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Original article

A combination of α-fetoprotein, midkine, thioredoxin and a metabolite for predicting hepatocellular carcinoma

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ABSTRACT

Introduction and objectives: The heterogenous nature of hepatocellular carcinoma (HCC) motivated this attempt at developing and validating a model based on combined biomarkers for improving early HCC detection.

Patients/materials and methods: This study examined 196 patients for an estimation study (104 patients with HCC, 52 with liver cirrhosis and 40 with liver fibrosis) and 122 patients for the validation study (80 patients with HCC, 42 with liver cirrhosis). All patients were positive for hepatitis C virus. Four markers were measured: Midkine and thioredoxin using ELISA, 1-methyladenosine and 1-methylguanosine using a gas chromatography–mass spectrometry (GC–MS). The results were compared with alpha-fetoprotein (AFP). The performance of the model was estimated in BCLC, CLIP and Okuda staging systems of HCC.

Results: The model yielded high performance with an area under ROC (AUC) of 0.94 for predicting HCC in patients with liver cirrhosis, compared with AUC of 0.69 for AFP. This model had AUCs of 0.93, 0.94 and 0.94 in patients who had only one single nodule, absent macrovascular invasion and tumor size <2 cm, respectively, compared with AUCs of 0.71, 0.6 and 0.59 for AFP. The model produced AUCs of 0.91 for BCLC (0-A), 0.92 for CLIP (0–1) and 0.94 for Okuda (stage I) compared with AUCs of 0.56, 0.58 and 0.64 for AFP. No significant difference was found between AUC in the estimation and the validation groups.

Conclusion: This model may enhance early-stage HCC detection and help to overcome insufficient sensitivity of AFP.

1. Introduction

The global status of liver cancer as the sixth most common malignancy and the third leading cause of cancer-related deaths [1,2], emphasizes the importance of detecting HCC at an early stage in an attempt to offer more curative therapies [3]. Diagnosis of HCC is based on laboratory investigations and imaging techniques [4,5], particularly ultrasound (US), triphasic computed tomography (CT) and dynamic magnetic resonance imaging (MRI), which form the basic standard diagnostic tools for HCC detection [6]. The simple, non-invasive, safe and highly accessible nature of US make it the imaging tool of first choice for HCC diagnosis, in spite of its accuracy being affected by limitations such as operator experience, the quality of the machine used and the patient's body constitution [7]. Nevertheless, US has been shown to detect early-stage HCC with sensitivity ranging from 25% up to 65% and specificity of 97% [3,5]. Conversely, use of alpha-fetoprotein for detection of HCC has been shown to have a lower sensitivity in the range of 18–60% [7,8]. However, utilization of this widely used biomarker in combination with US for diagnosis of HCC has demonstrated increased detection rates of HCC [9]. In addition, the combination of AFP with other biomarkers may improve these early diagnostic rates [10,11].

Midkine is a heparin-binding growth factor created in the liver during the fetal period and is minimally expressed in the normal adult liver [12]. Midkine is induced by inflammation with overexpression of this growth factor contributing to the development of tumor formation, therefore playing a crucial role in the early HCC detection [13,14]. Thioredoxin (TRX) is an antioxidant protein

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and forms a part of the TRX system which composed of NADPH, thioredoxin and thioredoxin reductase. It responds to biological stress due to oxidative damage or inflammation and plays a vital role in regulating redox signaling [15,16]. The supplemental role of thioredoxin in the regulation of cancer cell growth make it another critical determinant in the detection of early-stage HCC [8,16]. In addition, metabolomics is a powerful technique used for global analysis of small metabolites less than 2 kDa and can also be employed in the identification of novel biomarkers for early detection of HCC [17], based on the presumption that the rapid RNA turnover in malignant diseases is associated with an increase in methyltransferase activity leading to high concentrations of some modified form of nucleoside 1-methyladenosine and 1-methylguanosine [18,19].

