis. The best cutoff value of the predictive score was optimized for identifying significant fibrosis. On the other hand, the diagnostic performances of our developed score were compared with other common noninvasive liver fibrosis scores. $p < 0.05$ is considered significant. P value < 0.01 is considered highly significant, $p < 0.001$ is considered very significant and $p < 0.0001$ is considered extremely significant.

Results

The study cohort included 70 chronic HCV patients [mean age 40.9 ± 8.5 years, 70% men ($n=49$)]. According to liver histopathology, F0, F1, F2, F3, F4 were identified in 7, 37, 14, and 11, patients respectively. Patients were classified into significant liver fibrosis ($n = 26$) and advanced liver fibrosis ($n = 11$). Baseline demographic and laboratory values and mi-RNAs levels are shown in table 2 and figure 1(A-B). The mean $\pm$ SD, AUC of the predictive score, and common noninvasive liver fibrosis scores were summarized in table 3. For prediction of significant liver fibrosis, the AUC of common liver fibrosis scores; FIB 4, GUCI, Fibro-Q, APRI, BRC, AAR, Hui index, Fibro- Alfa and FI were 0.67, 0.62, 0.62, 0.62, 0.61, 0.60, 0.54, 0.53 and 0.46; respectively

Performance characteristics of biomarkers using areas under the ROC curves

Univariate analysis of all comparing biomarkers in the present study identified micro RNA 122, Fib4, bilirubin, and micro RNA 855 as independent markers for discriminating significant liver fibrosis. The AUC were 0.79, 0.67, 0.66 and 0.64, respectively (Table 4). The previous analysis indicated that micro RNA 122 was the most efficient index among other biomarkers.

Diagnostic performances of the score

The multivariate discriminant analysis (MDA) identified the best formula which included microRNA 122, FIB4, microRNA 855, and bilirubin as a novel score that was able to predict significant liver fibrosis noninvasively.

Cairo University Fibrosis Index (CUFI) = 4.4 + FIB4 x 0.19 – (micro RNA 122 x 0.086 + micro RNA 855 x 0.052 + bilirubin x 0.44), where 4.558 is considered a numeric constant. Levels of CUFI in significant liver fibrosis and advanced liver fibrosis were presented in figure 1C. The AUC of the score was assessed for identifying significant fibrosis showing an AUC of 0.85 (figure 2A). A cut-off level of 0.44 presented sensitivity of 77%, specificity of 79%, PPV of 68%, NPV of 86 %, and overall accuracy of 78% for identification of significant liver fibrosis (Table 3). Our predictive score at a cut-off value of 0.44 yielded an AUC of 0.81 for predicting advanced fibrosis (figure 2B). It showed 80 % sensitivity, 66% specificity, 35% positive predictive value, 95 % negative predictive value, and overall accuracy of 68 % for identification of advanced liver fibrosis (Table 4).

