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Predictive Role for Serum Aldo-Keto Reductase Family1 Member B10 for Early Detection of Hepatocellular Carcinoma in Egyptian Patients

Abdel-Naser Abdel-Atty Gadallah[1], Ehab Ahmed Abdel Atti[1], Tary Abdel Hamid Salman[2], Abd elSamie Othman Hassan[3]*, Hanan M. Bedair[4]

[1]Department of Internal Medicine, Faculty of Medicine, Menoufia University, Al Minufiyah, Egypt; [2]Department of Tropical Medicine, National Liver Institute, Menoufia University, Al Minufiyah, Egypt; [3]Department of Internal Medicine, Faculty of Medicine, Mansoura University, Al Minufiyah, Egypt; [4]Department of Clinical Pathology, National Liver Institute, Menoufia University, Al Minufiyah, Egypt

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***Correspondence:** Dr. Abd elSamie Othman Hassan, Faculty of Medicine, Mansoura University, Al Minufiyah, Egypt. E-mail: othmanhassanabdelsamie@gmail.com
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Abstract

BACKGROUND: Hepatocellular carcinoma (HCC) leads to a low rate of early detection. Aldo-keto reductase family 1 member B10 (AKR1B10) is associated with several types of cancer. The diagnostic significance of AKR1B10 measurement in the early stage of HCC has been poorly understood.

AIM: The aim of the study was to evaluate the diagnostic performance of serum AKR1B10 in hepatitis C virus (HCV)-related liver disorders and its unique role in diagnosing HCC.

METHODS: Serum AKR1B10 was detected by sandwich ELISA in 30 patients with HCV-related HCC, 30 patients with HCV-related liver cirrhosis, and 20 healthy controls. Both Serum AKR1B10 and α-fetoprotein (AFP) levels were analyzed, evaluated, and compared. HCV positive cirrhotic patients with and without HCC were included in the study. HCC patients with (underwent treatment, distant metastasis, advanced HCC, causes of chronic liver disease other than HCV, other liver tumors, age <18 years old, chronic inflammatory diseases, autoimmune diseases, hematological malignancy, and tumors of any organ, other risk factors for HCC) were excluded from the study.

RESULTS: Serum AKR1B10 was significantly higher in HCC patients compared to other groups. Sensitivity 86.7% and specificity 70% for HCC diagnosis with AKR1B10 were high at a cutoff value of 0.945 ng/ml, alpha-fetoprotein sensitivity 67%, and specificity 88% in early detection of HCC at a cutoff point higher than 17.9. ng/ml. Furthermore, concurrent measurement of alpha-fetoprotein and AKR1B10 had increased sensitivity to 97.6% and specificity 100% in early detection of HCC at a cutoff point higher than ≥150 ng/ml among studied groups.

CONCLUSION: AKR1B10 has a unique role as a biomarker for early-stage HCV-related HCC.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies and the third leading cause of cancer-related deaths worldwide among men aged between 40 and 59 years. HCC prevalence is high in Asia and western and central Africa. In the USA, the incidence of HCC increased during 1973–2011 on a year-on-year basis. The 5-year recurrence rate of HCC is 48.8%, and the mean survival time is between 54.4 and 70.0 months [1].

Hepatitis C virus (HCV) is responsible for 27–75% of HCC cases in Europe and the United States and >80% of the cases in Japan. Notably, HCV–positive patients present a 20-fold higher risk of developing HCC than do HCV-negative patients, which indicates a major carcinogenic role for persistent HCV infection [2].

Despite improved screening methods for the detection of potentially curable HCC, asymptomatic HCC is only sporadically diagnosed in patients. As a result, HCC is most commonly diagnosed in the advanced or terminal stage. HCC is one of the most lethal cancer types with the third highest mortality rate of all cancer types worldwide after lung cancer and gastric cancer [3].

Surgical tumor resection is considered to be an effective treatment for early-stage HCC providing a good outcome in the majority of cases. Unfortunately, due to suboptimal diagnostic methods available to detect individuals with a high risk of HCC, few HCC patients were eligible for curative therapy (transplantation, radiofrequency ablation, or resection) [3].

In the last decade, several studies have identified that many serum biomarkers have the potential to assist the early diagnosis of HCC. Alpha-fetoprotein levels were detected in the blood of many patients with advanced HCC. However, high AFP levels were unreliably detected in patients with early-stage HCC [4].

Aldo-keto reductase family 1 member Bl0 (Akr 1 Bl0), cancer-related oxireductase was originally identified as a gene whose expression was upregulated

in human HCC but was low in normal liver tissue. Akr l Bl0 upregulation was observed in several studies in certain chronic liver diseases such as chronic hepatitis B and C and steatohepatitis, which are widely recognized to represent a precancerous condition of HCC [2]. Therefore, this study aims to investigate the role of serum AKR1B10 as a screening test for early detection of HCC in HCV-infected patients.