2. Material and methods

2.1. Patients

The present case-control study examined 196 patients with chronic hepatitis C for an estimation study and 122 patients for the validation study. The estimation group included 104 patients with HCC and 92 with non-malignant liver diseases recruited from the Tropical Medicine Department at Mansoura University Hospitals, Mansoura, Egypt, between December 2016 and October 2017. Informed consent was signed by all patients in compliance with the ethical guidelines of the 1975 Helsinki Declaration. All patients were positive for HCV-antibody as well as HCV-RNA, and none of the patients had previously undergone HCV therapy. Noninvasive methods were used for HCC diagnosis, according to the American Association for the Study of Liver Diseases (AASLD) Practice Guidelines [20]. Tumors were measured using triphasic CT and/or dynamic MRI. None of the HCC patients had received prior treatment interventions such as transarterial chemoembolization (TACE) or radiofrequency ablation. Patients with kidney failure, cardiovascular disease, rheumatoid arthritis, autoimmune liver diseases, hepatitis A or B viruses, bilharzial infection or other causes of liver diseases were excluded. In addition, patients with other causes of thrombocytopenia, such as typhoid, leukemia and deficiency of vitamin B12, as well as other causes of HCC or the presence of other malignancies were also excluded from this study. The non-malignant group of patients included those with liver cirrhosis ($n = 52$) and liver fibrosis ($n = 40$). Liver cirrhosis was diagnosed based on biochemical, ultrasonographic and computed tomography imaging findings of splenomegaly or macronodular liver. The severity of cirrhosis was determined using the Child–Pugh score. Patients with chronic HCV infection in the absence of ultrasonographic and CT criteria for cirrhosis and HCC were diagnosed as having hepatic fibrosis. Patients in the non-malignant group were followed up for 6 months to ensure the absence of HCC. The validation group assumed similar clinical assessment, laboratory and pathological investigations and classifications, in addition to inclusion and exclusion criteria, as the estimation group.

The current study was mainly aimed at developing and validating a non-invasive model for improving early HCC detection by adequately distinguishing HCC from liver cirrhosis. This model consisted of incorporating the use of alpha-fetoprotein with one of the growth factors (midkine) in conjunction with one of the antioxidant proteins (thioredoxin) and one of the serum metabolites (1-methyladenosine). The model was then subsequently applied to three common staging systems; Barcelona Clinic Liver Cancer (BCLC) [21], Cancer of Liver Italian Program (CLIP) [22] and Okuda systems [23].

2.2. Blood samples and laboratory assays

Blood samples were collected from all patients after clinical diagnosis and fresh samples were examined for routine laboratory investigations, including biochemical profile [aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin and albumin] and creatinine using an automated biochemistry analyzer (BT1500; Biotecnica instruments S.P.A, Italy). Complete blood count was measured using an automated hematology analyzer (Micros 60; Horiba medical, Montpellier, France). Prothrombin-INR was estimated using a special apparatus (Coatron. M1; TECO, Neufahrn, Germany). AFP and HCV antibody were evaluated using an immunofluorescence assay (IFA) by auto-analyzer (Mini-Vidas; bioMérieux, Marcy L'Etoile, France). The presence of HCV-RNA was determined by quantitative real-time polymerase chain reaction (COBAS Ampliprep/COBAS TaqMan; Roche Diagnostics, Pleasanton). Serum thioredoxin and midkine levels were ascertained using a quantitative sandwich enzyme immunoassay technique (Elabscience Biotechnology Co, Ltd, USA). The analysis of candidate metabolites (M1A and M1G) was performed using a gas chromatography–mass spectrometry (GC–MS) (Agilent 7890B; Agilent Technologies, Santa Clara, CA). The retention time (RT) was 13.961 and 16.745 min for M1A and M1G, respectively. The mean absorbance value of patient samples was measured for each biomarker. Laboratory investigators were blind to the clinical data of each sample examined.

2.3. Statistical analysis

Data was statistically analyzed with SPSS software (SPSS Inc.) version 20.0. Variables were expressed as mean ± standard deviation (SD) or as median and interquartile range (IQR) for continuous non-normally distributed variables. Categorical variables were analyzed using the *Chi*-square test or Fisher's exact test when appropriate and expressed as numbers (%). Differences between variables were analyzed using analysis of variance (ANOVA) or non-parametric Kruskal–Wallis test for multiple comparisons, and Student's *t*-test or non-parametric Mann–Whitney *U* test when appropriate between two groups. The deviation of AFP was corrected using a log transformation of the data. A two-sided *P* value <0.05 was an indication of to be statistical significance.