Discussion

Liver disease is a considerable cause of mortality and morbidity [5, 28]. Accurate determination of the disease stage is mandatory for clinical decision-making. Still, liver histopathology is considered the golden benchmark for the diagnosis of liver fibrosis [29]. However, the invasiveness of biopsy, the potential associated complications, and poor sample quality urge the search for noninvasive diagnostic markers for liver fibrosis [30]. The need to find predictors of the hepatic fibrosis stage at the molecular level is increasing as hepatic fibrosis progression may result in hepatocellular carcinoma development [31, 32]. The miRNAs are now considered to be important indicators of liver fibrosis [33], and liver carcinogenesis [32]. In the current study, we analyzed the performance of microRNAs as hepatic fibrosis biomarkers. Patients suffering significant and advanced fibrosis showed a significantly lower value of MiR-122. Our study showed more decrease in serum miR-122 in patients with advanced hepatic fibrosis (F3,4) when compared with significant fibrosis (F2-4) which agreed with Trebicka et al who stated that loss of functional hepatocytes in the late stages of fibrosis (F3–4) might cause decreased serum levels of miRNA-122 [34]. Moreover, the suppressive function of miR-122 that hinders the proliferation of hepatic stellate cells and fibrogenesis is absent in the late stages of fibrosis. [35]. Also, many studies proved an inverse relationship between miR-122 and the stage of fibrosis in HCV-based liver disease [36, 37]. Furthermore, we formulated a novel score (CUFI) which shows a greater diagnostic value for pointing to significant and advanced hepatic fibrosis taking histopathology as the gold standard (AUR 0.85, 0.81 respectively). The score included microRNA 122, microRNA 855, FIB4, and bilirubin. It is well known that changes in miRNA-122 serum levels occur early in liver disease, suggesting that miRNA -122 can be a reliable predictor of hepatic injury [33]. Additionally, miR-122 induces HCV translation and stimulates viral genomic RNA replication [38]. Also, it is inversely correlated with fibrosis in HCV-infected patients [36, 37]. miR-122 levels were higher levels in the non-significant liver followed by reduced levels in significant liver fibrosis in response to inflammation and damage of liver cells confirming previous studies. *miR-885* was found to correlate with different liver pathologies [39-41]. Bilirubin is the lipophilic end product of heme breakdown [42]. Hyperbilirubinemia is common in chronic liver disease and serum bilirubin is

routinely included in the biochemical assessment of such patients, Serial measurement of serum bilirubin is might be indicative of liver disease progression being an indirect marker of hepatic fibrosis [43].

Our novel score CUFI which is composed of serum bilirubin, miRNA -122, and miRNA -855 performed even better than common liver fibrosis scores. Its AUC was 0.85 for documenting significant liver fibrosis and 0.81 for detection of advanced fibrosis which was the greatest score among common liver fibrosis scores. This could be cleared up by the fact that most of the common liver fibrosis scores depend on multiple biochemical and serum parameters which may differ with many factors such as blood sampling time, the blood sample delivery time, and liver functional status [44]. On the other hand, miRNAs are stably present in the blood, making them suitable as biomarkers [45]. The limitation of our study included a small sample, so our results should be validated on larger samples.

In conclusion, Cairo University Fibrosis Index is more accurate than the existing fibrosis scores as it gives more indicative results for judging fibrosis.

Abbreviations

AAR: AST/ALT ratio; ALT: alanine aminotransferase; APRI: aspartate to platelet ratio index; AST: aspartate aminotransferase; AUC: area under curve; BRC: Biotechnology Research Center; CHC: Chronic hepatitis C; CUFI: Cairo university fibrosis index; DAAs : direct-acting antivirals; FI: Fibrosis index; FIB-4: Fibrosis-4; GUCI: Goteborg University Cirrhosis Index; HBV: hepatitis B virus; HCV: Hepatitis C virus; HCV: Hepatitis C virus; MDA: multivariate discriminant analysis; Mi: micro; PCR: Polymerase chain; RNA: ribonucleic acid; ROC: receiver operating characteristics; RT-PCR: reverse transcriptase-polymerase chain reaction

Declarations

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Not applicable.

Authors' contributions

AH, OD, ZA, OM and EA designed the study. ZA measured the micro RNA. AM and EA collected the clinical data and the samples. OAM and OM analyse the data. AM shared in writing the manuscript. OM and OD wrote the manuscript. All authors revised and approved the manuscript.

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Availability of data and materials

Data not available due to [ethical/legal] restrictions: Due to research nature, the study participants did not agree for their data to be shared publicly, so supporting data is not available. The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was organized in accordance with the Declaration of Helsinki 1973 and Good Clinical Practice guidelines. The Ethics Committee of Endemic Medicine and the Hepato-gastroenterology Department reviewed and approved the protocol. Every patient signed informed consent before taking part in the study

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests to declare.