Patient and Methods

This study was carried out over a period of 12 months (from April 2019 to April 2020). The patients were randomly selected by randomized controlled trials from those attending outpatients' clinic and inpatients of Internal Medicine Department, Menoufia University hospital and Hepatology and Gastroenterology Department, National Institute, Menoufia University.

Ethical considerations

All procedures were carried out in accordance with the ethical standards from each subject and approval from the ethics committee of the Faculty of Medicine, Menoufia University was taken.

Inclusion criteria

HCV positive cirrhotic patients with and without HCC were included in the study.

Exclusion criteria

Patients underwent treatment for HCC, HCC patients with distant metastasis, advanced HCC patients (multilocular or PVT), patients with causes of chronic liver disease other than HCV, other liver tumors (e.g., adenoma), HCC cases on top hepatitis B virus and co-infection with hepatitis C and B, age <18 years old, cases with chronic inflammatory diseases, autoimmune diseases, hematological malignancy, and tumors of any organ other than the liver were excluded from the study and patients with other risk factors for HCC (e.g., DM and obesity).

Patient preparation

Patients were submitted to thorough history taking, clinical, examination, and investigations.

All the patients underwent

History taking included age, gender, history of blood transfusion, history of the treatment of HCV, HCC, history of hepatic encephalopathy, and GIT bleeding.

Clinical examination

Including BMI, hepatomegaly, splenomegaly, ascites, oedema lower limb, spider angioma, spider native, icterus, and pallor.

Liver cirrhosis was diagnosed based on clinical findings, as well as, imaging studies (abdominal ultrasound) and laboratory results. The severity of cirrhosis was graded according to different scoring systems.

Laboratory investigations

Complete blood count was done by Sysmex XT-1800i automated hematology analyzer (Sysmex, Japan), liver profile; serum assay of ALT, AST, GGT, alkaline phosphatase, total and direct bilirubin, albumin were done using Cobas e501 Auto analyzer (Roche-Germany), thyroid functions using Cobas e601 Autoanalyzer (Roche-Germany), prothrombin time and INR prothrombin time and INR were done on fully automated system ca-1500 that depends on clotting, chromogenic and immunologic assay by thrombosed S reagent (Siemens) quantification of HCV-RNA by Abbott real-time PCR technique (Abbott Molecular Inc., Des Plaines, IL 60018 USA). Viral markers: Including HBs Ag. By electro-chemiluminescence immunoassay (ECLIA) and anti-HCV by ECLIA were done using Cobas e601 Auto analyzer (Roche-Germany).

Determination of serum AKR1 B 10 was done by ELISA. This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for AKR1B10 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any AKR1B10 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for AKR1B10 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of AKR1B10 bound in the initial step. The color development is stopped and the intensity of the color is measured.

Imaging investigation

Abdominal ultrasound (Philips iu22), (liver, spleen, ascites, and focal lesion)**.** Triphasic CT (128 MDCT Semiens biograph, Germany) of the liver for HCC cases (tumor size, location, number) (GE healthcare 128 multislieces, USA).

Statistical methodology

Data were collected, tabulated, and statistically analyzed using an IBM personal computer with Statistical Package of the Social Science (SPSS) version 22 (SPSS

Inc., Chicago, Illinois, USA). Descriptive data were presented in the form of the mean (X^-), standard deviation (SD), range, and qualitative data were presented in the form of numbers and percentages. Analytical statistics: ANOVA (f) test, Chi-square, Kruskal–Wallis test (nonparametric test), and ROC (receiver operating characteristic) curves. Results were considered significant if $p \leq 0.05$ and highly significant if $p \leq 0.01$.

Results

In the current study, there was no statistically significant difference between the studied groups regarding their demographic and clinical data ($p > 0.05$) (Table 1).

Table 1: Comparison among the studied groups regarding their demographic data and laboratory data