The primary aim of this study was to construct a model to differentiate patients developing HCC from those with liver cirrhosis. Univariate and multivariate logistic regression analyses were carried out to screen the independent risk factors of HCC. Variables with a *P* value <0.05 at univariate analyses were entered into the multivariate model. The Hosmer–Lemeshow test was evaluated to determine the fitting goodness of the multivariate logistic model. A higher *P* value in this test suggested that the model was more meaningful. Odds ratios with 95% confidence intervals (CIs) were calculated. The area under the curve (AUC) was performed with 95% CIs for statistically significant variables in the multivariate analysis. The optimal cut-off value was selected using a ROC curve (higher values of sensitivity and specificity). The corresponding sensitivity, specificity, positive and negative predictive values were calculated using their standard formulae.

3. Results

3.1. Patient characteristics

The clinical and laboratory data of study patients ($n = 196$) are displayed in Table 1. A comparative study between the three groups showed a significant difference between all variables ($P < 0.05$). Most of the patients were male with a significant increase in

Table 1
Demographic and laboratory data of the estimation group.

Variable	Nonmalignant chronic liver disease ($n=92$)		HCC ($n=104$)	P value[a]
	Fibrosis($n=40$)	Cirrhosis($n=52$)		
Gender				
Male %	24.(60%)	32 (61.5%)	81 (77.9%)	0.121 [b]
Female %	16.(40%)	20 (38.5%)	23 (22.1%)	
Age (years)	52.6 ± 2.9	58.4 ± 8.5	61.1 ± 7.1	0.033
Alanine aminotransferase (U/L)	47.(28– 49)	49 (31–65)	53 (44–67)	0.087
Aspartate aminotransferase (U/L)	32.(19–60)	41 (27–64)	59 (36–64)	0.042
Total bilirubin (mg/dL)	0.8 (0.7–0.9)	1.3 (0.8–2.2)	1.4 (0.8–2.3)	0.995
Albumin (g/L)	40.2 ± 1.9	34.7 ± 7.3	32.1 ± 6.1	0.278
Prothrombin-INR	1.1 ± 0.1	1.3 ± 0.3	1.3 ± 0.2	0.418
Platelet count ($\times 10^9$/L)	151.(99–171)	105 (56–167)	88 (68–125)	0.774
Hemoglobin (g/dL)	13.0 ± 2.3	12.2 ± 2.3	11.8 ± 2.1	0.768
Total leucocytic count ($\times 10^9$/L)	5.8 (5.7–6.5)	4.9 (3.7–7.8)	4.9 (3.9–6.7)	0.335
Creatinine (mg/dL)	0.9 ± 0.1	1.1 ± 0.3	1.0 ± 0.4	0.095
Quantitative PCR (IU/mL)($\times 10^5$)	4.8 (0.8–13.3)	2.0 (0.1–12.1)	1.2 (0.8–2.2)	0.394
α-Fetoprotein (U/L)	4.3 (2.6–5.5)	5.1 (3.1–9.2)	38.5 (11.6–427)	< 0.0001
Log AFP	0.7 ± 0.1	0.8 ± 0.4	2.0 ± 1.1	< 0.0001
Thioredoxin (ng/mL)	83.9 (33.9–95.7)	84.5 (37–126)	129.5 (112–135)	< 0.0001
Midkine (ng/mL)	0.75 (0.46–0.98)	0.83 (0.31–1.8)	2.02 (1.12–2.4)	< 0.0001
1-Methyladenosine (ng/mL)	36.5 (34.5–38.5)	35.9 (34.8–37.5)	40.7 (36.4–46.8)	< 0.0001
1-Methylguanosine (ng/mL)	13.3 ± 2.6	16.5 ± 3.5	17.5 ± 4.3	0.113
Child A (n; %)	–	(26; 50%)	(49; 47.2%)	0.349 [c]
Child B (n; %)	–	(13; 25%)	(39; 37.5%)	
Child C (n; %)	–	(13; 25%)	(16; 15.3%)	

Variables were expressed as mean ± SD or median (IQR).
[a] P-values = the significant differences between variables in HCC and patients with liver cirrhosis; Categorical variables were analyzed using Fisher's exact test.
[b] or chi-square.
[c] $P > 0.05$ is considered nonsignificant; $P < 0.05$ is considered significant; $P < 0.001$ is considered very significant and $P < 0.0001$ is considered extremely significant.