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Tables

Table 1. Calculation of noninvasive hepatic fibrosis indexes

indexes	Calculations	References
Fi	$8 - (0.01 \times \text{platelets (10}^9\text{/L)}) - \text{albumin (g/dL)}$	[19]
Fibro-Alfa	$1.35 + \text{AFP (IU/ml)} \times 0.009584 + \text{AAR (AST/ALT)} \times 0.243 - \text{platelet count (10}^9\text{/l)} \times 0.001624$	[20]
Hui index	$3.148 + 0.167 \times \text{BMI} + 0.088 \times \text{bilirubin} - 0.151 \times \text{albumin} - 0.019 \times \text{platelet}$	[21]
AAR	- AST/ALT	[22]
BRC score	- $1.02 + 0.4 \times \text{AFP (U/l)} + 0.19 \times \text{Age (years)} - 0.02 \times \text{Platelet count (10}^9\text{/l)}$	[23]
APRI	- $\text{AST(IU/L)} / \text{upper limit of normal/platelet count (10}^9\text{/L)} \times 100$	[24]
Fibro-Q	$[(10 \times \text{age (years)} \times \text{AST} \times \text{PT INR}) / (\text{PLT} \times \text{ALT})]$	[25]
GUCI	- $\text{AST} \times \text{INR} \times 100 / \text{Platelet count (10}^9\text{/L)}$	[26]
FIB-4	- $[\text{Age [yrs.]} \times \text{AST [U/L]} / (\text{platelet count [10}^9\text{/L)} \times (\text{ALT [U/L]}^{1/2})]$	[27]

Table 2. Comparison between non-significant hepatic fibrosis (F0-F1) and those with significant (F2 - F4) and advanced hepatic fibrosis (F3–F4).

P value	Advanced fibrosis (n= 12)	Non advanced fibrosis (n= 58)	P value	Significant fibrosis (n= 26)	Non significant fibrosis (n= 44)	Variable
0.015	46.3 ± 5.5	39.9 ± 8.6	0.004	44.7 ± 5.54	38.8 ± 9.2	Age (years)
0.39	42.08 ± 14.5	38.6 ± 12.4	0.7	40.0 ± 13.85	38.8 ± 12.1	ALT (U/L)
0.13	42.83 ± 11.8	37.6 ± 10.3	0.06	41.6 ± 10.11	36.7 ± 10.7	AST(U/L)
0.03	0.91 ± 0.25	0.76 ± 0.20	0.001	0.9 ± 0.21	0.78 ± 0.1	Bilirubin (mg/dl)
0.33	43.50 ± 3.87	42.49 ± 3.4	0.54	42.9 ± 3.85	42.3 ± 3.3	Albumin (g/L)
0.85	13.73 ± 1.04	13.76 ± 1.4	0.62	13.8 ± 1.27	13.6 ± 1.3	Hb (g/dl)
0.68	288.6 ± 94.4	278.1 ± 78.1	0.96	280.5 ± 89.5	279.5 ± 75.7	PLT (×10 ⁹ /L)
0.91	86.3 ± 6.9	86.6 ± 6.8	0.61	86.0 ± 6.11	86.8 ± 7.3	PC (%)
0.89	1.14 ± .07	1.14 ± 0.01	0.74	1.14 ± 0.07	1.1 ± 0.08	INR
0.99	92.4 ± 13.7	92.4 ± 13.4	0.88	92.1 ± 13.61	92.6 ± 13.4	Glucose (mg/dl)
0.29	59,6 ± 99,6	105,7 ± 143,9	0.87	94,3 ± 138,0	99,8 ± 139	PCR (IU ml X10 ⁴)
0.50	6.2 ± 3.7	5.5 ± 3.2	0.75	5.8 ± 3.5	5.5 ± 3.13	AFP (U/L)
0.007	26.9 ± 1.8	29.1 ± 2.6	< 0.0001	27.1 ± 2.3	29.6 ± 2.33	Micro RNA 122
0.72	26.9 ± 1.9	27.2 ± 1.7	0.51	27.3 ± 1.7	27.03 ± 1.73	Micro RNA 192
0.04	25.1 ± 1.1	26.3 ± 1.9	0.01	25.3 ± 1.5	26.5 ± 1.99	Micro RNA 855
0.75	29.6 ± 3.8	29.9 ± 3.6	0.52	30.3 ± 3.3	29.7 ± 3.81	Micro RNA 375
0.66	31.7 ± 3.9	31.3 ± 3.1	0.18	30.6 ± 3.4	31.7 ± 3.07	Micro RNA 224
0.33	32.8 ± 3.4	34.4 ± 5.6	0.78	33.9 ± 3.4	34.3 ± 5.96	Micro RNA 221