Studied variables	Studied groups						Test of sig.	p-value
	Group A HCC patients (n = 90)		Group B Liver cirrhosis (n = 90)		Group C Controls (n = 60)			
	No.	%	No.	%	No.	%		
Age/years								p1:0.429
Mean ± SD	59.4 ± 6.00		57.9 ± 5.04		55.8 ± 11.1		F=1.47	p2:0.090
Range	50 – 70		50 – 66		40 – 70		P=0.18	p3:0.319
BMI (Kg/m^2)							F=1.12	p1:0.732
Mean ± SD	25.6 ± 2.81		24.8 ± 2.69		27.9 ± 3.19		P=0.53	p2:0.670
Range	19.4 – 26.6		21.1 – 26.5		21.6 – 33.2			p3:0.390
Sex								p1:0.071
Male	84	93.3	69	76.7	48	80.0	X^2 3.37	p2:0.155
Female	6	6.70	21	23.3	12	20.0		p3:0.780
Urea (mg/dl)								p1:0.001**
Mean ± SD	33.8 ± 13.0		54.2 ± 10.8		14.7 ± 3.27		K=54.9	p2:0.001**
Range	13.0 – 75.0		40.0 – 71.0		10.0 – 20.0		P=0.003*	p3:0.006**
Creatinine (mg/dl)								p1:0.031*
Mean ± SD	0.96 ± 0.19		0.87 ± 0.40		0.89 ± 0.08		K=15.4	p2:0.001**
Range	0.70 – 1.35		0.69 – 3.00		0.80– 1.00		P=0.001**	p3:0.001**
ALT (IU/L)								p1:0.107
Mean ± SD	56.4 ± 24.2		44.3 ± 29.2		21.7 ± 2.27		K=25.3	p2:0.001**
Range	17.0 – 119		11.0 – 129		20 – 25		P=0.006*	p3:0.001**
AST (IU/L)								p1:0.001**
Mean ± SD	57.5 ± 23.3		52.8 ± 37.8		21.9 ± 2.42		K=29.4	p2:0.001**
Range	18.0 – 90		12.0 – 183		20 – 25		P=0.001**	p3:0.001**
Total bilirubin (mg/dL)								p1:0.139
Mean ± SD	2.30 ± 0.51		2.00 ± 0.60		1.05 ± 0.18		K=5.48	p2:0.851
Range	0.32 – 2.60		0.70 – 3.40		1.00 – 1.80		P=0.037*	p3:0.009**
Direct bilirubin (mg/dL)								p1:0.643
Mean ± SD	0.42 ± 0.23		0.54 ± 0.49		0.46 ± 0.21		K=0.534	p2:0.530
Range	0.10 – 0.90		0.18 – 2.60		0.20– 0.80		P=0.82	p3:0.633
INR								0.310
Mean ± SD	0.85 ± 0.25		0.95 ± 0.050		0.99 ± 0.32		1.88	
Range	0.85 – 1.00		0.90-1.00		0.98-1.00			
Serum albumin (g/dl)								p1:0.886
Mean ± SD	3.70 ± 0.58		3.68 ± 0.59		4.11 ± 0.36		F=4.69	p2:0.009**
Range	1.60 – 4.50		2.20 – 4.80		3.40 – 5.00		P=0.016*	p3:0.006**
ALK (IU/L)								p1:0.001**
Mean ± SD	111.9 ± 30.3		87.6 ± 17.0		94.0 ± 15.6		F=9.03	p2:0.008**
Range	65.0 – 183		14.0 – 115		60.0 – 120		P=0.002*	p3:0.335
GGT (IU/L)								p1:0.001**
Mean ± SD	103.0 ± 50.5		45.5 ± 20.1		13.7 ± 4.55		F=57.9	p2:0.001**
Range	20.0 – 200		12.0 – 126		10.0 – 20.0		P=0.001**	p3:0.001**
Hb (g/dl)								p1:0.612
Mean ± SD	12.4 ± 1.49		12.6 ± 1.98		13.0 ± 1.10		F=1.10	p2:0.144
Range	9.90 – 15.0		7.30 – 15.0		11.3 – 15.0		P=0.25	p3:0.311
WBCs x 1000/mm^3								p1:0.137
Mean ± SD	5.81 ± 2.18		6.68 ± 1.39		5.41 ± 0.67		K	p2:0.199
Range	1.70 – 9.50		3.70 – 9.00		4.00 – 6.00		9.50	p3:0.001**
Platelet x 1000/mm^3								p1:0.002**
Mean ± SD	143.1 ± 51.2		111.7 ± 81.2		350.4 ± 54.0		K	p2:0.001**
Range	16 – 226		46 – 376		200 – 400		45.6	p3:0.001**

Significance level P < 0.05. **Highly significant, *Significant, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, INR: International normalized ratio, ALK: Alkaline phosphatase, GGT: Gamma-glutamyl transferase, HCC: Hepatocellular carcinoma, N: Number, Hb: Hemoglobin, WBC: White blood cells, CBC: Complete blood count, X^2: Chi-squared test, F: ANOVA test, K: Kruskal test, SD: Standard deviation, HCC: Hepatocellular carcinoma. P1: Comparison between group A HCC patients and group B Liver cirrhosis. P2: Comparison between Group A HCC patients and Group C Controls. P3: Comparison between Group B Liver cirrhosis and Group C Controls.