age with progression of liver disease from liver fibrosis to HCC. Serum levels of MDK, TRX, M1A and AFP were significantly higher in HCC than in patients with liver cirrhosis ($P < 0.0001$), whereas M1G showed no significant difference in HCC compared with liver cirrhosis ($P > 0.05$) and was consequently excluded from further analysis. Regarding tumor morphology in HCC cases, 43.3% of patients had a single tumor and 56.7% demonstrated multiple growths, with 26.9% of subjects showing macrovascular invasion while 73.1% did not. Furthermore, analysis of tumor size showed that 27.9% of patients had small tumors (<2 cm) while 72.1% had large ones (≥2 cm). Classification of HCC patients, according to different staging systems showed that according to the BCLC system, 30.8% of patients were found to have early HCC (stage 0-A), with 30.8% being classified as intermediate (stage B) and 22.1% as advanced HCC (stage C), while only 16.3% demonstrated criteria of end-stage HCC (stage D). Application of CLIP staging system demonstrated that HCC was identified as early stage (0–1 point) in 47.1% of patients, intermediate stage (2–3 points) in 43.3%, and advanced stages (≥4 points) in 9.6% of study subjects, while the Okuda staging system showed 45.2% of patients to have early stage HCC (stage I), with 49% having intermediate (stage II) and 5.8% having advanced HCC (stage III).

3.2. Performances of candidate biomarkers compared with AFP for predicting HCC

The multiple logistic regression analysis indicated that the increase in AFP, MK, TRX and M1A levels were significantly associated with the presence of HCC ($P < 0.05$) (Table 2). The significant P value of the Hosmer–Lemeshow test was 0.535, indicating the acceptable goodness of fit of the model. Based on the AUC of ROC analysis, the performances of the candidate biomarkers were evaluated for identification of HCC and illustrated in Table 3. At a cut-off 400 U/L, the AUC of AFP was 0.69 with absolute specificity and 29% (30/104 patients) sensitivity. MDK had a greater AUC than TRX and M1A for discriminating patients developing HCC from those with LC as demonstrated in Fig. 1 (A–D). According to their optimal diagnostic cut-off, the three biomarkers (MDK, TRX and M1A) had higher sensitivity and lower specificity compared with AFP. In HCC patients, MDK was positive in 71.6% (53/74 patients) with AFP values less than 400 U/L, TRX was positive in 66.2% (49/74) and M1A was positive in 74.3% (55/74).

3.3. Diagnostic model using candidate markers

For enhancing the performance of AFP, the significant variables in univariate analysis were inserted in a stepwise logistic regression analysis with consequent development of a novel model that combined the most discriminatory factors (AFP, MDK, TRX and M1A) for predicting HCC in patients with LC. The model is illustrated as follows: $0.533 + 0.146 \times \log$ AFP $+ 0.062 \times$ MDK (ng/mL) $+ 0.004 \times$ TRX (ng/mL) $+ 0.009 \times$ M1A (ng/mL). This version yielded AUCs of 0.94 and 0.96 for discriminating patients with HCC from those with LC and non-malignant liver disease, respectively, compared with AUCs of 0.69 and 0.7 for AFP. These values were higher than their individuals as presented in Table 3 and Fig. 1(E–F). The AUC (95% CI) of the model was 0.97 (0.94–1.0) for distinguishing patients with HCC from those with cirrhosis Child A, 0.92 (0.87–0.99) for Child B, and 0.85 (0.80–0.98) for Child C with sensitivity of 90% and specificity of 100%, 85% and 69%, respectively.

3.4. Diagnostic power of model in three common staging systems of HCC

The performance of the model increased with the increase in HCC severity and HCC progression (Table 4). This model had a good performance in predicting early HCC in cirrhotic patients with AUC of 0.93, 0.94 and 0.94 in patients having only one single nodule, absent macrovascular invasion and tumor size <2 cm, respectively, compared with 0.71, 0.6 and 0.59 for AFP (Fig. 2 (A)). Better performance was achieved in the prediction of HCC in the early stages in the three established staging systems with AUCs of 0.91 for BCLC

Table 2
Candidate blood markers independently associated with the presence of HCC.