Table 3. Comparison of our predictive score and common liver fibrosis scores for predicting significant and advanced fibrosis

AUC	P value	Advanced fibrosis (n= 12)	Non advanced fibrosis (n= 58)	AUC	P value	Significant fibrosis (n= 26)	Non-significant fibrosis (n= 44)	Variable
0.399	0.295	-38.39 ± 4.03	-37.18 ± 3.53	0.46	0.545	-37.73 ± 3.93	-37.18 ± 3.45	FI
0.485	0.937	1.19 ± 0.17	1.20 ± 0.16	0.53	0.434	1.22 ± 0.19	1.19 ± 0.14	Fibro-Alfa
0.561	0.740	-3.86 ± 2.29	-4.06 ± 1.81	0.54	0.956	-3.89 ± 2.17	-4.10 ± 0.26	Hui score
0.569	0.812	1.07 ± 0.27	1.04 ± 0.37	0.60	0.151	1.12 ± 0.38	0.99 ± 0.33	AAR
0.579	0.500	17.99 ± 5.76	16.61 ± 6.55	0.61	0.082	6.23 ± 2.99	5.0 ± 2.67	BRC
0.580	0.546	0.39 ± 0.12	0.37 ± 0.15	0.62	0.180	0.40 ± 0.13	0.35 ± 0.15	APRI
0.583	0.308	2.25 ± 1.47	1.87 ± 1.11	0.62	0.018	2.37 ± 1.53	1.68 ± 0.83	Fibro -Q
0.602	0.151	6.54 ± 3.39	5.24 ± 2.69	0.62	0.159	18.25 ± 5.99	16.01 ± 6.56	GUCI
0.632	0.02	1.2 ± 0.1	0.98 ± 0.31	0.67	0.001	1.21 ± 0.21	0.90 ± 0.44	FIB -4

Table 4. Diagnostic power of single and combined candidate biomarkers

Markers	AUC (95% CI)	Cut-off	Sen	Spe	PPV	NPV	Accuracy
Significant vs non-significant							
Micro RNA 855	0.64 (0.51-0.78)	26	46	57	38	65	53
Bilirubin	0.66 (0.51-0.81)	0.75	65	65	51	75	64
FIB-4	0.67 (0.53-0.81)	0.95	64	65	52	76	65
Micro RNA 122	0.79 (0.86-0.91)	26	72	76	64	83	75
Cairo University Fibrosis Index	0.85 (0.75-0.95)	0.44	77	79	68	86	78
Advanced vs non-advanced							
Micro RNA 855	0.62 (0.48-0.77)	26	50	58	20	85	56
Bilirubin	0.65 (0.44-0.86)	0.75	52	59	21	86	57
FIB -4	0.68 (0.53-0.83)	0.95	70	60	26	90	62
Micro RNA 122	0.79 (0.65-0.93)	26	80	66	32	94	68
Cairo University Fibrosis Index	0.81 (0.69-0.93)	0.44	80	66	35	95	68

Figures

Figure 1

Levels of candidate markers and Cairo Univeristy Fibrosis Index. A. Level of miro-RNA 122. B. Level of miro-RNA 855. C. Level of Cairo University Fibrosis Index.

Figure 2

ROC curves of Cairo Univeristy Fibrosis Index for identifying. A. Significant fibrosis B. Advanced fibrosis.