While we showed a highly statistically significant difference between studied groups regarding routine laboratory data such as urea, creatinine, AST, ALT, albumin, alkaline phosphatase, GGT, total bilirubin, and platelet count while there was no statistically significant difference among the studied groups regarding HB and INR ($p > 0.05$) (Table 1).

Table 2 shows a highly statistically significant increase in splenic size in the HCC group when compared to the liver cirrhosis group ($p < 0.001$). While there was no significant difference between studied groups regarding ascites, child-Pugh score, collaterals, PV diameter, and this implies a good selection of cases ($p > 0.05$).

Our study showed a significant difference between studied groups regarding AKR1B10, they were increased in the HCC group when compared to HCV cirrhotic patients and control group, p = 0.001 with mean ± SD 3837.7 ± 334.7, 1176.0 ± 187.7, and 664.7 ± 402.2, respectively, also AFP was significantly increased in the HCC group when compared to HCV cirrhotic patients and control group, p = 0.001 with mean ± SD 6173.0 ± 14080.8, 12.7 ± 5.5, and 7.20 ± 4.25, respectively (Table 3).

In the present study, there was a negative significant correlation between AKR1B10 and FIB4, INR, HB, and total-Bilirubin HCC patients ($p < 0.05$). While there was a positive significant correlation between AKR1B10 with a number of focal lesions, WBC, and AST ($p < 0.05$). On the other hand, there were no significant correlation betweenAKR1B10 and age, APRI score, MELD score, urea, creatinine, ALT, Alb, ALK, GGT, and Child-Pugh score ($p > 0.05$) (Table 4).

ROC analysis indicated that AKR1B10 had a sensitivity of 86.7% and specificity 70% in early detection of HCC among studied groups at a cutoff point higher than 0.945 pg/ml level.

Table 2: Comparison among the studied groups regarding their abdominal ultrasound and child-Pugh score

Studied variables	Studied groups				Test of sig.	p-value
	Group A HCC patients (n = 90)		Group B Liver cirrhosis (n = 90)			
	No.	%	No.	%		
Ascites					X^2	p: 0.278
Yes	18	20.0	9	10.0	4.88	
No	72	80.0	81	90.0		
Collaterals						p: 1.00
No	0	0.00	0	0.00	X^2	
Lieno renal	30	3.33	30	3.33	80.0	
Peri esophageal	30	3.33	30	3.33		
Gastro esophageal	30	3.33	30	3.33		
Splenic size (cm)					F	p: 0.001**
Mean ± SD	15.7 ± 0.80		11.7 ± 0.93		68.5	
Range	15 – 17		10 – 13			
PV diameter (cm)					F	p: 0.149
Mean ± SD	15.3 ± 0.99		15.0 ± 0.83		47.6	
Range	14 – 18		14 – 16			
child - Pugh score					X^2	p=0.79
Score A	72 (80)		81 (90)		1.46	
Score B	18 (20)		9 (10)			
Score C	0 (0.0)		0 (0.0)			

Significance level P < 0.05. χ^2: Chi-squared test, F: One way ANOVA test. **: Highly significant *Significant.

Table 3: Comparison among studied groups regarding AKR1B10 and fetoprotein levels

Studied variables	Studied groups			p-value ANOVA	p-value
	Group A HCC patients (n = 90)	Group B Liver cirrhosis (n = 90)	Group C Controls (n = 60)		
AKR1B10 level (pg/ml))					
Mean ± SD	3837.7 ± 334.7	1176.0 ± 187.7	664.7 ± 402.2	U=24.7	p1:0.001**
Median	882.5	662.5	490.0	p=0.001*	p2:0.001**
Range	700 – 16500	500 – 6370	300 – 1635		p3:0.001**
Fetoprotein (ng/mL)					
Mean ± SD	6173.0 ± 14080.8	12.7 ± 5.58	7.20 ± 4.25	F=17.8	p1:0.001**
Median	220	14.0	5.00	p=0.001*	p2:0.001**
Range	3.19-71000	5.00 – 25.0	3.00 – 18.0		p3:0.001**

Significance level P < 0.05**: Highly significant, AKR1B10: Aldo-keto reductase Family1 member B10, HCC: Hepatocellular carcinoma, U: Mann–Whitney test. P1: Comparison between Group A HCC patients and Group B Liver cirrhosis. P2: Comparison between Group A HCC patients and Group C Controls. P3: Comparison between Group B Liver cirrhosis and Group C controls.ww