Factor	Beta	Standard error	OR	95% CI	*P*-value
AFP	−0.053	0.021	0.949	0.910–0.989	0.013
MDK	−0.095	0.030	0.909	0.857–0.965	0.002
TRX	−0.026	0.010	0.974	0.956–0.993	0.005
M1A	−1.087	0.385	0.337	0.159–0.718	0.007

AFP, α-Fetoprotein; MDK, midkine; TRX, thioredoxin; M1A, 1-methyladenosine; OR, odds ratio.

Table 3
Performances of candidate metabolites and blood markers for predicting HCC.

Variable	AUC (95% CI)	Cut-off	Sensitivity %	Specificity %	Accuracy %	PPV %	NPV %	*P* value
HCC vs. liver cirrhosis								
AFP (U/L)	0.69 (0.59–0.77)	400	29	100	53	100	41	<0.0001
MDK (ng/mL)	0.81 (0.71–0.90)	1.0	76	71	74	84	60	<0.0001
TRX (ng/mL)	0.79 (0.69–0.89)	120	74	71	73	84	58	<0.0001
M1A (ng/mL)	0.75 (0.64–0.87)	36.5	74	75	74	86	59	<0.0001
Model	0.94 (0.90–0.99)	1.54	90	88	90	94	82	<0.0001
HCC vs. non malignant liver								
AFP (U/L)	0.70 (0.61–0.79)	400	29	100	62	100	55	<0.0001
MDK (ng/mL)	0.83 (0.75–0.91)	1.0	76	79	78	81	74	<0.0001
TRX (ng/mL)	0.81 (0.73–0.90)	120	74	84	79	84	74	<0.0001
M1A (ng/mL)	0.72 (0.62–0.83)	36.5	74	70	72	73	70	0.001
Model	0.96 (0.92–0.99)	1.54	90	93	92	94	90	<0.0001

AFP, α-Fetoprotein; MDK, midkine; TRX, thioredoxin; M1A, 1-methyladenosine; M1G, 1-methylguanosine; AUC, area under ROC curve; HCC, hepatocellular carcinoma; NPV, negative predictive value; PPV, positive predictive value.