Table 4: Correlation between AKR1B10 and different parameters in HCC patients

	AKR1B10	
	R	p-value
Age (years)	0.216	0.253 NS
BMI	0.057	0.763NS
APRI score	–0.186	0.326NS
Size of tumors (cm)	0.021	0.530NS
No. of focal lesion	0.367	0.039*
MELD score	0.081	0.671NS
FIB4	–0.456	0.014*
INR	–0.257	0.017*
HB (g/dl)	–0.345	0.032*
WBC	0.406*	0.026*
PLT	0.211	0.263NS
Urea (mg/dl)	–0.200	0.288NS
Creatinine (mg/dl)	–0.185	0.327NS
ALT (IU/L)	–0.150	0.429NS
AST (IU/L)	0.550	0.017*
ALB (g/dL)	0.015	0.938NS
ALK (IU/L)	0.038	0.840NS
GGT (IU/L)	0.150	0.430NS
AFP (ng/mL)	–0.043	0.034*
Total-Bil (mg/dL)	–0.375	0.042*
Child-Pugh score	0.081	0.671NS
BCLC stage	–0.109	0.566NS

NS: Non-significant correlation. *Significant correlation.

While alpha-fetoprotein had a sensitivity of 67% and specificity of 88% in early detection of HCC among studied groups at a cutoff point higher than 17.9.ng/ml (Figures 1-3), while combined alpha-fetoprotein and AKR1B10 had an increased sensitivity of 97.6% and specificity 100% for early detection of HCC among studied groups at a cutoff point higher than ≥150 ng/ml (Table 5).

Table 5: ROC analysis

ROC curve	HCC patients		
	AKR1B10 ng/ml	Alpha fetoprotein ng/ml	Alpha fetoprotein combined with AKR1B10 ng/ml
AUC	0.828	0.705	0.867
Cutoff point	0.945	>17.9	≥150
p-value	0.038*	0.041*	0.002*
Sensitivity (%)	86.7%	67%	97.6%
Specificity (%)	70%	88%	100%
PPV (%)	73%	77%	89%
NPV (%)	82%	81%	86%
Accuracy (%)	71%	80%	93%

Discussion

HCC is now the fifth most common cancer in the world and the third cause of cancer-related mortality as estimated by the World Health Organization. In African and Asian countries, the diagnosis of HCC at earlier ages is attributed to a synergy between HBV and dietary aflatoxins, which is thought to induce mutations in the TP53 gene.

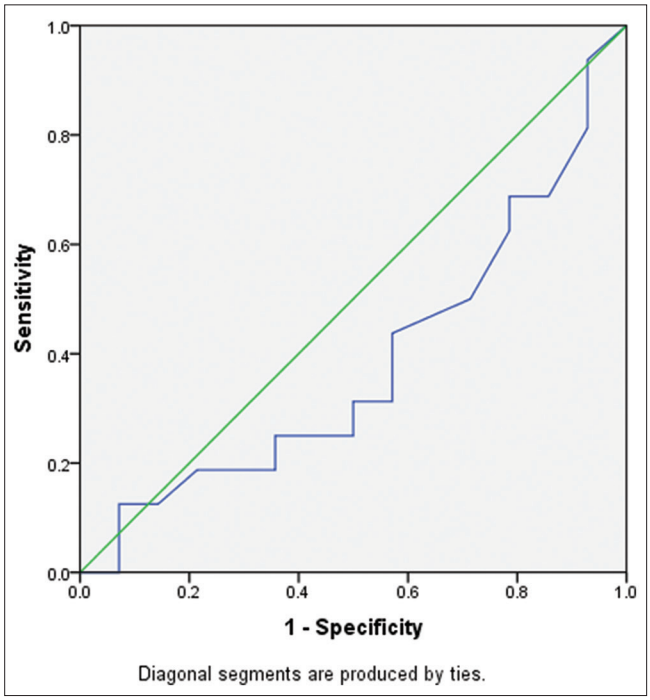

Figure 1: ROC curve of AKR1B10, for the detection of hepatocellular carcinoma

HCCs are not homogeneous and certain HCCs may have normal or only mildly elevated levels of AFP compared with healthy individuals. The highest sensitivity and specificity of AFP for diagnosis of HCC (60–80% and 70–90%, respectively) were achieved at a cut of value of 16 nm/ml, but AFP was not expressed