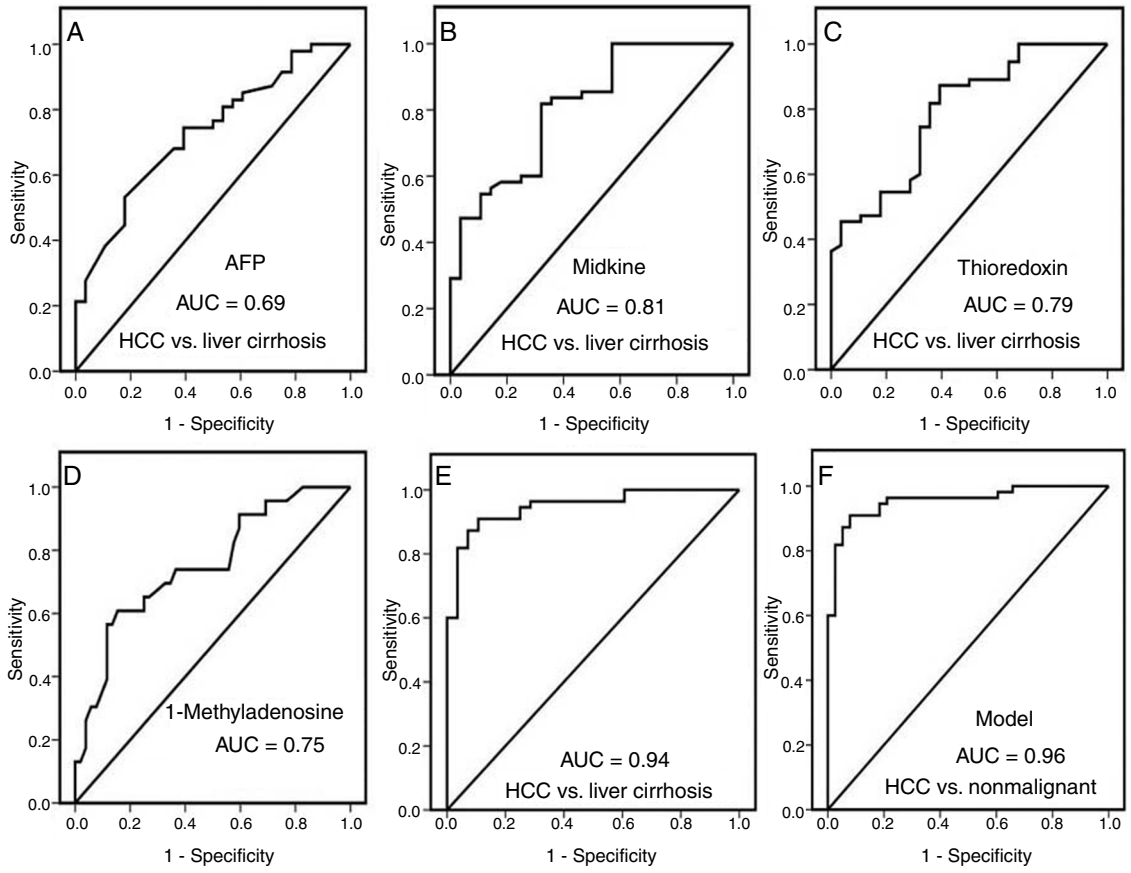

Fig. 1. Area under curve of single markers to discriminate patients with HCC from those with liver cirrhosis. (A) Alpha-fetoprotein. (B) Midkine. (C) Thioredoxin. (D) 1-Methyladenosine. Area under curve of the model to discriminate (E) patients with HCC from patients with liver cirrhosis (F) patients with HCC from patients with nonmalignant liver disease.

Table 4
Performance of model to discriminate patients with HCC from patients with liver cirrhosis and nonmalignant chronic liver disease.

Classification	AUC	HCC vs. liver cirrhosis					AUC	HCC vs. nonmalignant liver disease				
		Sen %	Spe %	PPV %	NPV %	AC %		Sen %	Spe %	PPV %	NPV %	AC %
Number of nodules												
Single	0.93	89	88	87	90	89	0.95	89	93	87	95	92
Multiple	0.95	92	88	90	90	90	0.96	92	93	90	95	93
Macrovascular invasion												
Absent	0.94	89	88	92	85	89	0.95	89	93	92	91	92
Present	0.97	93	88	81	96	90	0.98	93	93	81	98	93
Size of nodules												
<2	0.94	90	88	81	94	89	0.95	89	93	81	97	93
≥2	0.96	91	88	92	87	90	0.97	91	93	92	92	92
BCLC stage												
0–A (early)	0.91	81	88	91	88	96	0.92	81	93	81	93	90
B (intermediate)	0.95	91	88	83	94	89	0.95	91	93	83	97	93
C (advanced)	0.98	96	88	79	94	91	0.98	96	93	79	99	94
D (end-stage)	0.99	100	88	74	100	91	1.0	100	93	74	100	94
CLIP stage												
0–1 (early)	0.92	88	88	88	88	88	0.93	88	93	88	93	91
2–3 (intermediate)	0.97	91	88	87	92	90	0.98	91	93	87	96	93
≥4 (advanced)	0.98	100	88	63	100	90	0.99	100	93	63	100	94
Okuda stage												
Stage I (early)	0.94	87	88	87	88	88	0.95	87	93	87	93	91
Stage II (intermediate)	0.95	92	88	89	92	90	0.96	92	93	89	96	93
Stage III (advanced)	1.0	100	88	50	100	90	1.0	100	93	50	100	94

AC, accuracy; AUC, area under (ROC) curve; BCLC, Barcelona Clinic Liver Cancer; CLIP, Cancer of the Liver Italian Program; HCC, hepatocellular carcinoma; NPV, negative predictive value; *n*, number; PPV, positive predictive value; Sen, sensitivity; Spe, specificity.