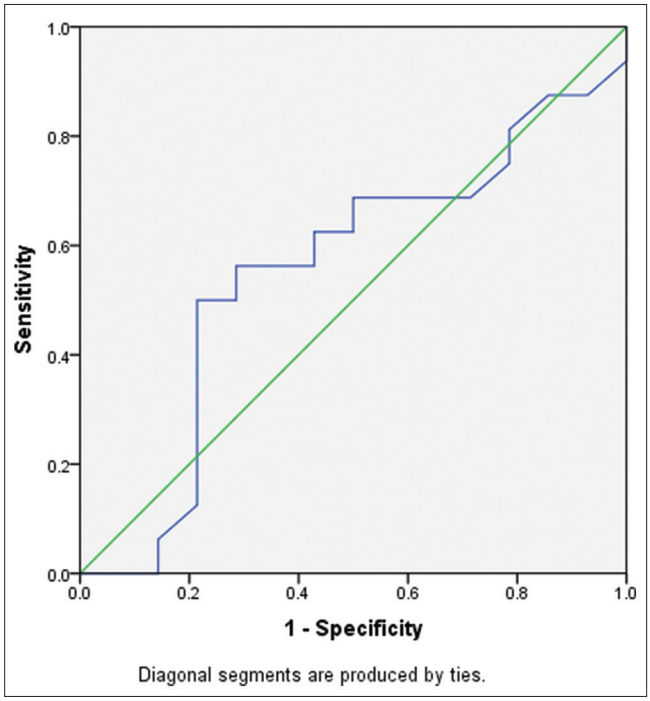

Figure 2: ROC curve of alpha-fetoprotein for detection of hepatocellular carcinoma and liver cirrhosis

in approximately 30–40% of patients with HCC, which makes this diagnostic approach less reliable [5].

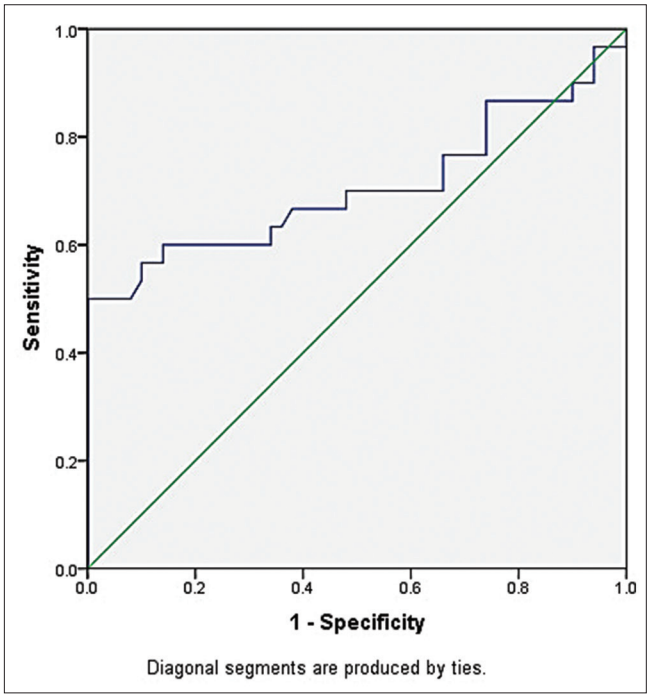

Figure 3: ROC curve of alpha-fetoprotein combined with AKR1B10 for detection of hepatocellular carcinoma

The present study was carried on 180 patients classified into 90 patients with HCV-related liver cirrhosis complicated with HCC (Group I), 90 patients with post HCV liver cirrhosis (Group II). The selected patients were compared to sixty age- and sex-matched healthy controls (Group III). Our results revealed no statistically significant difference between the studied groups regarding their demographic and clinical data. The mean age of the studied patients in Group A was 59.4 ± 6.00 years and it was 57.9 ± 5.04 years in Group B while it was 55.8 ± 11.1 years in Group C, also mean BMI in Group A was 25.6 ± 2.81 kg/m^2 and 24.8 ± 2.69 kg/m^2 in Group B versus 27.9 ± 3.19 kg/m^2 in Group C. These results were consistent with the study conducted by Sato *et al*. [6], on 40 HCC patient and 40 controlled demonstrated that, according to the case-match design, age and gender were similar in both groups, also there was no statistically significant difference between the studied groups regarding BMI. The additional study which was conducted by El-Moety *et al*. [7] found no statistically significant difference between the studied groups as regards their age, sex, and BMI.

The current study showed that there was a highly statistically significant increase in WBC and decrease in PLT count between the liver cirrhosis groups compared to the control group. Furthermore, the PLT count was significantly lower in the cirrhosis group than in the HCC group. While, there was no statistically significant difference among the studied groups as regard HB. In patients with cirrhosis, there is a redistribution of platelets, with up to 90% of the circulating platelet mass located in the enlarged spleen [8]. Our results agreed with that of Mori *et al*. [9] who demonstrated a statistically significant difference as regard PLT count. This is also in agreement with a study conducted by Wei *et al*. [10] who found a significant increase in PLT in group C, but there was no significant difference in the lymphocyte, RBC, hemoglobin, and among studied groups.