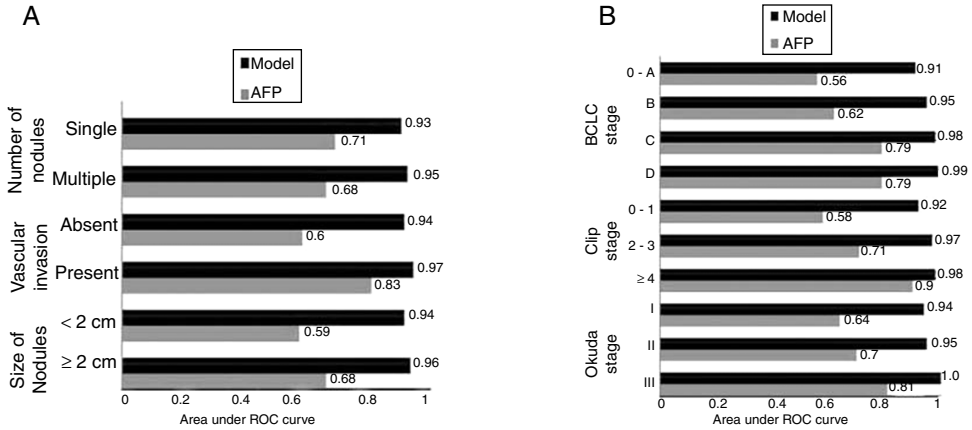

Fig. 2. Area under ROC curve of the model compared with AFP ≥ 400 U/L to discriminate HCC. (A) In patients with a tumor features as the number of nodules, macrovascular invasion and size of nodules. (B) In three common staging systems BCLC, CLIP and Okuda.

(0-A), 0.92 for CLIP (0–1) and 0.94 for Okuda (stage I), reaching optimal levels in advanced tumor stages, compared with AUCs of 0.56, 0.58 and 0.64 for AFP alone as shown in Fig. 2 (B).

3.5. Validation study

The model was evaluated in 122 new patients categorized into the HCC group ($n = 80$; 75% males and 25% females) and liver cirrhosis group ($n = 42$; 65% males and 35% females) to differentiate patients with HCC from those with liver cirrhosis. Patients in the validation study were held to the same criteria as the estimation study to test its accuracy and prove its clinical utility. There was no significant difference in patient characteristics and laboratory data between the estimated and validated groups (Table 5). ROC curve assessment of the performance of the model applied in the validation study evaluated AUC to be 0.95 (95% CI 0.92–0.99), with sensitivity of 91%, specificity of 90% and accuracy of 91%. The positive and negative predictive values were 95% and 84%, respectively. No significant difference was found between AUC in the estimation and the validation groups.

4. Discussion

HCC is the sixth most widespread tumor and the third leading cause of cancer-related death worldwide. As a result, it is important to identify blood markers that correlate with the pathological features and progression of HCC. To date, AFP is the most widely used blood markers to definitively establish the diagnosis of HCC [24]. However, AFP was found to be raised in 40% of patients with liver disease with values fluctuating in parallel with inflammatory activity [25]. In the current study, AFP at cut-off 400 U/L only had 29% sensitivity, a value that alone was unable to confirm the diagnosis of HCC.

The conjunctive use of AFP with TRX, MDK and M1A had not previously been studied, prompting this study group to attempt to develop and validate a non-invasive model to improve early HCC

Table 5
Laboratory data of the validation group.

Variable	Cirrhosis ($n=42$)	HCC ($n=80$)	*P*-value
Age (years)	57 9 ± 8.0	60.7 ± 6.8	0.047
Alanine aminotransferase (U/L)	52 (32–65)	53 (45–70)	0.111
Aspartate aminotransferase (U/L)	40 (29–65)	60 (36–64)	0.090
Total bilirubin (mg/dL)	1.4 (0.9–2.1)	1.4 (1.0–2.45)	0.869
Albumin (g/L)	33.8 ± 8.1	32.9 ± 6.3	0.360
Prothrombin-INR	1.3 ± 0.3	1.4 ± 0.3	0.305
Platelet count ($\times 10^9$/L)	101 (54–160)	87 (65–116)	0.838
Hemoglobin (g/dL)	11.6 ± 2.2	11.7 ± 2.2	0.843
Total leucocytic count ($\times 10^9$/L)	4.9 (3.6–7.6)	4.9 (3.5–6.7)	0.278
Creatinine (mg/dL)	1.11 ± 0.36	1.02 ± 0.27	0.132
Quantitative PCR (IU/ml))($\times 10^5$)	2 (0.2–11.4)	1.1(0.4–3.7)	0.427
α-Fetoprotein (U/L)	4.1 (3.0–8.4)	34(11.6–243)	<0.0001
Log AFP	0.73 ± 0.35	1.80 ± 1.01	<0.0001
Thioredoxin (ng/mL)	84 (43–126)	128 (114–134)	<0.0001
Midkine (ng/mL)	0.79 (0.29–1.7)	2.01 (1.04–2.58)	<0.0001
1-Methyladenosine (ng/mL)	36.1 (35.8–40)	41 (36.8–53)	<0.0001
1-Methylguanosine (ng/mL)	16.2 ± 3.7	17.4 ± 3.6	0.151