The current study revealed a highly statistically significant increase in creatinine, AST, AFP, ALK, and GGT levels in the HCC group compared to the liver cirrhosis group and control group. Furthermore, blood urea level and ALT level were significantly higher in the HCC group than in controls while albumin level was significantly lower in the HCC group than controls. This agreed with the study conducted by Hsu *et al*. [10], who revealed a statistically significant increase in the HCC group as regard ALT, AST, and AFP when compared to the healthy control group, also they found no statistically significant difference in total bilirubin between studied groups. However, there was a statistically significant increase in creatinine levels in HCC patients than in healthy control. Similarly, Sato *et al*. [6], demonstrated that serum ALT and AFP were significantly higher in HCC cases than in control cases.

Our results are in the same line with that conducted by Han *et al*. [3] indicated that AFP levels were below the threshold for HCC diagnosis. HCCs were not homogeneous and certain HCCs may have normal or only mildly elevated levels of AFP compared with healthy individuals. Furthermore, their study identified that 62% of patients with HCC (52/84) had serum AFP levels of <200 ng/ml, and 200 ng/ml is considered to be the diagnostic value of HCC in clinical practice [11].

A high AFP concentration ≥400 μg/L in HCC patients is associated with greater tumor size, bilobar involvement, portal vein invasion, and a lower median survival rate [10]. Another study was conducted by Han *et al*. [3], enrolled a total of 84 patients with HBV/HCV-related HCC, they found that (65.9%) of patients had serum AFP levels of <100 ng/ml. In accordance with our study Mori *et al*. [9] found a significant difference between studied groups as regard AFP.

There was a conflict with the study of El-Moety *et al*. [7], as they demonstrated that, serum albumin level showed that ALP level was significantly higher in patients with cirrhosis and HCC than patients with cirrhosis. Furthermore, there were no statistically significant differences regarding creatinine. Patients with advanced cirrhosis almost always have hypoalbuminemia caused both by decreased synthesis by the hepatocytes and water and sodium retention that dilutes the content of albumin in the extracellular space [8].

The current study showed that there was a highly statistically significant increase in the HCC group as regard spleen size than the liver cirrhosis group

($p < 0.001$). Furthermore, there was no significant difference regarding ascites, collaterals, PV diameter, and Child-Pugh score. A cohort study conducted by Chen *et al*. [11] showed that the spleen volume and spleen multidimensional index increased with increasing Child-Pugh class of cirrhosis. Many previous studies have introduced spleen size as a diagnostic criterion for cirrhosis [12]. Furthermore, Tsushima and Endo [13] reported that the spleen longitudinal diameter in patients with a non-alcoholic fatty liver disease was significantly higher than in healthy subjects. In addition, Murata *et al*. [14] showed that patients with primary biliary cirrhosis (PBC) tended to have a larger spleen, especially in PBC patients who developed symptoms. However, some reports indicated that the spleen size in patients with alcoholic cirrhosis was smaller than in those with hepatitis C and non-alcoholic steatohepatitis cirrhosis [12], [15]. On the contrary of our results, El-Moety *et al*. [7] found a statistically significant increase regarding Child-Pugh Score (Classes B and C) in patients with cirrhosis and HCC than patients with cirrhosis. Furthermore, Fattovich *et al*. [16] found in a surveillance program of a cohort of 313 Italian cirrhotic patients of different etiologies (Child-Pugh Classes A, 58%: B, 34%; and C, 8%) for early diagnosis of HCC based on ultrasonography determination at 6-month intervals. Child-Pugh Class B/C cirrhosis at entry was found to be an independent prognostic factor for HCC, with a 3-fold increased risk [17]. In another prospective study of 400 Chinese cirrhotic patients, Child-Pugh Class B cirrhosis (3-fold increased risk), or Child-Pugh Class C cirrhosis (8-fold increased risk) were independent prognostic factors for HCC [18].

The current study showed that there was a statistically highly significant increase of AKR1B10 level in the HCC group compared to both HCV liver cirrhotic and control groups, this stepwise increased level of AKR1B10 from cirrhosis to HCC might indicate its potential role in the early stage of hepatocarcinogenesis. Our results were consistent with the study conducted by Han *et al*. [3] who indicated that patients with HCC have a significantly higher level of serum AKR1B10 compared with patients with hepatic cirrhosis and chronic hepatitis. This suggests that the concentration of AKR1B10 associates well with the development of HCC and thus could be a potential biomarker for early HCC detection. This is consistent with the high expression of AKR1B10 in HCC tissues as previously mentioned [19], [20], [21].

This is also in agreement with the findings detected by Matkowskyj *et al*. [19] who found that serum AKR1B10 levels were significantly increased in patients with HCC when compared to patients with non-cancerous hepatic disease, including liver cirrhosis and chronic hepatitis. Furthermore, Tsuzura *et al*. [22] showed that AKR1B10 expression levels were significantly higher in livers with chronic hepatitis or cirrhosis, which are preneoplastic conditions underlying HCC than in normal livers, while, AKR1B10 expression was still higher in HCCs.

Poor outcomes for patients with advanced HCC are largely due to the lack of biomarkers to identify tumors in the early stages before progression to advanced stages and metastasis, resistance to pharmacological interventions, and a high degree of intratumor heterogeneity. The identification of biomarkers for the different pathophysiological stages of HCC is critical to improve early disease detection and enable the early implementation of chemotherapy or surgical resection to prevent progression to deadlier advanced stages and tumor metastases [23].

AKR1B10 is emerging as a promising biomarker for HCC, higher AKR1B10 expression is correlated with better long-term outcomes, such as increased survival rate and lower metastatic incidence. AKR1B10 expression is also higher in more advanced states of HCC, indicating that it would serve as a useful biomarker for a good prognosis in patients with HCC. AKR1B10 may likewise represent a potential therapeutic target for the treatment of HCC. A number of experimental studies have shown that silencing of AKR1B10 prevents tumor growth and metastasis and induces cell death [24].

The current study showed that ROC analysis indicated that AKR1B10 had sensitivity 86.7% and specificity of 70% in early detection of HCC among studied groups at a cutoff point higher than 0.945 pg/ml level. Furthermore, AKR1B10 had a sensitivity of 80% and specificity of 74% in early detection of liver cirrhosis among studied groups at a cutoff point higher than 0.737 pg/ml level. While, alpha-fetoprotein had a sensitivity of 67% and specificity of 88% in early detection of HCC among the studied group at a cutoff point higher than 17.9. Similarly, a multicenter study conducted by Ye *et al*. [25], who validated AKR1B10 as a useful marker for the detection of HCC, found that serum AKR1B10 levels were >18 times higher in HCC patients compared to healthy individuals (1567.3 ± 292.6 pg/mL vs. 85.7 ± 10.9 pg/mL). Similar results were detected by Han *et al*. [3] who found that AKR1B10 at a cutoff value of 1.51 pg/ml had 81% sensitivity and 60.9% specificity of serum AKR1B10 for diagnosing HCC.

A multicenter study conducted by Ye *et al*., which validated AKR1B10 as a useful marker for the detection of HCC, found that serum AKR1B10 levels were >18 times higher in HCC patients compared to healthy individuals (1567.3 ± 292.6 pg/mL vs. 85.7 ± 10.9 pg/mL) [25]. Our results were consistent with that of the study conducted by Han *et al*. as their results indicated that AKR1B10 at a cutoff value of 1.51 pg/ml had 81% sensitivity and 60.9% specificity of serum AKR1B10 for diagnosing HCC. This is also in agreement with Hsu *et al*. [10] who detected that optimal diagnostic cutoff of 267.9 pg/mL was determined using a training cohort of 519 participants. Serum AKR1B10 showed better diagnostic parameters of the area under the curve (AUC) 0.896, sensitivity 72.7%,

and specificity 95.7%, than AFP (AUC 0.816, sensitivity 65.1%, and specificity 88.9%).

In our results, ROC analysis indicated that alpha-fetoprotein combined with AKR1B10 had a sensitivity of 97.6% and specificity 100% in early detection of HCC among studied groups at a cutoff point higher than ≥150 ng/ml. A similar finding was detected by Han *et al*., who revealed that the combination of the two markers showed better diagnostic accuracy than either one alone. AKR1B10 also showed diagnostic potential for patients with early-stage HCC and AFP-negative HCC. Similar findings were recently reported in a study involving 78 HCC and 63 non-HCC patients [3].

An analysis by Kanno *et al*. [26] revealed that serum AKR1B10 levels and HCC incidence relative to fibrosis stage, severe fibrosis was found to be associated with high AKR1B10 levels in HCC and non-HCC patients while our study showed that there was a negative significant correlation between AKR1B10 and FIB4, INR, HB, and total-Bilirubin HCC patients ($p \leq 0.05$). While, there was a positive significant correlation between AKR1B10 with the number of focal lesions, WBC, and AST ($p < 0.05$). On the other hand, there was no significant correlation betweenAKR1B10 and age, APRI score, MELD score, urea, creatinine, ALT, Alb, ALK, GGT, and Child-Pugh score ($p > 0.05$).

Add strengths and limitations of this study.

Conclusion

We can conclude that theAKR1B10 has higher sensitivity and low specificity than alpha-fetoprotein for HCC detection, the unique expression of AKR1B10 in early-stage HCC makes it a stronger candidate biomarker compared with others for early detection of HCC, evaluating combined AKR1B10 and AFP levels has some promising clinical implications, since a high diagnostic accuracy for HCC by concurrently examining these two biomarkers appears to be achievable. Moreover, AKR1B10 may be used as a potential target for HCC-directed drug therapy that warrants further investigation.

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