detection. Tsuchiya et al. [26] reported that midkine and thioredoxin were elevated in patients with HCC more than those with liver cirrhosis and were considered useful biomarkers for HCC diagnosis. In this work, midkine was able to identify HCC patients with an AUC of 0.81, a finding corroborating previous reports of AUCs ranging from 0.70 to 0.99 for MDK in predicting HCC [12,13,27]. Thioredoxin is an antioxidant molecule which cells employ to regulate the free radicals and maintain the thiol-disulfide balance [15]. Thioredoxin in the current study had an AUC of 0.79 to identify HCC patients, while earlier studies reported that TRX had AUCs ranging from 0.85 to 0.90 [16,26]. This discrepancy in results regarding TRX may be explained by the diversity in population and ethnicity, genetic variations, use of different cut-off value, or variable etiology of HCC [28]. Metabolomics is a powerful technique used for the systematic analysis of abnormal metabolites linked to HCC development [17,29]. In this study, two metabolites were analyzed in serum samples using GC–MS. 1-Methylguanosine showed no significant difference in HCC patients compared to those with liver cirrhosis, whereas, 1-methyladenosine was found to be increased in patients with HCC compared to LC patients with an AUC of 0.75. This endorses previous findings of elevated 1-methyladenosine levels in patients with HCC [19,30].

Incorporating AFP with MDK, TRX and M1A led to the development of novel non-invasive model that maximizes the performance with a power higher than each individual value. AUCs were raised to 0.94 and 0.96 for distinguishing HCC patients from those of liver cirrhosis and non-malignant liver diseases, respectively. An AUC of 0.95 in the validation group confirmed the ability of the model to diagnose HCC. Several studies previously reported the sensitivity of combining AFP with des-γ-carboxy pro-thrombin to be 59%, with AFP-L3 to be 77%, and with dickkopf-1 and osteopontin to be 88.8% [31,32]. Therefore, the current proposed model appears to be superior to other models utilizing combined AFP and midkine (AUC = 0.85) [13] and midkine- talin-1 ratio (AUC = 0.88) [12]. Furthermore, this model demonstrated good performance in predicting early HCC in patients who had tumor size <2 cm with an AUC of 0.94 and a sensitivity of 90%. The sensitivity of HCC Alpha-fetoprotein routine test (HCC-ART) and simplified HCC-ART scores were 70% and 82% in the diagnosis of small-size HCC [11]. The combination of AFP and ultrasound exhibited 63% sensitivity for early HCC detection, whereas the sensitivity of CT and MRI in detection of early HCC was 62.5% and 83.7%, respectively [3]. In addition, the AUC of the validated HCC-ART was 0.95 [25], while that of the validated model combining AFP with PIVKA-II, gender, and age was 0.87 [33]. In conclusion, utilizing MDK, TRX, and M1A together with AFP may improve HCC detection in its early stage and help to overcome insufficient sensitivity of AFP.

Abbreviations

AFP	alpha-fetoprotein
BCLC	barcelona clinic liver cancer
CLIP	cancer of liver Italian program
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
M1A	1-methyladenosine
M1G	1-methylguanosine
MDK	midkine
TRX	thioredoxin

Author contribution

Mohamed M. Omran, Khaled Farid and Mona A. Omar conceived the idea of this paper. Mohamed M. Omran, Khaled Farid, Tarek M. Emran, Fathy M. Eltaweel and Ashraf A. Tabll supervised the findings of this work. Mohamed M. Omran, Tarek M. Emran and Mona A. Omar carried out the work. Khaled Farid examined the patients. Mohamed M. Omran and Mona A. Omar analyzed the data and drafted the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

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